Hongzhang Chen

Biotechnology of Lignocellulose

Theory and Practice

Chemical Industry Press

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Preface

Biotechnology of Lignocellulose focuses on biotechnologies in the application of natural lignocellulosic materials, which has not been fully utilized because of the concomitant formation of cellulose, hemicellulose, and lignin. In this book, the concept of natural lignocellulose has two meanings: One refers to renewable resources synthesized through photosynthesis, and the other refers to such resources that have a complex intertwined structure of cellulose, hemicellulose, and lignin.

Natural lignocellulosic materials are the most abundant renewable organic matter on Earth. Biomass in large numbers and a wide variety exists from the forest to the sea, and new biomass is constantly generated through photosynthesis. According to statistics, there are about 180 billion tons of biomass above the ground, 40 million tons in the oceans, and almost an equal amount in soil as there is above the ground. Most of the natural lignocellulosic materials are decomposed and transformed by a variety of microbes in the natural environment and ultimately converted into CO_2 and H_2O. This is an important part of the carbon cycle ecosystem but undoubtedly a huge waste for humans from the point of natural resources utilization.

Along with the rise of green chemistry, the proposal of recycling economy and sustainable development strategies, conversion, and research in the application of natural lignocellulosic feedstock are highly valued and widely used. The development of biotechnology especially provides a broad technology platform for the comprehensive utilization of natural lignocellulosic materials. The strategic task of green chemistry is to make biomass as raw materials for chemicals. Since the late 1960s, humans have gradually recognized the negative impact of the coal and petroleum chemical industry on the environment. Therefore, scientists have begun to consider how to reuse biomass instead of coal and oil to produce chemical substances that satisfy human requirements. Fortunately, lignocellulose biotechnology has achieved rapid development and expanded the research field significantly in recent decades. To communicate some accumulated experience acquired during these decades and recent progress in the biotechnology of lignocellulose with researchers around the world, *Biotechnology of Lignocellulose* was prepared using a large literature base and research progress in lignocellulose biotechnology concerning our own research field.

Biotechnology of Lignocellulose embodies the recent new ideas, new methods, and new progress of our research group. Concepts and ideas, such as primary refining of raw materials, fractionation and directional conversion of each component, and selective structural fractionation, were proposed because of the chemical and structural characteristics of lignocellulose. In these concepts and ideas, lignocellulose is considered a multilevel resource. Through bioconversion, lignocellulose can play roles in ecological agriculture, bioenergy, chemical industry, pulping and papermaking industry, and more and then be used for establishing a distinctive ecoindustrial park.

My achievements in this field were gained from 20 years of research under the conductive guidance of Professors Peiji Gao and Yinbo Qu from the State Key Laboratory of Microbial Technology at Shandong University and the engineering education and selfless help given by Professor Zuohu Li from the Institute of Process Engineering, Chinese Academy of Sciences. All these individuals encouraged me to obtain systematic training in lignocellulose biotechnology. Research on the biotechnology of lignocellulose has support from the National Key Basic Research Development Program of China (973 Project, No. 2011CB707401), the National Key Project of Scientific and Technical Supporting Program of China (No. 2011BAD22B02), and the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX1-YW-11A; KGCX2-YW-328).

In addition, the works of my doctors and masters were essential preconditions for publishing this book. Dr. Weihua Qiu, Master Xiaoguo Fu, Yuzhen Zhang, and Jianli Ding; and graduate students Xiang Zhang, Junying Zhao, Guanhua Wang, Lianhua Zhang, Wenjie Sui, and Zhimin Zhao participated in writing this book. Especially, Weihua Qiu and Guanhua Wang participated in the book's revision and review. Many references of our predecessors and colleagues are cited. I wish to express my sincere thanks to all of them.

Biotechnology of Lignocellulose is available for researchers engaged in lignocellulose science, scientific and technical personnel, and graduates. It also provides a reference for researchers and administrators engaged in the utilization and industrial development of agricultural resources.

Some errors may exist in this book. I sincerely hope to receive criticism and guidance from readers in this regard.

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Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline oxazole-6-sulfonic acid)
ADF	Acid Detergent Fiber
ADL	Acid-Insoluble Lignin
ADP	Average Degree of Polymerization
ADS	Acidic Detergent Solubles
AFR	Aerobic Fluorescence Recovery
AldOMT	5-Hydroxy Coniferaldehyde-O-methyltransferase
AOX	Absorbable Organic Halogens
BET	Brunauer-Emmett-Teller Method
[BMIM]Cl	Chlorinated 1-Butyl 3-Methyl Imidazole
BSC	Basal Synthesis Cellulase
CAD	Cinnamoyl Alcohol Dehydrogenase
Cadoxen	Cadmium Ethylenediamine
Cald5H	Coniferaldehyde-5-hydroxylase
CAS	Chinese Academy of Science
CBD	Cellulose-Binding Domain
CBH	Cellobiohydrolase
CBHI	CellobiohydrolaseI
CBHII	Cellobiohydrolase II
CBP	Consolidated Bioprocessing
CCH	Coumaroyl CO A-3-Hydroxylase
CCML	Cell Corner of Middle Lamella
CCoAOMT	Caffeoyl CoA 3-O-Methyltransferase
CCR	Cinnamoyl CoA Reductase
CD	Catalytic Domain
CH_4	Methane
C_3H	Coumarate-3-hydroxylase
C_4H	Cinnamic Acid-4-hydroxylase
CHS	Chalcone Synthase

4CL	4-Coumarate CoA Ligase
CLSM	Confocal Laser Scanning Microscopy
CMC	Carboxymethyl Cellulose
CML	Complex Middle Lamella
C/N	Carbon-to-Nitrogen Ratio
CO	Carbon Monoxide
Cooxen	Cobalt Hydroxide Ethylenediamine
CR-p	Plant-Conserved Region
CSTR	Continuous Stirred Tank
Cuen	Copper Ethylenediamine
Cuoxam	Copper Oxide Ammonia
DDGP	Di-β-D-glucopyranoside Permease
DES	Diethyl Sulfate
DMC	Direct Microbial Conversion
DNS	3,5-Dinitrosalicylate Method
DO	Dissolved Oxygen
DON	Deoxynivalenol
DP	Degree of Polymerization
dsRNA	Double-Stranded RNA
ED	Endoglucanase
EDA	Electron Donor-Acceptor
EM-EDXA	Electron Microscopy Energy Dispersive X-ray Analysis
EMP	Embden-Meyerhof Parnas Pathway
EMS	Methyl Sulfonic Acid Ethyl Ester
ETEK	Ethanol Teknik
EWNN	Iron–Tartaric Acid–Sodium Complex
F5H	Ferulic Acid 5-Hydroxylase
FPA	Filter Paper Activity
FPU	Filter Paper Activity Units
GLOX	Glyoxal Oxidase
G1u	Glutamic Acid
G-S-H	Guaiacyl-Syringyl-Hydroxyl-Phenyl Lignin
H_2	Hydrogen
HBT	1-Hydroxyphenyl Benzotriazole
HEC	Hydroxyl Ethyl Cellulose
HRGP	Hydroxyproline-rich Glycoprotein
H_2S	Hydrogen Sulfide
HVR	Hypervariable Region
Hyp	Hydroxyproline
IM	Interference Microscope
IPCC	Intergovernmental Panel on Climate Change
IPE	Institute of Process Engineering
IPN	Interpenetrating Polymer Network
ISO	International Organization for Standardization

LCC	Lignin-Carbohydrate Complexes
LG	1,6-β-D Glucopyranosyl
LM	Optical Microscopy
LMS	Laccase Mediator System
LORRE	Laboratory of Renewable Resources Engineering
L/W	Length-to-Width Ratio
ML	Middle Lamella
MWCO	Molecular Weight Cutoff
MWL	Milled Wood Lignin
NDF	Neutral Detergent Fiber
NDS	Neutral Detergent Solubles
NGGI	National Greenhouse Gas Inventories
NMR	Nuclear Magnetic Resonance
NMU	Methyl Nitrosourea
NREL	National Renewable Energy Laboratory
NTG	Nitrosoguanidine
OTR	Oxygen Transfer Rate
PAL	Phenylalanine Ammonia Lyase
PCL	Polycaprolactone
PEG	Polyethylene Glycol
PF/DMSO	Paraformaldehyde/Dimethyl Sulfoxide
PFT	Plug Flow
PHA	Polyhydroxyalkanoate
PLA	Polylactic Acid
PLFA	Phospholipid Fatty Acid
POD	Peroxidase
PU/B-KGM	Polyurethane/Benzyl Konjac Glucomannan
PVC	Polyvinyl Chloride
RNAi	RNA Interference
SAD	Sinapyl Alcohol Dehydrogenase
SCD	Sitosterol Cellodextrin
SCP	Single-Cell Protein
SECS	Steam-Exploded Corn Straw
SEM	Scanning Electron Microscopy
SHF	Separate Hydrolysis and Fermentation
SPE	Solid Polymer Electrolyte
SSCF	Simultaneous Saccharification and Cofermentation
SuDH	Sulfide Dehydrogenase
SUSY	Sucrose Synthase
TAL	Tyrosine Deaminase
TEM	Transmission Electron Microscopy
TS	Total Solid
UDPG	Uridinediphosphate-D-glucose
UF	Ultrafiltration

UV	Ultraviolet Absorption Spectroscopy
VIO	Violuric Acid
WPC	Wood-Plastic Composite
XDH	Xylitol Dehydrogenase
XR	Xylose Reductase
Zincoxen	Zinc Ethylenediamine

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Chapter 1
Brief Introduction to the Biotechnology of Lignocellulose

Abstract Biomass resources, from the point of bioconversion, mainly include energy storage substances (e.g., starch, fat, etc.) and the main components of plant cell walls, such as cellulose, hemicellulose, and lignin. Protein, starch, and fat have been utilized extensively and efficiently and have been reported in many times in literature. This book focuses on the application of biotechnologies in natural cellulosic feedstocks; this application has not been fully utilized because of the concomitant formation of cellulose, hemicellulose, and lignin. In this chapter, the concept of natural lignocellulose has two meanings. The first refers to renewable resources synthesized through photosynthesis; the second refers to such resources that have a complex intertwined structure of cellulose, hemicellulose, and lignin.

Keywords Natural lignocelluloses • Structural inhomogeneity • Biotechnology of lignocelluloses

1.1 Definition of Natural Lignocellulose

The concepts of biomass were first rooted in ecology, but now increasingly are defined from the point of utilization of resources and industrialization. According to the definition of the U.S. national energy security regulations, *biomass* refers to renewable organic materials, including agricultural products and agricultural waste, wood and wood waste, animal waste, urban wastes, and aquatic plants [1]. Biomass in the Japanese Industrial Standards is defined as the collection of organisms derived from organic material in Earth's biosphere recycling system [2]. Basically, biomass is synthesized through the photosynthesis of solar energy, water, and carbon dioxide. Because of its most important renewable features, biomass is also known as a renewable resource. Biomass resources are abundant. The solar energy captured by biomass is sufficient to supply the needs of 1.5×10^{11} people, more than 28 times the current world population [3].

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Biomass resources, from the point of bioconversion, mainly include energy storage substances such as protein, starch, fat, and the like and the main components of plant cell walls, such as cellulose, hemicellulose, and lignin. Protein, starch, and fat have been utilized extensively and efficiently and have been reported in many literature bodies. This book focuses on the application of biotechnologies in natural cellulosic feedstocks; these biotechnologies have not been fully utilized because of the concomitant formation of cellulose, hemicellulose, and lignin. Here, the concept of natural lignocellulose has two meanings: One refers to renewable resources synthesized through photosynthesis; the other meaning refers to resources with a complex intertwined structure of cellulose, hemicellulose, and lignin.

Natural lignocellulose is the most abundant renewable organic matter on Earth. A huge number and wide variety of biomass exists from the forest to the sea, and new biomass is constantly generated through photosynthesis. According to statistics, there are about 1.8×10^{11} tons of biomass aboveground, 4.0×10^{7} tons in the oceans, and almost equal amount in soil as there is aboveground [4]. Most natural lignocellulose is decomposed and transformed by a variety of microbes in the natural environment, and ultimately, it is converted into CO_2 and H_2O. It is an important part of the carbon cycle ecosystem, but undoubtedly is a huge waste to humans from the point of view of natural resources utilization.

China is rich in natural lignocelluloses. According to 2001 statistics, China's crop straw resources reached 7×10^{8} tons, with wood consumption for fuel of 2.13×10^{8} tons. Annual resources of about 1.36×10^{8} tons of dry human and animal feces exist, with about 3.7×10^{5} tons generated from intensive culture of livestock and poultry [5]. Rice straw, cornstalks, and wheat straw are the major straw resources in China, accounting for about 75.6 % of the total amount of straw. Rice straw accounts for 29.93 % of the total straw yield, followed by cornstalks, which account for 27.39 %; wheat straw production is in third place, accounting for 18.31 %. Other straw materials include beans, potato seedlings, and oil crop straw, accounting for 5.06, 3.47, and 7.99 %, respectively. In recent years, with the adjustment of crop structure, the proportion of economic crop residues in the total straw yield has increased significantly [6]. In addition, natural cellulose raw materials from forestry by-products, municipal waste, and industrial waste are also considerable.

1.2 Characteristics of Lignocellulose

For better development and utilization of natural cellulose materials, its characteristics should be understood:

1. Sources are extensive source, are in an enormous amount, and are renewable.
2. These materials are scattered in areas that meet certain conditions and can be seasonally produced; the supply of raw materials and the intensive production scale can be controlled flexibly.
3. They are in a multiform configuration, with cellulose, hemicellulose, and lignin having somewhat different structures and compositions.

4. They have a large specific volume.
5. These materials are cheap and currently are considered as waste.

These characteristics indicate that natural lignocelluloses have a great potential to solve some current problems, such as food shortages, energy crises, environmental pollution, and other issues facing the world. However, they also make the bioconversion of lignocellulose difficult.

Multiform utilization modes for straw exist in China, including utilization as energy, fertilizer, industrial raw materials, and substrates for edible mushrooms. Actually, the unitization mode of straw is differed from the economic development degree and industrial structure. According to field investigation and document analysis, in 2006, it is estimated that the main crop straw used for the consumption of straw as energy for rural residents, for direct application, as a feed for livestock, as a raw material for papermaking, as a substrate for edible mushrooms, and burned or as waste was about 108, 130 (containing the part not been collected), 79, 20, 10, and 86 million tons, respectively, accounting for 25, 30, 18, 4.6, 2.3, and 20 % of the theoretical amount of resources, respectively [7]. Thus, straw in China is mainly used as fuel or burned directly in the field. A high percentage of waste and a low percentage of use as industrial raw materials exists. This industry structure not only destroys the ecological balance, sterilizes the soil, causes a vicious agricultural cycle, but also results in serious environmental pollution and the potential for a fire disaster. Meanwhile, the low utilization ratio of straw burned for heat (below 10 %) is also a great waste of resources. Biodecomposition and transformation are effective for the high-value utilization of natural lignocellulose. They have great practical significance for solving environmental pollution, food shortages, and the energy crisis.

1.2.1 Composition Complexity of Lignocellulose

Cellulose, hemicellulose, and lignin constitute the main components of plant cell walls [8]. They are the major components of natural lignocellulose. The sum of these three components accounted for 80 % of the total weight of raw materials; the contents of cellulose, hemicellulose, and lignin are 30–35, 25–30, and 10 %, respectively. In addition to the three main components, lignocellulose contains protein, lipid, ash, water, pectin, low molecular weight carbohydrate, and other items. Ash content of straw is generally more than 5 % (up to 15 % of rice straw), and most of the ash is silica. The content of crude protein is low, in the range of 2–8 %, and the lipid content accounts for 1–2 % of straw. In mature straw, almost all vitamins are destroyed.

Cellulose is a macromolecular compound with the formula $(C_6H_{10}O_5)_n$, a water-insoluble homogeneous polysaccharide consisting of a linear chain of several hundred to over 10,000 β(1-4)-linked D-glucose units. Cellulose molecules often exist in the state of aggregation by arranging together. The structure of cellulose usually includes a crystalline region and an amorphous region. As for the crystalline

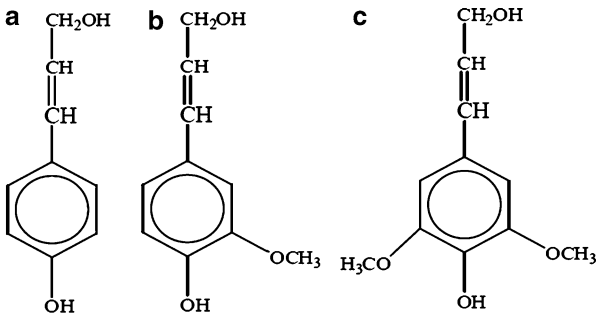

Fig. 1.1 Phenyl propane structural units of lignin: (**a**) p-hydroxyphenyl propane; (**b**) guaiacyl propane; (**c**) syringyl propane

region, the cellulose molecules are orderly arranged and are ruly, showing a clear X-ray image, and usually hinder the biodegradation of cellulose. The amorphous region of cellulose has a relaxed structure and is arranged irregularly. Generally, the polymerization degree of natural cellulose molecules is higher than 1,000.

Hemicellulose is a heterogeneous glucan with a short side chain composed of two or more monosaccharides. Sugar groups for hemicellulose are mainly xylose, glucose, mannose, arabinose, and galactose and their various derivatives. As for straw hemicellulose, it is mainly composed of poly-arab-4-O-methyl-glucuronxylans. Hemicellulose is an amorphous material with a low polymerization degree (less than 200, mostly 80–120). Generally, hemicellulose is the most complex of the components in the cell wall of lignocellulose because since it forms covalent bonds (mainly α-benzyl ether linkages) with lignin and an ester linkage with acetyl units and hydroxycinnamic acids.

Lignin is a phenolic polymer with a three-dimensional structure. It is derived primarily from three hydroxycinnamyl alcohols or monolignols (i.e., *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) by free radical generation, followed by chemical coupling processes [9] (Fig. 1.1). In accordance with its aromatic core, the phenyl propane structural units of lignin can be divided into three types: guaiacyl propane, syringyl propane, and *p*-hydroxyphenyl propane. The structure of straw lignin is dominated by guaiacyl propane and syringyl propane, with a few *p*-hydroxyphenyl propane. Cellulose and hemicellulose are mainly linked through hydrogen bonding. In addition to the strong hydrogen bonding internally in the lignin, there exists a stable lignin-carbohydrate complex.

Molecular chains arrange regularly in the cell wall structure to form protofibrils, which further form microfibrils, and then the microfibrils form fiber fines. Hemicellulose fills between protofibrils. Lignin and hemicellulose wrapped around the microfibrils, and a chemical connection exists between lignin and hemicellulose. So, in the cell wall, cellulose constitutes a cellulose skeleton in the form of microfibrils. Lignin and hemicellulose are cross-linked by a covalent bond to form a three-dimensional structure, enwrapping microfibrils inside. The outside of the cell wall (i.e., the intercellular layer) mainly is composed of lignin and pectin. Thus, cellulose, hemicellulose, and lignin are intertwined to form a complex cell wall structure to resist biodegradation.

The intertwined structure of cellulose, hemicellulose, and lignin determines that the degradation of any of the ingredients will be subject to the constraints of the other ingredients. For example, the steric hindrance of lignin on the enzymatic degradation of carbohydrates in the straw means the straw cannot be completely biodegraded. The main components of straw are chemically stable polymers; not only are they insoluble in water and common organic solvents, but also they cannot be hydrolyzed by diluted acid and alkali under normal temperatures. The enzymatic hydrolysis rate of straw without pretreatment is less than 10 %, even with excess loading of cellulase, because in untreated straw, cellulose, hemicellulose, and lignin, with a completely different chemical structure and properties, is cross-linked tightly and difficult to convert directly into high-value products.

1.2.2 Structural Inhomogeneity of Lignocellulose

As mentioned, lignocellulose has structural heterogeneity and a complex composition; it is structurally inhomogeneous. The distribution and content of the chemical composition and the structure of lignocellulose differ according to the species, tissues, origin, and growth period.

Lignocellulosic raw materials have a high degree of inhomogeneity on the organ, chemical, and tissue levels not only in different species but also the same species of lignocellulose.

1.2.2.1 Biological Structure Inhomogeneity

Biological structure inhomogeneity means that each stem, leaf, spike, sheath, and so on has a certain percentage of straw remarkably different in chemical composition and fiber morphology. For example, the fiber length of the internode and leaf sheath of wheat straw and stems of rice straw is similar to or even longer than the poplar fiber. Meanwhile, these fibers from straw are relatively narrow. Therefore, there is the potential for high-value utilization because of the high length-to-width ratio (L/W) (Tables 1.1, 1.2, 1.3, and 1.4). Usually, straw is harvested without separating the different organs, which results in coexistence of various organs and tissues. The part of wood utilized is the xylem of the trunk after the peeling process.

Structural characteristics and compositions of various organs, tissues, and cells of lignocellulose differ significantly, resulting in different conversion methods. For example, the straw skin includes epidermal cells in the outermost layer of cortex and cellulose in the subcutaneous fibrous layer. The latter is the main source of paper fiber, using the gramineae plant as a raw material for pulp making. In addition to less ash in the cortex, separating the outer layer of corn stover skin is bound to greatly increase the fiber content and consequently benefit the application of cellulose. Cornstalk cores are mainly vascular bundles surrounded by a large number of thin-walled cells. So, these cores have a relatively high content of hemicellulose,

Table 1.1 Chemical composition of lignocellulose (%, dry material) [10]

Species	Water-soluble composition	Cellulose	Hemicellulose	Lignin	Wax	Ashes
Wheat straw	4.7	38.6	32.6	14.1	1.7	5.9
Rice straw	6.1	36.5	27.7	12.3	3.8	13.3
Perennial ryegrass	4.1	37.9	32.8	17.6	2.0	3.0
Barley grass	6.8	34.8	27.9	14.6	1.9	5.7
Oat grass	4.6	38.5	31.7	16.8	2.2	6.1
Cornstalk	5.6	38.5	28.0	15.0	3.6	4.2
Corncob	4.2	43.2	31.8	14.6	3.9	2.2
Spanish grass	6.1	35.8	28.7	17.8	3.4	6.5
Sugar beet pulp	5.9	18.4	14.8	5.9	1.4	3.7
Bagasse	4.0	39.2	28.7	19.4	1.6	5.1
Oil palm fiber	5.0	40.2	32.1	18.7	0.5	3.4
Coke fiber	3.7	60.4	20.8	12.4	0.8	2.5

cellulose, and lignin. Meanwhile, the loose structure caused by the presence of a large number of thin-walled cells makes the cornstalk cores show a strong water absorption capacity. Therefore, cornstalk cores are suitable for use as a substrate for macrofungi fermentation and caramel production. Cornstalk leaves are mainly composed of epidermis and mesophyll. The ash content of the leaves is high because of the distribution of a large number of silicon cells in the epidermis. In the mesophyll, a vascular bundle is surrounded by mesophyll cells. Because of the low vascular bundle content in the leaves, the cellulose content in leaves also is relatively low. At the same time, the curling and stretching of the leaf is related to not only the motor cell but also the low degree of lignification. Therefore, compared to other parts of straw, the leaf is more appropriate for livestock feed because of its better palatability. The lignin content of the node is high because of its high lignification degree.

1.2.2.2 Significant Differences in Chemical Composition

Straw has a large amount of hemicellulose and a relatively high ash content (higher than 1 %). Taking rice straw as an example, the main components in the cell wall of dry rice straw are cellulose, hemicellulose, and lignin. In addition, there are minerals, cutin, and suberin in the cell wall. Their complex structure forms a tight recovery system for straw. In rice straw, the chemical composition also contains crude protein (mainly embodied in the cell wall in the form of structural proteins) and acids (including p-hydroxybenzoic acid, vanillic acid, coumaric acid, ferulic acid, etc). In general, the content of cellular components and cell wall material in the straw accounts for 21 and 79 % (including 33 % cellulose, 26 % hemicellulose, 7 % lignin, and 13 % silicide), respectively.

Table 1.2 Length-to-width ratio of fiber in different parts of wheat straw [11]

		Length (mm)				Width (μm)				
		Average	Maximum	Minimum	General	Average	Maximum	Minimum	General	L/W
Whole		1.32	2.94	0.61	1.03–1.60	12.9	24.5	7.4	9.3–15.7	102
Each organ	Stem	1.52	2.63	0.66	1.07–1.88	14.0	27.9	8.3	12.7–18.6	109
	Internode	1.21	2.39	0.39	0.85–1.56	11.5	24.5	7.4	8.8–15.7	105
	Leaf sheath	1.26	3.31	0.44	0.70–1.80	14.7	34.3	8.8	9.8–18.6	86
	Leaf	0.86	1.47	0.24	0.59–1.16	12.1	19.6	6.4	8.8–18.6	71
	Node	0.47	1.29	0.18	0.24–0.77	17.8	43.1	8.3	9.8–27.0	26

Table 1.3 Length-to-width ratio of fiber in different parts of rice straw [11]

		Length (mm)				Width (μm)				
		Average	Maximum	Minimum	General	Average	Maximum	Minimum	General	L/W
Whole		0.92	3.07	0.26	0.47–1.43	8.1	17.2	4.3	6.0–9.5	114
Each organ	Stem	1.00	2.13	0.47	0.75–1.17	8.9	20.6	4.3	6.5–12.9	112
	Internode	0.85	2.61	0.26	0.44–1.14	8.1	12.3	4.9	5.9–9.8	105
	Leaf sheath	0.64	1.21	0.18	0.39–0.88	6.7	9.3	4.9	5.9–8.3	96
	Leaf	0.33	0.68	0.14	0.20–0.46	9.9	14.7	4.9	7.4–13.7	33
	Node	0.58	1.38	0.18	0.29–0.88	10.1	17.2	5.9	8.3–13.7	57

Table 1.4 Morphological characteristics of several poplar fibers [12]

Species	*Populus diversifolia*	*Aspen wood*	*Populus tomentosa*	*Populus ussuriensis*	*Lombardy poplar*	*Populus* × *canadensis Moench cv. I-214*	*Populus euramericana San Martino-72*	*Populus derides 'Harvord'*	*Populus derides 'Lux'*	*Populus dakanensis*
Average length (mm)	0.94	1.38	0.82	1.32	0.90	1.13	1.11	1.04	1.04	1.19
Average width (μm)	22.8	18.5	20.8	24.0	21.6	24.0	24.0	22.0	23.0	25.0
L/W	41.2	74.0	34.4	55.0	42.0	47.0	46.3	47.3	45.2	47.6

Table 1.5 Chemical differences of wheat straw and wood [13]

		Wheat straw	Wood
Lignin	Phenolic hydroxyl (C9 phenyl propane unit)	0.4–0.45/C9	0.1–0.3/C9
	Phenolic hydroxyl in lignin	44/100 units	9–13/100 units of birch
	Carboxyl in lignin	13.8/100 units	
	Carboxyl in lignin	0.1–0.2/C9	–
	Proportion of ester in lignin	2.60 % ester bond 1–2.8 %	Almost absence of ester bond
	Ferulic acid in lignin	α-Ether bond	–
	Molecular weight of kraft lignin	7,500	18,200 (birch)
	Polydispersity		
	Proportion of lignin unit (V:S:H)	1:0.77:0.31	1:1.2:0.05 (hardwood) 1:0.02:0.009 (softwood)
	Lignin carbohydrate complexes (LCCs)	Accounting for 60 % of water-soluble lignin	Without LCC
Others	Polymerization degree of hemicellulose	84	120
	Structural composition of hemicellulose	90 % of xylan	80–90 % of xylan (hardwood) 60–70 % of mannose (softwood)
	Crystallinity of cellulose	40–60 %	60–80 %
	Organic extracts	Less than 1 %	4 % for softwood Less than 1 % for hardwood
	Ashes	8 % (60 % are SiO_2)	Less than 1 % (mainly contains CaO, K_2O, Na_2O)
	Characteristics of fiber	1.0–1.5 mm (length)	5 mm (length)
	Microfibrils and hybrid fiber	39 %	5 % (softwood) 25 % (hardwood)

1.2.2.3 Morphological Differences of Cellulose

Straw has a relatively high content of microfibrils and hybrid cell (about 40–50 %) and a low content of fiber cells (40–70 %). Wood has a low hybrid cell content and a high fiber cell content. For example, the fiber cell contents of hardwood and softwood are 60–80 and 90–95 %, respectively. Table 1.5 indicates that wheat straw and wood have obvious differences not only in chemical composition but also in the morphological structure of lignin and hemicellulose.

In sum, there is significant difference between straw and wood. Extensive research is necessary concerning the inhomogeneous composition and structure of straw and to separate the components of straw on the basis of their characteristics.

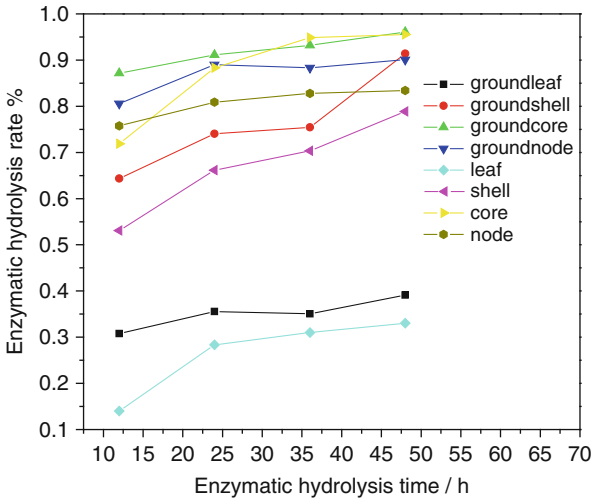

Fig. 1.2 Enzymatic hydrolysis rate of cornstalk in different parts

To resolve the problem of low-value utilization of straw used as a whole, my research group from the Institute of Process Engineering (IPE), Chinese Academy of Science (CAS) deeply studied the difference of each part of the cornstalk regarding chemical composition, physical properties, and cellulase hydrolyzation [14]. From the chemical composition, the cellulose content in the skin was the highest (36.66 %) and had a significant difference compared to other parts. Hemicellulose content in the leaves was the highest (33.86 %), and lignin was mainly concentrated in the skin and knot, with contents of 14.23 and 12.52 %, respectively. Ash was mainly concentrated in leaves (11.63 %) and was significantly different from the content of other parts. Straw fiber had a remarkable morphological difference. The content of microfibrils and hybrid cells was up to about 40–50 %, and fiber cell content was about 40–70 %. Wood has a low content of hybrid cells and a high content of fiber cells. For example, the fiber cell contents in hardwood and softwood were 60–80 and 90–95 %, respectively. The enzymatic hydrolysis rate of different parts of straw also showed inhomogeneity. The enzymatic hydrolysis rate of the core, after enzymatic hydrolysis for 24 h, was up to 88.32 %, which was 28.33 % of the leaves under the same hydrolysis conditions (Fig. 1.2). Overall, compared to wood, the cellulose and lignin contents of corn stover are relatively low, and the hemicellulose and ash contents are relatively high. Meanwhile, the distribution of chemical components differs from different parts of straw. In wood, the three major components are mainly concentrated in the trunk (xylem), with a high content of cellulose (higher than 45 %) and lignin (higher than 20 %) but a low hemicellulose (typically less than 20 %) and ash (lower than 1 %) content.

The inhomogeneity results in the chemical and structural complexity of natural lignocellulose and leads to obvious differences in the conversion process. The inhomogeneity on bioconversion performance is a key factor that makes the high-value utilization of lignocellulose difficult. However, the complex composition and inhomogeneous characteristics of natural lignocellulose provide it the potential to

become a current industrial raw material. Because of this, lignocellulose itself is a functional macromolecule with a complex composition, and different components generate different functional products. Therefore, the most important issue is how to utilize each component of lignocellulose and truly realize efficient and high-value conversion, which will be a difficult problem for researchers for a long period in the future.

1.3 Significance of Exploration on Lignocellulose Biotechnology

1.3.1 Role in Natural Material Cycle

Microorganisms play an important role in the natural material cycle. Microbial decomposition of organic matter and plant respiration will release CO_2, but more than 80 % of CO_2 is produced by microbial activity. Without the microbial decomposition of organic matter, the gradually increased organic matter on earth not only will cause the limited CO_2 in the air to be depleted quickly by plant photosynthesis, but also will result in interruption of the cycle of other inorganic elements in the plant ecosystems.

It is necessary that sustainable development of human society should keep harmonization between substantial human activities and the natural ecological cycle. This requires a fundamental change in the material life of human beings to return to the framework of the ecosystem. The ecological system of nature consists of a complex closed food chain. Plant biomass (fiber, starch, etc.) is synthesized by photosynthesis; this biomass transforms into animal biomass after consumption by animals. Then, these two types of biomass are decomposed into water and carbon dioxide by microorganisms. Various biological populations in this system make the waste and energy in the transformation process be efficiently used at all levels. Energy from the sun is gradually consumed; various elements of the organisms are recycled [15]. However, organic materials photosynthesized by green plants (the primary producers) must be degraded by microorganisms because about 80 % of them are natural lignocellulose, which is difficult for animals to digest and absorb. If these organic materials cannot break down in time, there will be a significant impact on natural carbon circulation. Most of the plant residues complete their energy and material recycling by embedding in the soil. This is a slow process for which humans cannot directly obtain the required energy and matter. On the other hand, direct burning of plant residues is a low-energy utilization with serious energy waste. Oxidation of nitrogen and phosphorus substances in plant residues into insoluble salts is also a serious loss.

How can the microbial transformation of natural lignocellulose be brought into the natural material cycle process to achieve the coordinated development of natural reproduction and economic reproduction? In the long history of human evolution, the relationship between human beings and nature has gone through several stages.

In the first phase, humans mainly rely on hunting techniques to directly obtained subsistence for life from natural ready-made plants and animals; nature has the ability to regenerate after consuming a certain amount of resources. In the second phase, humans interfered in the growth process of plants and animals to obtain subsistence for life mainly through agricultural production techniques. In the third stage, humans exploited nature on a large scale and created an unprecedented number of physical materials to obtain subsistence for life by processing natural substances. However, the negative factor from this industrial production technology system also increased rapidly because the system is contrary to natural ecological laws because it completely ignores the reproduction capacity of the natural resources.

In agroecosystems, just 50 % of organic compounds synthesized through photosynthesis from crops are available for direct human demand; the rest are mostly returned to nature through the synergistic effect of various microorganisms in the soil. This return process is slow and accompanied by the loss of material conversion and energy. For example, the water-soluble components in straw usually can be decomposed within a few days, but it takes several months for the degradation of cellulose and hemicellulose and perhaps several years for lignin. This is a huge waste for humans and is undoubtedly a huge contribution to humans survival and development if the lignocellulose can be converted into available products for humans through the artificially selected biological populations. This is also significant for the microbial transformation of natural lignocellulose.

Therefore, some new concepts about cleansing production processes emerged, such as biorefinery [16, 17], consolidated bioprocessing (CBP) strategy [18], fractionation, and oriented multilevel conversion [1, 19]. From point of view of the dynamics and ecology, making industrial production part of the circulation of natural substances will be the new development stage of the human-ecological industry. This means making natural circulation developed coordinately with economical circulation by accelerating natural circulation through industrial approaches. The so-called ecoindustrial ecology means conducting the production and consumption of matter and energy in nature by simulating the ecological principles in industrial production. In this industrial system, the waste emitted by one kind of biology is the nutrients of a different biology, resulting in multilevel utilization and continuous currency of matter and energy. It will make the best use of material, give full play to the production potential of a substance, promote the virtuous circle of nature, produce products that are more useful, and finally achieve the synchronous development of economy efficiency and ecology efficiency [20, 21].

1.3.2 Status and Functions in Green Chemistry and Engineering

Green chemistry originated in the late 1990s and is a measure to eliminate pollution from the source. It provides an effective means to resolve environmental pollution caused by chemical industries and achieve economically and socially sustainable

development. Green chemistry has become a hot topic of current chemical research and an important direction in the development of chemistry in the twenty-first century. Green chemistry is also known as environmentally harmless chemistry, environment-friendly chemical, or cleaning chemistry [22, 23]. It is a kind of chemistry that uses chemical technologies to eliminate or reduce the use or generation of raw materials, catalysts, solvents, reagents, products, and by-products that is beneficial for human health, community safety, and ecological environment. The shift from traditional chemistry to green chemistry can be seen as the shift from "extensive chemistry" to "intensive chemistry." *Atom economy* is the main feature of green chemistry; that is, "zero emissions" are realized by taking full advantage of each atom of raw material during all material transformation processes [24]. From a scientific point of view, green chemistry updates the basic contents of chemical science; from an environmental point of view, it eliminates pollution from the source; from an economic point of view, it utilizes the resources and energy rationally, reduces production costs, and is in line with the requirements of sustainable economic development. Since the U.S. president established the Presidential Green Chemistry Challenge Award in 1995, countries around the world have set up a number of awards for green chemistry, which not only promoted the development of green chemistry but also made more people recognize the significant effects of green chemistry on social progress, economic development, and the chemical industry itself. Academician Qingshi Zhu proposed that the trend of chemistry development in the twenty-first century was the chemical transformation of green plants and green chemistry [25].

The strategic task of green chemistry is to use biomass as chemical raw materials [26]. The majority of industrial organic chemicals 150 years ago were obtained from plant biomass, and a few were obtained from animal substances. Later, coal was used as a chemical raw material. After the invention of a cheap oil extraction method, oil became a major chemical raw material. Today, more than 95 % of organic chemicals are produced using oil as the raw material. However, the reserves of coal and oil on Earth are limited. Since the late 1960s, humans have gradually recognized the negative impact of the coal and petroleum chemical industry on the environment. Therefore, scientists have begun to consider how to reuse biomass instead of coal and oil to produce chemical substances to satisfy human requirements.

Sucrose and glucose extracted from biomass can be used as raw materials to produce chemicals with microorganisms. Biomass is mainly divided into two categories: starch and lignocellulose. Corn, wheat, and potato are representatives of starchy biomass; agricultural wastes (such as cornstalks, wheat straw, etc.), forest waste, and grasses are typical representatives of lignocellulose. Starch and lignocellulose are polymers containing carbohydrate; they can be used for fermentation by breaking down into monomers. For example, recently in the United States, the government provided substantial support and allowance for alcohol production using corn as a raw material. The starch in corn was turned into glucose by wet milling and dry milling technology, and then the glucose was used for ethanol fermentation. However, most of the biomass on Earth is lignocellulose, especially

the cellulose component. In fact, cellulose is the most abundant organic structure of biomass. Therefore, how to convert lignocellulose into low-cost raw materials for chemical industry is a key issue for substituting coal and oil for biomass.

Compared to starch, cellulose can also be used to produce glucose, but it is more difficult. The main reason is that the lignocellulose is more complex; cellulose, hemicellulose, and lignin are wrapped to form a more compact three-dimensional network spatial structure. It is necessary to adopt methods such as pretreatment and enzymatic hydrolysis to degrade cellulose into glucose. At present, a variety of pretreatment technologies can be used to fractionate natural lignocellulose into three components efficiently. Then, the obtained cellulose, hemicellulose, and lignin are further converted into various chemicals. For example, Professor Mark Holtzapple from Texas State University invented a simple technique to convert biomass into animal feed, chemical reagents, and fuel with simple processes such as lime pretreatment and bacterial fermentation. This work was recognized when he achieved the Academic Award of the 1996 U.S. Presidential Green Chemistry Challenge Award [27]. The Biofine Company won the Small Business Award of the 1999 U.S. Presidential Green Chemistry Challenge Award. The company developed a new technology to convert waste cellulose into levulinic acid, a key intermediate for the production of other chemical products. Using this technology, lignocellulose such as paper waste, municipal solid waste, nonrecycled waste paper, waste wood, and even agricultural residues can be degraded by diluted H_2SO_4 at 200–220 °C within 15 min. Meanwhile, a reactor used to eliminate side effects was also invented so that the reaction can be conducted in a direction in favor of the desired product. In this reactor, the levulinic acid yield was up to 70–90 %; at the same time, some valuable by-products (e.g., formic acid and furfural) can also be obtained. These pioneering works were successful attempts to open research on major issues in green chemistry.

However, although the petrochemical industry development has spent more than a century bringing the biomass-based chemical industry from a vision into reality, at least some decades of hard work are needed.

1.3.3 Status and Functions of Lignocellulose Biotechnology in Circulation Economy

As a reinforcing material for terrestrial plants, millions of years of evolution have made plant fiber materials have a strong self-protection function. Each of the three main components (cellulose, hemicellulose, and lignin) is a polymer with a complex structure Combined, they form complex supramolecular complexes and further various plant cell wall structures [8]. The complex three-dimensional spatial structure makes plants able to avoid attack by microorganisms and various physical and chemical factors. However, this recalcitrance is also the main reason that lignocellulose has never been utilized effectively. Thus, the main challenge in taking

full advantages of natural lignocellulose is how to overcome such recalcitrance. The encirclement of lignin is the biggest obstacle inhibiting the effective utilization of cellulose. For example, cattle and sheep can digest 80 % of forage (not lignified) in vivo by the microbial decomposition. But can only digest 20 % of wheat straw [28, 29]. Although various physical and chemical pretreatment methods have been developed to improve the digestibility of straw, they were not applied practically because of the problems of high energy consumption, high cost, serious environmental pollution, and more. To achieve this goal, a deep understanding of the role of relevant enzymes in the degradation of lignocellulose is necessary.

Another major obstacle to the practicability of lignocellulose is the low specific activity of the degrading enzymes of natural lignocellulose, resulting in the high production cost of enzymes and limiting their widespread application. Along with the thorough understanding of the three-dimensional structure and mechanism of these enzymes, as well as the rapid development of genetic engineering and protein engineering technology, it is possible to improve the enzyme-specific activity through transformation by bacteria. Much research has reportedly successfully improved the application performance of protein engineering bacteria.

In short, modern biotechnology is the best way to realize the effective conversion of biomass resources. Improving biotransformation efficiency is the key to achieve biotechnology success. There are still some resources and environmental problems to be solved to realize the bioutilization of lignocellulose, including the comprehensive association of researchers from biological, chemical, light industry, chemical industry, and other disciplines; the clarification of the structural basis for the self-protection function of biomass; the in-depth exploration of the degradation mechanism of biomass resources; the study of the biochemical engineering fundamentals for the processing of solid lignocellulose; and the exploration of new technologies for bioconversion of biomass and applicability of waste.

1.3.4 Strategic Transfer of Raw Materials to the Chemical and Fermentation Industries

Oil is one of the most important mainstays of modern society and a major raw material for the chemical industry. Presently, the status quo of the world includes the continuous increase of the population and people's living standards, the continuous growing demand for raw materials for energy and the chemical industry, the high energy consumption of the chemical industry, and serious pollution of the environment. Owing to rising oil prices, energy shortages, and increasing environmental protection consciousness, the chemical industry must grope for a new developmental orientation. It can be claimed with certainty that the twenty-first century will be a regularization period for the chemical industry with biomass as a raw material. The biochemical industry is the target of a strategic shift in the chemical industry.

The fermentation industry takes starch as the basic raw material and annually consumes hundreds of millions of tons of grain, with development limited because of competition with humans and livestock for grain.

Cellulose and hemicellulose in natural lignocellulose are polysaccharide polymers that can be generated into glucose, xylose, and other sugars after hydrolysis. By microbial fermentation, these sugars can be converted into ethanol, acetone, butanol, acetic acid, butanediol, and other liquid fuels and chemical raw materials. They also can be taken as the fermentation raw material for antibiotics, organic acids, single-cell protein, and enzyme preparations. Phenylpropanoid derivatives as the monomers of lignin can be transformed into other chemical products and further used as basic raw materials for the organic chemical industry.

In the 1990s, developed countries successfully exploited renewable chemicals to replace the oil feedstock and further accelerated the development of nonfood renewable resources, such as agricultural stalks and urban fiber waste. The U.S. Department of Energy proposed that by 2020 at least 10 % of the chemical-based products should be produced from lignocellulose, with that value increased to 50 % in 2050. In 2030, biomass should provide 5 % of the electricity, 20 % of transportation fuels, and 25 % of chemicals, equivalent to 30 % of current oil consumption in the United States. In order to realize this target, a billion tons of dry biomass feedstock would be consumed annually, five times the current consumption [30, 31]. This indicates the transition of fuel and chemical industries from the nonrenewable hydrocarbon era to the renewable carbohydrate era [32]. From an environmental perspective, the large biomass upconversion process has a slight effect on the balance between the various nature systems because that biomass can constitute an active basic element of Earth's ecosystem. In the growth of biomass, it absorbs CO_2 through photosynthesis, and the production and utilization process constitutes closed circulation of CO_2 [33]. Therefore, the biomass-based industrial structure is sustainable.

In recent years, with energy shortages and environmental problems, China has come up with several policies to support and protect the development of biomass energy and the biochemical industry. The *Twenty-First Centenary Agenda of China: White Paper on the Population, Environment and Development of Twenty-First Century China* clearly pointed out that to achieve rapid economic development, it is necessary to put development and utilization of new energy and renewable resources into the national energy development strategy preferentially and strengthen the development and utilization technologies of biomass-based energy, especially clean liquid fuels from biomass. China's Renewable Energy Law was formally implemented in January 1, 2006, and prescribed in a legal form that 5 % of primary energy in 2010 should be generated from renewable energy, and that this proportion would reach 10 % in 2020. The government not only arranged the overall industrial development of biomass but also provided tax support policies [34, 35]. It is not difficult to understand that, in the evolution of human material, natural lignocellulose will become the common substitute for the chemical and fermentation industries. The comprehensive utilization of natural lignocellulose in biotechnology will reconstruct the traditional industries relying on grains as a raw material and promote the strategic transfer of the chemical industry.

1.4 Lignocellulose Biotechnology Research and Development Progress

1.4.1 Research Development for the Bioconversion of Lignocellulose

Because the chemical composition and structure of lignocellulose have been mostly clarified, it is natural examine how to make the utilization of its constituent sugars possible. The techniques for the microbial conversion of these sugars into ethanol and feed are more mature. Therefore, the utilization course of natural lignocellulose is presented as the research and development course for the cellulose saccharification process.

1.4.1.1 Acid Hydrolysis Technologies

Acid hydrolysis technologies mainly include concentrated acid hydrolysis and dilute acid hydrolysis. The concentrated acid hydrolysis process is a single-phase hydrolysis reaction; the dilute acid hydrolysis process is a multiphase reaction [36]. Concentrated acid hydrolysis was put forward in the nineteenth century. Its principle is that the crystalline cellulose is completely dissolved in 72 % sulfuric acid, 42 % hydrochloric acid, and 77–83 % phosphates at low temperature, resulting in the homogeneous hydrolysis of cellulose. The high recovery rate of sugar is the advantage of concentrated acid hydrolysis; about 90 % of the sugar converted from hemicellulose and cellulose can be reclaimed. Farone et al. [37] invented saccharification technology for the concentrated acid hydrolysis of biomass with a 90 % recovery rate of the hydrolyzed sugar when crystalline cellulose in the biomass was hydrolyzed with 70–77 % sulfuric acid at a liquid-to-solid ratio of 1.25:1. The main problem of concentrated acid hydrolysis, on one hand, is severe corrosion of the equipment; on another hand, it is the separation and recycling of a large volume of acid from sugar. Recently, the equipment corrosion problem caused by the concentrated acid was solved by lining the reactor with acid-resistant polymer materials or ceramic materials. Commonly, acid was recovered by anion exchange membrane dialysis, which has the disadvantages of huge investment, high power consumption, and susceptibility to organic pollution of the membrane. Another method, namely, using nine sections of a simulated moving bed for acid and sugar separation, not only can separate sulfuric acid with sugar effectively but also can remove by-products such as acetic acid and facilitate follow-up sugar processing [38].

Traditional concentrated acid hydrolysis technology for lignocellulose has such disadvantages as severe reaction conditions, requirements for high temperature and low pH, serious sugar degradation, and more. Therefore, in the 1980s, with the emergence of cellulase as a catalyst for the saccharification of cellulose,

acid hydrolysis gradually became the main technology for enzymatic hydrolysis pretreatment [39]. The dilute acid hydrolysis technology mainly includes a high-temperature dilute acid hydrolysis process and a low-temperature, two-stage acid hydrolysis process. As traditional technology, the high-temperature dilute acid hydrolysis mainly has two modes of operation: percolation hydrolysis and the bolt-flow high-temperature dilute acid method.

The dilute high-temperature hydrolysis method has the following problems: (1) The long retention time of products in the reactor results in serious sugar degradation. (2) Under high-temperature and acidic conditions, the monosaccharides further degrade to generate uronic and 5-(hydroxymethyl) furfural and lower the sugar yield (40–48 %). On the other hand, detoxification treatment is necessary because furfural will also inhibit the yeast alcoholic fermentation. (3) Acid used in dilute acid hydrolysis is difficult to recover because of its low concentration. So, a large number of alkalis are used to neutralize acid, leading to huge consumption of acid and alkali and serious pollution of the neutralized products. (4) The sugar concentration after dilute acid hydrolysis of lignocellulose is relatively low (2.5–4 %), so a huge fermentation vessel is necessary, and the concentration of fermentation products is low. (5) Distillation is associated with costs.

The low-temperature, two-step concentrated acid hydrolysis process is the more advanced acid hydrolysis technology. Its principle is the hydrolysis of hemicellulose and cellulose according to their different hydrolysis conditions. Namely, first hemicellulose is separated under conditions of lower temperature and weak acid (or no acid), and then separate cellulose is separated at high-temperature and strong acidic conditions, thus avoiding the overlong retention time of the sugar obtained in the reactor and reducing sugar degradation [40]. The hydrolysis of hemicellulose and cellulose was conducted in different conditions twice with a low hydrolytic temperature, resulting in a low concentration of by-products. Thus, the sugar yield obtained from the hydrolysis of hemicellulose was up to 75–90 %, while part of the cellulose was dissolved with sugar to yield 50–70 % [41]. By further increasing the solid concentration, the monosaccharide concentrations in the prehydrolysate and hydrolysate can reach 12 % and 38 %, respectively. A high sugar concentration can greatly minimize the size of the fermentation equipment and reduce energy consumption.

At present, there still are many difficulties in the industrialization of the two-stage, low-temperature acid hydrolysis process: (1) Only about 75 % of the acid used can be recovered, and large consumption of sulfuric acid and lime causes environmental pollution. (2) This technology requires a huge engineering investment because of the large number of highly corrosion-resistant materials and acid recovery equipment. (3) This technology requires a larger production scale, which is contradict to the scattered distribution characteristics of lignocellulose resulting in high cost of transport and storage and consequent high investment in process equipment.

Thus, acid hydrolysis technology still has many problems (such as acid recovery, equipment corrosion, construction costs, etc.). In addition, acid hydrolysis has considerable inhibition effects on subsequent fermentation because of the generation

of a large number of by-products, such as formic acid, acetic acid, furfural, 5-hydroxymethyl furfural, and benzene compounds. Therefore, acid hydrolysis is gradually being replaced by biotechnology.

1.4.1.2 Enzymatic Hydrolysis Process

Enzymatic hydrolysis has many advantages, such as mild conditions, high specificity, fewer by-products, high sugar yield, simple equipment, and no need for corrosion-resistant materials and an acid recovery device; it is without pollution and is suitable for miniature local production where raw material is produced. Therefore, the study of the enzymatic hydrolysis process has aroused extensive attention and in-depth research recently.

As early as 1850, the phenomenon of microbial decomposition of cellulose has been observed. However, the study of cellulase gradually attracted the attention of the world after 1906, when it was found that cellulase in the snail's digestive juice could break down cellulose. Cellulase is a highly specific biocatalyst for the hydrolysis of lignocellulose and a general term for a group of enzymes that degrade lignocellulose to generate glucose. It is not a single enzyme but a multicomponent enzyme system with synergistic effects on each other. Cellulase can be produced by fungi, bacteria, actinomycetes, and so on. Generally, fungi are still the major producer of cellulase, especially *Trichoderma* spp. and *Aspergillus* spp., obtained through deep and thorough research. *Trichoderma viride* and *Aspergillus* spp. are recognized as the most stable and nontoxic strains for cellulase production. Cellulase can also be produced by bacteria, such as *Cytophaga* and *Cellulomonas* spp., and actinomycetes, such as *A. roseus* and *A. cellulosae*.

Bioconversion of renewable natural lignocellulose is one of the most advanced technologies with the ability to solve such current world problems as food shortages, energy crises, and environmental pollution because coal, oil, and other mineral raw materials will be exhausted in a few hundred years and environmental pollution is increasingly serious. But, the key factors to restrict the enzymatic hydrolysis of lignocellulose include an inefficient enzymatic hydrolysis rate and higher prices for the enzyme, resulting in higher production costs. Therefore, the focus of saccharification conducted by the enzymatic hydrolysis method is to improve the utilization efficiency of cellulase and reduce production costs. In searching for how to improve the efficiency of the enzymatic hydrolysis of lignocellulose significantly, scholars have carried out much research, mainly in the following areas:

1. Pretreatment technology before enzymatic hydrolysis: The compact structure of lignocellulose, constructed of cellulose, hemicellulose, and lignin, hinders the entry of the cellulase. Appropriate pretreatment technology is necessary to enhance the accessibility of cellulase.
2. Compounds of multiple enzymes: Multiple cellulases act synergistically on the cellulosic substrate to enhance the enzymatic hydrolysis rate by providing each other with new accessible sites, removing obstacles to eliminate product inhibi-

tion, and so on. In addition to the synergistic effect between various cellulases, compound cellulase with hemicellulase and pectinase can also improve the saccharification efficiency of lignocellulose.
3. Strengthening of enzymatic hydrolysis processing: Presently, cellulase accounts for about 50 % of the hydrolysis total investment. To achieve the economy of fuel ethanol production and reduce the invalid adsorption of cellulase, achieving full utilization and circulation of cellulase has an extremely important significance. On the other hand, to improve the enzymatic saccharification rate of lignocellulose, it is necessary to further develop new enzymatic hydrolysis techniques, design a corresponding enzymatic hydrolysis and saccharification reactor, and remove the inhibition of enzymatic saccharification.

1.4.2 Problems with and Prospects for the Microbial Transformation of Natural Lignocellulose

In the early 1970s, the world's oil crisis gave nascent biotechnology impetus for vigorous research on the comprehensive utilization of natural lignocellulose, and many specialized research institutions were established in some developed countries. In China, lignocellulose biotechnology research reached a climax in the period 1981–1990, but quickly was reduced because of secular technical and economic difficulties and the reality of short-term cost inefficiency. However, many countries around the world regard the bioconversion of natural lignocellulose as the major strategic task of the twenty-first century.

Lignocellulose is the most abundant carbon-containing organic resource in nature, but it still has not achieved efficient utilization. In theory, there is no difficulty on the natural biological degradation of the cellulose materials. However, the industrial utilization of lignocellulose still has technical and economic barriers; the key issue is the lack of understanding of the relationship between the structure and biotransformation of lignocellulose. Generally, insufficient basic research and lack of technological innovation are the main reasons that the microbial transformation of natural lignocellulose still cannot break through the economic and environmental difficulties, which are represented concretely by the following aspects:

1. Emphasis on the utilization of cellulose and treating other components as a waste cause of environmental pollution and waste of resources. This not only did not play an important role in improving economic efficiency but also became the burden to high efficiency. Utilization technologies for hemicellulose and lignin are still not mature.
2. Cellulase production still has many problems, including high cost, excessive use of enzyme for enzymatic hydrolysis, and immature technology.
3. Research on component separation technology, previously called pretreatment technology, is unsubstantial, and there is no comprehensive utilization of all components. The ineffective separation and recycling not only waste resources

but also increase the difficulty of cellulase hydrolysis, which have an impact on the concentration and purity of hydrolysate, making the consequent biotransformation process more difficult and reducing effectiveness.
4. Product-coupled recycling is insufficient, and energy consumption is too large.
5. Just the simple combination of the existing conventional techniques with equipments, lacking of systematic and optimum engineering researches.

In summary, the key scientific issue for the development of the biomass-refining industry is establishing a lignocellulosic biomass-refining process for a new generation of biological and chemical industries to achieve cost-effective conversion of the biomass and biorefining industry. The fundamental reason is that the biomass conversion process is a systematic project; relying on a single discipline or single technology makes it difficult to achieve high-value utilization of biomass. It is necessary to develop key innovative processes and technology platforms for biomass. In addition, long-term and short-term goals must be combined to stabilize the current biomass utilization industry with natural lignocellulose as a raw material to further improve technologies, such as clean technology for papermaking, straw protein feed production technology, and the application of cellulase in all relevant industries. The long-term goals, such as new clean energy and raw materials for biochemical engineering, will be achieved based on industrial-scale short-term goals.

1.4.3 Enzymes: The Key to Open the Biomass Resource Treasure

The utilization of plant resources needs to convert macromolecules such as starch, cellulose, hemicellulose, lignin, and the like into low molecular weight substances such as glucose for use as the raw materials for fuel and organic chemical engineering industries. The research methods include physical, chemical, and biological transformation.

Regarding physical and chemical methods, macromolecular components such as cellulose and lignin are degraded into low molecular weight hydrocarbons, flammable gases, and liquids by pyrolysis, fractionation, redox degradation, hydrolysis, and acid hydrolysis, which can be used directly or after the separation and purification as raw materials for the energy or chemical industries. Generally, these methods have disadvantages such as high energy consumption, low yield, and serious pollution. Therefore, they are usually used as auxiliary means of biotransformation because of lack of practicality when used alone.

The bioconversion method degrades lignocellulose into glucose biologically and then converts the latter into a variety of chemicals. Enzymes play a key role in a variety of conversion processes, such as the degradation of starch and cellulose into glucose, which is carried out successfully using amylase and cellulase, respectively. Then, glucose is further converted into various chemical products depending on a variety of microorganisms under the effects of different types of enzymes within the cells.

A catalyst is necessary for many chemical reactions. An enzyme is also a catalyst, although it is more complex than ordinary chemical catalysts. Enzymes are specific proteins in biological cells, and almost all chemical reactions in vivo are carried out by relying on enzyme catalysis.

Under the effects of enzyme or enzyme-containing microorganisms, biomass can be highly efficient and cleanly and economically converted into useful chemical substances that originate from mineral raw materials such as oil, natural gas, coal, and the like. Then, the application of renewable biomass resource has economic value. Therefore, it can be proposed that the enzyme is the key to open the high-value utilization of renewable resources.

This book focuses on the biotechnology principle and application in the development process of natural lignocellulose from the aspects of composition, structure, and physical and chemical characteristics of natural lignocellulose.

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Chapter 2
Chemical Composition and Structure of Natural Lignocellulose

Abstract The wide variety of natural cellulosic materials has complex and uneven components. Cellulose, hemicellulose, and lignin comprise the main composition of cell walls of plants and are important components of natural lignocellulosic materials. Cellulose molecules determine the cell wall framework, and pectin is located between the cellulose microfilaments of the cell wall. In addition, cellulosic materials contain rich cell wall protein, pigment, and ash. Understanding of the chemical composition and structure of natural lignocellulosic materials, characteristics of each component, and interrelationships between various components would contribute to the research and development regarding natural cellulose materials. This chapter mainly describes the chemical composition and structure of natural cellulosic materials.

Keywords Cellulose • Hemicellulose • Lignin • Cell wall protein • Biological properties

2.1 Main Components of Natural Lignocellulosic Materials

Cell walls of plants consist mainly of three organic compounds: cellulose, hemicellulose, and lignin. These compounds are also major components of natural lignocellulosic materials. Cellulose molecules arrange regularly, gather into bundles, and determine the framework of the cell wall. Fibers are filled with hemicellulose and lignin. The structure of the plant cell wall is compact. There is different bonding among cellulose, hemicellulose, and lignin. Cellulose and hemicellulose or lignin molecules are mainly coupled by a hydrogen bond. In addition to the hydrogen bond, there is the chemical bonding between hemicellulose and lignin, which results in the lignin, isolated from natural lignocelluloses, always contains a small amount of carbohydrates. The chemical bonds between the hemicellulose and lignin mainly refer to the chemical bonds between galactose residues, arabinose residues on the side chains of hemicellulose molecules and lignin, and carbohydrates, with this

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Table 2.1 Structure and chemical composition of cellulose, hemicellulose, and lignin in cell walls of plants [2]

	Lignin	Hemicellulose	Cellulose
Subunits	Guaiacylpropane (G), syringylpropane (S), p-hydroxyphenylpropane (H)	D-Xylose, mannose, L-arabinose, galactose, glucuronic acid	D-Pyran glucose units
Bonds between the subunits	Various ether bonds and carbon-carbon bond, mainly β-O-4 ether bond	β-1,4-Glycosidic bonds in main chains; β-1.2-, β-1.3-, β-1.6-glycosidic bonds in side chains	β-1,4-Glycosidic bonds
Polymerization	4,000	Less than 200	Several hundred to tens of thousands
Polymer	G lignin, GS lignin, GSH lignin	Polyxylose, galactoglucomannan (Gal-Glu-Man), glucomannan (Glu-Man)	β-Glucan
Composition	Amorphous, inhomogeneous, nonlinear three-dimensional polymer	Three-dimensional inhomogeneous molecular with a small crystalline region	Three-dimensional linear molecular composed of the crystalline region and the amorphous region
Bonds between three components	Contain chemical bond with hemicellulose	Contains chemical bond with lignin	Without chemical bond

knowledge gained through research on the separated lignin-carbohydrate complexes (LCCs) [1, 2]. Table 2.1 shows the chemical composition and structure of cellulose, hemicellulose, and lignin.

Cell walls mainly consist of cellulose, hemicellulose, and lignin in a 4:3:3 ratio. This ratio differs from sources such as hardwood, softwood, and herbs. Besides these three components, natural lignocellulosic materials contain a small amount of pectin, nitrogenous compounds, and the secret ash. For instance, the element content of wood is about 50 % carbon, 6 % hydrogen, 44 % oxygen, and 0.05–0.4 % nitrogen.

2.2 Biological Structure of Plant Cell Walls

One of the most important components in the plant cell wall is cellulose, which determines the wall structure. Cellulose is a natural high molecular polymer composed of glucose residues, with cellobiose as the basic coupling unit. It is the most abundant renewable resource in nature, and cellulose metabolism is an

important part of the biosphere's carbon cycle [3]. Gao et al. used cotton fiber as a raw material for research on the structure of cellulose in plant cell walls. Cotton fiber is the only natural pure cellulose; its cellulose content can reach 95–97 %, and its crystallinity is about 70 %. Scanning electron microscopy showed that the diameter of fibrils is about 500 nm, so it is the largest structural unit of cellulose. A fibril is composed of entwined microfibrils, which makes cellulose stronger than steel wire of the same thickness. Microfibrils would entwine into a network as the basic framework of the cell wall; their diameter is about 10–25 nm. The microfiber is formed with elementary fibrils arranged in parallel. The diameter of the elementary fibril is approximately between 2 and 4 nm, the structural unit of which is cellulose molecules linked by β-1,4-glycosidic bonds [3]. In some regions of the microfibrils, cellulose molecules are arranged in an orderly fashion, so the cellulose has crystal properties. This regular arrangement of cellulose molecules in microfibrils is called the *micelle*. Some noncellulose molecules also exist in the network structures of cellulose, including hemicelluloses, pectin, and so on.

Another important component in the cell wall is lignin. Except for cellulose, it is the most abundant large-molecule polymer in the cell wall. Botanically, lignin encloses the bundle cells, such as wood fibers and sclerenchyma cells. From a chemistry point of view, phenylpropanoid derivatives are the basic units of the lignin; they combine into high molecular substances by ether bonds or carbon-carbon bonds. According to the physical characteristics, lignin is hard, which increases the hardness of the cell wall. Commonly, the cell wall of plants with a supporting function and mechanical action always contains a high lignin content. The lignin content is about 27–32 % in woody plants and about 14–25 % in herbaceous plants [4].

The cell wall of protective tissue usually also contains cutin, suberin, wax, and other fatty substances. For example, the cell wall surfaces of the epidermic cell are covered with cutin; the cell walls of cork cells in secondary protective tissue contain suberin, cutin, and suberin, often combined with wax. These components greatly reduce water loss from the plants.

Depending on the time of formation and chemical composition, the cell wall can be divided into the primary wall and the secondary wall. Plant cell wall formation follows after cell division; the primary cell wall is formed in the new cell plate, and intercellular layers are formed between primary cell walls. As cells differentiate, secondary cell walls are formed inside primary cell walls and outside the protoplast; with the further differentiation of the cells, the structure of the cell wall gradually adapts to the function of the cells. The intercellular layer is formed outside the primary wall, but it is difficult to identify the boundaries between them, especially after the secondary wall has been formed. The intercellular layer is mainly composed of pectic substances, which are amorphous colloids and have strong hydrophobicity and plasticity. Multicell plants rely on the pectin substances to bond neighboring cells together. Pectins are easily broken down by acids or enzymes, resulting in the isolation of cells. When the cells are lignified, the sequence of lignification degree is middle lamella (ML), primary wall, then secondary wall [5].

In the process of cell growth, the primary wall is formed from some protoplast secretions. The main components of the primary cell wall are polysaccharides, proteins (such as the expansins), and many other enzymes, glycoproteins, and some ions (such as calcium). Main polysaccharides of primary walls are cellulose, hemicellulose, and pectin. Cellulose accounts for 15–30 % dry weight of the primary cell wall. The hemicellulose interacts with celluloses, forming a network with microfibrils. Pectin accounts for about 30 % of polysaccharide of the primary cell wall [6]. Those cells with an active division property usually do not have the primary wall, which is similar to those mature cells relating to photosynthesis, respiration, and secretory action. These cells without secondary cell walls can change specialized forms and restore the ability to divide and differentiate into different cell types. Therefore, these cells that only have primary walls are relevant to callus reaction and regeneration. Usually, when the primary wall grows, it thickens unevenly. There would be a thin field in the primary wall called the primary pit field. Plasmodesmata, which connect protoplasts of adjacent cells, tend to be concentrated in this field [5]. The main function of the primary cell wall is to provide structural and mechanical support, maintain and determine cell morphology, withstand cell swelling pressure, control the rate and direction of cell growth, promote plant morphogenesis, regulate material diffusion in ML, reserve carbohydrates, maintain resistance to pathogens, resist dehydration, and activate the interaction between source signal molecules and cells [6]. In plants, many cells only have primary walls, but many others have both primary and secondary walls. When cells stop growing and the superficial area of the primary wall no longer increases, the secondary wall begins to form. Some substances produced in the metabolic process of protoplasts deposit on the inside of cell walls, then form secondary cell walls next to the plasma membrane. The secondary wall cells are formed inside the primary cell wall and have some differences in composition compared to the primary cells. In addition to containing cellulose and hemicellulose, the secondary wall contains lignin. Lignin could highly cross-link with each other to enhance mechanical support for the plants to grow upward [6]. The secondary wall is particularly important for those specialized cells that are related to mechanical reinforcement and water transportation. The secondary wall has more celluloses than the primary wall but lacks pectin. Therefore, the secondary cell wall is harder, is less extended than the primary wall, and has no enzymes and glycoprotein. The basic component of the secondary cell wall is hemicellulose. It usually can be divided into three layers: inner layer (S3), middle layer (S2), and outer layer (S1). Different layers have differences in composition, structure, microfibrillar angle, and so on. A large amount of solar energy and carbon fixed by plants is stored in secondary walls. The accumulated biomass in secondary walls accounts for the vast majority of the total plant biomass, which is the main form of biomass on Earth, and are also fiber materials and bioenergy raw materials for human life.

The primary pit field is not covered by the secondary cell wall component, resulting in the formation of many sunken areas called pits. Sometimes, the pits can also occur in the absence of a primary pit field. Pits on the cell wall are often

opposite the pits on the adjacent cell walls; the intercellular layer between the two pits and two layers of primary walls make up the pit membrane, and two opposite pits and pit membrane make up the pit pair. Pits on the secondary wall have two types: the simple pit and the bordered pit. The basic difference between them is that the secondary thickened wall uplifts toward the central part, hangs over the pit cavity, and forms a dome-shaped edge so that the pit aperture is significantly smaller, but this kind of dome-shaped edge does not exist in the simple pit.

The growth of the cell wall includes an increase in surface area and thickness; the growth process is strictly controlled by biochemical reactions in the protoplast. The growth of the cell wall should be in a relaxed state and have a high respiration rate, protein synthesis rate, and water absorption rate. Most newly synthesized microfibrils are superimposed on the original cell wall, but a few insert into the original cell wall. In those cells that grow evenly, such as marrow cells, storage cells, and culture cells, cell wall microfibrils randomly arrange in various directions and form an irregular network. In contrast, in the extended-growth cells, the deposition direction of microfibrils on the side walls makes an acute angle with the extended direction of the cells. When the surface area of the cell is increased, the external microfibrils arranged direction gradually is parallel with the long axis of the cells. Substrates (such as pectin and hemicellulose) and glycoprotein are mainly transported to the cell wall by the Golgi vesicles. The type of substrate is dependent on the development stage of the cell. For example, at the expanding stage of cells, the pectin is predominant in the matrix; otherwise, hemicelluloses predominate at the shrinking stage [5].

2.3 Cellulose

Cellulose is the most abundant renewable organic resource on Earth and is widespread in higher plants, bacteria, marine algae, and other biomass. The total annual amount of cellulose is several billion tons, revealing the huge economic value of it. Cellulose is the main component of the plant cell. Although some animals (such as tunicates) and some bacteria contain cellulose, the content of cellulose in these species is negligible when compared with plants. Cellulose was first separated by Anselme Payen (1839) from timber that was alternately treated with nitric acid and sodium hydroxide solution. It is a β-1,4-linked linear polymer of glucose units and is insoluble in water, dilute acidic solutions, and dilute alkaline solutions at normal temperatures. Although the structure and composition of the cell walls of plants vary widely, the cellulose content usually accounts for 35–50 % of dry weight and, peculiarly, almost 100 % for cotton. Study of the supramolecular structure of natural cellulose showed that the crystalline and noncrystalline phases intertwine to form the cellulose. The noncrystalline phase assumes an amorphous state when tested by X-ray diffraction because most hydroxyl groups on glucose are amorphous. However, large amounts of hydroxyl groups in the crystalline phase

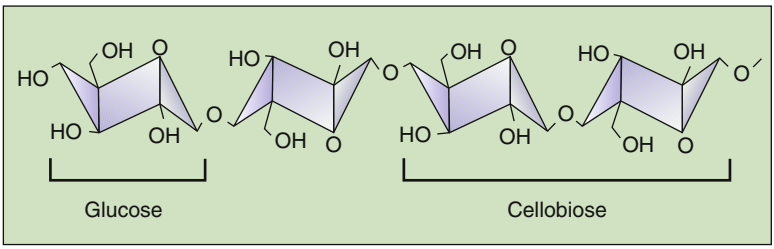

Fig. 2.1 Molecular chain structure of cellulose [8]

form many hydrogen bonds, and these hydrogen bonds construct a huge network that directly contributes the compact crystal structure [7]. In most conditions, the cellulose is wrapped by hemicellulose (dry matter accounting for 20–35 %) and lignin (dry matter accounting for 5–30 %). Cellulose has become an important raw material for the pulp and paper, textile, and fibrous chemical industries. Predictably, bioenergy generated from lignocellulosic materials will become clean energy in the future.

2.3.1 Chemical Structure of Cellulose

Cellulose is a linear homopolymer composed of D-glucopyranose units linked by β-1,4-glycosidic bonds. It mainly contains carbon (44.44 %), hydrogen (6.17 %), and oxygen (49.39 %). The chemical formula of cellulose is $(C_6H_{10}O_5)_n$; n, called the degree of polymerization (DP), represents the number of glucose groups, ranging from hundreds to thousands or even tens of thousands. In the twentieth century, it was proved that cellulose consists of pure dehydrated repeating units of D-glucoses (as shown in Fig. 2.1), and the repeating unit of the cellulose is called cellobiose.

Sodium hydroxide solution at different concentrations and different temperatures could dissolve cellulose with different DP. According to different solubilities under specific conditions, cellulose can be divided into three types: ① α-cellulose, which is dissolved in 16.5 % NaOH at 20 °C; ② β-cellulose, which is deposition extracted after neutralizing the acid solution and the remaining alkaline solution; and ③ γ-cellulose, which is the soluble remainder in the neutralized solution. Staudinger used a viscosity method to measure the DP of these three celluloses. The results indicated that the DPs of α-cellulose, β-cellulose, and γ-cellulose were more than 200, between 10 and 200, and less than 10, respectively. In industry, α-cellulose usually is used to express the purity of cellulose. Traditionally, β-cellulose and γ-cellulose are together called industrial hemicellulose. *Holocellulose* refers to all the carbohydrates in natural cellulose material, which also is the sum of cellulose and hemicellulose [2].

2.3.2 Physical Structure of Cellulose

The physical structure of cellulose refers to the spatial arrangement of different-scale structural units, including the chain structure and aggregation structure of the polymer. The chain structure, also known as the primary structure, shows the geometric arrangement of the atoms or groups in the molecular chain. The short-range structure is the first-level structure and refers to the chemical structure or stereochemical structure of one or several structural units in a single-molecule polymer. Remote structure is the second-level structure and refers to the size of a single-molecule polymer and a special structure. The aggregation structure, also called the secondary structure, refers to the inner structure of the whole polymer, including the crystal structure, noncrystal structure, orientational structure, and liquid crystal structure. The third-level structure term is used to describe how molecules in a polymeric aggregate accumulate each other, such as tangly clew structure and crystal structure formed with ordered folding chains. The chain structure of the polymer is the main structural hierarchy that reflects many characteristics of a polymer, such as melting point, density, solubility, viscosity, adhesion, and so on. The aggregation structure of a polymer is the major factor that determines the service performance of macromolecular compound products [9].

2.3.2.1 Filament Structure

A fibril is a small, stretching unit; these units aggregate and then constitute the structure of some natural and synthetic fiber materials (such as textile fibers, timbers, or fibrous protein); they also make long molecular chains gathered into bundles in one direction. Because the inequality in size of fibrillar aggregation, current terminologies include elementary fibril, microfilament, macrofilament (also called a microfilament bundle) [10]. Natural cellulose has 10,000 glucose units, and the fibril contains approximately 60–80 cellulose molecules. Hydrogen bonds are formed between adjacent molecules. In a certain range of space, hydrogen bonding can be shown in the X-ray pattern when it reaches a certain number. This space is called the crystalline region, and the rest is called the amorphous region. Microfilament is composed of elementary fibrils and is fixed in size. Macrofilament is has more than one microfilament, and its size varies with the sources or processing conditions of raw materials.

The structural model proposed by Fengel for the cell wall of timber is the representative model for the microfilament structure of each layer of the cell wall. He reported that the elementary fibril with a diameter of 3 nm is the most basic structure unit; 16 (4×4) elementary fibrils form a fibril with a diameter of 12 nm, then 4 fibrils form a relatively thick microfibril with a diameter of 25 nm, and more than one microfibril form macrofilament. Hemicellulose is filled between the adjacent elementary fibrils; the microfilaments are wrapped with lignin and hemicellulose. A multilayer of several hemicelluloses is filled between fibrils 12 nm

in diameter; the monolayer of hemicelluloses is filled between 3-nm elementary fibrils. Because the microfilament is formed before the lignification of the cell wall, lignin is surrounded by only microfilament with a diameter of 25 nm [10]. It is generally thought that the movement of the liquid in the cell wall occurs mainly at the elementary fibril level of hemicellulose; usually, contraction and swelling processes also mainly occur at this level.

In recent years, elementary fibrils with a diameter of 1.7 nm have been found with high-resolution electron microscopy. Because the fibrils are surrounded by hemicellulose and the microfilaments are surrounded by a large amount of lignin, the microfilament can be observed after delignification, and the elementary fibril can be found only after the hydrolysis of hemicellulose. The measurement results for elementary fibrils according to most investigators indicated that the diameter of elementary fibrils is between 30 and 35 Å, and an elementary fibril is composed of 40 cellulose macromolecular chains. The ordered region of cellulose macromolecules is the crystalline region; irregular regions form an incomplete crystalline structure. When the crystal diameter is about 3 nm, a monolayer of hemicellulose would exist around the crystal, and several cellulose crystals combine to form the cellulose crystal beam called the nanofiber. The diameter of a nanofiber is about 2–3 nm, and hemicellulose and lignin are around it. In summary, the fiber cell walls are composed of many fibers, and larger microfibrils always consist of smaller elementary fibrils.

2.3.2.2 Aggregation Structure

The aggregation state of cellulose, also called the supramolecular structure of cellulose, mainly refers to how cellulose molecules arrange to form crystal and amorphous structure, then elementary fibril, fibril, and microfibril structures. X-ray diffraction studies showed that, in the aggregates of cellulose macromolecules, molecules in crystal structure arrange regularly and display a clear X-ray pattern, so the density of cellulose in the crystalline region is high (1.588 $g \cdot cm^{-3}$). Molecular chains in the amorphous region arrange irregularly and loosely, so the distance between molecules is large. The density of cellulose in the amorphous region is low, 1.500 $g \cdot cm^{-3}$. However, the molecule chain is almost parallel with the main spindle of cellulose. The cellulose crystallinity, generally between 30 and 80 %, refers to the percentage of all the cellulose occupied in the crystalline region [2].

The crystallization of cellulose shows pleomorphism. There are five kinds of crystal modification in solid cellulose, whose characteristics can be reflected by characteristics of their unit cells. Under certain conditions, cellulose crystals can be converted into many crystal variants. Type I is the crystal form of the natural cellulose. Types II, III, IV, and X are those crystal forms of "artificial" cellulose under artificial processing. Now, the commonly accepted cell structure of type I is the monoclinic unit cell model introduced by Meyer and Misch in 1937 [9]. Extensive chemical treatment and heat treatment will change the crystal form; for example, ball milling can destroy crystal lattice completely. There is no distinct boundary from the crystalline region to the amorphous region. Each crystalline

region is called a microcrystal (also called a micel or micella). Since free hydroxyls at position 2, 3, and 6 of glucosyl in cellulose microcrystal regions have formed hydrogen bonds, only amorphous regions contain some free hydroxyls.

2.3.3 Physicochemical Properties of Cellulose

2.3.3.1 Chemical Properties of Cellulose

Every glucosyl ring of cellulose has three active hydroxyls: one primary hydroxyl group and two secondary hydroxyl groups. Thus, cellulose may have a series of chemical reactions related to hydroxyl. However, these hydroxyl groups also can form hydrogen bonds between molecules, which has a profound influence on the morphology and reactivity of cellulose chains, especially the intermolecular hydrogen bond formed by oxhydryl at C3 and oxygen at an adjacent molecule ring. These hydrogen bonds not only can enforce the linear integrity and rigidity of the cellulose molecule but also can make molecule chains range closely to form a highly ordered crystalline region [10]. The accessibility of cellulose refers to the difficulty reagents have in arriving at the cellulose hydroxyl. In heterogeneous reactions, the accessibility is mainly affected by the ratio of the cellulose crystalline regions to the amorphous regions. The reactivity of cellulose is the reactive capability of the primary hydroxyl and the secondary hydroxyl at the cellulose ring. Generally, because of the smallest steric hindrance, the reactivity of the primary hydroxyl groups is higher than for the secondary hydroxyl groups, so the reactivity of hydroxyl at C6 with a bulky substituting group is higher. For example, esterification of toluenesulfonyl chloride chiefly occurs in the primary hydroxyl. The reversible reaction occurs mainly in the hydroxyl group at C6, and an irreversible reaction always occurs in the hydroxyl group at C2. Thus, for the esterification of the cellulose, the reactivity of the hydroxyl group at C6 is the highest, but for the etherification, C2 is the highest [10].

The degradation of cellulose is an important reaction that can be used to produce cellulose products. Acid degradation, microbial degradation, and alkaline degradation are mainly to break the glycosidic bonds between two adjacent glucose molecules; an alkali peeling reaction and oxidation-reduction reaction of cellulose usually act on reducing ends of celluloses, and the oxidative degradation of the cellulose occurs mainly in dissociating hydroxyls at C2, C3, and C6 of the glucosyl ring. Cellulose molecule chains will form carbonyls at C2 when oxidized to some degree and then be degraded in the following alkali treatment process by the elimination reaction of β-alkoxy. After disconnecting the glycosidic bond, the reaction product is formed and then degraded to a series of organic acids [9].

Esterification and etherification reactions of cellulose act on three alcoholic hydroxyls of cellulose molecule monomer. They can greatly change the properties of cellulose, thereby producing many valuable derivatives of cellulose, such as sulfonic ester, cellulose acetate, cellulose nitrate, and cellulose ether (carboxyl

methyl cellulose, methyl cellulose, ethyl cellulose). To enhance the reactivity of ester and the ether bond of cellulose in multiphase medium and improve the quality of cellulose ester and ether, some pretreatments need to be performed. The main methods include the following: ① Preswelling treatment of celluloses can weaken the hydroxyl-binding forces between cellulose molecules to increase the reagents' diffusion velocity in cellulose, such as being immersed in concentrated caustic solution, activated by glacial acetic acid, and so on. ② The elimination of crystallinity by the ethamine can only change the DP by 20 % (usually, the concentration is higher than 1 %) when the concentration of ethamine is more than 71 %. Therefore, it was analyzed that ethylamine only enters into the microfilaments, only makes the amorphous region swell, and does not greatly change crystallization regions. ③ Cellulose derivatives with a high degree of substitution and many hydroxyl groups are substituted substantially, so the total free hydroxy declines and water absorbability decreases. So, actually some cellulose derivatives with a low degree of substitution have higher water absorbability, such as methyl, ethyl, hydroxyethyl, hydroxymethyl cellulose ether, and so on. These groups lead to the swelling of the cellulose structure and binding force decrease in macromolecules. They further result in the increase of water absorbability, degree of hydrolysis, and wrinkle resistance. The improvement of wrinkle-resistant property can be used to enhance the stiffness and moisture resistance of cardboard; also, it can improve the burst strength and the dimensional stability of paper [9].

2.3.3.2 Physical Properties of Cellulose

Free hydroxyls of cellulose have a strong attraction to many solvents and solutions, but adsorbed water only exists in the amorphous region, not the crystalline region. In the moisture sorption process, the hydrogen bonds of the amorphous region in the dry cellulose constantly could be broken; the hydrogen bonds in the cellulose molecules are replaced by the hydrogen bonds between cellulose molecules and water molecules, even forming new hydrogen bonds, and some hydrogen bonds in cellulose molecules remain. In the desorption process, because of the obstruction from inside, hydrogen bonds between cellulose molecules and water molecules cannot be broken completely and reversibly, resulting in hysteresis. Some water absorbed by cellulose enters into the amorphous region of cellulose and forms the water bound by hydrogen bonds, called bound water. Molecules of bound water attracted by hydroxyl of cellulose are arranged in a certain direction and have a high density, making swelling the cellulose and generating a heat effect. When the celluloses absorb water that reaches the fiber saturation point, water molecules continue to enter into the cell lumina and pores of cellulose to form a main layer adsorbed water or capillary water, which is called free water. No heat effect and swellability of cellulose exist when absorbing free water [9].

When solids absorb liquids, the configuration homogeneity does not change, but solids become soft with the decrease of the inner cohesive force and increased volume. This phenomenon is known as the *swellability*. Swellability of cellulose

is divided into swellability in the crystalline regions and swellability between crystalline regions. The former refers to the fact that the swelling agent can only reach the surface of crystalline and amorphous regions, and the X-ray pattern of cellulose does not change. The latter refers to the fact that the crystallization regions of microfilaments are permeated with the swelling agent and then swell to generate new crystalline lattice and display a new X-ray pattern. Unlimited swelling of the cellulose is dissolution. The hydroxyl groups in the cellulose have polarities. As a swelling agent, the greater polarity the liquid has, the greater degree of swelling the cellulose has. The metal ion in the alkali solution is usually in the form of aquo ions, which is more favorable for entering the crystallization region. Usually, 15–20 % NaOH will cause swelling within crystalline regions. If the alkali concentration is increased, the radius of aquo ions is reduced because the ion density is too high, resulting in the drop of swellability. Except for alkali, the swellability of other swelling agents, sorted from strong to weak, is as follows: phosphoric acid, water, polar organic solvents, and so on. Cellulose is saturated in a concentrated solution of NaOH to generate alkali cellulose. Although alkali cellulose is washed with water and dried, such changes cannot restore it to its original condition. Alkali cellulose may have a crystalline form of hydrate cellulose that is more stable than that of natural cellulose, which would increase its absorbability and make it easy to react with a variety of reagents. Using alkali to impregnate celluloses is also called *mercerization*. In addition, alkali cellulose is the important intermediate product for the production of viscose fibers and derivatives of cellulose ether [9].

Characteristics of polymer compounds are high molecular weight and a strong cohesive force. They have movement difficulties in the system and a poor diffusion capacity, so they cannot be dispersed in a timely manner in the solvent. The solvent dissolved with celluloses is not the real cellulose solution, but the mixed product is obtained by mixing celluloses and components in liquids. The solvents of the cellulose can be divided into two categories: aqueous and nonaqueous. Aqueous solvents include the following: ① Inorganic acids, such as H_2SO_4 (65–80 %), HCl (40–42 %), H_3PO_4 (73–83 %), and HNO_3 (84 %) can lead to the homogeneous hydrolysis of cellulose. Concentrated HNO_3 (66 %) does not dissolve the cellulose but forms an addition compound with cellulose. ② Lewis acids, such as LiCl, $ZnCl_2$, $Be(ClO_4)_2$, thiocyanate, iodide, bromide, and others, could dissolve celluloses with a low DP. ③ Inorganic bases, such as NaOH, hydrazine and sodium zincate, NaOH, and others can only dissolve cellulose with a low DP. ④ Organic bases, such as quaternary ammonium bases $(CH_3)_4NOH$, amine oxides, and others, are also aqueous solvents. The application of amine oxide solvent to dissolve cellulose can be used to manufacture the man-made fibers. ⑤ Complexes, such as copper oxide ammonia (cuoxam), copper ethylenediamine (cuen), cobalt hydroxide ethylenediamine (cooxen), zinc ethylenediamine (zincoxen), cadmium ethylenediamine (cadoxen), and the iron–tartaric acid–sodium complex (EWNN, an aqueous alkaline solution of iron sodium tartrate) are included as aqueous solvents [9].

A nonaqueous solvent of cellulose refers to a nonaqueous or less-aqueous solvent that is based on the organic solvents. It consists of activators and organic liquids. The organic solvents can be used as a component of the active agent and as a

solvent of the activator, which can make the solvent have a larger polarity to dissolve cellulose. Therefore, the mechanism of cellulose dissolved in a nonaqueous solvent system cannot be easily explained by swelling theory, as in aqueous solvents. The detailed mechanism of this process can be expressed as follows: ① An oxygen atom and a hydrogen atom of cellulose hydroxyl participate in the interaction of the EDA; the oxygen atom and the hydrogen atom act as a π-electron donor and a δ-electron acceptor, respectively. ② The active agent in the solvent system has an electron donor center and an electron receiving center; the spatial location of these two centers is suitable for interaction with the oxygen atom and hydrogen atom of cellulose hydroxyl. ③ There is necessarily a suitable scope for the interaction strength of the EDA, causing the centers of the donor and acceptor to interact in polar organic solvents. When the hydroxyl charge separates to some extent, the complex of cellulose molecular chains is separated and dissolved.

Several different systems of nonaqueous solvents of cellulose exist: ① Paraformaldehyde/dimethyl sulfoxide (PF/DMSO) is an excellent new solvent system that is not biodegradable. PF resolves into formaldehyde by heating, and then formaldehyde reacts with the hydroxyl group of cellulose to generate hydroxymethylcellulose, which is dissolved in DMSO. ② Dinitrogen tetroxide/dimethylformamide (N_2O_4/DMF or DMSO) is an intermediary derivative of the reaction of N_2O_4 with cellulose to generate nitrite esters; it can be dissolved in DMF or DMSO. ③ Amine oxides directly dissolve cellulose without the intermediate derivatives. ④ Liquid ammonia/ammonium thiocyanate restricts the dissolution of the cellulose; the solvent consisting of 72.1 % (w/w) NH_4SCN, 26.5 % (w/w) NH_3, and 1.4 % (w/w) H_2O has the maximum dissolving ability. ⑤ Lithium chloride/dimethylacetamide (LiCl/DMAC) also directly dissolves cellulose without the intermediate derivatives. At room temperature, the LiCl/DMAC solution is stable and can be used for reeling off raw silk and film forming. Recently, research on nonaqueous solvents of cellulose has been active; they not only can be used to produce artificial fiber and films but also can be available for processing cellulosic materials and for the use of cellulose in homogeneous conditions to produce cellulose derivatives. The problems of cellulose solvents are the low solubility of cellulose, high price and low recovery of solvents, and environmental pollution.

Thermal decomposition of cellulose is in the narrow temperature range of 300–375 °C. Different products depend on different temperatures. Heated at a low temperature (200–280 °C), cellulose mainly dehydrates into dewatering cellulose and then forms charcoal and gas products. Heated at higher temperatures, cellulose separates into flammable volatile products (tar). The most important intermediate product of cellulose high-temperature thermal degradation is laevoglucose, which can be further degraded into low molecular products and tar-like products. Tar-like products can be polymerized into an aromatic ring structure similar to graphite structure at high temperature (400 °C or higher). Mechanical degradation of the cellulose occurs because cellulose in the mechanical process can effectively absorb

mechanical energy, causing changes of morphology and microstructure; these changes are shown as decreased DP and crystallinity and significantly increased accessibility [10].

2.3.4 Biosynthesis of Cellulose

2.3.4.1 Cellulose Synthesizing Site

Some research has already forecast that assembling of cellulose microfilaments is finished in the enzyme complex located in the extending top of the cellulose. Then, scientists hypothesized that a cellulose synthase complex was made up of many subunits, and each subunit synthesized single-chain glucose, then polymerized it to the ordered particles of cellulose. But, until 1976, through the freeze-etching technique, the complex located in the end of the cellulose microfilament was first observed in green algae. This verified the hypothesis that assembling of cellulose microfilaments is finished in the enzyme complex located in the extending top of the cellulose. The subunits of the complex are arranged linearly in three lines and form the linear enzyme complex where cellulose is synthesized [11]. The alternating self-aggregation and dispersion of the complex determine that the microfilament arrangement direction changes periodically, resulting in different levels of microfilaments arranged perpendicular to each other. Later, similar terminal complexes were observed in bacteria, mosses, ferns, green algae, and microtubule plants, but in corns, spherical complexes were found [12].

A terminal complex like a rosette has been observed in higher plants and concentrates in the cellulose gathering place. Each six cellulose synthase subunits of rosette synthesizes 6 glucose chains and then forms microfilaments with 36 chains. The microfilament directions are mutually different in the different levels of the cell wall, which makes the cell wall in any direction have high mechanical strength [13]. The rosette complex not only has the function of synthase but also can bring glucose chains to the surface of cytoplasm. A complete rosette complex is essential for the synthesis of crystalline cellulose. The terminal complex would disappear or be changed when EDTA (ethylenediaminetetraacetic acid) is used to handle oysters or Congo red is used to handle banana cells, further causing the interruption or disturbance of cellulose biosynthesis. Once the cellulose synthesis recovers, the terminal complex reappears. The mutation of the *Ces*AI gene in *Arabidopsis* heat-sensitive mutants (RSWL) will lead to the reduction of the cellulose content, the content of antacid β-glucan, and the number of rosette complexes in the cell membrane, perhaps because the mutation of this enzyme disrupts the structure of the rosette complex. This indicates that the RSW1 (a radial swelling phenotype) maybe one component constituting the rosette complex [14]. Kimura et al. [15] used the polyclonal antibody technique on the central area in cotton *CesA* protein to verify that the plasma membrane has a rose-like structure, which was the complex of cellulose synthase, and was *Ces*A protein located in rosettes. This finding demonstrated that

the *Ces*A gene has an important role in cellulose synthesis and provided direct evidence for the hypothesis that cellulose biosynthesis takes place in the terminal complex of the rosette [11].

It is uncertain whether the rosette complex is composed of identical subunits or different subunits. Key information on assembly also still cannot be clarified. The bacteria linear terminal complex goes through the cell membrane and lipopolysaccharide layer and mainly synthesizes the 1α types of cellulose I, which is the metastable monoclinic system. But, the rosette terminal complex in plants is part of the cell membrane, which mainly synthesizes the 1β type of cellulose I, which is a stable monoclinic form. Therefore, it is generally believed that cellulose synthesis in cotton also occurs in the cellulose synthase complex connected with the plasma membrane.

In the process of cellulose biosynthesis, in addition to the terminal complex, another polypeptide with a molecular mass of 18 kDa also plays an important role. This polypeptide does not exist in the plasma membrane but is loosely connected with the plasma membrane. So, it is unlikely to be the component with catalytic activity in the cellulose synthase complexes. However, it may have regulating effect because it can combine with 2,6-dichloro-phenyl nitrile, which is the inhibitor of cellulose synthesis.

2.3.4.2 Substrate for Cellulose Synthesis

Identifying the substrate of cellulose synthesis has been difficult. Previous studies reported that callosum generated in the translating period was the substrate of cellulose synthesis. This result was derived from the fact that, with in vivo conditions, the speed of synthesizing callosum from the substrate that can supply a radioactive label is higher than the predictable accumulation level, and the conversion of callose radioactivity is consistent with the change of cellulose. The conversion may occur because of the transglycosylation of β-l,3-glucose polymerase; the discovery that the cell wall had the activity of β-l,3-glucose polymerase also supports the hypothesis mentioned. Callose is a homopolymer of β-1,3-linked glucoses. It plays an important role in the regulation of vital processes, such as metabolism of the sieve tube, the development of the gametophyte, and so on. The composition and resolution of callose are directly related to the normal growth of plant metabolic processes [16].

The precursor for the biosynthesis of cellulose is uridinediphosphate-D-glucose (UDPG). However, in the past, UDPG was thought to be obtained by the catalysis of UDPG pyrophosphorylase. Now, with research on cotton fiber development, it has been found that the catalytic reaction by the sucrose synthase could also provide UDPG. In the formation stage of the secondary wall of the cotton fibers, sucrose synthase is connected with cellulose synthase, which may be used as carbon path. Two sets of evidence support this view. It has been proved that in vitro biosynthesis of cellulose takes cellulose synthase from the cell membrane of cotton fiber as the enzyme source and UDPG as a substrate. The products are always β-1,3-glucan and

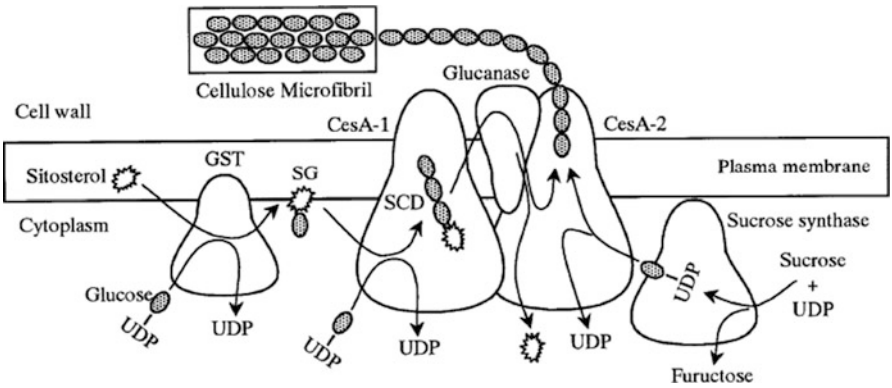

Fig. 2.2 Biosynthetic pathway of plant cellulose [18, 19]

β-1,4-glucan, a few calloses, and a small amount of cellulose. When taking sucrose as a substrate, the synthetic rate of cellulose is equal to the synthetic rate of callose, and sometimes is more than the synthetic rate of callose; the absence of Ca^{2+} is more conducive to cellulose synthesis. This shows that cellulose synthase can only use the UDGP directly from the catalytic reaction by sucrose synthase, but callose synthase can directly use the free UDPG. The other evidence is that, in the mutant of cotton without fibers, there is no gene expression of sucrose synthase in the ovule epidermal cell, but there is large gene expression of sucrose synthase in wild-type fiber cells; this shows that sucrose synthase has a close relation to the development of cotton fiber [17].

2.3.4.3 Cellulose Synthesis Process

Currently, there are different hypotheses about the mechanism of cellulose synthesis. One hypothesis suggests that the extension of the glucan chain is caused by the moving glycosyltransferase catalyzing several glucosyl residues to connect to the end of the growing cellulose chain. The synthesis of acetobacter cellulose may belong to such a mechanism. The other hypothesis claims that some short glucan could polymerize with lipid or protein and form the mature cellulose polymer. Peng et al. (2001) found that *Ces*A protein adhered to the end of noncrystalline fibers in fiber cell wall fragments treated with herbicide. Meanwhile, a small amount of the attached glucose chain was detected in these fragments, indicating that *Ces*A glycosyltransferase probably took sitosterol-β-glucoside (SG) as primers to start glucan polymerization. First, the SG and UDP-glucose is used as the substrate to generate sitosterol cellodextrin (SCD) and continue polymerization by the cellulose synthase, then enters into the crystallization process of cellulose (Fig. 2.2) [18, 19]. Schrick et al. [20] studied the relationship of biosynthesis of *Arabidopsis* sitosterol and biosynthesis of cellulose; they found that sitosterol is important in cellulose

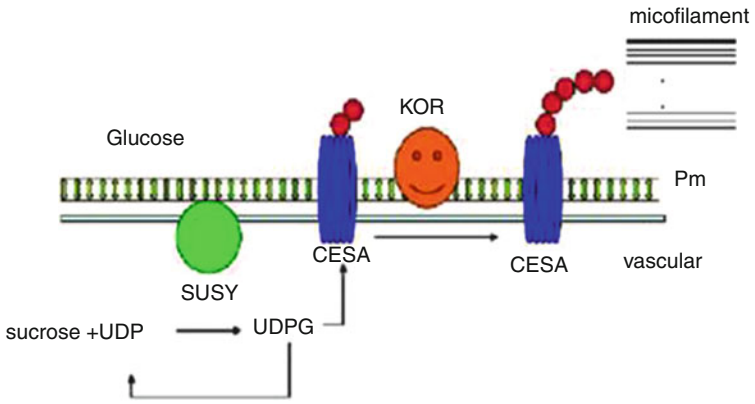

Fig. 2.3 Biosynthetic model of plant cellulose [21]

biosynthesis in *Arabidopsis*, and SG was not the only primer of cellulose synthesis. But, recent research showed that the mechanism of cellulose synthesis in higher plants may belong to the second hypothesis.

Cellulose synthesis in plants is the initiation, elongation, and termination of β-1,4-glycosidic chains. Under in vitro conditions, it is difficult to prove that UDP-Glu could directly synthesize cellulose, but it is generally considered that UDP-Glu is the substrate for it. A recent study found that SG from cotton fiber can be used as an initial extension when cellulose synthase catalyzes glucoside chains and then forms oligosaccharide linked with lipid, which is called SCDs. In the process of cellulose synthesis, cellulase (KOR) cut down SG from SCD so that the β-1,4-glucoside chain could extend more effectively. Further analysis showed that the catalytic subunit of *Ces*A is in one side of the cell membrane; the catalytic subunit of KOR is in the other side. This agrees with the following mechanism: *Ces*A accepts UDP-Glu from hyaloplasm to synthesize glucan, and then glucan crosses the plasma membrane to be further converted under the effect of cellulose. This is another significant discovery in understanding the molecular mechanisms of cellulose biosynthesis [11].

Therefore, the current anticipated process of plant cellulose biosynthesis has three steps: (1) Sucrose synthase associated with plasma membrane guides the UDP-glucose to provide a substrate for the synthesis of cellulose. (2) Hexagonal polymer is organized by the coexpressional multiple *Ces*A, and glucose monomers are polymerized to form a glucan chain; in the meantime, the discharged UDP is recycled to sucrose synthase. (3) KORRIGAN (KOR), a kind of cellulase relating to the membrane, is regarded as the editor or monitor of the cellulose microfilament and can cut defective glucan chains (Fig. 2.3). Therefore, *Ces*A, sucrose synthase, and the KOR protein interact with each other to coregulate the biosynthesis of cellulose in plants [21].

2.3.4.4 Cellulose Synthase

In 1996, by adopting complementary DNA (cDNA) random sequencing and series analysis, Pear et al. first cloned the β-1,4-glucosyltransferase gene *Cel*A, which encoded the catalytic subunit of cellulose synthase from cotton and rice [22]. Cellulose synthase has a polygene phenomenon; moreover, it constitutes a huge gene family with proteins such as cellulose synthase. Studies have shown that cellulose biosynthesis requires the participation of multiple cellulose synthase genes. Furthermore, different cellulose synthase may be related to the different tissues and cellulose synthesis of different cell wall layers.

Since the *Ces*A gene was cloned from cotton, cellulose synthase genes in many plants have also been separated and cloned in succession. The complete genomic sequence of *Arabidopsis thaliana* has been completed, so research on the *Arabidopsis Ces*A gene is the clearest. The length of the cellulose synthase gene is probably in the range of 3.5–5 kb and contains 9–13 introns. The length of the transcription product is between 3.0 and 3.5 kb. The length of the coded peptide chain is about 985–1,088 amino acids. Introns and exons of these genes are arranged conservatively; the presence of introns determines the most important differences between genes. Taking the homology comparison in the amino acid sequence of *Ces*A protein in *Arabidopsis*, corn, cotton, rice, and poplar, it has been found that each has three Asp residues and eight transmembrane domains. The three Asp residues are linked closely and together with the conserved sequence of QXXRw to form active sites used to connect with substrates on one side of the cytoplasm membrane [11]. In addition, the n-terminal of *Ces*A protein contains two conserved zinc finger domains or LIM domains (cysteine-rich zinc-binding domains) which contain four conserved and tandem cysteine residues (X3Cx2-4CX12-15CX2C). This sequence can be combined with DNA and may have a relationship with interaction between subunits of *Ces*A protein. Compared with *Ces*A protein of bacteria, there are two characteristic areas: the plant-conserved region (CR-p) and the hypervariable region (HVR) [23].

The number of amino acids in the *Ces*A protein family is approximately between 985 and 1,088; their homology 53–98 %. Studies indicated that in *Arabidopsis*, except for the identified *Ces*A gene family, there is a category of genes containing the conserved sequence of D, D, D, QXXRW similar to the structure of *Ces*A genes. However, they all belong to the glycosyltransferase family 2 (GT family 2) and do not have a specific sequence in *Ces*A genes. Meanwhile, amino acid sequences encoded by them have 7–35 % homology with the protein sequence of *Ces*A, so they are called cellulose synthase-like (Csl) protein [24]. Furthermore, they all have complete structural features of the membrane, and there are three to six transmembrane structures and one or two transmembrane domains in the C-terminal and N-terminal, respectively. So far, the function of these genes is unclear, and they may have a relationship with the synthesis of other β-polysaccharides in the cell wall.

Biosynthesis of plant cellulose requires the participation of multiple cellulose synthase genes. Analysis of gene expression showed that two or more *Ces*A proteins

are involved in the biosynthesis of cellulose in the same developmental stage of the same cell. Phenotypes of the IRX1 mutant are similar to IRX3; both appeared to have irregular xylem and a declining cellulose content; it also has been found that expression sites and periods of these two genes were identical. Ptr*Ces*A2 and Ptr*Ces*A1 of poplar are homologous with IRX1 and IRX3. Their expression sites and period are the same. They both are expressed at the secondary cell wall synthesis of xylem. So, it was suggested that these two genes may be expressed in the same cell and related to the formation of the second wall [21]. Currently, as most studies involved with cellulose biosynthetic genes are focus on the *Ces*A gene, the *Ces*A gene has been cloned from microbes and many plants. Further study showed that the mechanism of cellulose biosynthesis was complicated. Except for cellulose synthase, sitosterol glycosyltransferase, cellulase, sucrose synthase (SUSY), cytoskeletal protein, and Rac13 proteins were likely related to cellulose synthesis. Sucrose synthase is related to the supply of substrate from cellulose biosynthesis. The experiments proved that, in three different heterotrophic systems, sucrose synthase could improve the efficiency of cellulose biosynthesis. It can catalyze the reaction of sucrose and UDP to produce UDP-glucose and fructose, which can directly offer substrate to improve the biosynthesis of the cellulose. In 2004, Konishi et al. [25] further confirmed that sucrose synthase could use sucrose to synthesize UDP-glucose, which could be used directly for cellulose synthesis.

In short, cellulose biosynthesis is a highly complex biological process; thorough clarification of its mechanism will require extensive research.

2.4 Hemicellulose

Hemicellulose is another main component in plant fiber materials. In 1891, Schulz [26] thought that polysaccharides that easily separated from plant tissue were semifinished products of cellulose or precursor molecules of cellulose, so they were named hemicellulose. He also found that this component was easy to be hydrolyzed to monosaccharides in hot, dilute mineral acid or cold 5 % NaOH solution. To hemicellulose, this concept is vague in terms of both chemical structure and biological function. In recent years, people learned more about hemicellulose with improvements of polysaccharide purification as well as application of various types of chromatography, spectroscopy, nuclear magnetic resonance, mass spectrometry (MS), and electron microscopy. Aspinall in 1962 defined that hemicellulose was derived from polysaccharides of plants and included the basic chain containing residues of D-xylose, D-mannose, D-glucose, or D-galactose and other glycosyls as branched chains linked to this basic chain. The purification of hemicelluloses was conducted according to the different alkaline solubilities with cellulose. So, in 1978, Whistler thought that hemicellulose was the polysaccharide extracted by an alkali solution, except cellulose and pectin. Unlike cellulose, hemicellulose is a copolymer composed of different amounts of several saccharide molecules [2].

2.4.1 Chemical Structure of Hemicellulose

The content and structure of hemicellulose in various plants are different. The research on the chemical structure is mainly about the composition of the main chain and branched chains of glucans in hemicellulose. The main chain may consist of one or more types of glycosyls, and the connections between glycosyl are also different. Raw materials from different producing areas and different parts have different glycan compositions. Therefore, to illustrate the chemical structure, glycans must be classified first. It is generally believed that hemicellulose is the glucan in the matrix of the cell, and the main components are xylan, xyloglucan, glucomannan, manna, galactomannan, callose, and so on [27].

2.4.1.1 Chemical Structure of Xylan Hemicelluloses

Almost all plants contain xylan. D-Xylosyls are linked with each other to form homopolymer linear molecules as the main chain. Xylan hemicellulose is the glucan with a backbone of 1,4-β-D-xylopyranose and with branch chains of 4-oxymethyl-glucuronic acid.

The hemicellulose of hardwoods and gramineous forbs is mainly composed of this kind of polysaccharide. The hemicellulose of Gramineae also contains L-arabinofuranose linking to the main chain as branch chains. The number of branch chains depends on different kinds of plants. The typical molecular structure of hemicellulose of Gramineae is chiefly composed of β-D-xylopyranosyl, which is linked by β-1,4-glucosidic bonds. Branch chains consist of L-arabinofuranosyl and D-glucuronopyranosyl, respectively, on C3 and C2 of the main chain; there are also branch chains composed of xylosyl and acetyl (xylosyl acetate). The DP of hemicellulose in Gramineae is less than 100. Xylan hemicellluloses in timber are composed of linear xylans linked by β-1,4-glucosidic bonds, with some different short-branch chains linked to the main chain, similar to the Gramineae. However, average polymerization is higher than 100. In addition, hemicellulose from softwoods and hardwoods also has the distinction. Hemicellulose of hardwood is chiefly acidic xylans that have been partly acetylized; for example, the content of this hemicellulose in birch is about 35 %, while this content in *Euonymus bungenus* is only 13 % [28]. Xylan hemicellulose in softwoods is 4-O-methyl-glucuronic acid arabinose-xylan with almost no acetyl, while O-acety-L-4-O-methyl-glucuronic acid xylan is the most important hemicellulose in hardwoods [2].

2.4.1.2 Chemical Structure of Mannan Hemicellulose

Softwoods contain the highest content of mannan hemicellulose; some hardwoods also have mannan hemicellulose, but grass has little. Mannose and glucose are linked by β(1→4) bonds to form inhomogeneous polymer as the main chain.

The main chain of mannan hemicellulose in hardwoods is composed of glucose and mannose; the proportion of these two glycosyls is 1.5–2:1, and the average DP is 60–70; whether it is acetylated remains unclear. For the mannan hemicellulose of softwoods, glucose and mannose in a ratio of 3:1 arrange randomly to form the main chain, galactosyl is linked to the glucose or mannose of the main chain by α(1→6) bonds, and acetyl seems to be evenly distributed on the C2 and C3 of mannose. The average DP is more than 60; some can reach 100 [2].

2.4.1.3 Chemical Structure of Xyloglucan

The main chains of xyloglucan and cellulose are composed of D-glucopyranose linked with β-(1 → 4) bonds. The difference is that 75 % glucosyl residues are replaced by α-D-xylopyranose at *O*-6 on the main chain of the former. Xyloglucan mainly contains glucose, xylose, and galactose, and its residual ratio is approximately 4:3:1. According to differences in family, xyloglucan in plants may also contain fucose and arabinose. The main chain of xyloglucan in the dicotyledon is β-(1 → 4) glucans. α-Xylose residues are linked to the *O*-6 of the β-glucose residues. The terminal galactose is linked to the *O*-2 site of xylose residues with β bonds. If fucose is contained, it is linked to the *O*-2 site of galactose residues with α bonds. Sometimes, arabinose exists in xyloglucan, but the amount is small. The content of xyloglucan in the monocotyledon is much different; generally, terminal galactose does not exist, and the contents of xylose and galactose are lower than in the dicotyledon.

2.4.1.4 Chemical Structure of Mannan

Mannan compounds include mannan, galactomannan, glucomannan, glucuronic acid mannan, and so on. Mannose residues are connected by a β-(1 → 4) bond to form mannan, but they form galactomannan if linked to galactose residues by an α-(1 → 6) bond. The backbone of glucomannan is composed of glucose and mannose, which are linked by β-(1 → 4) bonds with the residual ratio of 1:3. Glucomannan also contains one galactose residue as a branch chain. Therefore, it is sometimes called galactoglucomannan. Furthermore, the hydroxyl group of mannose residues may also be acetylated. Glucuronic acid is prevalent in the cell wall, but its content is low. Mannose residues linked by α-(1 → 4) bonds and glucuronic acid residues linked by β-(1 → 2) bonds exist alternately in the main chain of glucuronic acid, whose side chains not only have β-(1 → 6)–linked xylose or galactose but also have 1 → 3–linked arabinose [2].

2.4.1.5 Chemical Structures of Galactan and Arabinogalactan

Galactose residues are connected by β-(1 → 4) bonds to form the backbone of galactan; galactose residues, as the side chain, are attached to the *O*-6. There are

two types of arabinogalactan: The common type has terminal galactose residues linked at *O*-3 or *O*-6, galactose residues linked at *O*-3 or *O*-6, and arabinofuranose residues linked at *O*-3 or *O*-5. Another type has galactose residues linked by *O*-4 or *O*-3 and *O*-4 bonds and arabinofuranose residues linked terminally or by an *O*-5 bond. Arabinogalactan may also be oligosaccharide constituted by several arabinose residues. Further, ferulic acid may be linked to some arabinose and galactose residues. Arabinogalactan in the cell walls may be an independent molecule or as the side chains on the polysaccharide molecules of pectin [2].

2.4.1.6 Chemical Structure of Arabinan

Arabinan entirely consists of arabinose, and α-L-arabinofuranosyl residues are linked with each other at C-5, forming the main chain. Arabinan contains many branched chains; some side chains of arabinofuranosyl are linked to *O*-2 or *O*-3 or simultaneously connected to *O*-2 and *O*-3, and some side chains are composed of arabinose.

2.4.2 Chemical Properties of Hemicellulose

Because of the low DP and few crystalline structures, hemicellulose is more easily degraded in acidic medium than cellulose. But, the category of glycosyl in hemicellulose varies, including the pyran type, furan type, α-glycoside bond-linked type, β-glycoside bond-linked type, L- configuration type, D- configuration type, and so on. The ways of linkage between glycosyls are various, such as 1-2, 1-3, 1-4 and 1-6 links [2]. Most studies showed that hydrolysis of methyl-rabopyranose is the fastest; the others are arranged in decreasing speed as follows: methyl-D-galactopyranoside, methyl-D-xylopyranoside, methyl-D-mannopyranoside, and methyl-D-galactopyranoside, which is the most stable. The β-D type of glycoside is easier to hydrolyze than the α-D type. Generally, the hydrolysis rate of the furan type is faster than that of the pyran type. The hydrolysis rate of glucuronide is 40,000; perhaps the carboxyl has positive control in the glucoside bond.

Hemicellulose is an inhomogeneous glycan composed of a variety of glycosyls, so the reducing ends have many kinds of glycosyls and some branch chains. Similar to cellulose, hemicellulose can have a peeling reaction under mild alkaline conditions. At high temperature, it would have alkaline hydrolysis. Research showed that the speed of alkaline hydrolysis of furan glycosides was many times faster than that of pyran glycosides. Hemicellulose can dissolve in both alkali solution (5 % Na_2CO_3 solution) and acid solution (2 % HCl solution). It has a relative affinity to water, which can make it form a viscous state or become a gelling agent. In rheologic studies of the viscosity of hemicellulose, this phenomenon can be well observed. For example, when the concentration of hemicelluloses in water reaches 0.5 %, the aqueous solution of hemicellulose has a certain consistency

that is the same as in human saliva; when the concentration is 2 %, the solution cannot flow because of the viscosity generated. When the concentration reaches 4 %, the solution is to be regarded as a gel. The affinity of hemicellulose is closely related to its pentose; for example, arabinose and xylose are responsible for fixing water masses on to different structures of the hemicellulose. The greatest benefit brought by this characteristic is to apply pentose in food technology. This feature also illustrates that if the percentage of the pentose in the hemicellulose is too low, the spatial organization keeps pentose away from water, resulting in low affinity of hemicellulose to water [2].

2.4.3 Biosynthesis of Hemicelluloses

Research showed that, in the plant cells obtained, the Golgi apparatus controls the biosynthesis of hemicellulose. Proteins synthesized on the ribosome of the endoplasmic reticulum of the plant cell can be transferred to the Golgi apparatus and form glycosides; the hemicellulose produced is contained in the Golgi vesicles and moved to the cell surface (moved to the cell membrane). In the cell membrane, the Golgi vesicles inosculate to the continuous plasma membrane, further causing the hemicellulose to be stuck to the cell wall. The Golgi apparatus can produce hemicellulose because it can produce the enzymes needed for its synthesis [2].

At the initial stage of polysaccharide biosynthesis in the cell wall, a certain primer is required to accept sugar residues under the effect of polysaccharide synthase. It can be speculated that the primer of polysaccharide biosynthesis in cell wall is a protein because that protein is the biosynthesis primer of many polysaccharides, such as starch, glycogen, and so on. First, the reducing end of the first sugar residue is linked to protein. Then, the protein accepts monosaccharide residues from sugar nucleotides under the effect of polysaccharide synthase, extending the polysaccharide chain. Actually, at the initial stage of the biosynthesis of glucorono-xylan in the pea and xyloglucan biosynthesis in the suspension culture of bean, it was found that some protein primers indeed participated. Inositol can be used as a primer at the initial stage of callose biosynthesis. However, regardless of the circumstance mentioned, it is not clear whether these primers have been "cut" before polysaccharide enters the cell walls or enters the cell wall together with polysaccharide. Therefore, this interesting issue should be studied further.

2.4.4 Physiological Function of Hemicelluloses

Although some data indicate that some hemicellulose, like starch, exerts a function as a storage polysaccharide, combination with lignin and cellulose molecules increases the resistance to enzymatic degradation of the cell wall and the insolubility

of components in the cell wall. Some reports also indicated that xyloglucan is related to plant morphogenesis. However, the generally accepted view is that the main function of hemicellulose is to take part in building the cell wall structure and the regulation of the cell growth process.

2.5 Lignin

Lignin is one of the most abundant organic polymers in plants, just behind cellulose. It is the exclusive chemical composition of gymnosperm and angiosperm. The content of lignin in wood and Gramineae is 20–40% and 15–20 %, respectively. Lignin is the name of a group of substances; their inhomogeneity is manifested in different species of plants, length of growing season, and different parts of the plants. Even in the different morphologies of cells of the same xylem or different cell wall layers, the structures of lignin are not the same [29].

Lignin is a complex composed of complicated phenylpropane units nonlinearly and randomly linked; three main monomers are coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Because of the different monomers, lignin can be divided into three types (Fig. 2.4): syringyl lignin polymerized by syringyl propane, guaiacyl lignin polymerized by guaiacyl propane, and hydroxy-phenyl lignin polymerized by hydroxy-phenyl propane. Usually, gymnosperm mainly contains guaiacyl (G) lignin; the dicotyledon mainly contains guaiacyl-syringyl (GS) lignin; the monocotyledon mainly contains guaiacyl-syringyl-hydroxy-phenyl (GSH) lignin [30].

At one time, lignin in plant was divided into softwood, hardwood, and grass lignins. Based on the structure of lignin, Gibbs divided lignin into G lignin and GS lignin. G lignin is chiefly formed through dehydrated oligomerization of coniferyl alcohol, and its structure is homogeneous. This kind of lignin has negative Maule reaction because less than 1.5 % of syringaldehyde and about 5 % of p-hydroxybenzaldehyde were generated when oxidized by nitrobenzene. Most lignin in softwood belongs to G lignin, which is copolymerized by guaiacyl and has a positive Maule reaction. GSH lignin is the result of the dehydrated oligomerization of coniferyl alcohol and sinapyl alcohol; the content of lignin is 17–23 %. The ratio of syringyl propane to guaiacyl propane is 0.5∼0.1; it also contains 7–12 % ester groups. p-Coumaryl alcohol in it is linked to lignin in the form of ester [10].

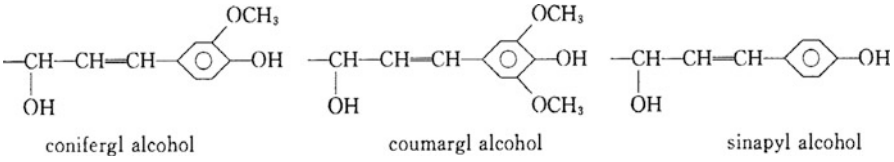

Fig. 2.4 Basic structural unit of lignin

2.5.1 Distribution of Lignin

Early studies indicated that the lignin concentration in the complex middle lamella (CML) layer is above 50 % (mass ratio), while it is about 20 % in the second wall (S layer). However, because the volume of the S layer is far greater than the volume of the ML layer, most of lignin still presents in the second wall. The lignin concentration of the cell corner of the middle lamella (CCML) layer is generally higher than that of the CML layer, even more than 70 % [31]. The lignin density of the CCML layer is about four times that of the S layer; that is, the CCML layer is full of lignins. The ultrastructure of *Salix psammophila* and the lignin distribution in various layers was researched by Xu et al. using optical (or light) microscopy (LM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), electron microscopy/ energy dispersive X-ray analysis (EM-EDXA), and confocal laser scanning microscopy (CLSM). The results showed that the cell wall of *Salix psammophila* was divided into the primary (P) wall, ML, and secondary (S) wall layer; the lignin concentration ratio in the CCML, CML, and S2 was 1.96:1.33:1. CLSM pictures (530 nm) demonstrated that the lignin concentration in the vessel and ML was higher than that in fiber cells [32]. Wu et al. proposed that the composition of the lignin molecule was affected by cell type, the site of the same growth ring, vessel arrangement, and the producing area in the 25 kinds of hardwood studied [33].

Because lignin structural units differ according to timber assortments and measurement regions, the measurement of lignin distribution is uncertain and difficult. Lignin measurement methods include ultraviolet (UV) absorption spectroscopy, EM-EDXA, the interference microscopy (IM) method, CLSM, and so on. The UV method determines the distribution of lignin in the cell wall because that lignin has a typical absorption at 270–280 nm in the UV spectra. The EM-EDXA method can provide distribution information for lignin in different regions. Simultaneously using UV and EM-EDXA to analyze samples not only could provide information on lignin microdistribution but also offer information on the ratio of G-type and S-type structural units in the different microareas. The IM method measures the optical path difference first by the inference microscope and then calculates the refractive index; finally, it obtains the volume percentage and mass percentage of lignin. The CLSM method can be used quickly to measure the biological structure of samples and obtain good measurement results in the *Z* direction [31].

2.5.2 Structure of Lignin

Lignin is a polyphenolic polymer with a three-dimensional network. Because almost all of the delignification process includes covalent bond rupture of natural lignin, with different separation methods and separation conditions, the lignin structure

would have great differences. Therefore, a structural model was usually used to present the structure of lignin. This kind of structural model only describes a hypothetical structure inferred from the average results. Further, different plant sources, or even lignin isolated from the same plant but in different ways, would have different categories of linkages and composition of functional groups, resulting in the complicated lignin structure. Through nearly two decades of research on lignin structure, a dozen structural models have been proposed. Figure 2.4 is a structural model of lignin from softwoods. It can be seen that the lignin has a complicated structure [34].

Through the study of various types of lignin structural models, lignin is a complicated amorphous polymer with three-dimensional network, which is basically composed of phenylpropane units linked to each other by the irregular coupling of C–C and C–O. Lignin includes three basic structural monomers: p-phenyl monomer (H type) derived from coumaryl alcohol, guaiacyl monomer (G type) derived from coniferyl alcohol, and syringyl monomer (S type) derived from sinapyl alcohol (Fig. 2.4). The structural formula is as follows:

Although lignin only has three basic structures, the quantity proportions of these basic structures vary greatly in different families of plants. Lignin of hardwood includes large amounts of syringyl units. In the UV-photodegradated production from *Eucalyptus urophylla* lignin, ω (syringyl-type compounds) is 58.10 %, and ω (guaiacyl-type compounds) is 18.75 %. Compared with eucalyptus lignin, the pyrolysis products of sulfate pulp lignin are rich in guaiacol. In the sulfate pulp lignin, M (syringyl)/m (guaiacyl) is 4.3:1; in the eucalyptus lignin, this ratio is 6.4:1. Structural units of softwood lignin are mainly guaiacyl-type units; a small amount of p-hydroxyphenyl-type units remains. Wheat straw lignin mainly consists of noncondensed guaiacyl units, noncondensed syringyl units, and other condensed units; the ratio of n (noncondensed guaiacyl units) to n (noncondensed syringyl units) to n (condensed units) is 1.44:1:3.24. The content of the $–OCH_3$ group in bamboo lignin is similar to the content in hardwood lignin, for example, in Bai jia bamboo (ph, nidularia Mu) lignin, the ratio of n (guaiacyl units) to n (syringyl units) to n (hydroxy-phenyl units) is 1:1.15:0.54 [35].

The coupling modes between each basic unit include β-O-4, β-5, β-1, and so on. Figure 2.5 is a partial section of a softwood lignin structure.

Ether bonds in lignin include phenol-ether bonds, alkyl-ether bonds, dialkyl bonds, diaryl ether bonds, and so on. About two thirds to three quarter phenylpropane units of lignin are linked to the adjacent structural units by ether bonds; only a small part is present in the form of free phenolic hydroxyl. Phenol-ether bonds account for 70–80 % in these groups, guaiacyl glycerol-β-aryl ethers (β-O-4) account for about half of phenol-ether bonds, followed by the guaiacyl glycerol-α-aryl ethers (α-O-4), also containing other types of ether bonds. Lignin in softwood and hardwood mainly contains aryl glycerol-β-aryl-(β-O-4) ether bonds, approximately reaching half of the lignin in softwood and more than 60 % in hardwood. In the C–C bonds of lignin, the dominant coupling type is β-5, β-β linkage, followed by β-1, β-2, 5–5, and so on [36].

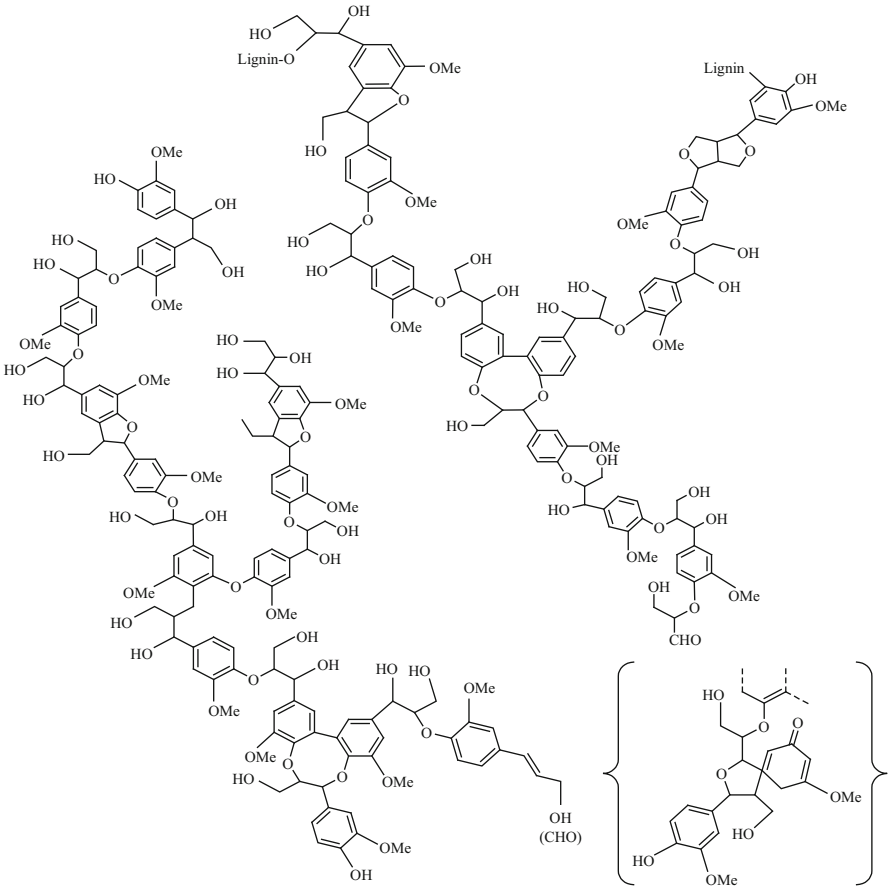

Fig. 2.5 Structural model of a section of cork lignin [34]

The main bond types of grass lignin are the same as those of lignin in wood. The main bond type of the structural unit is aromatic glycerol-β-aryl-ether bonds; these are fewer than hardwood and are similar to softwood. The proportion of carbon–carbon bonds, such as β-5 and β-β in structural units, is higher than in hardwood. In the structure of grass lignin, a considerable part of p-hydroxyphenylpropane units connects with phenylpropane units in its ester form. Taking straw as an example, 60 % p-hydroxyphenylpropane units are connected in the form of its esters. In addition, grass lignin still contains a small amount of ferulic acid esters.

Because the types and positions of functional groups in different types of lignin are different, lignin of gymnosperms has different chemical characteristics. Lignin in gymnosperm mainly contains guaiacyl lignin (G), the G-structure unit of which has a methoxy group, and is hard to remove in the papermaking process because a stable C–C linkage was formed by linkage with other monomers. Monocotyledon mainly contains GSH lignin [37].

2.5.3 Physicochemical Properties of Lignin

2.5.3.1 Chemical Properties of Lignin

The chemical properties of lignin include halogenation, nitration, and oxidation reactions on the phenyl ring; reactions on the benzyl alcohol, the aryl ether bond, and an alkyl ether bond in the side chain; lignin-modified chromogenic reaction; and so on. The chemical reactions of the lignin structural unit are divided into two major categories: nucleophilic reactions and electrophilic reactions.

(1) Chemical reactions of lignin structural unit on the side chain
Reactions on the lignin side chains are associated with pulping and lignin modification; the reaction is a nucleophilic reaction. The following reagents can conduct nucleophilic reactions with lignin:

① In alkaline medium, the effect of HO-, HS-, and S2-nucleophilic reagents leads to the cleavage of the main ether bond (e.g., α-aryl ether bond, a phenol-type α-alkoxy ether bond, and phenol-type β-aryl ether bond) and fragmentation and partial dissolution of macromolecule lignin. In alkaline medium, the phenol-type structural unit is separated into phenolate anions, and an oxygen atom that affects the benzene ring by induction and a conjugative effect, which activates their *ortho-* and *para-* positions and thereby affects the stability of the CO bond and breaks the α-aryl ether bond, then generating a methylene quinone intermediate and resulting in the aromatization of methylene quinone to generate a 1,2-diphenylethene structure.
② In neutral medium, reaction with nucleophile HSO_3^- or SO_3^{2-} leads to breaking of the ether bond and brings SO_3^{2-} groups in the degradation of lignin fragments.
③ Acidic media mainly relate to the lignin fragmentation reaction of the acidic sulfite pulping process. SO_2 aqueous solution is taken as an affinity reagent, leading to the breakage of phenol-type and nonphenolic α-aryl ether bonds, sulfonation of α-carbon, and increased lignin hydrophilicity. Phenol-type and nonphenolic α-alkoxy ether bonds may also have a similar reaction. In addition, C1, C5, and C6 on the high electron density centers of the aromatic ring could also have a condensation reaction with methylene quinone intermediates [10, 36].

(2) Chemical reaction of the aromatic ring in the lignin structure
Chemical reactions of the aromatic ring in the structural unit of lignin are closely related to the lignin-bleaching process and its modification and have been divided into electrophilic and nucleophilic reactions.

① Electrophilic substitution reaction: This mainly refers to substitution and oxidation reactions. Electrophilic reagents include chlorine, chlorine dioxide, oxygen molecule, ozone, nitro cation, nitroso cation, and so on. The electrophilic reagent replacement breaks the side chains of lignin and leads to the oxidative cleavage of β-aryl ether linkages. The aliphatic side chain is oxidized into a carboxylic acid,

and the aromatic ring is oxidized into the compound of the o-quinone structure, which will finally be oxidized into dicarboxylic acid derivatives.

② Nucleophilic reaction: Nucleophilic reagents that can react with the aromatic ring of lignin include hydroxide ions, hypochlorite ions, and hydrogen peroxide ions. These nucleophilic reagents can react with the chromophoric groups in the degraded lignin fragments, breaking the chromophoric structure to some extent [10].

(3) Lignin chromogenic reaction

① The condensation reaction of lignin with concentrated inorganic acids is mainly related to the coniferyl aldehyde structure in the lignin structure.

② Mäule chromogenic reaction: Hardwood lignin becomes reddish violet when firstly treat with $KMnO_4$ and HCl and then ammonia. The syringyl ring generates methoxy o-dihydroxybenzene under the effect of $KMnO_4$ and HCl, then generates a methoxy-o-quinone structure, which is purple after ammonia treatment.

③ Cross-Bevan reaction: Timber without extractives is treated with chlorine in the wet state; lignin will be converted into chlorinated lignin after sulfinic acid and sodium sulfite treatment, turning hardwood lignin red-purple [29].

2.5.3.2 Physical Properties of Lignin

(1) Molecular weight and polydispersity

Any type of separation method may cause some partial degradation and changes in lignin. Accordingly, the molecular weight of the original lignin is unsure. The molecular weight of the separated lignin varies with the separation method and conditions. Its molecular weight distribution can range from several hundred to several million. Under the effect of mechanical action, enzymes, or chemical reagents, the three-dimensional net structure is degraded into different size lignin fragments, which leads to the molecular weight polydispersity of lignin [29]. The molecular weight of separated lignin varies greatly; taking milled wood lignin (MWL) from spruce as an example, depending on the grinding time and extraction methods, its weight average molecular weights include 2,100, 7,100, and 11,000. The weight average molecular weight of the high molecular weight fraction of MWL from spruce and hardwood is 40,000 and 18,000, respectively, and the low molecular weight fraction is between 3,700 and 5,000. The molecular weight of lignosulfonate ranges from 10^3 to 10^5; the maximum is beyond 10^6. Kraft lignin has a much lower molecular weight [10]. Determination of lignin molecular weight includes the osmometric method, light-scattering method, supercentrifugation, gel permeation chromatography, high-pressure liquid chromatography, and so on. As for insoluble lignin, such as lignin obtained by acid hydrolysis, their molecular weights are measured depending on the linear relationship between $\lg M_w$ (the base-10 logarithm of molecular weight) and the heat-softening temperature T_s [10].

(2) Solubility
Hydroxyls and many polar groups exist in the lignin structure, resulting in strong intramolecular and intermolecular hydrogen bonds, and making the intrinsic lignin insoluble in any solvent. Condensation or degradation make the separated lignin able to be divided into soluble lignin and insoluble lignin; the former has an amorphous structure, and the latter is the morphological structure of the raw material fibers. The presence of phenolic hydroxyl and carboxyl makes the lignin able to be dissolved in alkaline solution. Separated Brauns lignin and organosolv lignin can be dissolved in dioxane, DMSO, methanol, ethanol, acetone, methyl cellosolve, and pyridine. Alkali lignin and lignosulfonate usually can be dissolved in a dilute alkali, water, and salt solution. Brauns lignin, phenol lignin, and many organosolv lignins can be completely dissolved in dioxane. Acid lignin is not soluble in any solvents. The best solvents for most separated lignin are acetyl bromide and hexafluoroisopropanol in acetic acid [29].

(3) Thermal properties
Lignin is an amorphous thermoplastic polymer. It has slight friability under high temperature and cannot form film in solution. It also has glassy transfer properties. Under the glassy transfer temperature, lignin is in the solid glass phase; it begins to move when it is above the glassy transfer temperature. The lignin is softened to become sticky and has adhesive force. The glassy transfer temperature of separated lignin varies with the raw materials, separation method, molecular weight, and water content. The softening temperature of absolutely dried lignin ranges from 127 to 129 °C, which remarkably decreased with increased water content, indicating that water acts as a plasticizer in lignin. The higher the lignin molecular weight is, the higher the softening point is. For example, for lignins with a M_W of 85,000 and 4,300, the softening points, respectively, are 176 and 127 °C [29].

(4) Relative density
The relative density of lignin is roughly between 1.35 and 1.50. Values vary with the liquid for measurement if measured by water. The relative density of sulfuric acid lignin isolated from pine is 1.451 and is 1.436 if measured by benzene. The relative density of dioxane lignin is 1.33 when measured by water at 20 °C and is 1.391 by dioxane. Lignin prepared by different methods has different relative densities, such as the relative density of pine glycol lignin is 1.362, but it is 1.348 for pine hydrochloride lignin [29].

(5) Color
Intrinsic lignin is a white or nearly colorless substance; the color of lignin we can see is the result of the separation and preparation process. For example, the color of lignin isolated by Brauns and named after him is light cream, and the colors of acid lignin, copper ammonia lignin, and periodate lignin vary from fawn to deep tan [29].

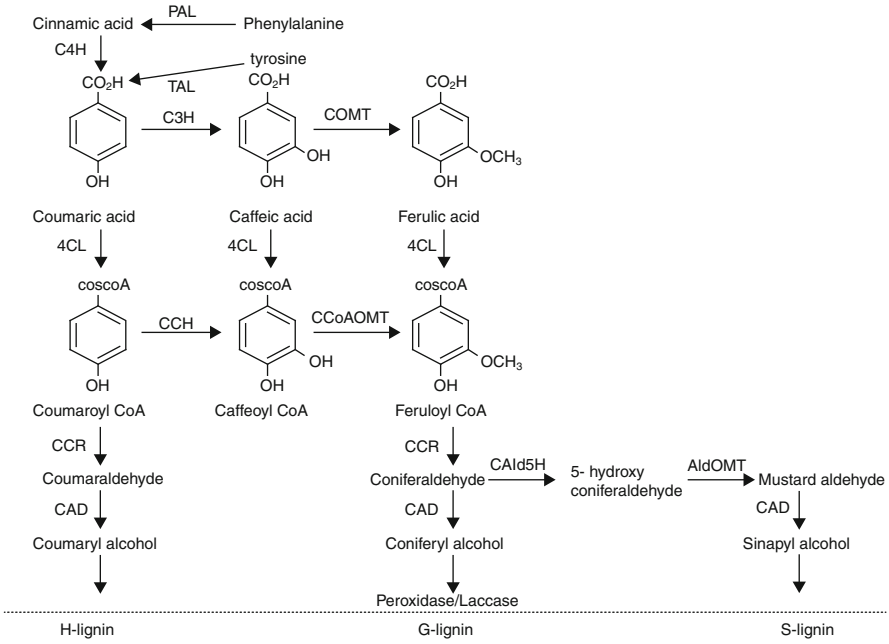

Fig. 2.6 Biosynthetic pathway of lignin monomer [38]. *PAL* phenylalanine ammonia lyase, *TAL* tyrosine deaminase, *Cald5H* coniferaldehyde-5-hydroxylase, *C4H* cinnamic acid-4-hydroxylase, *CCH* coumaroyl CoA-3-hydroxylase, *4CL* 4-coumarate CoA ligase, *CCoAOMT* caffeoyl CoA 3-O-methyltransferase, *C3H* coumarate-3-hydroxylase, *CCR* cinnamoyl CoA reductase, *AldOMT* 5-hydroxy coniferaldehyde-O-methyltransferase, *CAD* cinnamoyl alcohol dehydrogenase

2.5.4 Lignin Synthesis

2.5.4.1 Biosynthesis of Monolignols

Biosynthesis of monolignols is conducted through the phenylpropanoid pathway. In the shikimate pathway, glucose generated from the carbon dioxide by photosynthesis first is converted to the final intermediate: shikimic acid. Then, through the prephenic acid, the shikimic acid is converted into the final products of the shikimate pathway: phenylalanine and tyrosine. These two amino acids, which are widely present in plants, are starting materials for the cinnamic acid pathway. Under the effects of various enzymes, three monomers of lignin are finally synthesized after a set of reactions, such as deamination, hydroxylation, methylation, reduction, and so on (Fig. 2.6) [37, 38].

Cinnamoyl CoA reductase (CCR) catalyzes the first step of the redox reaction of lignin biosynthesis, which may be the rate-limiting step, controlling lignin synthesis and the pathway from which carbon can go into lignin biosynthesis. Cinnamyl alcohol dehydrogenase (CAD) catalyzes the redox reaction of another step in the lignin synthesis process, which may control the reduction of coniferaldehyde. Sinapyl

alcohol dehydrogenase (SAD), ferulic acid 5-hydroxylase (F5H), and bispecific caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT) are immunolocalized in cells and tissues that have S lignin. CAD is located in tissues with precipitation of G lignin. It is speculated that the last step of redox reactions of different types of lignin may be passed through different synthetic pathways and catalyzed by different enzymes [39].

Two hydroxylases are found in the phenylpropanoid pathway. C3H can catalyze coumaric acid to caffeic acid, and the other hydroxylase is F5H. *Arabidopsis fah1* mutant without F5H activity is almost full of S lignin. Now, it has been recognized that F5H can catalyze the hydroxylation of coniferaldehyde and coniferyl alcohol, converting them to monomers of S lignin.

Lignin monomer synthesis needs to go through two-step methylation reactions at the 3′and 5′ site, respectively. COMT and CCoAOMT (caffeoyl CoA 3-O-methyltransferase) are methylases on two different substrate levels. 5-Hydroxyferulic acid can be converted into sinapic acid under the methylation effect of COMT to involve in the synthesis of S lignin. Much research is more inclined to support that, in lignin biosynthesis, COMT can catalyze the methylation of caffeic acid, 5-hydroxyl coniferyl aldehyde, and 5-hydroxyl coniferyl alcohol into ferulic acid, sinapic acid, and sinapic alcohol, respectively, and CCoAOMT can catalyze the methylation of caffeoyl-CoA to generate feruloyl-CoA.

2.5.4.2 Macromolecular Synthesis of Lignin

It has been proven that the polymerization of lignin macromolecules by phenylpropane units is dehydro-oligomerization. The German scientist Freudenberg first produced synthetic lignin by coniferyl alcohol and laccase collected from mushrooms under aerobic conditions. Later, peroxidase (POD) was found to catalyze this polymerization reaction effectively. But, there is no direct evidence to prove that POD and laccase could catalyze the polymerization of various lignols [38]. Hans et al. discovered that POD and manganese ion could be used to synthesize lignin in vitro, with their structures similar to natural lignin. In this process, redox shuttles of Mn^{3+}-Mn^{2+} played an important role in lignin biosynthesis. POD did not directly react with lignol to participate in the synthesis of lignin. It catalyzed Mn^{2+} oxidized to Mn^{3+}. The latter (Mn^{3+}) may contact many kinds of lignin precursors by diffusion to catalyze the formation of free radicals in terminal groups of lignin. The latter also would participate in the formation of various covalent bonds in lignin molecules [39].

There have been several different hypotheses to explain lignification; one is a random coupling model, which proposed that lignol molecules are gradually connected to the lignin polymer by oxidative coupling, and the formation of lignin is adjusted according to the categories, amount, and chemical coupling characteristics of lignol at the terminal of the lignin molecule. The random coupling hypothesis is reasonable because it explains the plasticity of lignin biosynthesis in mutants and transgenesis research. Another hypothesis involves the dirigent-like protein model,

which considered that the lignified process is strictly under the manipulation of dirigent-like protein by controlling the formation of particular chemical bonds of the lignin molecules. The hypothesis showed that the metabolism of lignin should be an orderly life process, which explains the large numbers of *O*-4 linkages in lignin molecules. Dixon et al. believed that the formation of sinapyl alcohol and coniferyl alcohol was a relatively independent process by which enzymes involved in sinapyl alcohol synthesis were associated with each other to form a multienzyme complex. This multienzyme complex was combined in the endomembrane system through C4H, C3H, C5H, and other P450 proteins, which made a series of reactions associated with mustard alcohol synthesis pathway run in an orderly fashion. The synthesis of coniferyl alcohol is conducted in the cytoplasm another way. The phenylalanine ammonia lyase (PAL), caffeoyl CoA methyltransferase (CCOMT), CCR, and CAD distribute in the cytoplasm, catalyzing the synthesis of coniferyl alcohol [39].

Peroxidase is widespread in plants with multiformity. So, how it participates in lignin monomer polymerization needs further studies. So far, it remains uncertain whether the POD or laccase catalyzes the polymerization of lignin monomers in the plant or if they work synergistically [38].

2.5.4.3 Deposition of Lignin in Plant Tissues

Lignin biosynthesis in vascular plants is controlled by morphogenesis and the environment. The lignification process is the deposition process of lignin in plant tissues. Fluorescence detection indicated that, in the normal development of *Arabidopsis* stems, lignin mainly was deposited in the fiber cell wall between the vessel cell wall and vascular bundles. Dystopia anchorage genes ELP1 were isolated from lignin deposited mutants of *Arabidopsis* pith. In mutant stems, lignin could be deposited in the usual parts and ectopically deposited in the pith of parenchyma cells. Such dystopia anchorage would exist in both young stems and mature stems. Associated with this dystopia anchorage, enzyme activity related to lignin synthesis would increase, and COMT could be ectopically expressed in the central cells of the pith. These results suggest that ELP1 genes inhibit lignin synthesis in the pith. Lignin deposition is related to coniferyl alcohol because that methylated ester generated by coniferyl alcohol can be covalently coupled with hydroxyproline-rich glycoproteins (HRGPs), resulting in the deposition of lignin in the cell wall [40].

2.5.4.4 Factors Influencing Lignin Synthesis

Different parts of plants would have a different lignin content and composition. For example, the lignin content and structure are significantly different in the node and internode of reed (*Arundo donax*); the node has a higher density than the internode because of the high content of phenolic acids (p-coumaric acid and ferulic acid). The lignin content in different parts of lettuce is different depending on the harvest-

ing time. Compared with the normal harvest, lignin in the middle of overmature harvested lettuce buds is increased most obviously, secondarily increased in the basal area, and not significantly increased at the top [40]. The reasons for this difference are varied. For instance, methyl jasmonate can significantly improve the POD activity of seedlings and the lignin content. Both jasmonic acid and gaseous methyl jasmonic acid could induce the expression of chalcone synthase (CHS), thereby increasing the lignin content. In general, overuse of nitrogen fertilizer will reduce the lignin content and thus postpone the lignification of the plant. However, nitrogen nutrition can increase the lignin content in the mulch covering of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. Phosphate fertilizer will greatly limit the period of rapid growth of plants but increase the lignin content [40].

2.5.4.5 Regulation of Lignin Biosynthesis

Lignin biosynthesis may be regulated by changing the activities of different enzymes two main ways. One is to regulate the synthesis process of lignin monomers by reducing enzyme activities that participate in the synthesis process of lignin, such as those of CCR, and CAD, and by reducing the lignin content. The second way is to regulate certain particular enzyme activity to influence the composition and chemical structure of lignin. It is generally believed that the degradation of GS lignin, which is composed of guaiacyl monomers and syringyl monomers, is easier than that for G lignin, which is simply composed of guaiacyl monomers. Therefore, activate the enzyme activities of CAld5H and AldOMT for the coniferaldehyde substrate to compete with CAD, make additional syringyl lignin monomers synthesized via sinapyl aldehyde, and finally relatively reduce the synthesis of guaiacyl G monomer.

In the gene regulation of lignin biosynthesis, the search for and separation of biological enzymes related to lignin synthesis is the first task, and then the amino acid sequence of zymoprotein is analyzed to obtain the sequence of its messenger RNA (mRNA), which is the coding sequence of the functional gene exon. Gene transfer methods include agrobacterium-mediated indirect conversion and the gene gun technique. The antisense technology is also known as antisense DNA and antisense RNA. It can transfer the constructed or amplified antisense DNA or antisense RNA to the living body, then combine the exogenous antisense mRNA or antisense mRNA transcribed from antisense DNA with positive sense mRNA to form the duplexes, which could prevent the transportation of mRNA from the nucleus and combination with ribosome, consequently inhibiting the natural gene expression of endogenous mRNA and achieving the regulation of target genes. Antisense technology can be utilized for regulation of forest lignin. First, an oligonucleotide sequence is constructed that is antisense to the lignin synthase; then, the sequence is transferred into plants in direct or indirect ways, and it interacts with genes in plants to influence the translation and reduce the activity of zymoprotein. This is currently the most common and effective transgenic breeding technology in genetic engineering of lignin regulation. Finding and discovering a natural mutant in plants is also an equally effective and direct means [41].

2.5.5 *Biological Function of Lignin*

2.5.5.1 The Relationship Between Metabolism of Lignin and Cell Differentiation

Lignin metabolism in plants has physiological significance, which was mainly present as the close relationship between changes of its enzyme activity, the increase of intermediate and lignin contents and cell differentiation, the resistance to pathogen infection, and other physiological activities in plant development [37]. Plant cell differentiation is a core problem in developmental biology. The process of cell differentiation is essentially the changing process of cell physiology and morphology; cell differentiation and organ growth have some relation with phenylpropanoid metabolism and its products. Since the 1970s, there have been many reports about the relationship between lignin metabolism and cell differentiation; Oven found that when the plant was injured, the callus first formed lignified parenchyma cells, followed by formation of short vessels and traumatic resin canals. This process required the participation of the phenylpropanoid metabolites. Mala et al. thought that phenylalanine metabolism had a closer relationship with further development of an embryo.

The structure of cell walls from outside to inside is as follows: ML, primary wall, the first secondary wall (S1), the second secondary wall (S2), and the third secondary wall (S3). The structure of S1 and S2 of the wild-type tobacco cell wall is dense. However, the structure of the anti-CCR transgenic tobacco cell wall varies with types of cells. For example, in fiber cells, the fiber framework of S2 becomes significantly loose, but in vessel cells, the structures of S2 and S3 are changed. However, in the anti-COMT gene tobacco plants, structural changes of the cell wall are not detected. In the hybrid generation of these two transgenic plants, only the S3 structure of the vessel cell wall has slight changes. The immunocytochemistry analysis of the lignin subunit showed that the G and S units of wild-type plants concentrated on S2 in the fiber cells, G and S units of wild-type plants decreased in the hybrid generation, and cell walls became thin. In the vessel cells, G and S units in wild-type plants were unevenly distributed in the S1, S2, and S3, mostly in S2. However, G and S units of hybrid generation were distributed in S1 and S3. Corresponding with this microscopic change, plants with antisense CCR gene grow shorter and have smaller leaves. But, plants with anti-COMT gene have slight changes in height and leaf size, the height and leaf features of hybrid generation are between those of CCR gene plants and anti-COMT gene plants.

Many studies have confirmed that the formation of tracheary elements is accompanied by the synthesis of lignin. Therefore, research on the metabolism physiology of lignin is an important part in research on the differentiation physiology of tracheary elements. The results for the differentiation of wheat root tip (meristematic zone, elongation zone, maturation zone) showed that the tracheary element is mainly formed in the maturation zone. There was only preliminary differentiation and a small amount of lignin deposition in the elongation zone. Lignin is the basic factor

that causes the differentiation of totracheary elements from the apical cell of wood. In the construction of cambium and catheter, the middle structure of a nonlignified cell wall contains an irregular fine network structure, which may contain pectin and hemicellulose. After the lignification, the middle structure quickly becomes dense, fully lignified surface structure becomes more compact, and parts of surface structure are partially covered with a spherical structure [40].

2.5.5.2 Relationship Between Metabolism of Lignin and Fruit Development

Phenolics in the fruit can be mainly divided into water-soluble phenolic substances and water-insoluble phenolic substances. The former includes a variety of phenolic acids, water-soluble tannin, anthocyanin, and so on. They are soluble in both water and ethanol, but after oxidation, they cannot be redissolved in water, only in ethanol. The latter consists of condensed tannin and lignin. Lignin is the main ingredient for the formation of the secondary cell wall. The peel and pith of apple are vigorous metabolic areas of phenolics; anthocyanin is mainly synthesized in the flesh cells of peel and adjacent to peel. The large amount of lignin is in the fruit core, and the phenolics content is the lowest in the flesh.

According to reports, the content of lignin would change with the developmental stage of the apple [42]. Simple phenols and flavonoids (anthocyanin) are synthesized in the young fruit period of apple, and lignin is gradually synthesized with the beginning of the cell enlargement period. Lignin content in the peel is positively correlated with the development time of the fruit and the activity of POD [43]. In contrast, the lignin content in the yellow apple peel is slightly more than that of red apple. In addition, a low temperature has a significant impact on the lignin content in peel [40].

The harvest time also affects the lignin content. Ju et al. [44] found that the lignin content in the late-harvested Laiyang pears was the highest because many phenolics would be converted to lignin during the period of fruit development. Also, the conversion rate in flesh is higher than that in the pith tissue. Bagging also affects the lignin content. PAL is a photo-induced enzyme; its activity is positively correlated with light. However, after bagging the fruit, the activity of PAL decreases, and the lignin content in peel and pulp can both be reduced by 32 %.

2.5.5.3 Relationship Between Metabolism of Lignin and Plant Disease Resistance

Many studies have shown that, when the plant is infected or resistance is induced, the activity of enzymes related to lignin synthesis and the content of lignin would both increase, thereby enhancing the resistance of plants. Research on *Erysiphe graminis* f.sp. *tritici* showed that there was a positive correlation between the resistance of different types of wheat and the increase of lignin content that

depended on the types of wheat. Wheats different in disease resistance all have the PALactivity peak after the inoculation of powdery mildew 12 hours later. The activity peaks of enzymes related to disease resistance are high and have a long duration. The activity peak of susceptible cultivars would decline quickly and become close to the normal level after 24 h. PAL and POD activity increased sharply after bacterial infection in the leaves of mung bean. After inoculating Verticillium dahliae toresistant varieties of cotton in SBSI (Seabrook Sea Island) and susceptible varieties in Rowden, the expression of mRNA, encoding PAL and COMT, was higher than that of the reference substance. After the cotyledon, leaf, and stem of *Arabidopsis thaliana*, *Piplotaxis muralis*, *Piplotaxis tenuifolia*, and *Raphanus raphanistrum* and the cotyledon of *Sisymbrium loeselii* were inoculated with *Leptosphaeria maculans*, they all had strong resistance characteristics to tissue browning and lignin deposition.

In recent years, research on the metabolism of lignin and plant resistance has been plentiful. Pot-planting experiments of three poplar clones from *Aigeiros* sp. under waterlogging conditions showed that, in comparison to their respective controls, the activities of PAL and the contents of organic components in the basal stem phloem of all clones tested had obvious changes. When the waterlogging time was prolonged to 40 days, the metabolism within the plant was confused resulting in the abnormal contents of organic components, the PAL activity increased, and the plant growth decreased significantly. Through research on the overwintering shoots, as the temperature decreased, the PAL activities of the terminal bud, cortex, and xylem steadily increased until December, when it reached peak activity. The PAL activity of different strains in the coldest month was different [45].

In the interaction with phytopathogenic organisms, the lignification of the secondary cell wall is one of the characteristics of the disease resistance response. The lignin, as an important physical antibacterial substance in plants, can be deposited in the HRGP of cell wall as structural barriers, reinforcing the cell wall and protecting cells from infection by phytopathogenic organisms. The infection of ulcer germs can induce accumulation of HRGP and lignin deposition in the cell wall of poplar. The accumulation of different varieties of HRGP and lignin in the poplar cell wall is different and has a positive correlation with disease resistance. Treating tobacco seedlings with jasmonate acid not only can improve the ability of seedlings to be resistant to anthracnose but also can significantly increase seedling POD activity and lignin content. POD does not play a critical role in the disease resistance induced by methyl jasmonate, but lignin is closely related to the disease resistance. In addition, the lignin content in leaves of grape and cucumber has a relationship to disease resistance. Xu et al. reported that, when watermelon was infected by *Fusarium oxysporum*, lignin in seedling leaves and stems/roots, HRGP content, and POP and PAL activity would increase in different degrees with the infection in resistant varieties, and the increase of resistant varieties was significantly higher than for the susceptible varieties.

The biosynthetic pathway of lignin is a branch of the phenylpropanoid material synthesis pathway of a plant. There are reciprocal chiasmata on enzymes involved in these synthesis pathways. Therefore, in those susceptible and under waterlogging,

low-temperature, and other adverse conditions, lignin synthesis is correlated with synthesis of other phenylpropanoid substances. Chen et al. [46] reported that the total phenolic content and the pisatin and phaseoline content in the flavonoids were significantly higher than those in susceptible varieties. Zhou et al. [47] reported that, under noninfective *Verticillium* wilt, PAL activity and the content of phenol and lignin of the rootstock and grafted strains were obviously higher than those of the control strains. After infection with *Verticillium* wilt, the increasing amplitude of PAL activity, phenol content, and lignin content was higher than that of the control group. PAL activity and phenol content have some relevance to disease resistance to *Verticillium* spp. In buckwheat seedlings and different tissues, the flavonoid content always varies with the PAL activity, and this relationship is also reflected in the time sequence. As can be seen, in disease resistance and adversity resistance, nonlignin metabolism has some relevance for lignin metabolism in the phenylalanine metabolism. But, relatively little research currently is available on the degree of relevance in the different plants and the degree of relevance under normal circumstances.

2.5.5.4 Relationship Between Metabolism of Lignin and Plant Growth and Development

The growth and differentiation of plants are closely related to the activities of the cell wall, and lignin is one of the main components of plant cell walls. Since the 1970s, there have been many reports on the relationship between lignin metabolism and plant growth and development. Suzuki and Itoh [48] analyzed the lignifications of bamboo terminal buds and 2- to 14-year-old stem nodes, results of which indicated that the lignification of terminal buds was prior to the extension growth of stems; the stem node growth was closely related to the degree of lignifications. Through the study in the clones of the annual cutting alnus, they found that the sequence of the PAL activity of different clones corresponded to the sequence of the high growth of clones. Throughout the entire growth period, the PAL activity of alnus clones had a significant positive correlation with the high growth.

Lignin, as a secondary metabolite in plant growth and development, has important biological functions in the growth and development and disease resistance of plants. In the cell wall lignification process, lignin penetrates into the cell walls and fills in their framework, which increases the hardness of the cell wall, enhancing the mechanical and the compressive strengths, promoting the formation of mechanical tissues, and consolidating the plant body and water conduction. Because of the chemical characteristics of the lignin, such as the insolubility and complex phenolic polymer, lignin has hydrophobicity of the cell wall and higher resistance to pests. Clarifying the physiological effects of lignin would contribute to better understanding of many biological phenomena of the plant and provide a scientific basis for industrial and agricultural production, but further studies are needed in this field.

2.6 Ash

In plants, except for carbon, hydrogen, oxygen, and other basic elements, there are many kinds of other elements that are indispensable materials in living plant cells, such as nitrogen, sulfur, phosphorus, calcium, magnesium, iron, potassium, sodium, copper, zinc, manganese, chlorine, and more. When the sample is dried at 105 °C and further processed at 750 °C in a high temperature furnace, elements such as carbon, hydrogen, oxygen, nitrogen, sulfur, etc. disappear in the form of gaseous compounds, and the residue is the ash which contains many types of mineral elements in the form of oxide. Different types of plants and growth environment would lead to different types and contents of elements. These elements are absorbed from soil and generally exist as ions. It is well known that different parts (organs) of the plants have different functions in the process of plant growth, causing different distributions of metal ions. Residues from reed and bamboo are mainly parenchyma cells. Table 2.2 shows that, except Mn and Fe, the content of other metal elements in the residues of reed and bamboo are all significantly higher than those of wheat and bagasse. In the process of standard sieve-screening classification of bagasse, with the increase of the mesh number, the particle size becomes finer, the fiber content is reduced, the content of parenchyma cells increases (cells through the 80 mesh are mainly parenchyma cells), and the metal contents rapidly increase. The metal elements and silicon content in the joints, leaves, and ears of wheat straw are significantly higher than in the internode stem [2].

In the combustion process, a small part of the mineral elements may also be lost through gasification. The content and elemental proportion of ash vary with the varieties of raw materials, the kind of plant organs, the age of the plants, and so on. The elements in the growth environment of the plant would also have influence on the content and elemental composition of ash. Generally, wood has a low ash content, usually less than 1 % (the majority have 0.3–0.5 %, dry raw material). The

Table 2.2 Comparison of metal ion contents from several grass families [2]

Sample		Mn	Cu	Fe	Ca	Mg	Al	Na	Si	Co	Ni
Reed	Reed chip	460.2	9.9	481.4	1,569	625	763	134	9,413	<0.1	<0.2
	Reed residue	305.2	102.2	243.9	4,285	1,569	1,628	805	24,285	<0.1	12.1
Bamboo	Bamboo chip	251.3	2.5	36.4	593	440	39.1	21	8,674	<0.1	0.5
	Bamboo residue	379.8	11.1	2,181.9	2,901	1,110	353	98	17,810	0.5	4.7
Wheat	Internode stem	9.1	5.4	64	2,325	1,535	63.3	122	14,005	<0.1	1.78
	Joint	16	4.27	106	3,995	3,937	170.9	294	17,827	<0.1	1.61
	Leaf	40	4.61	329	5,061	4,376	54.1	357	4,032	<0.1	2.35
	Panicle	30	3.0	279	1,421	1,421	662.5	132	19,422	<0.1	1.21
Bagasse	>20 mesh	65	4.4	90	582	1,060	71.9	44	2,644	<0.1	0.56
	20/40 mesh	98	6.3	267	929	1,490	204.4	57	7,366	<0.1	0.65
	40/80 mesh	161	19.1	547	1,124	1,895	360.4	68	11,551	<0.1	0.78
	Over 80 mesh	312	118.1	3,004	3,084	3,075	138	209	4,408	<0.1	9.08

ash content in Gramineae and bark is high, with most having 2–5 % (straw ash content is 10–15 %) [2]. It is worthwhile to note that, compared with other plant materials, SiO_2 accounts for more than 60 % of straw ash, even more than 80 % in rice straw.

Silicon is a necessary nutrient in corn, rice, and other grass families [49, 50] There are several forms of silicon in the soil, but the silicon that can be directly used by higher plants mainly is the monomeric silicic acid [$Si(OH)_4$] in the molecular state. After $Si(OH)_4$ enters plants, the hydroxyl can form the SiO(OH) units through weak intermolecular forces with a variety of the hydrophilic components; then, the units are hydrolyzed to silica sol-gel. Finally, the $SiO_n(OH)_{4-2n}$ spherical nanoparticles are assembled into the silicon in any shape [51, 52]. It is considered that, according to the Si content, the cultivated plants can be divided into three categories. The first is plants that contain a high amount of Si, with the Si content ranging from 5 to 20 %, such as in rice. The second category involves plants that contain a moderate amount of Si, with the Si content ranging from 2 to 4 %, such as in wheat, barley, oats, and the like. The third category has plants with a low amount of Si; the Si content is below 1 %, such as in legumes and dicotyledonous plants [53]. The Si content in plants often is calculated using the percentage (%) of SiO_2 to the plant's dry weight; different plant species have different Si contents. For example, Takahashi et al. analyzed components of 175 plants cultivated on the same soil; the results showed that Si has the largest difference between plant species (the difference of Si content was as high as 200 times between the top 10 high-Si-containing plants and the top 10 low-Si-containing plants) [2].

The presence of silicon in plant materials may influence their subsequent use. For example, a large amount of Na_2SiO_3, generated from silicon of raw material in the alkaline pulping process, dissolved in alkali waste liquid would increase the viscosity of the waste liquid, lower the extraction rate of black liquor in pulp washing, and bring adverse effects in the evaporation, combustion, cauterization, and lime mud recovery processes and so on [2]. Chen et al. reported that the presence of ash in the raw materials would affect enzymatic hydrolysis and fermentation of straw. Therefore, according to the distribution characteristics of SiO_2, Chen and colleagues introduced a fractionated processing technology that included steam explosion, carding, and screening (referred to as steam-exploded fractionation) to realize the high-value utilization of lignocellulose. First, the abundant nanosilica was isolated without destroying the chemical composition of lignocellulose. Then, the lignocellulose that remained was used to prepare ethanol, butanol, and other high-value-added products through biological fermentation [54, 55]. In addition, studies showed that pulps from any source would contain a complex system composed of a variety of metal ions, with part of the metal ions coming from plant materials. In these ions, transition metal ions such as Cu, Fe, or Mn will exert an adverse influence on pulp color; decompose H_2O_2, O_2, O_3, and other bleaching agents; produce some •OH radicals; degrade carbohydrates; and finally reduce the effect of bleaching. Alkaline earth metal ions represented by Ca and Mg could have a stabilization effect on bleaching agents and protect carbohydrates. However, excessive alkaline earth metal ions would stabilize the lignin, thereby reducing

brightness [2]. Therefore, some appropriate pretreatments need to be performed to improve ion distribution in the pretreated materials, further improving the utilization efficiency of the raw material.

2.7 Pectin

Pectin is a major component of the plant cell wall; it is located between the cellulose microfilaments of the cell wall, mainly condensed by galacturonic acid and its methyl ester and composed of rhamnose and arabinose. The ML of plant cells is mainly composed of pectin, which can combine adjacent cells. According to the conditions of combination and physicochemical characteristics, the pectin can be divided into pectic acid, pectin, and protopectin. Pectic acid is a straight chain linked by an α-1,4 – bond by 100 galacturonic acids. Pectic acid is soluble in water and easily acts with calcium to generate the gel of pectate calcium. Pectin is the main component of intercellular substances in the higher plants; it is a long-chain polymer compound linked by α-1,4-glycosidic bonds by galacturonic acid ester and a small amount of galacturonic acid. The molecular weight of pectin ranges from 25,000 to 50,000, with each chain containing more than 200 galacturonic acid residues. Pectin can be dissolved in water and mainly exists in the middle and primary wall; some even is present in the cytoplasm or vacuole [56]. The molecular weight of protopectin is higher than that of pectin acid and pectin. The protopectin mainly exists in the primary wall and is insoluble in water, but it can be converted to soluble pectin under the effects of dilute acid and protopectinase. Because of the formation of calcium bridges between pectin substances, pectin molecules cross-link with each other and form a network structure that plays a bonding role as the middle layer between the cells and allows water molecules to pass through freely. The gel formed by pectin substances has viscosity and elasticity. The increase of the calcium bridge will cause decreased fluidity of the cell wall matrix. Along with the increase of the esterification degree, the formation of the calcium bridge would be reduced, and the elasticity of the cell wall would increase [57].

Pectinase is a complex enzyme system consisting of pectate lyase, pectin esterase, polygalacturonase, and so on. Polygalacturonase is also known as the pectinase and includes exonuclease and endonuclease. These can catalyze the hydrolysis of α-(1,4)-polygalacturonic acid by breaking pectin molecules down into small molecules [58]. A study found that polygalacturonase was related to the maturity of fruits; its main function is to decompose the ML of the cell wall. Many mature fruits accumulated a large number of polygalacturonases. Pectin esterase could deesterify the esterified part of polygalacturonic acids and change them to acidic pectin molecules so that the calcium bridge is formed in the polygalacturonic acid chains, making the pectin firm and strengthening the cell wall structure. Therefore, the esterification and deesterification of the polygalacturonic acid may affect the expansion of the wall. Pectinase not only exists in fruit tissue but also

exists in leaves. It is related to physiological processes such as fruit ripening and softening and leaf abscission. Pectinase is also the earliest enzyme secreted when a fungus invades the plant cell wall, which plays a key role in the utilization of natural fibrous materials by microorganisms [59].

2.8 Pigment

Pigment is also an important substance of lignocellulosic materials. Wood rays exist in the cambium, which is the meristematic tissue of wood existing between timber and bark. The function of cambium is to transport horizontally and store tissues, which makes the wood have various color changes because of the susceptibility to colored bacteria. Up to 1 year of age, tissues near the center of the xylem would be gradually replaced by branches, tannins, pigments, and other substances and then degrade to dead cells with the lost of physiological activity. Many types of heartwood have special pigments; for example, ebony heartwood is black, mahogany is red, and pine is brown [2].

Plant pigment is generally generated from lignin and some lignin degradation products with benzene ring. Lignin contains the chromophore and auxochrome, but the current understanding of the lignin chromophore is not uniform. It is generally believed that the lignin chromophore mainly contains quinone structures and conjugated systems of carbonyls and double bonds. The results of studies showed that the content of the lignin quinoid structure is less than the content of catechol. However, as the optical absorption coefficient of the quinone is higher than that of α-carbonyl (680,000 and 800 $m^2 \cdot kg^{-1}$, respectively), the quinoid structure has great optical absorption; the optical absorption in the visible region accounts for 35–60 % of the total absorption. Therefore, Kringstad et al. reported that the quinoid structure was the most important chromophore in lignin. The formation and degradation of lignin are involved with formations of paraquinones and *O*-quinone. The *O*-quinone is red, and the paraquinones are yellow; they are the major chromogenic structures of lignin. Paraquinone structures are mostly dissolved in the unit form instead of condensed units form except for a few condensed units, but *O*-quinone structures mainly remain in the lignin macromolecules without dissolution [60, 61].

Plants also contain small amounts of flavonoids and anthocyanin. In the transformation of plant materials, these pigment-like substances will be extracted, affecting the further conversion of lignocellulosic materials. In the pulp and papermaking process, the blue stain of pine would exacerbate the wood color and having adverse impacts on wood products and the papermaking process. The presence of pigments would cause a certain degree of inhibition of the properties of enzymolysis and fermentation. Therefore, pretreatments of lignocellulosic feedstock usually need to remove pigments in the hydrolysate; these treatments include macroporous resin adsorption, ion exchange, electrodialysis, and precipitation.

2.9 Cell Wall Protein

In addition to the main components mentioned, the cell wall is enriched with proteins. According to physiological function, the cell wall proteins can be roughly divided into two categories: structural protein, which is the main portion of the cell wall proteins; zymoprotein, including dozens of proteins with various enzyme activities [62].

2.9.1 Structural Proteins

(1) Extensin
Extensin is the most important structural protein in the cell wall and a subtribe of HRGP. It approximately is made up of 300 amino acid residues, with the content of hydroxyproline (Hyp) is especially high, typically 30–40 %. In addition to the Hyp, the extensin contains serine, valine, threonine, histidine, tyrosine, and so on [63]. The main sugar components in extensin are arabinose and galactose, accounting for 26–65 % of the glycoprotein, and sugars connected to the amino acid play an important role in maintaining the conformation of extensin. The extensin also can cross-link with hemicelluloses and pectin, conducive to the formation of the network structure in the cell wall and consequently increasing the rigidity and strength of the cell wall [64].

(2) Proline-rich protein
Proline-rich proteins in plant cell walls contain a repeated proline-proline sequence, which can make proline-rich protein molecules cross-link themselves or with extension and interact with acidic pectin through ionic bonds.

(3) Glycine-rich protein
The characteristics of glycine-rich protein cell wall protein are a high content of glycine (up to 70 %), highly repetitive primary structure units, and a similar protein structure as the structural protein-pectin in animals and cocoon silk protein in the silkworm. Arabinogalactan protein is also found in the plant cell wall, where the main sugars are arabinose and galactose. In the corn and other grass families, threonine- and Hyp-rich glycoproteins and histidine- and Hyp-rich glycoproteins are also found. These two glycoproteins contain threonine and histidine, respectively; the composition and content of the remaining amino acids and sugars are similar to the stretching protein [65].

2.9.2 Zymoprotein

Active proteins in the cell wall mainly refer to various enzymes and lectins. The primary wall of higher plants is enriched with a variety of enzymes that coalesce to the cell wall through ionic, covalent, and hydrogen bonds and so on. To date, dozens

of enzymes have been found in the cell walls, such as cellulase. In the plant growth and development process, the degradation of cell wall polysaccharides is mainly synergistic, completed by exoglucanase, endonuclease, and β-glucosidase [66]. POD is involved in the polymerization and cross-linking of various components in the cell wall and regulates the expansion of the cell wall. Therefore, POD plays an important role in the composition and plasticity of the cell wall [67]. Extensin is a class of cell-wall-specific apoenzyme in the cucumber hypocotyl; it was discovered by Cosgrove in 1989. It can break the hydrogen bonds among the cell wall polymers, inducing the relaxation and acidic growth of the cell wall. At present, more than 100 types of extensins have been separated and identified, and they may be prevalent in the dicotyledon and monocotyledon [68]. Studies have shown that the expression of extensin genes is subject to strict regulation in time and space. Extensin is involved in almost the entire development process of the plant [69]. Enzymatic analysis indicated that extensin has no cellulase activity but has a synergistic effect with cellulase in the enzymatic hydrolysis of microcrystalline cellulose, increasing hydrolysis efficiency of microcrystalline cellulose by 50 %. Extensin also can loosen structures of the filter paper, crystalline cellulose, hemicelluloses, and so on [70].

From the analysis of the plant cell structure mentioned, a large number of structural proteins and zymoproteins were found in the plant cell wall and cell membrane, where enzymes play an important role in cell wall growth, structural changes, and other aspects. Although in the aging stage of plants the protein content would decrease, as the corn stems stay green while seeds mature, the fresh cornstalk after harvest is still rich in protein. Currently, the utilization of straw mainly focuses on the utilization of cellulose, lignin, hemicellulose, and other major components but neglects the application of cell wall proteins. After harvest, straw often is dried and stored under natural conditions, which would significantly reduce its protein content. Some pretreatments can improve the accessibility of the substrate. But, the operating conditions of most pretreatment methods are intense, such as for steam explosion, acid or alkali treatment, or organic solvent treatment, denaturing the cell wall proteins and causing loss of active ingredients.

Han and Chen [71] separated the β-glucosidase from corn straw cell wall protein; the specific activity reached 13.5 IU·mg^{-1}, and the molecular weight was 62.4 kDa, which was similar to the β-glucosidase found in corn by other organizations. ESI-MS (Electron Spray Ionization) analysis showed that the β-glucosidase from corn straw had the highest similarity to the β-glucosidase from soybean (AAD09291). However, their species source and the molecular weights were different, indicating that they were different proteins. The best reaction temperature of β-glucosidase is 37 °C, and the optimum pH is 4.8, which also indicates the important applications in simultaneous saccharification and fermentation of cellulose because simultaneous saccharification and fermentation can react at the optimum temperature of the β-glucosidase and yeast. Studies of cornstalk simultaneous saccharification and fermentation found that cornstalk β-glucosidase was more suitable for ethanol fermentation than *Aspergillus* β-glucosidase. Through ammonium sulfate precipitation, hydrophobic chromatography, and CM-Sephadex G75, β-exoglucanase with a molecular weight of 63.1 kDa can be isolated from fresh corn straw cell wall

proteins. ESI-MS analysis found that this enzyme had the highest similarity with β-(1–3,1–4)-exoglucanase (AAF79936) in maize coleoptiles. The comprehensive comparisons of species, molecular weight, enzyme activity, and other characteristics illustrated that they may be the same protein. The optimum reaction conditions for this enzyme are 40 °C and pH 6.0, and this characteristic enables potential applications in simultaneous saccharification and fermentation of cellulose because it relieves the inconsistent temperature of β-exoglucanase and yeast. β-Exoglucanase can promote the simultaneous saccharification and fermentation and enzymatic hydrolysis, especially simultaneous saccharification and fermentation.

2.9.3 Hydrophobic Protein

Fresh cornstalks contain various proteins; in addition to the structural proteins and zymoproteins, they have hydrophobic proteins. Han and Chen obtained several kinds of hydrophobic proteins from the total proteins of fresh cornstalks; these proteins had strong adsorption of lignin. The molecular weights of these proteins ranged from 15 to 35 kDa. ESI-MS analysis of protein with a molecular weight of 18.5 kDa showed that the protein and corn lipid body-associated protein L2 (P21641) had a high degree of similarity. It can be concluded that they were the same protein by comprehensive analysis of the protein characteristics, species source, and so on. Structural analysis showed that the protein had an amphiphilic structure, 46–122 amino acids consisted of the significant hydrophobic region, while 1–46 amino acids at N-terminal and 122–187 amino acids at C-terminal were hydrophilic. Therefore, it can be inferred that, in the hydrophilic-hydrophobic interface, the protein will self-assemble into amphiphilic film. Hydrophobic protein is without cellulase activity. It can be adsorbed onto lignin and steam-exploded straw but cannot be adsorbed onto microcrystalline cellulose. It has a synergistic effect with cellulase in the enzymatic hydrolysis of steam-exploded straw but has no effect on the enzymatic hydrolysis of microcrystalline cellulose. Principle analysis showed that hydrophobic protein may play a role in the enzymatic hydrolysis of steam-exploded straw through the combination with lignin, reducing the nonspecific adsorption of cellulase on the substrate, particularly exonuclease and beta-glucosidase.

In view of the plentiful proteins in plants, as well as the possible synergy between the plant zymoprotein and exogenous cellulase in the enzymatic hydrolysis of lignocellulose, Chen et al. studied the application of vegetable protein in cellulose degradation for several years and had some success. Di Lu performed some research on the change of content of the apoplast protein of corn straw during storage and the impact on *Penicillium expansum* cellulase activity; it was found that the apoplast protein and *Penicillium expansum* cellulase had obvious synergies, with the new harvest straw apoplast protein having the most obvious synergies [72]. Han and Chen [71, 73] found that in vitro autolysis producing glucose as the main product also happened in the fresh corn straw; autolysis substrates accounted for 2.56 % of the straw dry weight; these autolysis substrates were mainly cellulose and the

β-(1–3, 1–4) glucan of hemicellulose. According to this characteristic, he developed the new technology of straw enzymolysis and fermentation based on the use of cell wall proteins in fresh corn straw; glucose and ethanol production increased by 61.2 and 112.7 %, respectively, compared with the control. Fresh cornstalks were used as the substrate for enzymatic hydrolysis and fermentation; the cell wall proteins also played an additive effect on *Trichoderma viride* cellulase.

With further research and development of the cell wall proteins in the future, the application of the plant cell wall proteins in straw biotransformation will increasingly be broadened, achieving prominent and significant results.

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Chapter 3
Biological Fundamentals for the Biotechnology of Lignocellulose

Abstract Lignocellulose is mainly composed of cellulose, hemicelluloses, and lignin. Microorganisms (fungi, bacteria, and actinomycetes) and animals can be used to degrade lignocellulose. However, the degradability and degrading mechanisms differ for the two groups. Clarifying the biological fundamentals for the biotechnology of lignocellulosic materials is the groundwork for the bioutilization of lignocellulose. This chapter introduces microbes for the degradation of natural lignocellulose; the animals capable of decomposing natural lignocellulose; the properties, hydrolysis mechanism, and application of cellulase; hemicellulose biotransformation; lignin biotransformation; microbial degradation of lignocellulose; and the ecological fundamentals of cellulose biotechnology.

Keywords Degradable microorganisms • Degradable animals • Enzymatic hydrolysis • Degradation mechanisms • Ecological fundamentals

3.1 Microbes for the Degradation of Natural Lignocellulose

Lignocellulose is mainly composed of cellulose, hemicelluloses, and lignin. The cellulose-degradable organisms are able to use lignocellulose as feedstock for growth, reproduction, and bioconversion. Microorganisms and animals can both be used as cellulose-degradable organisms (e.g., fungi, bacteria, and actinomycetes). The level of cellulase production from cellulose-degradable bacteria is not high; most of cellulases are composed of endoglucanases and are not active in crystalline cellulose. They are intracellular enzymes or adsorb in the bacterial cell walls, with a few secreting extracellularly. Little research has been done on cellulase production from actinomyces, which have extremely low cellulase activities. Fungi are well-characterized as the main groups of cellulose-degradable microorganisms with high-level cellulase production. The cellulases produced from fungi are mainly extracellular hydrolytic enzymes and provide more reasonable cellulolytic enzyme systems, including hemicellulase, pectinase, amylase, and so on. Therefore, fungi

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are the main species for cellulose degradation. At present, the study of rumen microbial cellulase has become a research focus for animal molecular nutrition. Microbes in the rumen can degrade cellulose mainly because of the existence of fungi and bacteria that can secrete cellulase, such as *Ruminococcus albus*, *Ruminococcus flavefaciens,* and *Bacteroide succinogenes* [1]. The synergistic effects between rumen microbes allow the rumen to be able to degrade cellulose well [2]. Therefore, the rumen of ruminants is regarded as a natural fermentor and has aroused increasing research interest.

According to the appearance in different stages of lignocellulose degradation, the microbes can be divided into the following four categories:

1. The microbes appear rapidly in herbaceous plant leaves. This kind microorganism can only use the secretions of leaves, the dung of insects and small animals, and some compositions that are easy to decompose in the leaves, such as starch and pectin. They have poor degradation ability on cellulose. The microbes include *Cunninghamella* spp., *Mortierella* spp., *Muwor* spp., *Aureobasidium* spp., *Cladosporium* spp., *Epicoccum* spp., and so on. Some of them grow on the leaves before they fall, and others grow in soils. They seldom appear in the end of lignocellulose degradation. The use of *Muwor* spp. fungi in the biodegradation of lignocellulose depends mainly on their fast growth and strong proliferation ability, which can make them occupy resources quickly before other microbes. However, they will disappear soon with the gradual reduction of available nutrients and the invasion of other microbes.
2. The microbes appear in the early and middle stages of the degradation process, including genera of *Chaetomium* spp. and *Deuteromycota* spp., which can grow on cellulose and hemicelluloses.
3. Microbes are almost not detectable in the early stage of the degradation process; these mainly include lignin-degradable Basidiomycota, such as *Mycena* spp., *Marasmius* spp., *Lepiota* spp., *Collybia* spp., and so on. They have a strong ability in the utilization of complex organic compounds, low population densities, and high stability. Many actinomycetes and Basidiomycota, which are typical representatives, can grow and reproduce stably in environments composed mainly of lignin, chitin, and humus.
4. The microbes that can be detected during the entire process of lignocellulose degradation include *Cladosporium* spp., *Trichoclerma* spp., *Penicillium* spp., *Aspergillus* spp., and others. Some yeast, bacteria, and actinomycetes also participate in the entire process of degradation of fallen leaves.

According to the degradation differences on lignocellulose components, the microbes can be divided into the following three categories:

1. Cellulose-degradable microorganisms, which include fungi, actinomycetes, bacteria, protozoa, and so on. The decomposition ability of fungi is especially strong, including some ascomycetes, adelomycetes, and basidiomycetes. Some cellulose-degradable microorganisms include strains from *Polyporus* spp., *Agaricales* spp., *Trichoderma* spp., and *Myrothecium* spp.; also included are *Sporocytophaga myxococcoides, Streptomyces antibioticus*, and so on.

2. Hemicellulose-degradable microorganisms. In this type of microbe, fungi dominate at the early stage of the degradation of hemicellulose, but actinomycetes are dominant at the later stage. Many groups of fungi can decompose hemicellulose; their number is much greater than that for cellulose degradation.
3. Lignin-degradable microorganisms. These mainly are Basidiomycota and *Aphyllophorales* spp. fungi, such as *Fomes* spp., *Polyporus* spp., *Polystictus* spp., and so on.

3.1.1 Cellulose-Degradable Microorganisms

In nature, only cotton fibers and a few other lignocellulosic materials are composed of single-cellulose molecules. Most of the natural lignocellulosic materials are a mixture of cellulose, hemicellulose, lignin, and other materials. The degradation lignocellulose by microbes is complex. Some microbes have complete cellulolytic enzymes and can degrade three components of lignocellulose. Some of the microbes can only degrade one of them; some only participate in a single reaction process. Cellulose-degradable microorganisms are a type of physiodeme with degrading ability on natural lignocellulose and do not include the microbes that can only degrade the degradation products of natural lignocellulose and water-soluble cellulose, although they also have an important role in the process of cellulose degradation. Cellulose-degradable microorganisms include different groups of bacteria, actinomycetes, and fungi [3].

3.1.1.1 Mesophilic Aerobic Bacteria

Aerobic bacteria play a significant role in the rapid degradation of cellulose in the surface of neutral and slightly alkaline soil. They can effectively degrade the unlignified lignocellulose, such as twigs and cotton, but it is difficult for them to degrade lignified lignocellulose. The excreted cellulolytic enzymes of aerobic bacteria adhere to the cell wall and cell membrane. These enzymes are not extracellular enzymes with low activity and are difficult to detect unless the cells are broken down.

Cytophaga spp. and *Sporocytophaga* spp. are aerobic cellulose-degradable bacteria commonly existing in soil. When they grow on filter paper, the surface of the filter paper appears pale yellow or other colors colonies. Macroscopically, filter paper dissolves and is attenuated in the area where colonies have grown. By observation with an optical microscope, it can be found that the bacteria in the colonies of various developmental stages exhibit different morphologies. In the early growth of bacteria, the cells bend, and two ends are sharp; later, the cells become shorter and thicker, and the cells arch just like *Cytophaga* spp. Some bacteria, such as *Sporocytophaga* spp., can form small cysts and produce heat-resistant spores [4].

(1) *Cellyibria* spp. and *Cellulomonase* spp. These are gram-negative mesophilic, strictly aerobic bacteria. They are small rods, with both ends circular; they have a single flagella and can move actively. Most of the species can produce yellow or brown pigments on cellulose. However, their ability to degrade cellulose is always weak, and extracellular cellulase activity is relatively low.
(2) *Cellfacicula* spp. *Cellfacicula* spp. are gram-negative bacteria. They are rods that are slightly curved, have a spindle on both ends, are amphitrichous, and can produce green, light yellow, or light brown mucus when they grow on cellulose silicate medium. They are a class of mesophilic aerobic bacilli with strong cellulolytic ability, which can make filter paper expand and stick.
(3) *Cytophaga* spp., *Sporocytophaga* spp., and *Polyangium* spp. The degradation ability of this category of lignocellulose is the strongest among neutral aerobic bacteria, and different colors can be produced on the filter. Their cell morphologies are special, and they belong to the glide bacteria. They can make filter paper, cotton, cotton fabric, and other raw fibers expand and form mucus that converts cellulose completely to extrapolysaccharide. They may be used to produce single-cell protein and polysaccharide on an industrial scale. They have been the donor for the genetic engineering of cellulase genes.

    Cytophaga spp. have some properties. For example, they are single cell, short-extending variable rods or are filamentous, with a spindle on both ends, a thin cell wall, and gliding movement; they are Gram negative, chemoorganotrophic, strictly aerobic, or facultative aerobic and contain yellow, orange, or red carotenoid pigments. The nutritional cells of *Sporocytophaga* spp. can differentiate into small sporangia, which are outer ciliated like capsular resting bodies, similar to the spores. They are circular or elliptical with diameters of approximately 1 μm. They can regerminate into nutritional cells. The nutritional cells of *Polyangium* spp. are columnar with an exactly circular end. Their small sporangia can be arranged in various shapes of fruiting bodies. Sporangia are sessile, alone or in piles. Myxosporium are similar to nutritional cells. All bacterial colonies are etching, are able to penetrate agar growth, and do not adsorb Congo red [5].
(4) *Pseudomonas* spp. *Pseudomonas* spp. are widely distributed in soil. They have the ability to decompose cellulose, but mainly soluble short-chain cellulose. They are single-cell microbes, are straight or curved rod shaped, have solitary or peritricha flagella, and are Gram negative.

    There is little research on the mesophilic aerobic bacteria because it is difficult to obtain pure culture, which consequently restricts their application. They can be isolated by the traditional method, namely, an inorganic salt tablet plus filter paper. However, the cellulose-degradable bacteria always mix with non-cellulose-degradable bacteria, which makes the sugars or metabolites produced by the former always consumed by the latter. This phenomenon may be formed in the long-term evolution of nature [5].

3.1.1.2 Thermophilic, Anaerobic, Cellulose-Degradable Bacteria

At the beginning of the twentieth century, thermophilic, anaerobic, cellulose-degradable bacteria were discovered and were the earliest known cellulose-degradable microorganisms. But, a pure culture was not obtained until the 1950s. Thermophilic, anaerobic, cellulose-degradable bacteria play a major role in the degradation of cellulose in compost and garbage disposal, and their metabolites are mainly organic acids and alcohols. Generally, under natural conditions, raw fiber materials can be decomposed quickly by these thermophilic, anaerobic, cellulose-degradable bacteria. However, the isolated pure strains lose the degrading ability under natural conditions, and the process slows. In the late 1870s, researchers began to study thermotolerant cellulose-degradable bacteria and separated them from compost successfully. There are several thermophilic, anaerobic, cellulose-degradable bacteria, such as *Clostridium thermocellum*, *Clostridium stercorarium*, and *Clostridium thermocopriae*; among these, *Clostridium thermocopriae* has the strongest ability to decompose cellulose.

1. *Clostridium thermocellum*: *C. thermocellum* can only grow on cellulose or its derivative as a carbon source. It has high extracellular cellulase activity and can break down cellulose effectively and convert cellulose into alcohol in one step. Therefore, it is one of the most promising strains for the industrial conversion of lignocellulose. Because of facile genetic manipulation, it is an excellent prokaryote donor.
2. *Clostridium* sp. EVA. In the cellulose degradation process, there is strong adhesion of microbes on insoluble cellulose. There is a corrugated apophysis structure around the transparent ring of the bacteria involved in the degradation of cellulose. The adhesion sites between bacteria and cellulose are electron-dense regions. The cellulase produced by bacteria is an extracellular enzyme linked with the cells and shows the maximum cellulase activity at 70 °C.
3. *Clostridium butyricum. C. butyricum* is basophilic and rod shaped, has peritricha flagella movement, and has oval or spherical spores.

Lin et al. [6] obtained several special thermophilic, anaerobic, cellulose-degradable bacteria strains through repeated enrichment and screening from a substantial amount of samples collected from springs, wells, and other sources in a region of heat. Multiple strains of bacteria had strong thermal stability and can grow well at 50–70 °C and have significantly higher vitality than mesophilic bacteria, showing maximum activity at 80 °C. *Clostridium* sp. JC3, a new strain isolated by Syutsubo et al. [7] was the main cellulose-degrading bacteria in thermophilic methanogenic sludge, which can degrade lignocellulose under high temperature (55 °C). Analysis of the microbial community of thermophilic cellulose-enriched culture medium showed that the new *Clostridium* sp. were the dominant bacteria participating in the degradation of cellulose.

3.1.1.3 Mesophilic Anaerobic Bacteria

Rumen microbes have been studied in detail; a class of the mesophilic anaerobic bacteria lives in a ruminant's rumen. For a long time, because of the quantitatively absolute advantage and diversified metabolic pathways of rumen bacteria, they were considered to play a major role in the degradation of the plant cell wall. Rumen microbes are strictly anaerobic gram-positive bacteria, mainly including *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivivrio fibrisolvens*, *Bacteriodes succinogenes*, and so on [8]. The first two bacteria can decompose lignocellulosic materials most remarkably. The others show cellulolytic ability only in mixed cultivation. The major products of cellulose decomposition by *Ruminococcus* spp. are O_2, acetic acid, and ethanol. Their nutritional requirements are also complex; for example, vitamins, organic nitrogen, and so on are required for the culture medium, and strictly anaerobic conditions and low oxidation-reduction potential are necessary for growth. Therefore, it is difficult to obtain their pure culture. Volatile fatty acids formed by *Ruminococcus* spp. after breaking down the cellulose are the base for ruminants to utilize straw [5].

3.1.1.4 Cellulose-Degradable Actinomycetes

Many soil actinomycetes have the ability to decompose cellulose. The ability to decompose filter paper is usually used to classify and identify actinomycetes. Many thermotolerant actinomycetes were isolated from compost in recent years, such as *Thermomonospora curvata* and *Thermomonospora fusca,* which have the ability to break down natural lignocellulose. Some strains of thermotolerant streptomyces, such as *Chepfomyces thermodiastatticus,* also have cellulolytic ability. However, few studies of these bacteria have been performed. In fact, in terms of compost, there are potential applications in developing strains that can both fix nitrogen and degrade cellulose. Moreover, it is meaningful to isolate strains with high yields of antibiotic and the ability to utilize lignocellulose because antibiotics are dominantly produced by actinomycetes, which consume a large volume of grain [5]. Song and Yang [9] isolated and screened an actinomycete that can degrade cellulose from the feces of herbivores. The strain was preliminary identified as *Streptomyces* spp., and the carboxymethyl cellulose (CMC) enzyme activity produced could reach 4.5 U mL^{-1}. It was also found that the strain rarely had pathogens and could produce antibiotics to inhibit bacterial growth, so XW5 was considered a cellulose-degradable bacteria with prospects for development and utilization. Wu et al. [10] isolated a cellulose-degradable actinomycete from thermophilic straw compost. It was identified as thermophilic streptomyces through phenotypic characteristics, chemical characteristics, and 16S ribosomal RNA (rRNA) gene sequence analysis.

3.1.1.5 Filamentous Fungi

Fungi play an important role in the decomposition of lignocellulose; they can generally be divided into mesophilic fungi and thermophilic fungi. Most mesophilic fungi grow well at 5–37 °C, with an optimum temperature range of 25–30 °C. Thermophilic fungi have strong decomposing ability on cellulose, hemicellulose, and lignin. They can secrete extracellular enzymes, and their mycelia have the function of mechanical interpenetration; these two things combine to degrade refractory organics (such as cellulose and lignin) and promote biochemical reactions [11].

Filamentous fungi are idiomatic expressions, which refers to the mycelia group of low fungi growing on solid media. It is easy to obtain a pure culture for such cellulose fungi; they have simple nutritional requirements, exist in various ecological environments, and have high extracellular cellulase activity. The understanding of the mechanisms of enzymatic degradation of cellulose started from this type microbe. They mainly include *Sclerotium rolfsii*, *Phanerochaete chrysosporium*, *Trichoderma* spp., *Aspergillus* spp., *Schizophyllum* spp., *Penicillium* spp., and so on. Among them, *Trichoderma* spp. are the most extensively studied cellulase-producing microbes, and 20 % of commercial cellulase is produced from *Trichoderma* spp. and *Aspergillus* spp. [12, 13]. *Trichoderma* spp., *Penicillium* spp., *Myrothecium* spp., *Chaefomium* spp., *Neurospora* spp., and others can produce extracellular cellulase with high filter paper activity; cellulase produced by *Aspergillus* spp. and *Rijopus* spp. have low β-glucan enzyme activities [5, 11].

Basidiomycetes play an important role in the biodegradation of lignocellulose. These fungi can be divided into three categories: brown-rot fungus, white-rot fungus, and soft-rot fungus. Brown-rot fungi, including *Coniphora puteana* and *Trichoderma viride,* mainly decompose cellulose and hemicellulose components, having almost no effect on lignin. White-rot fungus, including *Poria subacida*, *Polyporus versicolor*, and *Pleurotus ostreatus,* acts first on lignin and scarcely degrades fiber polysaccharides. Soft-rot fungus (e.g., *Cheatomium globosum*) can degrade both cellulose and lignin in hardwood and softwood, but the degradation rate is slow [11].

Trichoderma spp. and *Penicillium* spp. are suitable strains for producing cellulase; when they grow on the surface of lignocelluloses, their degradation effect is carried out by the secreted cellulase, not by the penetration of mycelia into the cell wall of fiber material. The mycelia of *Chaetomium* sp., *Ceratocystis sensu lato,* and *Xylaria* sp. can penetrate into the cell wall of lignocellulosic materials and degrade them more thoroughly. Their extracellular enzyme activity is low, so they are not suitable for the production of cellulase; they are suitable for the production of feeds by converting lignocellulosic materials into protein. Plant pathogenic fungi such as powdery mildew, anthrax, and *Cladosporium* are immersed in the plant tissue during the period of plant growth. They all have pectinase activity and endocellulose activity. *Chaetomium thermophile, Sporotrichum thermophile*, and *Thermonascm aurantiacus*, which are isolated from compost, can decompose cotton, filter paper, and other items efficiently at 50 °C, but their extracellular cellulase activity is not high [5].

Currently, cellulase is derived commercially from filamentous fungi. For example, Cellulase-Onojuka, Cellulosin AC, Macerojume, and Toyo-cellulose are generated by *Trichoderma viride*, *Aspergillus niger*, *Rhizopus arrhizus*, and *Fusarium moniliforme*, respectively.

Filamentous fungi generally can degrade cellulose and hemicelluloses but not lignin. Degradation of lignin in nature is mainly by white-rot fungi. The majority of white-rot fungi can degrade both hardwood and softwood. They have more speed and efficiency than other strains for degrading lignin. Lignin-degradable white-rot fungi include *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus,* and *Pycnoporus* spp. [11]. But, they grow slowly. Although they can strongly degrade cellulose, extracellular cellulase activity is low, which is almost undetectable with the traditional enzyme activity detection method. They generally do not produce asexual spores and can only rely on the mycelial breeding, which makes it difficult to scale up cultivation. They have a low propagation coefficient and a long culture period.

However, among the current commercial cellulases, there are at least two enzymes from basidiomycetes. One is driselase, produced by *Irpex lacteus*; another is Cellulase A-12-C, produced by *Trametes sanguinea*. Therefore, the basidiomycetes are also important as breeding strains for industrial production [3].

In short, the types of microorganisms that can synthesize complete cellulase components and secrete extracellularly to degrade cellulose are limited. Many species of bacteria, actinomycetes, and basidiomycetes can grow on natural lignocellulose or have a strong ability to decompose lignocellulose, but the extracellular cellulase activity is usually low. For example, the cellulase from myxobacteria is a surficial enzyme that binds to the surface of cells and decomposes cellulose when the bacteria contact cellulose [3].

3.1.2 Hemicellulose-Degradable Microorganisms

Almost all known majority of cellulose-degradable filamentous fungi can decompose hemicellulose and have high extracellular hemicellulase activity. However, hemicellulose-degradable microorganisms do not necessarily have the ability to decompose cellulose. In addition, some well-known amylase-producing bacteria often have a hemicellulose decomposition ability. Because hemicellulose is a generic name for a group of copolymers, the decomposition of such substances requires a variety of enzymes and microbes. Now, the hemicelluloses are classified according to different types of sugar residues contained in the main chain.

(1) Poly-L-arabinose

In the 1960s, it was found that bacillus, tumor endophytic fungus, plant pathogenic fungi, rumen bacteria, protozoa, and others could degrade poly-L-arabinose. Typical microorganisms include *Butyrivibrio fibrisolvens*, *Clostridium felsineum,* and *Aspergillus niger*.

(2) Poly-D-galactose
It has been found that some strains (*Bacillus subtilis*, rumen bacteria, and fungi) can degrade poly-D-galactose. Typical strains include *Sclerotium rolfsii, Rhizopus niveus,* and *Bacillus subtilis.*

(3) Poly-D-mannose
There are plenty of microorganisms capable of degrading poly-D-mannose, such as human intestinal bacteria, rumen bacteria, protozoa, saprophytic fungi, and plant pathogenic fungi, also including *Bacillus subtilis, Aspergillus niger*, *Rhizopus niveus*, and so on.

(4) Poly(1-3)-β-D-xylose
The microorganisms that can degrade poly(1-3)-β-D-xylose are mainly marine bacteria, green algae, brown algae, red algae, and terrestrial fungi. Typical microorganisms are marine bacteria and *Chaetomium globosum*.

(5) Poly-(1-4)-β-D-xylose
Poly-(1-4)-β-D-xylose is the dominating form of hemicellulose; therefore, many microorganisms can degrade such substances, for example, marine and terrestrial bacteria and rumen fungi. Typical strains include *Bacteroides ovatus*, spore myxomycetes, *Mucus cocci*, *Aspergillus niger*, *Trichoderma viride*, rough *Neurospora*, *Myrothecium verrucaria*, and *Coniophola cerebella*.

In short, the microbial degradation of hemicellulose is rapid in nature. Prior to cellulose decomposition, much hemicellulose generally has been decomposed. But, in terms of final decomposition, cellulose can be decomposed completely, but hemicelluloses only partially. There is almost no cellulose in humus or peat; however, a large amount of hemicellulose is often left because general microorganisms are devoid of the ability to decompose polygalactose [14].

3.1.3 Lignin-Degradable Microorganisms

Because of the complicated structure and connection between the structural units, which mostly ether bonds or a carbon-carbon bond, lignins are not suitable for degradation by hydrolysis. So, microbial degradation of lignin is significantly different from the enzyme-catalyzed hydrolysis reaction of general biopolymers, which is a series of enzyme-catalyzed and non-enzyme-catalyzed, nonspecific redox processes. Many studies have confirmed that lignin cannot be used as the sole carbon source of microorganisms. The degradation of lignin always occurs in the process of secondary metabolites, and the energy required for the synthesis of enzymes used in the degradation of lignin is provided by the hydrolysis of carbohydrates.

Therefore, lignin-degradable microorganisms can also decompose cellulose and hemicelluloses, which is the result of the evolution of biological adaptation. Therefore, lignin microbial degradation inevitably involves the decomposition of cellulose and hemicellulose. Lignin-degradable microorganisms generally refer to those that

enable complete decomposition of lignin to CO_2 or partially modify or degrade lignin. In the past, fungus was regarded as the only microorganism that had the ability to degrade lignin. Since the application of ^{14}C-labeled lignin and coniferyl alcohol dehydrogenation polymer, many lignin-degradable microorganisms have been found (actinomycetes and bacteria).

In nature, lignin is degraded completely by fungi, bacteria, and the corresponding microbial communities. Once lignocellulosic materials are degraded by wood-rot fungi when the land's humidity conditions are suitable, other microorganisms (e.g., bacteria) can further rapidly degrade lignin that has been decomposed to a certain degree and aromatic compounds derived from the lignin degradation. Moreover, part of decomposed lignin can bind with the amino of the amino acid, one of the microbial metabolites, and form humic acid and humus, which are excellent fertilizers. However, usually only white-rot fungi can completely decompose lignin into CO_2 and water. Generally, other rot bacteria and actinomycetes can only partially change the structure of the lignin molecules [14], such as by demethylation. According to different ways microbes decompose timber, lignin-degradable microorganisms can be divided into soft-rot bacteria and fungi, stain fungus, brown-rot fungi, and white-rot fungi.

3.1.3.1 Soft-Rot Microorganisms

Microorganisms that cause wood to soften and rot include bacteria, ascomycete fungi, and imperfect fungi. This rot usually occurs in wet timbers. Soft-rot bacteria are more likely to grow on hardwood than softwood, which may be because of differences in the structure of lignin. Soft-rot fungi preferentially degrade the polysaccharide of lignocellulosic materials; the metabolism of lignin is slow and incomplete, which usually results in softening of the wood surface, hence its name, and is considered to be involved in the formation of humus. The study of soft-rot bacteria is usually from the perspective of plant pathology [15]. Soft-rot fungi are mostly ascomycetes and adelomycete; for example, some strains of *Papulospora* spp., *Graphium* spp., *Thielavia* spp., and *Paecilomyces* spp. can decompose lignin. *Chaetomium* spp. genus also has slight lignin-decomposing ability.

3.1.3.2 Stain Fungus

One category of staining is caused by fungi. The mycelium deeply penetrates into the parenchyma of wood and directly stains the wood. Ascomycetes and adelomycete, such as *Ceratostomella ips* Rumbold and *Endoconidiophora bunae,* cause wood staining. The other category of staining is caused by the growth of fungi that only grow on the surface of the wood, such as *Mulor* spp., *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp., *Ascospore myxomycetes*, *Fusarium oxysporum*, and so on [5].

3.1.3.3 Brown-Rot Fungi

Brown-rot fungi can break down cellulose and hemicelluloses. They are more sensitive to polysaccharides, such as cellulose, than lignin. As a result, cellulose is broken down; most lignin is left and forms a brown residue composition of the mesh structure, namely, brown rot [15]. The brown-rot fungus can only slightly modify the lignin molecules, such as by demethylation or oxidation, hydroxylation, and so on, instead of substantial degradation. Because brown-rot fungus cannot efficiently decompose the aromatic ring of lignin, or even break the aromatic ring, it cannot further degrade lignin into fragments [5]. After brown rot of wood, the content of lignin changes little, but the structure changes to a certain extent. The decomposed wood takes on a pale or dark brown color because of the residual lignin, and the texture of wood takes on a granular, powdered, or box crack appearance. Basidiomycetes, such as *Gloeophyllum habeum* and *Poria cocos,* are the main fungi that cause the brown rot of wood. The number of species is less than for white-rot fungi. During the decomposing process of brown-rot fungi, the initial speed of cellulose decomposition is faster than that of decomposed products, resulting in the accumulation of oligosaccharides as well as a decrease in mass and strength of the wood.

3.1.3.4 White-Rot Fungi

White-rot fungi are filamentous fungi, which are the main lignin-degrading fungi. First, these fungi degrade the lignin component in lignocellulose without producing pigment. Most of the white-rot fungi belong to basidiomycetes, and a few belong to some species of Xylariaceae in ascomycete. Currently, the extensively studied white-rot fungi include *Phanerochete chrysosporium*, *Berkandera adusta, Thametes versicolor, Pleurotus ostreatus, Dichomitus squalens, Ceriporriopsis subvermis-pora,* and so on [16].

These fungi preferentially degrade lignin and hemicellulose, and cellulose is degraded only in the late stage. At the beginning of decomposition, the thickness of the cell wall remains unchanged. Prior to the removal of lignin, cellulase produced by white-rot fungi cannot degrade lignified cellulose directly, only after the degradation of lignin and hemicellulose. The decomposed wood is white because the dark lignin component is mainly degraded. The secondary wall of wood gradually thins as decomposition proceeds. Wood texture changes into fibrous or spongy. White rot often appears in hardwood.

In medium containing gallic acid or tannic acid, more than 90 % of the white-rot fungi can secrete extracellular enzymes, which can oxidate such organic acids to form a brown ring around the colonies. This phenomenon is called a Bavendamm reaction. But, such a phenomenon does not occur in brown-rot fungus. So, it is inferred that extracellular enzymes are associated with the decomposition of lignin.

In addition, there are some prokaryotes (mainly actinomycetes, such as *Strep-tomyces* spp., *Arthrobacter* spp., *Micromonospora* spp., *Nocardia* spp., and so on)

and some anaerobic bacteria (such as *Clostridium, Pseudomonas*, *Acinetobacter*, *Bacillus,* etc.) that can also produce lignin-degrading enzymes. However, the lignin-degrading enzymes secreted by these prokaryotes are intracellular enzymes, which determines that the studies of this kind of microbe are in a relatively minor status in the lignin-degrading bacteria [16].

3.1.4 Cellulose-Decomposing Microbial Community

Life in the ecosystem includes the communities of various species of plants, animals, and microorganisms, and the most effective ecological community should have a combination of animal, plant, and microbial communities. The microorganisms and other coexisting biologies together constitute the biological communities. On one hand, microbes are influenced by the physical, chemical, and other symbiotic organisms of their living environments. On the other hand, microbes exchange materials with the surrounding environments by their proliferation and metabolic activity and change the physical and chemical conditions of the environments to maintain the balance of the entire ecosystem. A cellulose microbial community refers to the collection of cellulose-degradable microbial populations living in a certain space within a certain period of time. Various cellulose microbial species regularly coexist in the ordered state. The formation and development of a microbial community must be via mutual adaptation between the microorganisms and the adaptation to the environments. A microbial community is not simply a collection of populations. The populations that can be combined form communities depending on two conditions: the inorganic environment in which they must work together to adapt; the coordination and balance of the their internal relationships. The composition of a cellulose-degradable microbial community and the relationship between the numbers of microbial species have a certain amount of balance and structure. Community structure demonstrates a layered property in space (including above- and belowground), trophic structure between the species, the ecological structure, and time seasonal changes. For lignocellulosic materials from different zones, the structure of the microbial community also differs with the types of celluloses. The structure of the cellulose microbial community in the tropical rain forest is the most complicated, and is the simplest in the Arctic. In the cyclic process of natural cellulose, a cellulose-degradable microorganism plays a role as a community structure [4].

Cellulose-degradable microbial ecology has become the theoretical basis to elucidate biological evolution, to promote microbial taxonomy, to develop and use microorganisms and their genetic resources, and to clarify the matter and energy transformation mechanism. It has been widely used in agriculture, health care, environmental protection, and geochemical and other fields. Microorganisms in the environment constitute communities that are interdependent and mutually restraint. They are controlled by the species; quantity and composition of physiological and biochemical characteristics of the species; the proliferation rate of

metabolic activity; environmental adaptability; and microbial interactions. The adaptation mechanism and interaction are important in microbial ecology. The role of environmental factors in cellulose microorganisms is to constitute comprehensive environmental systems (including macro- and microsystems) of microorganisms or microbial communities. The effect of the cellulose-degradable microbial community on environmental systems is realized by nutrient metabolism (namely, food chain) to complete the energy flow and biogeochemical cycles of elements. In a particular environment, both the interrelationship of the cellulose microorganisms and the relationship between cellulose microorganisms and the environment need mutual adaption and interaction, which take on not only the kinetic process but also a dynamic balance state. The overall concept of cellulose microbial ecosystems includes the cellulose-degradable microbial community structure and their metabolic functions to the environmental system, which constitute the core content of cellulose microbial ecology [4].

In the forest ecosystem, for example, fungus is a complex and important part that occupies a significant position in the structure and function of the ecosystem as a whole. For the structure of the cedar mycoflora, the lower the cedar canopy is, the higher the amount of cedar leaf-dwelling fungi composition species and fungi. Cedar leaf-dwelling fungi species composition and the number in winter are higher than in autumn. For autumn and winter, cedar leaf-dwelling fungi are the dominant species, but their habitats are quite different. Arboreal fungi refer to all fungi that live in trees (e.g., fungi that live in tissue or organs of live or dead trees). Many fungi living together in a tree form complex arboreal fungal communities. Arboreal fungal communities on different organs and apparatus of a host contain a variety of nutritional types of arboreal fungi. In the study, an arboreal fungi community is usually divided according to the site in the tree (tissues, organs) that is the habitat of fungi, such as bud habitat fungi, leaf habitat fungi, stick habitat fungi, dry habitat fungi, root habitat fungi, and floral habitat fungal community. Fungi, heterotrophic organisms, can be divided into three groups according to their state of life substrate provided by the dependent body (host) and choice manner (the lifestyle groups): the biotrophic type, saprophytic type, and facultative biotrophic type. Therefore, arboreal fungi can also be correspondingly divided as follows:

1. Biotrophic arboreal fungus. These fungi can only obtain the necessities of life from the living tissue of a living body; examples include *Cronartium ribicola Fisher* and *Coleosporium cimicifugatum* Thum.
2. The saprophytic-type arboreal fungi. These are fungi living in dead tissues of live trees to obtain the nutrients required for their lives. Some fungi live on the bark's surface.
3. The facultative biotrophic-type arboreal fungi. Between the two groups mentioned groups, there are many intermediate types. Some usually live on dead tissue and occasionally infringe the debilitating organization (called facultative parasitic fungi). Some first kill host tissue and then growth rapidly (known as facultative saprophytic fungi). Various arboreal fungal types are not composed of one single special population of fungi but by different populations composing

different arboreal fungal communities. For example, there is a wide variety of fungal communities on cedar leaf; these are in an ecological condition in which they confront with each other and keep in relative balance.

Microbial community structure has an important role in the material transformation of the prairie ecosystem and energy flow. A subsystem of decomposers is an important component in grassland ecosystems. Cellulose-degradable microorganisms play a main role in a decomposition system. Material circulation and energy transformation can proceed by the effect of cellulose microorganisms. There is a dynamic process during the microbial decomposition of plant residues with cellulose. When the *Leymus chinensis* vegetation regresses to grassland soil, microbial decomposition is initiated. At the initial stage of decomposition, the number of microorganisms on the surface of different plant residues is significantly increased by 10–300 of that at the beginning, especially bacteria and fungi. Different stages of plant and litter decomposition have obvious differences. This is related to the chemical composition of the plant. In the process of plant residue decomposition, the appearance of various types of cellulose fungi groups has an obvious phenomenon of alternate dominant populations. The pioneer populations at the early stage are *Mucor* and *Trichoderma*. Then, the number of *Mortierella chaetomium* mildew and *Trichothecenes bacteria* increases quickly. The compositions of fungal populations become more complex and diverse; most have a strong cellulolytic capability. With thorough study of the decomposition process, the number of cellulose actinomycetes increases rapidly, which is supposed to be mainly involved in the decomposition of substances that are difficult to decompose [4].

3.2 Animals Capable of Decomposing Natural Lignocellulose

At first, the general view is that the animal itself does not contain cellulase. The wood-eating arthropods and herbivores can take plant as food sources because a large number of symbiotic bacteria that can hydrolyze cellulose exist in the body. Their cellulose digestion primarily relies on their symbiotic microorganisms and protozoa in the gastrointestinal tract. However, with thorough research on cellulase, this theory has been challenged. It was constantly found that there might be the possibility of endogenous cellulase from the animal's body, such as snails, termites, wood cockroaches, or nematodes. In 1998, with molecular biological methods, Smant obtained the complementary DNA (cDNA) of four types of endo-β-1,4-glucanase (EG) from two different species of plant-parasitic nematodes. In the same year, Watanbae used antiserum of the endo-β-1,4-glucanase from termites to immunologically screen the cDNA library by rapid amplification of cDNA (RACE) and obtained a cDNA library of endo-β-1,4-glucanase, which further proved that endogenous cellulase exists in animals [18]. Wang et al. [19, 20] cloned the genomic fragment consisting of nine exons and eight introns from the apple snail's ovary, which so far was the first potential valuable multifunctional cellulase found in animals.

3.2.1 Protozoa

Protozoa are members of the most primitive and the lowest groups in the animal kingdom. There are 31,000 types in existence. Most of the individuals are small and only can be observed with the aid of a microscope; the smallest is only 2–3 μm. The protozoans are widely distributed in oceans, lakes, rivers, canals, stagnant rainwater, and moist soil. There are also a large number of protozoa in the digestive tracts of higher herbivores and invertebrates. Protozoa can secrete cellulase and cellobiase (CB), so wood fiber can be broken down into glucose and other products.

3.2.2 Invertebrates

Invertebrates that can degrade lignocelluloses include arthropods, annelids, and mollusks. Invertebrates degrade cellulose by the cellulase secreted mainly by the concomitant symbiotic bacteria, fungi, and protozoa in their digestive tract as well as by themselves. Termites, earthworms, and *Ampullarum crossean* are introduced as examples next.

3.2.2.1 Termites

Termites, insect-feeding cellulosic feedstocks, cause serious harm and economic loss in many areas, including city buildings, gardens, and trees. But, they are also insects with a powerful life force and wide distribution; they are easily carried and spread and are important to the natural world's material cycle. In nature, termites, especially soil-dwelling termites, decompose dead plants and deciduous cellulosic feedstocks and accelerate material recycling around the clock in a large number of individuals. The growth of several kinds of termite species is beneficial for the growth of edible fungi. Termites can play a role in medicine, including providing vitality, strengthening the kidney and spleen, providing dehumidification, and as a sedative. In addition, *Macrotermes barneyi* Light and *Odontotermes formosanus* exist in the soil of the copper mining district and are conducive to prospecting for copper because they are indicative of copper.

Termites are a class of social insects. There is strict division of labor within the population. Their abilities to produce cellulase are different. They are attributed to soil-dwelling termites and soil-wood-amphibious termites. There are dry-wood termites with lower water requirements; soil-wood-amphibious termites; and water-dependent strongly soil termites that have the major part of the population often hidden below the surface, eating underground grass, roots, or vegetation. Many species of termites have a strong vitality and can survive a harsh environment, even adapting to environmental changes voluntarily and changing their living habits to obtain survival [21].

The termite population and the number of individuals are huge (some groups have hundreds of millions of termites) with a wide variety (now known worldwide as having more than 2,000 types) and a wide distribution (distributed outside in most areas except for extremely cold areas and higher elevations of the mountains). Cellulase activities differ from different populations of termites. In nature, termites, especially ground-living termites, decompose the cellulose of dead plants rapidly with a tremendous number of individuals and plays an irreplaceable role in the cycle of substances on Earth [21].

Termites, with lignocellulose as a diet, play an important role in the mass cycle of nature. There is a wide range of food, including timber (intact or decomposed), plant leaves, humus, paper debris, herbivore feces, and so on. Live nutrients of termites mainly depend on the participation of endogenous and exogenous cellulase degrading lignocellulose into nutrients that can be completely absorbed and utilized. A study showed that the major digestive enzymes for cellulose in the digestive system of termites were endo-(1-4)-β-glucanase and β-glucosaminidase, which have high activities. β-Glucosaminidase can quickly decompose cellobiose and other low molecular oligosaccharides into glucose and enhance the absorption of the termite, which will benefit decomposition and utilization of cellulose.

The reason for termites to take cellulose as food is that its body contains many kinds of microorganisms and bacteria. There is a symbiotic relationship between microorganisms and termites. Only with the assistance of these microbes can termites digest cellulose completely [22].

3.2.2.2 Earthworms

Among all invertebrates in temperate soil, the earthworm has the largest biomass. In recent years, the straw used for no tillage and minimum tillage of soil provided favorable conditions for the activities and reproduction of earthworms. The earthworms swallow much soil and surface fallen residues. Through feeding, mining, and excretory activities, organic debris and mineral soil are mixed, and the degradation of organic matter is accelerated. Thus, the transformation of soil organic matter and nutrient cycling in earthworms plays an important role. Earthworms are also widely used for the treatment of organic waste. The Lausanne Experiment Station of England, using earthworms to treat agricultural waste, garbage, and sludge, has achieved industrialization and commercialization of earthworms. In addition, earthworms abound in protein and are fed as protein additives. Earthworms feed on lignocellulose and can take glucose generated from cellulose hydrolysis as the energy substances of growth and development.

Because its gastrointestinal system is different from that of herbivores, the hydrolysis of the cellulose enzymes of earthworms may be different from that of rumen microorganisms and even perhaps animal enzymes. It is necessary to

conduct an in-depth study of earthworm cellulase. Because of the abundance of cellulose, it is promising to explore the purification methods and characteristics of earthworm cellulase C_X, an enzyme that can convert noncrystalline cellulose into monosaccharides [23].

3.2.2.3 *Ampullarum crossean*

Ampullarum crossean is also known as snails belonging to Mesogastropoda Ampullariidae *Ampullarius*. It originates in the Amazon River basin in South America, where people eat snails. It is delicious and has a large body and flesh; is high in protein and low in fat; and has other characteristics. *Ampullarum crossean* feeds on plants, grows rapidly, and contains many digestive enzymes, especially cellulase with a high content and complete enzyme component [24]. Cellulase separated from *A. crossean* contains three enzyme components: endoglucanase, exoglucanase, and β-glucosidase. They can interrupt or loosen cellulose chain separately, then hydrolyze the cellulose into dextrin cellulose, cellotriose, cellobiose, and finally sugar and D-glucose [25]. Wang et al. purified obtained a purified glucosidase from the gastric juice of herbivorous mollusks *A. crossean,* which is ranked as the tenth in the glucosidase family. They also pointed out that the enzyme is an endo-β-1,4-xylanase containing exo-β-1,4-glucanase and endo-β-1,4-glucanase, called the multifunctional cellulase EGX. Thereafter, gene encoding of the enzyme was cloned from the *A. crossean* genome and was named egxa; it has a the full length of 1,683 bp, of which the encoded protein is a multifunctional cellulase having a cellulose-binding domain and the catalytic domain.

An internal multifunctional cellulase called EGX was isolated and purified from the gastric juice of *A. crossean* with a molecular weight of 41.5 kDa. The cellulase can hydrolyze denitrification of alkylphenol cellobioside (pNPC), microcrystalline cellulose (MCC; Sigmacell), CMC, birch wood soluble xylan, oat spelt xylan, and so on. It is a multifunctional cellulase with three enzyme activities: exo-β-1,4-glucanases, endo-β-1,4-glucanase enzymes, and endo-β-1,4-xylanase. After that, the gene of the multifunctional cellulase was cloned from the *A. crossean* gene group, named egxa. In the yeast expression system for methanol, recombinant multifunctional cellulase EGXA is obtained that has a similar nature to EGX. Amino acid sequence analysis showed that this enzyme belongs to the glycoside hydrolase family 10 [19, 20]. By comparison of the amino acid sequence of multifunctional cellulase EGXA with dual-function cellulase (exo-β-1,4 cut of β-1,4-xylanase C_{ex}) from bacteria (*Cellulomonas fimi*), it was found that the homology of multifunctional cellulase and EGXA with C_{ex} reached 35 % [26].

In summary, the activity of cellulase from *A. crossean* is high, and it can digest the yeast cell wall. The viscera of *A. crossean* are a good source for exploring the enzyme system for cellulose degradation.

3.3 Properties and Hydrolysis Mechanism of Cellulase

3.3.1 Enzyme Properties

3.3.1.1 Multienzymatic System of Cellulase

Because of the complicated structure of cellulose, it is difficult for any single enzyme to efficiently hydrolyze lignocellulose. Generally, cellulase for hydrolysis of natural lignocellulose is a complex multienzyme system. In 1993, Grassman studied the fungal cellulase system and distinguished two components. In 1950, Reese proposed the C_1-C_x hypothesis of cellulase hydrolysis. From that, the studies of cellulolytic enzyme were initiated. In the 1950s, with the development of separation technology, knowledge of the components and acting mode of cellulase achieved great progress [27].

Because cellulase from bacteria, actinomycetes, and basidiomycetes is mostly incorporated in the cell membrane and usually is extracellular enzyme with low activity, so far elucidation of the entire components of whole cellulase has failed. The activity of the extracellular enzyme from filamentous fungus is strong. Therefore, most cellulase study uses filamentous fungi as the object.

Based on a large volume of research on the separation and purification of the cellulase system, it has been determined that cellulase is a multienzyme system composed of multiple components. According to substrate specificity, cellulase may be divided into the following components: exo-β-1,4-glucanase, endo-β-1.4-glucanase, endoglucanase (EG), EC 3.2.1.4, and β-1,4-glucosidase (BG, EC 3.2.1.21).

(1) Exo-β-1,4-glucanase
The exo-β-1,4-glucanase enzyme is primarily β-1,4-glucan cellobiohydrolase (CBH) (EC 3.2.1.91). A large number of studies showed that C_1 enzyme proposed by Reese is CBH according to its role in nature. It can catalyze the generation of cellobiose from the nonreducing sugar end of cellulose by hydrolyzing the β-1,4-glucoside bond. It has also been reported that some strains produce another type of exoglucanase, β-1,4-glucan glucohydrolase (EXG, EC 3.2.1.74). It can catalyze the generation of glucose from the nonreducing end of cellulose by hydrolyzing the β-1,4-glucoside bond [28]. There are fewer reports about EXG are than for CBH. When CBH hydrolyzes natural cellulose alone, reducing sugar is almost undetectable. There is also only a weak effect on the substituents of cellulose, such as CMC. With the synergistic effect with endoglucanase, natural lignocellulose can be effectively broken down. CBH has a strong substrate specificity; the degradation capacity decreases with the shortening of the oligosaccharide chain.

(2) Endo-β-1.4-glucanase (EG, EC 3.2.1.4)
Endocellulase or endoglucanase (EG), that is, 1,4-β-D-glucan-4-glucan hydrolase (EC 3.2.1.4), accounts for 20–30 % of the protein of cellulase preparation. EG hydrolyzes the nonamorphous region of phosphate-expanded cellulose, CMC,

and amorphous cellulose by hydrolyzing β-1,4-glycosidic bonds irregularly to form glucose, cellobiose, cellotriose, and different sizes of fiber dextrin, which have more available ends for cellobiohydrolase enzymes [11]. Owing to the multicomponent cellulase and the differentiation of components in different strains, it is also known as the Cx enzyme. Because most CMC is used as a substrate to assay its vitality, it is also called the CMC enzyme. The specificity of EG is not strong, which means it could hydrolyze water-soluble cellulose; the substituents have little effect on enzyme activity.

(3) β-1,4-glucosidase (BG, EC 3.2.1.21)
β-1,4-Glucosidase mainly hydrolyzes cellobiose and glucose residues from the nonreducing end of the small cellulose dextrin to produce glucose. BG is usually known as CB with a low substrate specificity. Many noncellulolytic microbes can produce a large number of such components. The hydrolysis rate of BG increases with decrease of the size of the substrate, and cellobiose has the highest hydrolysis rate. In enzyme preparation, the enzyme protein content of BG accounts least compared to other cellulase components, only about 1 %; especially, there is even less extracellular BG from *Trichoderma* sp. With the development of genetic engineering techniques, it was found that BG also has another effect, that is, a transglycosylation role, so that during the hydrolysis process, glucose maintains a β-conformation. Strictly speaking, β-glucosidase cannot be considered a cellulase, but it can reduce the feedback inhibition of cellulase caused by cellobiose [11].

(4) Other components
In addition to these three major groups, other enzymes involved in cellulose degradation process are cellobiose dehydrogenase (CDH), cellobiose quinone oxidoreductase (CBQ), phosphorylase, cellulosome, and so on.

① Cellobiose dehydrogenase
Cellobiose dehydrogenase, also known as cellobiose oxidase (CBO) (EC 1.1.99.18) is a heme flavoprotein and is mainly synthesized by filamentous fungi that can hydrolyze lignocellullose. It can oxidize cellobiose and cellooligosaccharide to generate the corresponding lactone. Flavin adenine dinucleotide (FAD) and heme are taken as its prosthetic group. CDH is stable in pH 3–8 and below 50 °C. CDH has a wider substrate range, including cellotriose, fiber tetrasaccharide, cellooligosaccharide with five polymerization degree, lactose and cellulose, except cellobiose, but it cannot oxidize glucose, which is controlled by CB.

Taking cellobiose as an electron donor, CDH can reduce a variety of substances, such as cytochrome C, Fe^{3+}, O_2, and so on. CDH is called multidehydrogenase because the oxygen reduction speed with CDH is much slower compared to other electron acceptors. CDH can reduce Fe^{3+} and O_2 to generate Fe^{2+} and H_2O_2. A Fenton reaction occurs between Fe^{2+} and H_2O_2 to generate a hydroxyl radical (• OH) with strong oxidizing power. Therefore, it is speculated that CDH plays an oxidation and degradation role in the cellulose degradation process [29].

② Cellobiose quinone axidoreducfade
CBQ is a flavoprotein, containing only FAD, and its other physicochemical properties are similar to CDH. However, there is no report on the oxidizability of CBQ on cellulose. The biggest difference between CBQ and CDH is that CBQ cannot reduce cytochrome C. CDH and CBQ were isolated from *Phanerochaete chrysosporium* first, and later were found in *Sporotrichum thermophile*, *Aspergillus niger*, *Sclerotium rolfsii*, *Fomes annosus,* and *Moniliaceae* spp.

③ Phosphorylase
Some bacteria contain cellobiose or phosphorylase of fiber dextrin, which make cellobiose or cellulose phosphorylated and finally metabolized maximally, which is an imaginable metabolic pathway of cellooligosaccharide.

④ Cellulosome
In the study of *Clostridium thermocellum*, it is found that different kinds of cellulase synergistically catalyze hydrolysis of cellulose by the complexus with certain modes which is called cellulosome.

3.3.1.2 Molecular Structure and Function of Cellulase

In 1986, Tilbeurgh digested exoglucanase I (CBH I) of *Trichoderma reesei* with papain limitedly and obtained two domains with independent activity. One part is called the CD because it has a catalytic function and can only hydrolyze soluble cellulose with weak adsorption but cannot hydrolyze a larger core of insoluble cellulose with a molecular weight of 56 kDa. Another part is called CBD, which can be adsorbed on the cellulose surface and is small fragments with a molecular weight of 10 kDa. The two parts are connected by a hinge region (called a linker or hinge), forming the familiar tadpole-like structure. Subsequently, a similar structure is found with a similar manner in a variety of bacterial and fungal cellulases. CBD in the cellulase is located in the amino terminal or carboxy terminal, which is connected to the catalytic region through a highly glycosylated connection bridge (linker) [30]. The linker of bacterial cellulase is enriched in Pho and Thr. The linker of fungal cellulase is enriched in Gly, Ser, and Thr. The angles between CBD and CD of bacterial cellulase and fungus cellulase are 135° and 180°, respectively. There are two restriction sites in bacterial cellulase that can cut out the CBD and linker, respectively, but one restriction site is for fungal cellulase [31]. In short, with X-ray diffraction, the structural study of CBD from *T. reesei* CBH I showed that natural CBH I protein is python-like, including an isotropic head (CD zone) and wedge-shaped tail (CBD region).

From the terms of chemical reactions, the enzymatic hydrolysis of cellulose involves only hydrolysis of the β-1,4-glycosidic bond and breakage of the hydrogen bond, which is a simple reaction process. However, cellulose, as the plant cell wall structural material, is an inert polymer that is directionally arranged in parallel through hydrogen bonding of the sugar chain composed of the cellobiose unit. A synergistic effect of the cellulase system is required for complete degradation

to glucose. Compared with starch, which is composed of a maltose unit containing an α-1,4-glycosidic bond to form space spiral sugar chain, the enzymatic hydrolysis efficiency of cellulose is 100 times lower than that of starch [32]. Therefore, how to improve the enzymatic hydrolysis efficiency of cellulose becomes the key issue in cellulose utilization. First, the molecular structure and function of cellulase should be studied. Second, understanding is needed of how cellulase makes a bundle of fibers detach from unsoluble cellulose polymer, how the synergistic effect happens, and many other fundamental problems.

With regard to the function of the catalytic zone, Sinnott discussed the catalytic mechanism of glycosyltransferase with cellulase from the terms of the chemical mechanism angle. He pointed out that the removement of aglycon is the substitution reaction related to the acid-base catalysis of two amino acid residues, which is similar to that of lysozyme. Based on numerous studies of site-directed mutagenesis techniques and enzyme-specific inhibitors, it was proved that glutamic acid is located in the active sites of endoglucanase, exoglucanase, and glucose celecoxib enzyme from both bacteria and fungi. The catalytic reaction is completed at the site of the anomeric carbon atom by the retention and conversion of configuration. A double-replacement hydrolysis mechanism with two conserved carboxyl amino acids as proton donors and nucleophile was proved [30].

After removing the CBD from the cellulase molecule, the degradation activity of the soluble substrate changes a little, and the adsorption and hydrolysis activity on crystalline cellulose decreases significantly. By chemical mutagenesis and site-directed mutagenesis techniques, it was shown that the aromatic amino acid plays an important role in adsorbing with crystalline cellulose because its mutation will reduce the adsorption capacity of CBD on crystalline cellulose sharply. At present, it is proposed that CBD may absorb on cellulose by an accumulation force formed between the aromatic ring and the glucose ring. The rest of the hydrogen bonds in CBD form residues that form hydrogen bonds with adjacent glucose chains to break down the surface of single glucose chains, which facilitates the hydrolysis effect of the catalytic zone [30, 33].

3.3.1.3 Regulation and Control of Cellulase Synthesis

Regulation of cellulase biosynthesis includes two mechanisms: induction and glucose inhibition. There is microbial synthesis of cellulase under the presence of an inducer. But, in the culture containing glucose and glycerol, which are easy to utilize as carbon sources, cellulase synthesis will be inhibited even when sophorose is used as an inducer. The synthesis of cellulase will begin only when the glucose is exhausted. This phenomenon is called *glucose repression* or the *glucose effect*. According to this, antirepressor strains can be isolated to improve cellulase enzyme activity.

Induction and inhibition of cellulase synthesis is significantly affected by the concentration of the substance. For example, early physiological studies found that *A. niger* and *T. reesei,* which are two typical representative strains, can synthesize

cellulase in the lignocellulosic materials. Cellobiose is the main intermediate of cellulose degradation, but it can induce strains to produce cellulase only at lower concentrations (<10 mM), but inhibits at a concentration higher than 20 mM. This critical concentration value for the shift of induction and inhibition effect greatly differs from species to species. Gao et al. found that β-glucosidase isozymes had three different subcellular localizations: intracellular, extracellular, and plasma membrane bound. They summed up the "subregulatory network" model to understand multisubcellular localization of β-glucosidase enzyme isozyme involved in the formation of inducer in the process of conversion. Multisubcellulars localize β-GLase isozyme to constitute a subregulatory network that can further adjust cells' tolerable dosage of cellobiose and critical threshold values for generating direction, rate, and degree of strong inducer through further energy metabolization and transglycosylation. The difference degree of species of the β-GLase isozyme decides the difference of the inducing and inhibition effect of the same concentration of cellobiose on cellulase synthesis [34, 35].

(1) Cellulase-inducing synthesis

In the presence of cellulose, many of the filamentous fungi can synthesize cellulase that is ten times higher than that of the glucose culture. Culture medium has a significant effect on enzyme synthesis. So, it is speculated that the degradation products of cellulose can be the inducer of cellulase synthesis, such as cellobiose, lactose, sophorose, sorbitol, gentiobiosyl, methyl-β-glucosidase, and cellobiose sugar lactone. They can improve the enzyme synthesis of many cellulose microorganisms [36, 37]. In *Escherichia coli*, lactose induces β-galactosidase synthesis regularly. But, the inducing effect of fungi as an inducer compatible with substrate is not so regular.

Generally, it is believed that oligosaccharides and derivatives released from the polymeric substrate are truly inducers of cellulase expression. Sophorose is regarded as a natural soluble inducer for *T. reesei* cellulase, which is synthesized by the transglycosylation effect of β-glucosidase on the cellobiose [38]. However, the synthesis of cellulase can be induced by sophorose powerfully only for specific fungi, such as *T. reesei*, *Aspergillus terreus,* and *Penicillium purpurogenum*, but has no inducing effect on other cellulase-producing fungi, such as *Penicillium janthinellum*, *Penicillium chrysosporium*, *Aspergillus nidulans,* and *A. niger.*

The adjusting effect of cellobiose has a dual nature. Previous studies have shown that cellobiose induces the synthesis of cellulase for most fungi. But, when the concentrations of cellobiose are different, the cellulase-inducing effect will be in contradiction. Cellobiose has an inducing effect at low concentrations on cellulase synthesis but an inhibiting effect when the concentration is higher than a certain value. Cellobiose can be hydrolyzed by β-glucosidase to generate glucose and inhibit the synthesis of cellulase and can induce the synthesis of cellulase by the transglycosylation effect of β-glucosidase to generate sophorose. The balance of the final result depends on cellobiose regulation of β-glucosidase enzyme hydrolysis and transglycosylation [39]. In the natural process of cellulose hydrolysis, the

inhibiting effect of cellobiose and glucose on cellulase synthesis is not obvious because they cannot be accumulated to high concentrations.

For cellulase synthesis of *T. reesei*, lactose is the only soluble and inexpensive carbon source that can be easily obtained. It is hydrolyzed into glucose and galactose by β-glucosidase. Studies showed that the regulating effect of lactose is at least partially through a metabolic pathway, unlike sophorose induction [40].

(2) The inhibiting effect of a degradation product

The inhibiting effect of a degradation product is another regulation means on cellulase synthesis that is easily inhibited by simple metabolic substrates such as glucose, glycerol, and the like. When filamentous fungi are cultivated in the glucose-containing medium, most expression of the cellulase gene is inhibited. For many filamentous fungi, if they are grown in medium containing an easy-to-use carbon source (including glucose, glycerol, etc.), cellulase synthesis is inhibited even in the presence of an inducer, such as sophorose. Cellulase synthesis restarts only when the easily metabolized carbon source in the medium is nearly exhausted. The glucose repression effect is effective. When glucose is exhausted, cellulase gene transcription can be detected in the culture of *T. reesei*. Such expression in the basal level without an inducer guarantees fungal survival under natural conditions. In a basic medium without a carbon source, there is no induction of cellulase gene transcription, which indicates that the absence of a carbon source inhibits the gene expression of cellulase. A derepression phenomenon after the depletion of glucose is widespread in the synthesis of cellulase and hemicellulase [40]. Generally, the construction of a mutant that can resist a degradate-repressing effect is becoming an important way to breed strains from all original strains and most mutants.

As also mentioned, some carbon sources that can induce cellulase synthesis will be inhibitors of cellulase synthesis in a high concentration. All known inducers of the cellulase enzyme have a dual function, namely, as the inducer and carbon source for growth.

3.3.1.4 The Role of Basal Cellulase in the Regulation of Cellulase Synthesis

As early as the mid-twentieth century, Mandelst and Reese proposed that there might be basal synthesis cellulase (BSC) with at a low level. Its role is to identify extracellular cellulose substrate and be limitedly hydrolyzed to produce a small molecule soluble product, such as cellobiose. These soluble products can enter the cell to induce the efficient expression of cellulase gene. However, as subject to the restriction of a cellulase detection method, such a low level of constitutive cellulase cannot be detected. When the monoclonal and polyclonal antibody technique was applied to the detection of cellulase, this difficulty was scientifically validated, thereby confirming the existence of BSC.

In bacteria, when *Cellutomonas flavigena* is cultivated in a medium with a sole carbon source, such as glycerol, xylose, or cellobiose, base level CMC enzyme,

filter paper activity, and xylanase activity could be detected in culture supernatant. This indicated that the BSC generally exists in both cellulose-degrading fungi and bacteria. BSC is synthesized constitutively without the influence of a carbon source in the medium; the cellulose-induced cellulase synthesis capacity of the strain is limited by the synthesis capacity of BSC. However, different from "constitutive enzyme," cellulase synthesized constitutively refers to a low-level cellulase that plays an important role in the inducing synthesis of cellulase. Constitutive enzyme has a high synthesis level and can be secreted extracellularly.

BSC has a variety of basic enzyme components. Because all of the studies have focused on the transcriptional level and the difference of experimental conditions, some research results were not the same. For example, Wang and Cao proposed CBH II and EG II as BSC in *T. reesei* that plays a key role for the formation of inducer. The research results of Carle-Urioste and Hnrique-Silva showed that CBH I gene exists in basic transcription with only a lower amount [40].

Among a variety of soluble products produced by the effect of cellulase synthesized constitutively on cellulosic substrates, only cellobiose is considered to be a potential inducer of cellulase gene expression, but it needs to be transferred into the cells. In this process, there are a series of changes for cellobiose to become a true inducer, suggesting that the β-glucosidase enzyme plays a decisive role in this process. Austria's Kubicek group unexpectedly discovered constitutive di-β-D-glucopyranoside permease (DDGP) existed in the membrane of *T. reesei* during research for ethanol mutant strains, and it has a strong affinity for cellobiose. DDGP can assist cellobiose to enter a cell. Although DDGP is a constitutive enzyme, addition of sophorose to the medium can significantly increase its expression level. Glucose (exclusive of other monosaccharides) has an inhibition effect on DDGP. The unexpected finding provides further experimental evidence for the speculation that cellulose can produce soluble inducers for the expression of cellulase gene with the effect of low-level cellulase synthesized constitutively [40].

3.3.2 Enzymatic Hydrolysis Mechanism of Cellulase

3.3.2.1 Enzyme Catalytic Mechanism

Water-insoluble reactants (cellulose) and water-soluble cellulase determine that the cellulase-catalyzed reaction is a heterogeneous reaction. The natural cellulose hydrolysis process can be divided into three stages. The first is the accessibility of the cellulose to cellulase, followed by cellulase adsorption and diffusion processes and finally self-assembling composite body (C_1) of CBH-CMCase and β-Gase synergistically degradating the crystalline region of cellulose, while the amorphous region of cellulose is randomly hydrolyzed by the CBH-CMCase and β-Gase. The structural features of cellulosic material and the cellulase reaction mode affect the reaction speed. The crystallization region in natural lignocellulose that is highly resistive to cellulase as well as the limited binding sites of enzyme activity determine

that the reaction speed is relatively slow. The enzymolysis degree of lignocellulose is largely determined by the difficulty level of the enzyme available to the binding sites because it will determine the difficulty level of enzyme adsorbed to the solid substrate.

Several different mechanisms have been proposed about how cellulose is converted into glucose. The initial concept was proposed by Reese, which was the (C_1/C_x) hypothesis. In 1950, Reese et al. illustrated that cellulase produced by any strain could not decompose natural cotton cellulose, but cellulase produce by some kinds of strains could decompose noncrystalline cellulose, such as swelling cellulose, cellulose-induced body, and so on. Therefore, the C_1-C_x hypothesis was proposed that natural cellulose must be synergistically decomposed by different enzymes because of its specificity. Its basic hydrolysis pattern can be summarized as follows:

$$\text{Crystal cellulose} \xrightarrow{C_1} \text{Amorphous cellulose} \xrightarrow{C_x} \text{Cellulose} \xrightarrow{\beta\text{-glucosidase}} \text{Glucose}$$

This hypothesis can be explained as follows: At the beginning of cellulose hydrolysis, C_x enzyme (endoglucanase) hydrolyzes the cellulose crystalline regions, making it convert into a noncrystalline form that can be hydrolyzed by C_1 (exoglucanase). C_x randomly hydrolyzes noncrystalline cellulose. Then, β-1,4-glucosidase hydrolyzes cellobiose into glucose. It shows that the effect of C_x is a prerequisite for C_1 hydrolysis [41]. Fungi can secrete C_x so they can break down natural cellulose. However, other microorganisms do not secrete C_x so they can only break down the derivatives of cellulose. The catalytic mechanism of C_x is unclear, so there are various kinds of speculation. For example, it was proposed that C_x may have an effect on hydrogen bonding between the cellulose chains, on a few β-1,4-glucoside bonds in cellulose, or on other irregular bonds, weak keys, and so on, but no purified C_x was isolated.

Wood et al. analyzed the hydrolysis process of cellulose by cellulase from *T. reesei* and *P. funiculosum*. C_x was separated and identified, changing the concept of nonhydrolysis of C_x and indicating that C_x enzyme is a hydrolytic enzyme. It is difficult to act on CMC, but capable of acting on crystalline cellulose, phosphoric expansion methyl cellulose, and so on, with the main product of cellobiose, which proves that C_x enzyme is one type of β-1,4-glucan cellobiohydrolase [42]. Chen pointed out that C_x is a self-organizing complex of CMC enzymes, CBH enzymes, and CB enzyme that hydrolyzes the crystalline regions of cellulose [43].

Currently, the generally accepted hypothesis of the hydrolysis mechanism is the synergy model as shown in Fig. 3.1. EG (C_x) enzyme randomly hydrolyzes and cuts the amorphous region of cellulose molecule chains, resulting in more cellulose molecule end groups appearing in the crystalline region of cellulose and benefiting the enzymatic hydrolysis of CBH (C_1). Then, CBH and EG combine to hydrolyze cellulose and obtain cellobiose, which is further hydrolyzed into glucose by BG hydrolysis.

Another hypothesis is the synergistic effect of exoglucanase and exoglucanase as shown in Fig. 3.2. Exogluconase I (CBH I) cuts the reducing end of the cellulose

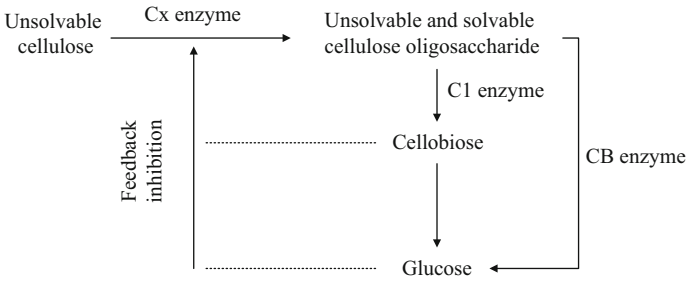

Fig. 3.1 Synergetic degradation model [44]

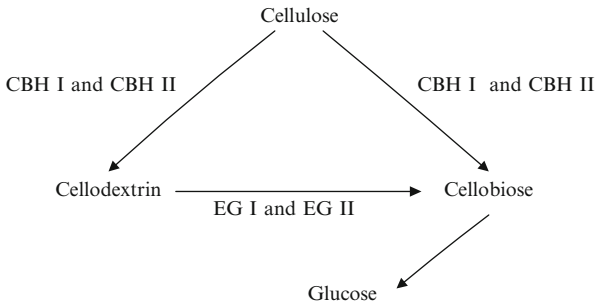

Fig. 3.2 Possible ways of enzymatic hydrolysis of cellulose [45]

chain, and exoglucanase II (CBH II) cuts the nonreducing end of the cellulose chain. The synergistic effect occurs only in the hydrolysis of natural cellulose, but not CMC and hydroxyethyl cellulose (HEC). Enzymatic hydrolysis of endogluconase and exogluconase is a heterogeneous reaction, and the hydrolysis of cellobiose catalyzed by β-glucosidase is a homogeneous reaction. The enzymatic hydrolysis efficiency of endogluconase is high for CMC but low for MCC. However, exoglucanase has high enzyme activity for MCC but low activity for CMC [45].

A hydrogen bond is an important force for maintaining the high-level structure of protein and cellulose, and it is also the initial point of cellulose degradation by cellulase [46]. At different pH environments, the proton state of hydrophilic layer group of enzyme molecule will change, affecting the hydrogen-bonding state and the higher-order structure of the enzyme molecule, leading to changes in the binding activity of enzyme and substrate or the catalytic activity [47]. Gao et al. pointed out that although considerable research on the cellulase degradation mechanism has been carried out on a molecular level, the degradation mechanism of natural crystalline cellulose has not been clarified because the effect of aggregation structure on the degradation of the supramolecular structure of cellulose molecules has been neglected [48]. Now, in the most popular theory, it is considered that first under the effect of nonhydrolyzable enzymes that can hydrolyze a cellulose chain or hydrogen bond, intrachain and interchain hydrogen bonds in the natural cellulose chain are

broken, and noncrystalline cellulose is formed. Then, cellulose is hydrolyzed into fiber dextrin and glucose with the synergistic effect of the three enzymes [48].

With the development of biotechnology, it is possible to elaborate enzymatic mechanisms from the molecular level. Cellulase molecules usually include catalytic domain (CD), cellulose-binding domain (CBD), and a connecting bridge (linker) between CD and CBD. The CBD of cellulase molecules are adsorbed to the cellulose by accumulation force forming through the aromatic ring on aromatic amino acid and the glucose ring. Then, residues formed by the rest of the hydrogen bonds of CBD connect with the adjacent glucose chains to form a hydrogen bond that will cut down single glucose chains from the fiber surface and facilitate the hydrolysis of the catalytic region. Glutamic acid (G1u) of the CD is located in the active sites of endoglucanase, exoglucanase, and glucosidase from both bacteria and fungi. A catalytic reaction is completed at the site of the anomeric carbon atom by the retention and conversion of the configuration. A double-replacement hydrolysis mechanism with two conserved carboxyl amino acids as a proton donor and nucleophile is proved. But, there is no reasonable explanation how CBD absorbs on the surface of cellulose and interacts with the CD to degrade cellulose.

3.3.2.2 Cellulase Adsorption and Desorption on the Surface of Solid Substrate

The enzymatic hydrolysis of cellulose is a solid-liquid heterogeneous reaction. This reaction is usually divided into three steps: the combination of cellulase and cellulose, the enzymatic hydrolysis of cellulose to produce fermentable sugars, and desorption of cellulase. A critical step is that cellulase adsorbs the insoluble cellulose substrate to form a cellulase-substrate complex. Cellulase adsorption is related not only to the nature of the enzyme itself, but also to the characteristics of the substrate. Cellulase adsorption ability is closely related to its sugar content and hydrophobic property. Whether the adsorption process is reversible depends on the specific type of enzyme. The cellulose adsorption ability of CBH II and EG3 from *T. viride* is a reversible process, while it is an irreversible process for CBH1. It was also found that there is no linear relationship between adsorption and hydrolysis efficiency of the enzyme components in addition to CBH I and CD [49].

Enzyme adsorption is reflected by a variety of factors, such as the component and structure of substrate, types and characteristics of enzyme, proportion of enzyme and substrate, temperature, pH and buffer systems, and so on [50]. Both the adsorption of cellulase and the formation of cellulase/substrate complexes are considered to be critical steps of cellulose hydrolysis. The adsorption of cellulase on insoluble cellulose is irreversible, reversible, and half-irreversible, which mainly depends on the physicochemical characteristics of the substrate, the cellulase components, the enzymatic reaction temperature, and the mass transfer rate of the system. Only when the pore size of the substrate reaches 0.51 nm (i.e., the approximate dimensions of cellulase) will the cellulase have the possibility of attraction to the substrate [50]. Compared with the enzymatic hydrolysis of

cellulose, the adsorption of cellulase on substrate is fast; it has been reported that the adsorption equilibrium state can be reached within 0.5 h. Considering the factors affecting enzyme adsorption, the specific surface area of the substrate and degree of crystallinity are the most important. The adsorption of the cellulase on the substrate increases with the reduction of particle size (i.e., the increase in surface area). The adsorption amount decreases when cellulase adsorbs the high-crystallization zone or low-affinity district. There are specific adsorption sites on the substrate for different cellulase components, which reduces with substrate continuous degradation [51]. Studies about adsorption of cellulase on substrate illustrated that, within a certain cellulase concentration range, the hydrolysis rate increases with the increase of the amount of cellulase. When the cellulase concentration keeps increasing, the magnitude of the enzymatic hydrolysis rate decreases. It proves that, under certain conditions, a certain amount of cellulose has limited binding sites. When these binding sites are all occupied by cellulase, increasing the amount of cellulase cannot enhance enzymatic hydrolysis [52].

Woodward et al. studied the saturated and unsaturated adsorption of cellulase components on MCC with the Scatchard analysis method. The saturated adsorption capacities of EG I, EG II, CBH I, and CBH II on MCC were 4.77, 4.04, 3.68, and 6.57 $mg \cdot g^{-1}$, respectively. EG I or EG II had a good synergy effect with CBH I and CBH II under unsaturated adsorption but had a poor synergistic effect under saturated adsorption [44, 53]. Tomme et al. found that the saturated adsorption amounts of CBH I and CBH II on MCC were up to 70 and 64 $mg \cdot g^{-1}$, respectively, which were much larger than that reported by Woodward et al. Tomme et al. pointed out that the adsorbing rate of cellulase on MCC was slow. However, as Woodward et al. reported, the adsorption time of cellulase on substrate was only 2 min, which was not enough for equilibrium, leading to low amount of saturated adsorption [54]. The cellulase component must be added simultaneously and in a nonsaturated concentration to achieve maximum synergistic effects. It was presumed that each component of cellulase not only competes with the same adsorption sites, but also first forms a "loose complex" in the solution before adsorption, which agreed with some other scholars.

Ryu et al. found that endogluconase and exoglucanase presented competitive adsorption, and when either enzyme component was added, the adsorption of another cellulase component would be partially replaced. When the composition of cellulase was close to the composition of cellulase stock solution, there was the most obvious competitive adsorption. In the interpretation of this phenomenon, Ryu et al. considered that endoglucanase and exoglucanase, respectively, had different adsorption sites. Exonucleases influenced the active adsorption sites of endoglucanase. Random hydrolysis of endoglucanase accelerated the effect of exoglucanase on the activity site of endoglucanase and initiate desorption of endoglucanase. Endogluconase affected active adsorption sites of exoglucanase. Exoglucanase hydrolysis can enhance the effect of endoglucanase on active site of exoglucanase, while exoglucanase carries out desorption. The coordinating role of the adsorption and desorption caused by the competitive adsorption of cellulase components significantly improves the enzymatic hydrolysis of cellulose. Exoglucanase has

a strong adsorption affinity. The content of exoglucanase in the cellulase stock solution is highest, followed by endoglucanase, and β-glucosidase only accounts for a small part. However, on some binding sites of cellulose, no enzymatic hydrolysis occurred [55, 56]. Chanzy et al. marked CBH I with colloidal gold and found that CBH I was incorporated throughout the whole cellulose chain, rather than just at its reducing end of the enzymatic action sites.

The high degree of crystallinity of natural cellulose reduces the adsorption of cellulase and availability of cellulose. The competitive adsorption of cellulase will reduce the utilization efficiency of cellulase on cellulose [57]. The hydrolysis process of cellulose catalyzed by cellulase is a dynamic system because in addition to hydrolyzing the surface of cellulose, as the reaction proceeds, cellulase also diffuses into cellulose internally. The movement of cellulase on a complex substrate is the main factor affecting the efficiency of cellulase hydrolysis. A high lignin content will hinder the diffusion rate of cellulose, causing inhibition of the final product and reducing the conversion yield of cellulose [58].

3.3.2.3 Cofactors of Cellulase Hydrolysis

For a long time, the study of cellulase mainly focused on glucosidase. Although considerable research has been conducted, it still failed to clarify the degradation mechanism of natural crystalline cellulose because of the neglect of the supramolecular structure of cellulose molecules. From the view of matter and energy, it was found that the dense structure of cellulose formed by the hydrogen bonds was the bottleneck for its efficient degradation. The first stage of cellulose hydrolysis is the separation of free oligomeric cellulose from the surface of solid cellulose, which is a rate-limiting step of enzymatic hydrolysis. In the enzymatic hydrolysis of cellulose, in addition to the synergistic effect of the three components mentioned, there are also some proteins without cellulase activity but capable of promoting the enzymatic hydrolysis of cellulose; these are called the cofactor of cellulase.

In 1950, Reese proposed that there was a hydrogen bond enzyme that could destroy crystalline cellulose; this improved the accessibility of cellulose by destroying the structure of the substrate. Cosgove found an expansion protein from a plant that is considered to be the most likely to act as a “hydrogen bond enzyme” protein. In the process of plant growth, expansion protein induces the looseness and irreversible stretch of the cell wall in a reversibly nonhydrolysis way, which results in breaking of the hydrogen bond in the polymer network and promotes the transfer of polymers. The expansion protein itself has no glycosidase activity, but it is able to weaken the strength of the filter paper. In the enzymatic hydrolysis of filter paper, the expansion protein and cellulase enzymes have a synergistic effect. In 2002, Saloheimo found the Swollenin protein in *T. reesei* that had similar gene sequences as the plant expansion protein. It was found that the Swollenin protein could destroy the structure of filter paper and cotton without reducing sugar formation [59]. Shandong University of China also explored the Swollenin protein

effectively, including gene cloning, protein structure analysis, and heterologous expression [60, 61].

In addition, the researchers found several nonhydrolyzed cellulase cofactors. In 2002, Gao et al. isolated a protein from the filtrate of *Trichoderma pseudokoningii* that could weaken the hydrogen absorption intensity of the cotton fiber and expand the cotton fiber and chitin without reducing sugar protein. It was pointed out that this protein was consistent with the feature of hydrogen-bonding enzyme [62]. By studying the CBD structure of cellulase, it was found that non-CDs of the cellulase could promote the degradation of cellulose. Xu et al. investigated the CBD of CBH I from *Conradson trichoderma* and *Penicillium janthinellum,* which indicated that the glycosidic bond of cellulose was not hydrolyzed because when the CBD was adsorbed on cellulose, the supramolecular structure of cellulose would be destroyed without generating reducing sugars [63]. Qiu et al. [64] used *Sclerotium* laccase (LaC) to improve the enzymatic hydrolysis efficiency of lignocellulosic materials through the synergistic effect of LaC and cellulase, which increased the reducing sugar content by 37.9 % compared with a control group and improved the fermentation ethanol yield by 13.8 %. Analysis of the mechanism indicated that surface lignin of lignocellulose was partially degraded by LaC, of which the ring-opening reaction of the benzene ring caused the formation of a mesh-like structure in the surface of the raw material, thereby improving accessibility of raw material for cellulase.

3.3.2.4 Factors Affecting the Activity and Hydrolysis of Cellulase

It is usually considered that cellulase is easy to inactivate under the shearing force of liquid, and different enzymes have different deactivation rates. In a stirred liquid environment, the presence or absence of the substrate has great influence on the inactivation of the enzyme. In general, cellulase will be more mechanically stable in the absence of substrate. Accelerating the stirring speed will promote the inactivation of enzyme. When the enzyme is at the air/liquid interface, inactivation will also be intensified.

Matsuno et al. [65] reported that the reversible inactivation of cellulase might be caused by the effect of steric hindrance of cellulose on the cellulase components. Fan et al. [66] and Holtzapple et al. [67] pointed out that cellulase inactivation was caused by the formation of a complex by the combination of cellulase and substrate or product. Therefore, the amount of inactivated enzyme should be proportional to the amount of adsorbed cellulase or to the cellulase concentration in the solution if under another enzymatic hydrolysis mechanism.

It is well known that cellulose enzymatic hydrolysis is repressed by the mutual inhibition of cellobiose, glucose, or both. Inhibitive pathway studies have been conducted for a long time, and a number of different views have been put forward. It is not clear whether this inhibition is based on the dominant role of competition, noncompetition, or collaboration, which should have different inhibitory effects for different enzymatic systems. However, for most cellulase systems, cellobiose has

Table 3.1 Key factors considered to establish comprehensive mechanical dynamics of cellulose enzymatic hydrolysis

Cellulase system	Cellulose system	Interaction between enzyme and cellulose	Inhibition and inactivation of enzymes	Mass transfer
Different sources and composition	Composition differences of lignocellulose	Combination and desorption of enzyme and cellulose	Inhibition of cellobiose on endoglucanase and exoglucanase Inhibition of glucose on β-glucosidase	External mass transfer resistance
Mechanical and synergistic effect of different enzyme components	Structural differences of lignocellulose	Enzyme-cellulose complex formation mechanism	Product inhibition mechanisms (competitive and noncompetitive); other factors causing enzyme inactivation (shear force)	Internal enzyme diffusion resistance

been deemed to have stronger inhibition than glucose on enzymatic hydrolysis of cellulase. In addition, the study found that CB was inhibited by glucose, endoglucanase, or 1,4-β-D-glucan cellobiohydrolase was inhibited by cellobiose, while exoglucanase was not affected by the inhibition of the final product. At the beginning of enzymatic hydrolysis, addition of glucose to the enzymatic system will reduce the rate and efficiency of enzymatic hydrolysis. At the same time, the presence of cellobiose could also strongly reduce the initial velocity of glucose production. Holtzapple et al. pointed out that all of the enzymes (adsorbed enzyme, free enzyme, and complex enzyme) in the enzymatic process would be inhibited by products. They believed that if most of the enzymes in the enzymatic system exist in the form of enzyme-substrate complex, then the inhibition effect of the final product will only exhibit competitiveness [67].

The high complexity of the enzymatic hydrolysis mechanism of cellulose is inevitable because cellulose has a complex structure and composition and is water insoluble. Moreover, the hydrolysis of cellulosic material requires a synergistic effect of several enzymes in a heterogeneous reaction system. All of these make it extremely difficult and imprecise to establish a mathematical model for this system.

Table 3.1 lists five categories of factors that need to be considered when establishing a comprehensive kinetic model of cellulose enzymatic hydrolysis and saccharification. Historically, the cellulase kinetic model was more successful if it was established to predict the initial speed of the enzymatic hydrolysis reaction. However, the establishment of a kinetic model used for the prediction of reducing sugar yield in the long-term batch enzymatic hydrolysis of lignocellulose encountered many difficulties. The main reason for this result is that the kinetic model varies with the enzyme inactivation and the structure of the substrate.

In batch enzymatic process, enzymatic speed increases logarithmically at first, followed by the rapid increase of soluble sugar yield; then, the degradation of cellulose decreases stably. The data reflecting that initial enzymatic reaction kinetics trend coincide with the classic Michaelis-Menten equation model. Study showed that, in the early stage of a fast reaction, enzyme adsorption and mass transfer rates do not influence the reaction rate. The active binding sites of enzyme on the substrate almost remain constant, ensuring a high substrate-to-enzyme-ratio. However, this model is not suitable for the later stage of enzymatic hydrolysis because it ignores the inhibition of enzyme, the change of cellulosic structure, and the dynamic changes of interaction between enzyme and substrate interface.

Many enzymatic kinetics models have been proposed, but most are for a specific enzymatic system or a certain enzymatic system and will not be suitable if the substrate or enzymes change. This is because the reaction kinetics of enzymatic hydrolysis are influenced by many interrelated factors, making it difficult to quantify each individual factor.

3.4 Cellulose Enzymatic Hydrolysis and Saccharification

Along with economy development, population growth, and increasing energy consumption, lignocellulose as the most abundant and cheapest renewable resource on Earth has received considerable attention. However, because of the complex structure of the cellulosic biomass, its chemical properties and mechanical properties are relatively stable. Generally, it is extremely difficult to dissolve in solvent, and can be utilized further only when it is hydrolyzed into monosaccharides.

The enzymatic saccharification of cellulose has many advantages, such as mild conditions, simple equipment, low energy consumption, and less pollution. However, the industrial applications of enzymatic hydrolysis and saccharification of cellulose are limited because of the expense of enzyme preparation, the slower rate of hydrolysis, lack of an effective response system to meet the complex heterogeneous interface catalytic reaction, and so on.

3.4.1 Factors Affecting Cellulose Enzymatic Hydrolysis

The main factors affecting cellulase include substrates, enzyme activity, and reaction conditions (temperature, pH, and other operating conditions). To improve enzymolysis efficiency and yield, much research has been performed to optimize the hydrolysis conditions and improve enzymatic activity.

3.4.1.1 The Effect of Cellulose Structure on Enzymatic Hydrolysis

The sensitivity of cellulosic substrates to enzymatic hydrolysis is affected greatly by their structure, including the crystallinity degree, polymerization degree, specific surface area, and lignin contents in lignocellulose [68]. The pretreatment methods for different raw materials are substantially to improve enzymatic efficiency and productivity by changing the structure of cellulosic materials, such as decreasing the crystallinity and polymerization degree of cellulose, improving the specific surface area of the substrate, and removing the lignin content of substrates. Gharpuray et al. [69] treated wheat straw with physical and chemical methods and studied the specific surface area, crystallinity, and changes in the chemical composition of the treated wheat straw. The results showed that the specific surface area is the main factor affecting cellulase enzymolysis, followed by the lignin content and finally the degree of crystallinity. Inhibitory effects of lignin on enzymatic hydrolysis include two aspects. On one hand, lignin shields cellulose, and the higher the lignin content, the greater the shielding effect. On the other hand, lignin adsorbs on cellulase invalidly. So, removal of lignin would greatly improve the enzymatic hydrolysis rate [70].

3.4.1.2 Effect of Enzyme on Enzymolysis

Cellulase is a complex enzyme. There are significant differences in the composition of cellulase synthesized by different microorganisms, and their ability for enzymatic hydrolysis of cellulose is quite different. Cellulase-producing microbes include bacteria, actinomycetes, and filamentous fungi; microbes with strong cellulose-degrading ability are mainly strains that include *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., and *Acremonium* spp. The cellulase produced by *Trichoderma reesei* is currently the most widely used enzyme. The enzymes usually contain high activity of endoglucanase and exoglucanase but low activity of CB. Many strains from *Aspergillus* spp., such as *A. niger,* can produce high activity of CB. Usually, enzymes produced by *T. reesei* and *A. niger* when mixed in a certain proportion can have a better effect.

3.4.1.3 Effect of Operating Conditions on Enzymolysis

Most of the activity of cellulase is affected by environmental temperature and pH. The enzyme reaction has a maximum speed under the optimum pH, which for cellulase is generally in the range of pH 4.5–5.5. Temperature is also an important factor that affects enzymolysis of cellulose. Usually, the optimum temperature range of cellulase is 40–60 °C. Therefore, to improve enzymatic hydrolysis of cellulose, it is necessary to select the optimum pH and optimum temperature according to different enzymes and reaction conditions.

Substrate concentration is a main factor that affects the efficiency and initiation rate of enzymatic hydrolysis. At a low substrate concentration, increase of the substrate concentration usually benefits improved efficiency and initiation rate of enzymatic hydrolysis [71]. But, a high substrate concentration will reduce the enzymatic hydrolysis rate because of substrate inhibition, which is related to the ratio of the total amount of substrate and the amount of enzyme. Huang and Penner [72] found that substrate inhibition occurred when the ratio of microcrystalline substrates (Avicel pH 101) to cellulase was higher than 5 g/FPU (grams substrate/filter paper activity units). Penner and Liaw [73] found that the optimum ratio of microcrystalline substrate (Avicel pH 105) to cellulase was 1.25 g/FPU.

The amount of cellulase is also a factor that affects enzymatic hydrolysis. In a certain cellulase concentration range, increasing the cellulase amount can improve the yield and rate of enzymatic hydrolysis but enhance the cost increase. In laboratory studies, the amount of cellulase is often 10 FPU·g^{-1} of cellulose. It was reported [74] that the enzymatic hydrolysis rate increased rapidly along with the increase of cellulase added when the cellulase dosage was less than 100 mg in the hydrolysis process of 5 g wheat straw. When the cellulase dosage was higher than 100 mg, the enzymatic hydrolysis rate increased slowly with the increase of cellulase. It indicated that, in certain conditions, the number of sites binding the cellulose molecule and enzyme molecule was limited. When all these sites were occupied by cellulase molecules, enzymatic hydrolysis did not increase with the additional cellulase dosage. In terms of enhancing accessibility of cellulase and lignocelluloses, exploring the binding mechanism, and increasing the binding sites, Chen and Xu [75] invented strengthening techniques for enzymatic hydrolysis, namely, repeated adsorption and desorption of cellulase and substrates, which could reduce the lowest enzyme dosage to 6 IU·g^{-1} substrate; usually, the lowest enzyme dosage standard in the industrialization of lignocellulose ethanol was 10 IU·g^{-1} substrate.

After enzymatic hydrolysis, a cellulase-recycling system is another effective way to reduce the cost of production. The cellulase may be recovered from the supernatant and the solid substrate. Generally, the cellulases that remain on cellulosic substrates account for 40–90 % of the original enzyme amounts because of the differences in enzyme-to-substrate ratio and enzymatic time. Therefore, most enzyme recycling focuses on the cellulase remaining on the substrate, such as recycling by washing with a generous amount of water, flushing with dilute Tween 80, and crushing the residual cellulose. Reese [76] reported that it was effective to elute cellulase with a range of chemical reagents, such as guanidine hydrochloride, dimethyl sulfoxide, and n-propanol under conditions of pH 5.0 and 30 °C. However, because of the high concentration (3 M or higher), it is not suitable for industrial production. The potential way to obtain higher activity recovery is to use low concentrations of alkali. Otter et al. [77] recovered 60 % of cellulase activity within 10 min using different base and controlling the appropriate pH (5.0) and temperature (0–30 °C). Some researchers obtained about 50–60 % of the cellulase activity by controlling the pH near 7.0 [78].

3.4.1.4 Effect of Inhibitors and Activators on Enzymatic Hydrolysis

Cellulase can be competitively inhibited by enzymatic reaction products and certain substances similar to substrates, such as cellobiose, glucose, and methyl cellulose, which are usually competitive inhibitors of cellulase. Some phenol, tannin, and anthocyanidin in plants are natural inhibitors of cellulase. Halides, heavy metals, detergents, dyes, and so on can also inactivate cellulase. Especially in the enzymatic process, the cellobiose and glucose produced can cause obvious feedback inhibition on the entire reaction and reduce the efficiency of enzymatic hydrolysis.

Based on the results of years' research, it was also found that many substances could activate cellulase, such as Mg^{2+} $CoCl_2$, Ca^{2+}, $Ca_3(PO_4)_2$, and neutral salts. When the conditions for enzymatic hydrolysis were changed, some substances could be converted between the inhibitor and activator. For example, cellobiose is the inhibitor in most cases, but seven types of cellulase can be activated by cellobiose when cellulase acts on CMC.

3.4.2 Cellulase Enzymolysis

There are many ways of cellulase enzymolysis, such as batch enzymolysis, consecutive enzymolysis, and simultaneous saccharification and fermentation. Cellulase enzymolysis is seriously inhibited by the product. So, according to the removal of product inhibition under various processes, cellulase enzymolysis can be divided into two categories: The product inhibition is not completely removed but just alleviated; the product inhibition is removed completely. The former mainly includes the direct enzymatic saccharification process and a synchronous process of enzyme production and enzymatic hydrolysis. The latter mainly includes simultaneous saccharification and a coupling process of enzymatic hydrolysis and membrane separation.

3.4.2.1 Direct Enzymatic Hydrolysis and Saccharification Technology

In an earlier period, enzymatic hydrolysis of lignocellulose used the method of single feeding [79]. But, the products of reaction exhibited significant inhibition of the entire reaction and resulted in low efficiency of enzymatic hydrolysis. Later, this was improved using batch enzymatic hydrolysis technology, by which the substrates were fed to the reactor in a batch. It was reported that the enzymatic hydrolysis yield when substrates were fed in a batch was higher than that when fed once when the concentrations of substrate and enzyme dosage were the same. When the substrate concentration was 15 % (w/v), the hydrolysis yield of batch-fed substrates increased by 10 %. Because of the low concentration of CB in cellulase produced by *T. reesei*, in cellulase enzymolysis CB cannot act synergistically with C_1 and C_x, which results in the accumulation of cellobiose. It has a strong inhibitory effect on the cellulase and causes the inactivation of parts of the enzyme and the decline of the hydrolysis yield.

Using the method of batch feeding can reduce substrate concentration and mass transfer resistance. It can relatively increase the enzyme dosage of substrate, especially the amount of cellobiose. So, it can reduce the inhibition caused by the accumulation of cellobiose generated and thus increase the hydrolysis yield. But, this technology can only improve enzymatic efficiency to a certain extent.

In addition, it is also possible to adopt continuous enzymatic technology, by which material is fed in and fed out at a certain speed simultaneously for a long time. The dilution D is defined as the ratio of the flow rate F and liquid volume V, that is, $D = F/V$. Compared with enzymolysis in batches, continuous enzymatic technology could obtain relatively high productivity. However, this technology needs to add not only the substrate but also the cellulase, which increases the cost of the enzyme [80].

The major drawback of direct enzymolysis is that it cannot completely eliminate the inhibitory effect of glucose on the reaction, and the substrate residues and enzyme cannot be reused.

3.4.2.2 Simultaneous Cellulase Production and Enzymatic Hydrolysis

To reduce the cost of cellulase, a simultaneous process of cellulase production and enzymatic hydrolysis was developed. This process is the coupling of cellulase production and cellulose enzymatic saccharification, which are carried out in the same reactor. Previous studies have shown that the immobilized *Trichoderma reesei* mycelia growing in the specific limiting conditions can be batch cultured repeatedly, which allows cellulase production coupled with enzymatic saccharification in one reactor. In the intermittent feed conditions, the amount of cornstalks reaches 120 g L^{-1}, and saccharification reaches 89.20 % after 7 days. The cost of this process is low, but there are strict sterilization demands for equipment. It also cannot completely eliminate the inhibition of glucose to the reaction.

3.4.2.3 Simultaneous Saccharification and Fermentation

The so-called simultaneous saccharification and fermentation process is that the reaction of saccharification and ethanol fermentation process happen in the same tank simultaneously. The enzymatic hydrolysis of cellulose takes place simultaneously and continuously with the saccharification process in the same apparatus. The hydrolyzate, glucose, is used by the continuous fermentation of bacterial cells, which can eliminate the feedback inhibition to cellulase. The advantages of this process include reducing product inhibition, promoting enzymatic hydrolysis of cellulose, decreasing the amount of enzyme, simplifying the equipment, and enhancing production efficiency. But, there are also many problems, such as the differences between temperatures of hydrolysis and fermentation. In the saccharification process, the optimum temperature of cellulase is about 50 °C; the optimum fermentation temperature of yeast is 31–38 °C, which causes a problem in that the enzymatic hydrolysis cannot be carried out at the optimum temperature.

The following measures can be taken to solve the problem mentioned: (1) The fermentation conditions of the bacteria, such as *Lactobacillus* spp., including temperature, pH, and anaerobic or microaerobic fermentation characteristics, should be similar to the enzymatic hydrolysis conditions of cellulase. And there is not CO_2 produced during the fermentation process. So the saccharification and lactic acid fermentation can be coupled simultaneously. (2) The saccharification and ethanol fermentation can be operated independently to avoid the differences between simultaneous saccharification and fermentation reaction conditions. A circular conveyor system between the two reactors is established to couple the saccharification and alcohol fermentation. At the same time, because the glucose can be separated from the saccharification bioreactor, the cellulose enzymatic reaction and the product separation online can also be completed. Therefore, the glucose concentration in the reactor can be regulated through the cycle and the amount of the circulating fluid. In 1998, researchers proposed a simultaneous saccharification and fermentation process for the nonisothermal method. It was achieved by separating hydrolysis and fermentation in different reaction zones so that the two reactions could be carried out at their optimum temperatures. The yeast was fixed in the fermentation zone to avoid heat inactivation.

3.4.2.4 Coupling of Enzymolysis and Membrane Separation

With the development of membrane technology, the application of membranes in biochemistry is no longer limited to the separation and enrichment of the product. In the early days, because of the selected separation performance, the ultrafiltration (UF) membrane was used to study the reaction mechanism of enzymatic hydrolysis of cellulose. Then, people began to study a membrane reactor that could couple enzymatic hydrolysis and membrane technology.

The so-called enzyme-membrane bioreactor introduces an appropriate membrane module into enzymatic hydrolysis and separates the permeable solution from the reaction system using the driving force across the membrane. This reactor combines biocatalysis, product separation, concentration, and the recovery of enzyme into a single operating unit. There are many classifications of membrane-enzyme reactors. (1) According to structural and hydrodynamic characteristics, they can be classified into continuous stirred tank reactors (CSTRs) and plug flow reactors (PFR). (2) According to different ways of coupling the reaction and separation, they can be classified into cycled-type and integrated-type reactors. (3) According to the contact mechanism of enzyme and substrate, the reactors can be classified into direct contact type, diffusion contact type, and multiphase contact type. (4) According to the state of enzyme in the reactor, they can be classified into free-enzyme-membrane reactors and immobilized-enzyme-membrane reactors. (5) According to the difference of the driving force between substrate and product transfer, they can be classified into enzyme-membrane reactors promoted by pressure differential and concentration differential.

These reactors can also be classified according to the different characteristics of membrane material, such as the nature, structure, shape, and so on, giving the enzyme-membrane reactor the following characteristics: (1) Continuous operation makes it possible to greatly limit the use of enzymes, which not only can improve the yield but also reduce costs. (2) The products can be continuously separated from the reaction system, resulting in improved reaction speed in the enzyme-membrane reactor and the conversion rate of the substrate. (3) The process is without emulsification, so the reaction system avoids enzyme inactivation and power caused by high-speed stirring emulsification. (4) Convection diffusion replaces the molecular diffusion way of mass transfer.

The enzyme-membrane reactor also has its drawbacks. For example, the deactivation of the catalyst requires additional fresh enzyme at appropriate times. The reduction of reactor efficiency caused by the decrease of transfer mass efficiency makes improvement of operating conditions necessary to effectively control the concentration differential polarization and blockage of membrane.

When choosing the film in the design of an enzyme-membrane reactor, the size of the enzyme molecules should be considered, as should the solution characteristics of the substrate and the product and the characteristics of the film itself, such as materials, flux, rejection rate, and other factors. The ideal film has a product rejection coefficient of zero, ensuring that the product can be completely penetrated through; the rejection coefficient of the enzyme should be 100 % to ensure that the enzyme is completely retained in the reaction system. But, it does not mean that when the substance molecules are smaller than the pores of membrane the molecules must not be trapped. In addition to the size factor, although some molecules are slightly smaller than the pores of membrane material, there may be electrostatic repulsion. And, membrane chemistry can affect solute permeability because of nonspecific interactions (electrostatic, hydrophobic, and hydrophilic interactions), causing an additional layer (gel layer) to be formed on the surface of solute to reduce the permeability (the concentration polarization phenomenon). When the separation of enzyme and chemical reaction occur, the enzyme concentration polarization is particularly evident.

A UF membrane is used most often in cellulose hydrolysis. Generally, the molecular weight cutoff (MWCO) of the UF membrane is between 500 Da and 100,000 Da, and the size of the cellulase is between 40,000 and 60,000 Da. So, a membrane whose MWCO is nearly 10,000 Da is usually chosen.

Membrane fouling and cleaning in the use process require attention. To reduce film pollution, film material should be selected that has less adsorption on cellulase, such as polysulfone, polyethersulfone, and the like. Yang et al. took a polyethersulfone membrane with a molecular weight of 10,000 Da as the research object, and they studied the effects of membrane fouling. This showed that under the proper cleaning conditions, the membrane flux of pure water recoverable rate could still be 90–95 % after use for more than 6 months.

Enzymatic hydrolysis coupled with membrane separation technology could discharge the suppression of the product completely, improving enzymatic hydrolysis efficiency and yield by isolating online small molecules obtained by enzymatic

hydrolysis from enzymatic hydrolyzate. Yang et al. [81] took steam-exploded straw as a substrate and studied the impact of enzymatic hydrolysis coupled with a membrane separation system on the enzymatic hydrolysis. The study showed that, under the same reaction conditions, the substrate conversion rate could improve from 23 to 38 %, and the enzymatic time could be shortened to 24 h.

3.5 Hemicellulose Biotransformation

3.5.1 Hemicellulose Compositions and Structural Characteristics

Hemicellulose is a polysaccharide polymer interpenetrated between cellulose and lignin in the plant cell wall. It can usually be extracted from the original or delignination materials with water or an alkali solution. Its structure is complicated. Hemicellulose consists of heterogeneous glycans concluded from the hydrolyzate, while it is complex sugar compounds composed of different structural units. The structural unit comprises D-xylosyl, L-arabino, D-glucosyl, L-mannose, 4-*O*-methyl-glucuronic acid, D-semigalacturonic acid, D-glucuronic acid, and so on [82]. Hemicelluloses from different sources have different proportions of various structural units, but xylose, one of the components, distributes relatively stable and is linked by β-1,4-glycosidic with high branching.

3.5.2 Hemicellulose Degradation Mechanism

Hemicellulase represents the specificity of a group of enzymes to degrade hemicellulose, which belongs to the glycanhydrolases (EC 3.2.1). These enzymes do not include glucosidase, which not only has the ability to degrade low molecular weight glycosides but also can hydrolyze the hemicellulose main chain branch. The typical hemicellulase includes D-xylan-glucosidase, L-arabinanase, D-semiarabinogalactan enzyme, and D-mannan enzyme [83]. Xylan is not only the most abundant hemicellulose but also one of the most abundant resources in nature besides cellulose. To make full utilization of these resources, scientists carried out research on its major degrading enzyme, β-D-1,4-endo-, exocutting xylanase. The mechanism of degradation of hemicellulose is analyzed by sequence analysis of different sources of xylanase, the enzyme molecule responsible for different functions of the regional and enzyme activity points.

Compared with cellulose, the complex structure of hemicellulose determines that its degradation needs the synergy of multiple enzymes. In addition, the microorganisms producing hemicellulase can generally also produce cellulase; that is, they secrete a mixture of two types of enzyme. Therefore, traditional microbiology and

biochemistry methods encounter many difficulties, but molecular biology provides a new approach to the development of the method to solve these problems. The application of recombinant DNA technology enabled the biochemistry and molecular biology study of coding hemicellulose by focusing on xylanase.

Xylan has different sources. There are different side-chain substituents, such as acetyl, galactose, arabinose, or glucuronic acid residues. Xylan exhibits many forms in nature. The most abundant xylan forms are acetyl xylan in hardwood and arabinoxylan in softwood. Complete hydrolysis of xylan requires the synergistic effect of a variety of enzymes, and xylanase is the most important one involved in degrading enzymes. Xylanase includes a group of enzymes that can degrade xylan to oligosaccharides and xylose, including β-1,4-xylanase, β-1,4-endoxylanase enzyme, and β-xylosidase. The β-1,4-exoxylanase takes a single xylose as the cutting unit, which acts on the nonreducing end of xylan so that the reduction of the reaction system increases. Endo-1,4-β-D-xylanase enzyme cleaves from the interior of the main chain of the xylan β-1,4-glycosidic bond, which can break down xylan backbone randomly, producing xylooligosaccharide and reducing the degree of polymerization (DP). Then, xylooligosaccharides and xylobiose are decomposed to xylose by exonuclease β-xylosidase [84]. The presence of the side-chain substituent will inhibit the activity of xylanase and therefore needs different glycosidase to hydrolyze the glycosidic linkage between xylose and side-chain-substituted groups, such as α-L-arabinosidases, α-D-glucuronidase, acetylesterase, and ferulic acid esterase. Studies showed that these specific glycosidases can decompose xylan efficiently in a synergistic effect together with the endoxylanase and beta-xylosidase. Similar to endo-1,4-β-D-glucose enzyme, endo 1,4-β-D-xylanase enzyme also has diverse enzyme components. It is usually explained that a few xylanase genes can form numerous xylanase components with similar catalytic performance and different molecular weights and isoelectric points through the shear processing of pre-mRNA in the transcription, posttranscriptional glycosylation and protease-limited hydrolysis after secretion, and so on.

Most 1,4-β-endo-xylanase of fungi and bacteria are a single-subunit proteins, with molecular weight between 8,500 and 85,000 Da and an isoelectric point of 3.6–10.3. Systematic statistics of dozens of molecular weights and isoelectric points of xylanases indicated that molecular weights of 70 % acidic 1,4-β-endo-xylanases were higher than 30,000 Da, while the molecular weights of alkaline 1,4-β-endo-xylanases were lower than 30,000 Da. The optimum pH value of the majority of the 1,4-β-endo-xylanases was less than 6.0, and the optimum reaction temperature was between 45 and 75 °C. Most endoglucanases can hydrolyze oligoxylan, whose DP value is more than 2, leading to its affinity decrease with the decrease of DP value [85].

Substituent groups on the main chain of xylan inhibit the hydrolysis process. However, it is unsure whether this is caused by steric hindrance. If these substituent groups are removed in advance, the degree of apparent combination between enzyme and substrate would be strengthened. Some xylanases have strict requirements for the substituents on the main chain of xylan, and it seems that these substituents contribute to the orientation of the enzyme catalytic group, and

they position at specific restriction sites. For example, there is no arabinose in the catalyzed products of the two xylanases from *A. niger* (pI 8.0 and pI 9.6), and the xylanase has little or no effect on oligosaccharide removal of the arabinose residues. So, it can be proposed that the action requires the presence of arabinose residues near its sites.

β-Xylosidase has different forms, such as monomer, dimer, or tetramer. Its molecular weight is between 26,000 and 360,000 Da. Its isoelectric point value is between 3.3 and 6.3. In bacteria and yeast, β-xylosidase is mainly present in the cell. It mainly acts on the oligoxylan, which belongs to the exonuclease. Its affinity with the substrates decreases with the increase of DP values; therefore, it almost does not embody the vitality to the polyxylan. In various oligoxylans, dual xylans are the preferred substrate for β-xylosidase. β-Xylosidase is a multifunctional enzyme. Xylosidase derived from *Trichoderma* spp. also has the activity of arabinosidases. β-Xylosidase derived from *A. niger* can digest xyloside and the α-arabinoside bond, β-glucoside bond, and β-galactosidase bond. Many β-xylosidases have 1,4- and 1,3-xylosidase transferase activity, particularly in the case of a high concentration of substrate, so they can transfer the oligomeric xylan to xylan polysaccharide. As the main enzymes of xylan degradation, β-xylosidase enzyme and β-1,4-endoxylanase can degrade the xylan to oligosaccharides or xylose [85].

Another type of xylan-degrading enzyme is alpha-L-arabinofuranosidase. The study of this enzyme encountered many difficulties because of the lack of suitable natural substrate and other reasons. Therefore, in the past 20 years, few reports have appeared on the separation of the enzyme. Now, known arabinosidases usually exist in monomeric form, and they also have dimer, tetramer, hexamer, and octamer forms. Molecular weights range from 53,000 to 4,995,000 Da, and isoelectric point values range from 2.5 to 9.3. The optimum pH value is between 2.5 and 6.9 [85].

Xylan in nature mostly is a heteromeric polysaccharide. There are various substituent groups on the main chain and side chain of glycosylation, such as acetyl, glucuronic acyl, and so on. The removal of these groups requires the involvement of different enzymes. The enzyme molecule and the substrate combine with each other to play a role; the enzyme and substrate molecules often require full three-dimensional coordination to ensure an efficient reaction. Otherwise, the enzyme molecule is invalid, as shown in Fig. 3.3.

Currently, more than 300 kinds of different sources of the xylanase genes have been reported, and more than 100 kinds have been cloned and expressed in a suitable host. Bacterial xylanase gene has been studied more intensively than others. Many thermophilic xylanase genes were cloned into *E. coli,* such as XynA and XynB of *Thermotoga neapolitana*, which both were cloned and expressed in *E. coli* with lower expression levels. In recent years, nonconventional yeasts became an ideal system for expression of foreign genes, with high expression, high stability, and high secretion characteristics.

But, there have been rare reports about the secretion and expression of xylanase genes in nonconventional yeast. By means of constructing appropriate expression vectors and suitable host, some xylanase genes have been highly efficiently expressed, and the products are secreted extracellularly. However, study is still in

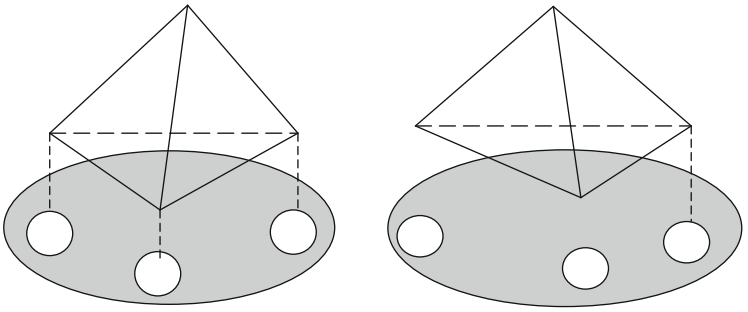

Fig. 3.3 Relationship between structure and efficiency of the three-dimensional structure of enzyme and substrate molecules. *A* reaction can be carried out efficiently, *A′* reaction cannot be carried out

the basic research stage. Some scholars studied the secretion and expression of thermostable xylanase gene by *Bacillus subtilis*, *Trichoderma reesei,* and *Streptomyces* spp., and they also optimized the merger code, the choice of signal peptide expression strategy research. A successful case still has not been reported. It is obvious that the highly efficient expression of the xylanase gene and extracellular secretion mechanism remain for further deep research [86].

3.6 Lignin Biotransformation

Lignin is an important component of plant cell walls, accounting for about 20 % of the plant's dry mass content in nature, which is just after cellulose. It is estimated that about 6×10^{14} t lignin are produced annually worldwide, mainly in the form of paper industry wastewater and straw of crops. Lignin, cellulose, and hemicellulose are combined through covalent bonds in plant tissue. The cellulose molecule is entrapped there to form a sturdy natural barrier so that general microorganisms have difficulty entering them to decompose cellulose. So, degradation of lignin is also important for the effective application of cellulose [87]. Compared with cellulose, lignin constituent units and spatial structure are more complex. The structural complexity determines that the degradation of lignin needs the synergistic effect of different enzymes. In nature, there are few species that can thoroughly degrade lignin. Fungi play a major role in lignin degradation, but most degrade lignin slowly.

The lignin content and composition differ according to plant species or plant growth stage. Because of the absence of regularity and orderliness of the repeating unit, so far entire lignin molecules still have not been isolated in an originally intact state. Therefore, its structure is difficult to discern.

3.6.1 Related Lignin-Degrading Microorganisms

The lignin-degrading bacteria mainly include *Clostridium* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Micrococcus* spp., *Pseudomonas* spp., *Amphibacillus* spp., *Xanthomonas* spp., *Mycoplana* spp., *Branhamella tarrhalis* spp., *Brochothrix* spp., and *Bacillus* fir-*mus.* The lignin-degrading actinomycetes mainly include *Streptomyces* spp., *Thermoactinomyces* spp., *Arthrobacter* spp., *Micromonospora* spp., *Nocardia* spp., and *Thermonospora fusca* spp. Molds play a major role in the lignin degradation process. There are mainly three categories of lignin-degrading fungi: white-rot fungi, brown-rot fungi, and soft-rot fungi. Among them, the lignin-degrading ability of white-rot fungi is relatively stronger than the other two categories. They do not produce pigment during the process of lignin degradation by secreting extracellular oxidative enzymes. There is a bright future for white-rot fungi. The white-rot lignin-degrading fungi mainly include *Phanerochete chrysosporium*, *Coridus versicolor*, *Thametes versicolor*, *Phlebia radiate*, *Pleurotus pulmononanus*, *Pycnoporus cinnabarinus,* and so on. *Phanerochete chrysosporium* has been studied most thoroughly. In addition, there are other categories of molds with lignin-degrading ability, such as *Phomopsis* spp., *Penicillium* spp., *Fusarium link* spp., *Aspergillus* spp., and *Trichoderma* spp. Moreover, Elena Sláviková discovered a kind of yeast (*Trihosporon pullulans*) that also has the ability to degrade lignin [87].

3.6.2 Related Enzymes of Lignin Degradation

The lignin-degrading enzymes are a complex system. Recently, there have been many studies about the catalytic decomposition mechanism of the lignin-degrading enzyme system. These enzymes mainly include extracellular peroxidase, such as lignin peroxidase (LiP), manganese peroxidase (MnP), and extracellular phenol oxidase, such as LaC. In addition, many other enzymes participate in the degradation of lignin or have some impact on its degradation, such as aryloxide oxidase (AAO), glyoxal oxidase (GLOX), glucose oxidase (glucose-L-oxidase), the phenol-oxidizing enzyme, catalase, and so on. In addition, the bacteria can produce two new types of enzymes, ferulic acid esterase and coumaroyl esterase, which can produce ferulic acid and p-coumaric acid, respectively. These two types of enzymes with the glycanase can decompose the hemicellulose-lignin conjugant synergistically but do not mineralize the lignin [87].

3.6.2.1 Lignin Peroxidase and Manganese Peroxidase

LiP and MnP are both iron extracellular heme proteins with a sugar group, also known as heme peroxidase. LiP is a glycoprotein, constituted by ten length proteins

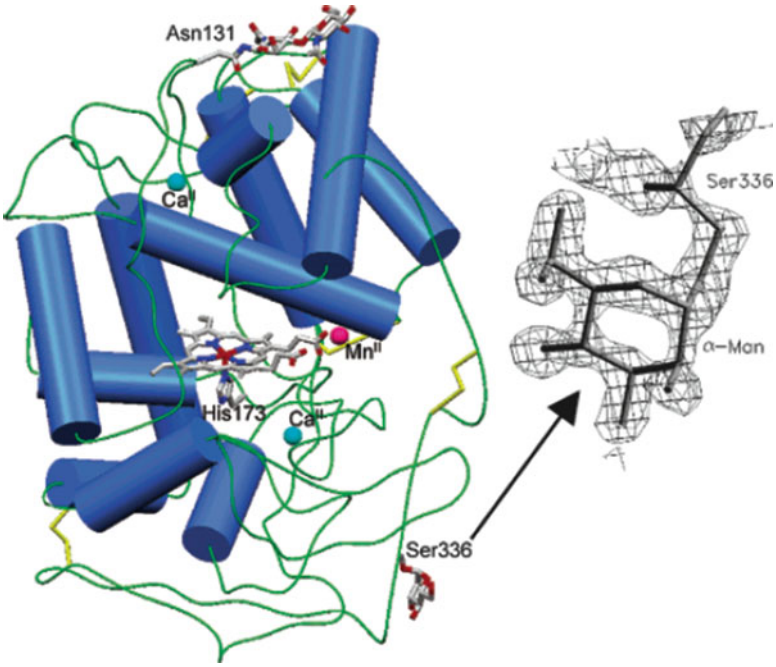

Fig. 3.4 Molecular structure of MnP [16]

and a single short chain. It is similar to other peroxidases, such as horseradish peroxidase, in that they perform a typical catalytic cycle of peroxide. MnP is also a glycoprotein and is constituted by ten long proteins and one single chain (Fig. 3.4) [16].

The LiP activity center is composed of a heme base as well as two Ca^{2+} with the role of stabilizing the structure. The MnP activity center is essentially the same as that of LiP, but there also exists Mn^{2+} (Fig. 3.5). The main difference between the two is that the C-terminal of LiP is between the two propionates of heme, while the C-terminal of MnP is separated from the heme. In addition, MnP has five disulfide bonds, while LiP only has four. The first four disulfide bonds of MnP and LiP are the same. The fifth disulfide bond of MnP is in the C-terminal of protein, suggesting that it is related to the active center of the Mn^{2+} [16]. In the lignin degradation process, LiP and MnP in the reaction capture an electron from the benzene ring of the phenol and nonphenolic compound, leaving the latter to form a cationic group, leading to the breakdown of primary bonds in the lignin molecules. LiP oxidates phenol to generate a phenoxy residue. MnP catalyzes the oxidation of Mn^{2+} and H_2O_2 to generate Mn^{3+}, and then Mn^{3+} oxidates phenol to produce phenoxy residues. But, the specific role of each enzyme in lignin degradation has not been fully elucidated [88].

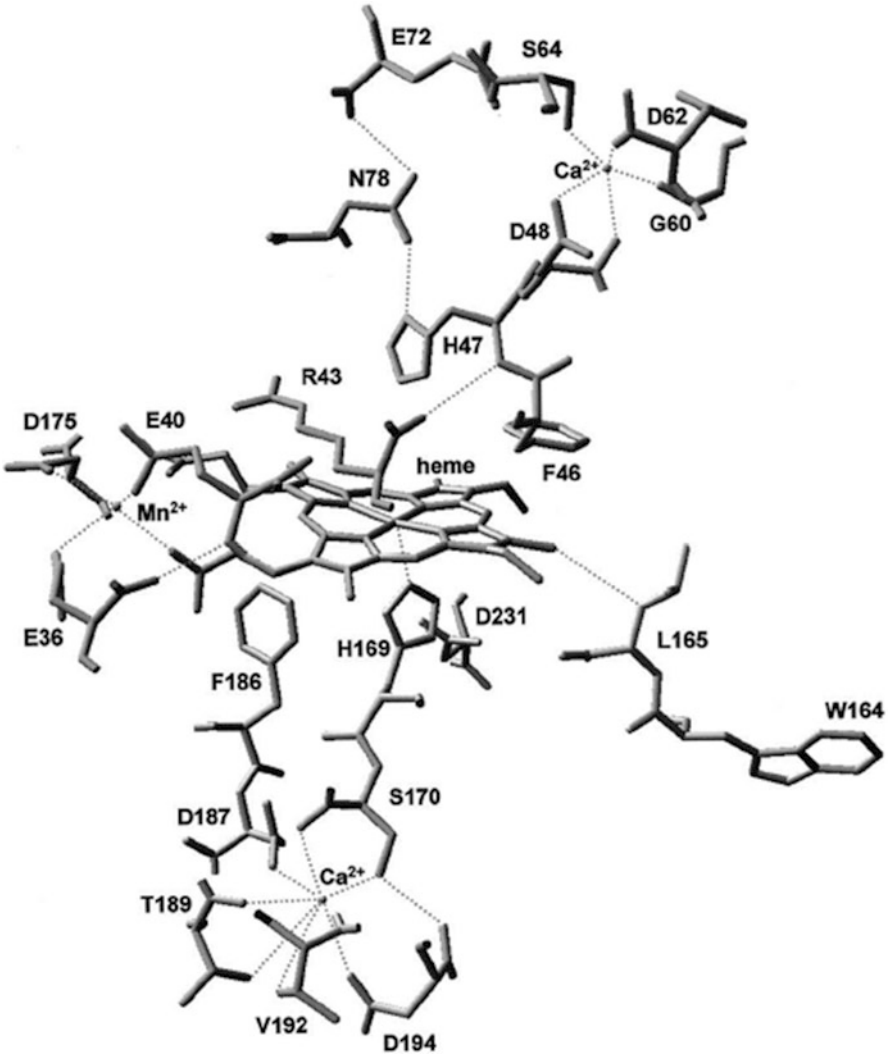

Fig. 3.5 The structure of the active center of LiP and MnP [17]

3.6.2.2 Laccase

Laccase is a polyphenol oxidase containing copper, which is divided to two categories, *Rhus* LaC and fungal LaC, mainly derived from the lacquer and fungi, respectively. Because the fungal LaC had a higher sugar content, LaC crystals were prepared from *Coprinopsis cinereus* bacteria until 1998, which provided a more detailed understanding of spatial structure (Fig. 3.6) [89]. A single LaC molecule is made up of three cup-shaped domains, which are closely connected to form a

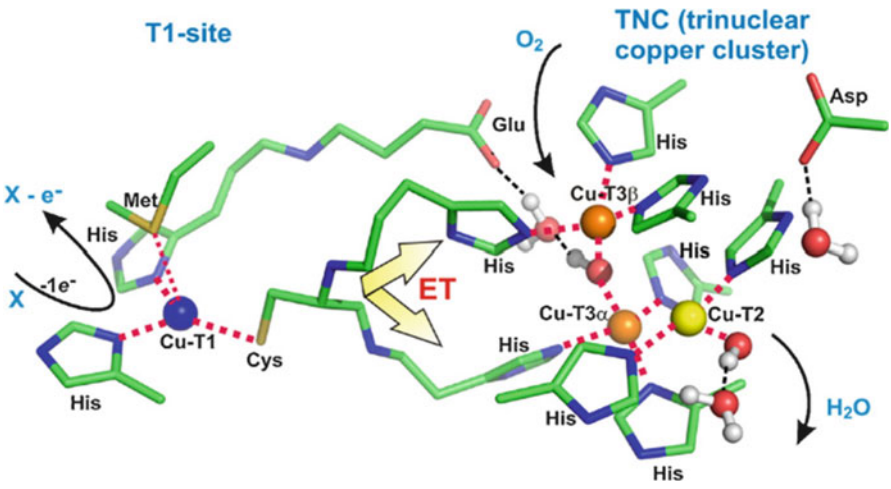

Fig. 3.6 Crystal structure of laccase and its copper ion sites [89]

spherical structure. The β-barrel structure is similar to the other ceruloplasmin [90]. Each of the LaC protein molecules contains four copper ions, which can be divided into three categories depending on the magnetism and spectroscopy properties. At least it has one I-type copper ion, which becomes the center of single-core binding with amino acid residues. It makes the enzyme blue and has absorption at 600 nm. In addition, it contains type II copper atoms and two type III copper atoms, which together constitute the trinuclear center. Two III-type copper atoms link to a hydroxyl bridge to form a dual core. This structure causes the disappearance of the electron paramagnetic resonance effect (Fig. 3.7) [91]. These copper ions play a decisive role during the catalytic reaction of LaC.

It is possible for LaC to directly use O_2 as the second substrate by converting O_2 to H_2O in the absence of H_2O_2 and other secondary metabolites. LaC forms radicals by removing the hydroxyl group on the electron or proton. It may also make lignin from phenoxy radical, which is so unstable that further copolymerization or homopolymerization rapidly occurs. These properties make LaC attract increasing interest in the comprehensive utilization of lignin [92].

3.6.2.3 Other Enzymes

Other enzymes, like glucose oxidase, GLOX, mellow oxidase, and catalase, are involved in the degradation of lignin, but so far, the specific role of each enzyme in lignin degradation has not been entirely clarified.

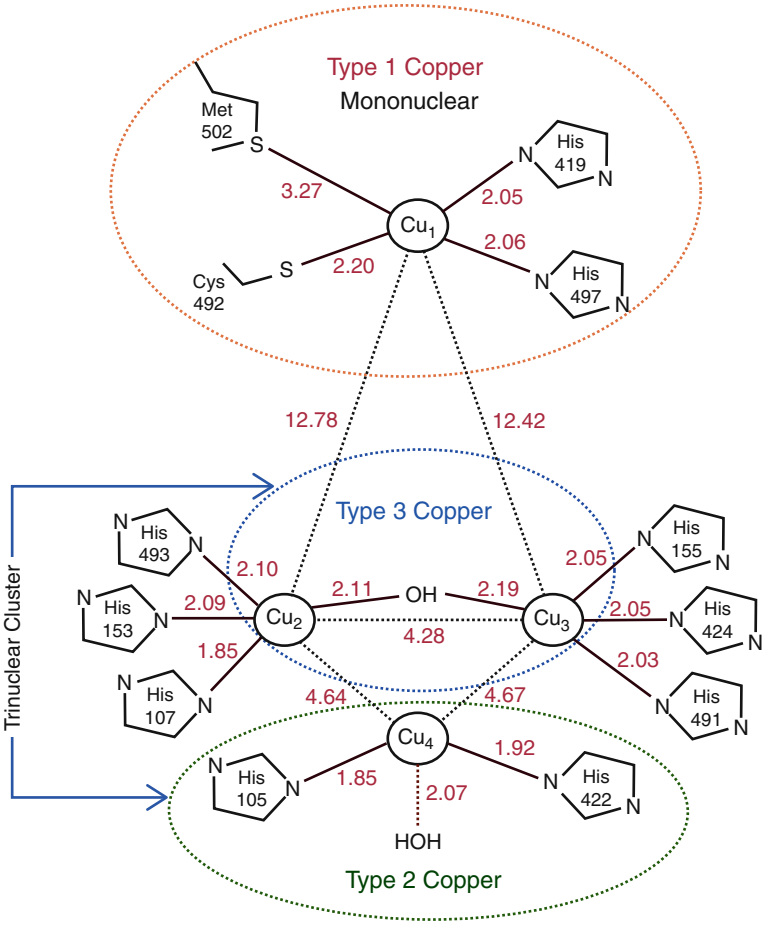

Fig. 3.7 Schematic representation of copper coordination centers, including interatomic distances among all relevant ligands [91]

3.6.3 Mechanism of Lignin Degradation

In the process of lignin degradation, because of the large-scale structure of the lignin polymers, the lignin-degrading enzyme system of white-rot fungi is outside the cell. The lignin structure has an interior unit C-C bond and an ether bond. Therefore, compared with the degrading enzyme system of cellulose and hemicellulose, the catalytic mechanism of the lignin-degrading enzyme is oxidation rather than hydrolysis. Furthermore, because of the irregularity of the complex three-dimensional structure of lignin, the lignin-degrading enzyme should decompose the lignin structure nonspecifically. The lignin is oxidized to produce unstable free radicals, and then it may experience a series of nonenzymatic catalytic spontaneous cleavage

reactions [93], causing the oxidation and fracture of the lignin polymer. Usually, LiP oxidates nonphenolic compounds, causing the aromatic ring cleavage of C_α-C_β. LaC catalyzes the degradation of β-O-4 and β-1 dimer of lignin through the Cα oxidation, C_α-C_β fracture, and alkyl-aryl fracture. Among them, the typical reaction includes demethoxy, dehydroxylation, C-C bond breakage, and so on [94]. MnP can oxidize phenolic and nonphenolic structures. The catalysis of LiP, MnP, and LaC, as well as the generation system of reactive oxygen, would cause the generation of aromatic radical cations, C_α-C_β bond cleavage, C-β oxidation, aromatic ring cracking, monomethoxy aromatic oxidation, and quinine and hydroquinone formation, by which the products can further convert to CO_2 by different metabolic pathways [16].

The molecules of LaC, MnP, and LiP are too large and cannot enter the cell wall pore of wood that is not rotten. The degradation of lignin in the cell walls of lignocellulosic feedstock by these three enzymes must be caused by a number of small molecular oxidants, which can be diffused into the cell wall of wood. These small molecular substances are much smaller than the enzyme to decompose lignin. They are part of the lignin-degrading enzyme system. Studies showed that the three enzymes mentioned can react with the low molecular weight intermediary and have the capacity to attack the lignin structure, which causes the oxidation reaction of lignin indirectly, resulting in the decomposition of lignin. It has been proposed as the lignin degradation mechanism [93]. In 1990, Bourbonnais and Paice [95] proposed that if an appropriate dielectric body is added to the oxidation reaction system of LaC, it could lead to the oxidation of nonphenolic lignin, which made the laccase mediator system (LMS) extensively used in lignin conversion areas. Mediator will form intermediates with certain stability under the action of the enzyme. These reactive intermediates can capture electrons from oxygen molecules transferring to the substrate, degrading the substrates. The presence of mediator increases the oxidation-reduction potential of the system and thus enables the oxidization of nonphenolic lignin units. This is also strong evidence of the mechanism of LMS bleaching and delignification. The LMS is capable of not only catalyzing the degradation of lignin β-O-4 and β-1 structure but also oxidizing the nonphenolic 5-5′and β-5 lignin structure in the presence of a surface active agent [96]. Currently, effective small molecule mediators include 2,2′-azino-bis(3-ethylbenzothiazoline oxazole-6-sulfonic acid) (ABTS) and some compounds containing N═O or N–OH such as 1-hydroxyphenyl benzotriazole (HBT), violuric acid (VIO) [97–99], and others.

3.7 Lignocellulose Microbial Degradation Process

Natural cellulose materials are the main substrate in natural ecology for microbial decomposition and also the most important substrate with the most potential for solid-state fermentation. Study of the ways of microbial invasion and decomposition of lignocellulose will facilitate research on the relationship of microorganisms and solid insoluble substrate. Whether bacterial cells grown on the surface of natural

cellulosic raw material can spread to the interior of the raw material to decompose cellulose depends not only on the raw material structure itself but also on the decomposition ability of the different strains. Some basidiomycetes, including brown pathogens and white pathogens, are the origin to initiate the decomposition of lignocellulosic biomass. Brown pathogens mainly degrade cellulose and hemicellulose without impact on lignin. They contain a strong hydrolytic enzyme to decompose cellulose, demethylate, and destruct the aromatic ring. White pathogens first affect the lignin, almost without the decomposition of cellulosic polysaccharides. They destroy the lignin to form a white substance, removing the methyl group through the oxidation process, and forming diphenol, which is then decomposed by oxygenases. Soft-rot fungi, such as *Cheatomium globosum,* decompose both lignin and cellulose. In addition, ascomycetes, such as *Penicillium* and *Aspergillus*, and *Adelomycete*, such as *Fusarium altornaria*, have the same degradation ability. Other microbes also participate in the degradation of lignocellulose, such as *Streptomyces* spp. and *Thermomonospora* spp. of actinomycetes*, Achromobacter* spp., *Agrobacterium* spp., *Acinetobacter* spp., *Nocardia* spp., *Pseudomonas* spp., *Xanthomonas* spp., and *Micrococcus* spp., which belong to bacteria. Their abilities to decompose the lignin are weak, but the colony effect is huge [88, 100].

3.7.1 Nutritional Properties of Lignocelluloses

Carbon-containing substances in raw cellulose materials are structurally stable and hardly decomposable materials (i.e., cellulose, hemicellulose, and lignin). Easily degrading organic materials such as starch, soluble sugars, lipids, pectins, amino acids, and so on are low in content. A nitrogen source is one of the important nutrients for microorganisms. Usually, C/N in microbial cells is 8:1–12:1. In the growth of the microorganism, 50 % of the carbon supplies the breathing energy, and 50 % of the carbon composes the microbial cells; thus, an ideal C/N in growth medium is 16:1–24:1, making sure that there is enough carbon for the biological use of nitrogen [101]. Overall, the nitrogen content of natural cellulose material is low. The C/N of natural lignocellulose means the ratio of an available carbon source to the nitrogen source. In natural lignocellulose, because of the compact structure constructed by cellulose, hemicellulose, and lignin, most of the carbon source is difficult to utilize. If only an easily degradable carbon source and nitrogen source are considered, the C/N of natural lignocelluloses is estimated as significantly low.

In the process of degradation of natural lignocellulosic materials, the C/N keeps changing. During the microbial degradation process of natural lignocellulosic feedstock, many carbon sources are released in the form of CO_2 into the atmosphere through microbial respiration. The nitrogen source experiences constant enrichment, and inorganic nitrogen is converted into organic nitrogen, so that a limited source of nitrogen in the debris of plants is utilized. Thus, the C/N of natural lignocellulose decreases gradually, dropping to about 25:1 eventually.

Microorganisms need to obtain carbon and energy to grow from insoluble natural lignocelluloses. Thus, the accessibility of substrate is an important parameter for microbial degradation of natural lignocelluloses. For natural lignocelluloses, the accessibility of the matrix depends on their particle surface area and porosity. The second limiting factor that affects microbial nutrients obtained from natural lignocellulose is that insoluble substances must be transformed first by microbes. The conversion process includes depolymerization, hydrolysis, and other chemical reactions. The crystallinity of the lignocellulose and the mass transfer characteristics of the surface of natural lignocellulose affect the transformation process.

3.7.2 Invasion of Microbes into Lignocellulose

The invasion means of microorganisms into natural lignocelluloses are generally classified into the following two kinds: The first is to invade into lignocellulose through the intrinsic orifices. For example, in wood, the radial direction is arranged by ray parenchyma cells, in which the fiber direction is arranged by the tracheid (softwood), catheter (hardwood), or pits of plant cell walls. The invasive capacity of most filamentous fungi to the plant cell wall is lower than for the basidiomycete. Generally, they invade into the cell through pits. The second way is the direct penetration of plant cell walls. For example, basidiomycetes act just like pathogenic fungi; the mycelia contact the cells and hydrolyze the secondary wall by cellulase and hemicellulase to make it more susceptible to microbial decomposition.

Most research showed that the microbes decompose layer S2 of the secondary wall, which is much easier decomposed in the plant cell wall. Layer S2 is often decomposed by the wood-rotting fungi in precedence. According to the differences of invasion of the plant cell wall, it can be divided into the cavitary and erosion types. When the mycelia reach the S2 layer, they grow parallel to the long axis of cells, that is, in the direction of the fiber axis. They form clusters in the cell wall with the S3 layer of the secondary wall not decomposed. Some microorganisms grow in the cell wall and secrete enzymes. They first break down the S2 and S1 layers through the S3 layer by diffusion, and then the S3 followed. Generally, the middle layer, which is highly lignified (intercellular layer on both sides of the primary wall), is not decomposed. Although the mycelial invade the cell wall, they are rarely found in the cell wall. For example, the mycelia can be found during the decay process of wood but cannot be found later. There is no necessary correlation between the degree of wood decay and hyphae. Cavitary-type decomposition is usually the way of degradation of cell wall by soft-rot fungi and brown-rot fungi. Generally, cellulose and hemicellulose are degraded, while lignin is just chemically modified, such as by demethoxylation. Erosion-type decomposition is usually the degradation that first decomposes the S3 layer of the cell wall from the cell cavity, followed by the S1 and S2 layers and finally the middle layer. At the beginning of the white-rot fungus decomposition process, lignin and hemicellulose in the cell wall are first degraded, with the cell wall thickness completely unchanged. Before the remove of lignin,

the lignified lignocellulose cannot be degraded by the cellulase produced by white-rot fungi. Only after the lignin and hemicellulose are removed can the cellulose be degraded. Other types of microorganisms can degrade all the components of the cell wall at the same speed, and the cell wall is gradually rarefied. Cavitary-type decomposition generally occurs in the lignocelluloses, which have a high content of lignin and are difficult to invade. Erosion-type decomposition generally occurs in materials with less lignin that are easily penetrated. For example, white-rot fungus generally exists in broad-leaved wood.

Some microorganisms, such as *Panus conchatus,* invade the cell cavity through the stomata on the surface of grass straw, and then they grow in the cavity and parenchyma cells. Parenchyma cells are first disintegrated. *Panus conchatus* first degrades regions of the fiber secondary cell wall and intercellular layer, keeps the fibers intact and makes the fiber bundles separated with each other.

Because of the poor venting quality of cell walls, many fungi grow rapidly in the surface of raw cellulose materials, and they could secret enzymes extracellularly. At the same time, part of the mycelia grows within the cell cavities and cell walls. Generally, in solid-state fermentation, the mycelia are hard to overgrow in the materials. If the ventilation is strengthened using periodic pressure, it will make the mycelia overgrow internal materials, and the fermentation period will be significantly shortened.

In summary, the action mode of microorganisms on the raw lignocellulosic materials is closely related not only to the types of raw cellulose materials but also to the types of microorganisms and culture conditions.

3.7.3 Mechanism of Microbial Degradation of Lignocellulose

3.7.3.1 Degradation Mechanism of Filamentous Fungi

The strains of *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Cladosporium* spp., and in particular *Trichoderma viride* and its closely relative strains, are able to produce the enzymes capable of decomposing cellulose, hemicellulose, and pectin. Therefore, small filamentous fungi are mainly used for the production of cellulolytic enzymes. Most studies showed that the purified cellulase can digest pure cellulose or amorphous cellulose but has low digestibility of the crystalline form or a combination of cellulose and lignin. There are fewer enzymes to decompose the ester bond between cellulose, hemicellulose, and lignin and the wax substances on the straw surface and lignin [102]. The mechanism of degradation by filamentous fungus is commonly regarded as synergism. Fungal cellulase has two forms of synergistic effect. One is synergy between endoglucanase and exoglucanase. Endo-1,4-β-D-glucanase first inner cuts amorphous cellulose to produce a new end (the reducing end or nonreducing end); then, exo-1,4-β-D-glucanase from the reducing end or the nonreducing end cutting cellulose chain produces cellobiose (or glucose). Another is synergy between exonuclease and

exonuclease. Cellobiohydrolase I degrades the reducing end, and CBH II degrades the nonreducing end of the cellulose chain. The second synergy occurs only in the hydrolysis of natural cellulose, not in the hydrolysis of CMC and HEC. The enzymatic hydrolysis of endo-1,4-β-D-glucanase and exo-1,4-β-D-glucanase is a heterogeneous reaction, while the reaction of β-glucosidase hydrolysis cellobiose is a homogeneous reaction. Endo-1,4-β-D-glucanase has high enzyme activity when it degrades CMC, but enzyme activity is low when it degrades MCC. Exo-1,4-β-D-glucanase has high enzyme activity when it degrades MCC. Enzyme activity is low when it degrades CMC.

The effects of synergy are associated with the nature of the substrate. As the degree of crystallinity increases, the synergistic effect is also enhanced. The synergistic effect is small when the degree of crystallinity is low and the substrate is soluble. Synergies between CBH and EG components generally consider that BG hydrolyzes the inhibitory product of the CBH end and thereby enhances the functional capacity of the CBH or EG. Cross synergy of the different sources of cellulase components has been confirmed. If CBH is added to many false cellulose microorganisms that do not generate CBH, they can degrade natural cellulose.

However, the exact role of the cellulase components in the cellulose hydrolysis process is still not fully informed, and the synergy theory never shows how the synergy reaction is carried out; especially, the degradation mechanism of the cellulose crystalline regions is still not clear. Chanzy et al. [103] proved that CBH I can be combined by cellulose molecular chains randomly through colloidal studies. Henrissat et al. [104] found that, except for the homogenization of MCC, the enzymolysis capacity of EG I to MCC is low. There is no enzymatic activity of CBH I on CMC, but there is high degradation ability to MCC. EG I and CBH I can synergistically degrade filter paper, MCC, homogenized MCC, and bacterial MCC, but they cannot synergistically degrade *Valonia* cellulose and CMC. CBH II and EG I or EG II can synergistically degrade insoluble cellulose. CBH I and CBH II also can synergistically degrade insoluble cellulose. However, it is different from the best synergy ratio between various enzymes [104]. CBH I can even hydrolyze barley β-glucan via endoenzymatic hydrolysis. Because 90 % of barley β-glucan is connected with cellotriose, cellulosic tetrasaccharide bonded by β-1,3-glycosidic linkages, and it also has several amounts of polysaccharide chain composed of ten consecutive β-1,4-glycosidic bonds. Therefore, β-glucan is similar to CMC. The difference is that the hydroxyl group of glucose is not replaced by carboxymethyl. The studies of Nisizawa et al. found that the high degrees of substitution of CMC had a greater resistance than the low degrees of substitution of the CMC to the exocellulase. They showed that low activity of CBH I to CMC may be associated with substituent [105].

3.7.3.2 Degradation Mechanism of Basidiomycetes

Some types of basidiomycetes are the main cause of wood decay and can be divided into two groups: brown pathogens and white pathogens. Brown pathogens

are brown-rot fungi, which decompose cellulose and hemicellulose components and almost no lignin. These fungi include *Coniphora puteana*. These fungi have many unique points. First, brown-rot fungi can degrade timber significantly. However, in the early growth stage, they can reduce the DP of cellulose significantly but do not cause weightlessness of timber. Second, brown-rot fungi have great capability to degrade cellulose, but their cellulase composition is not yet complete. They do not have exoglucanases, which are critical for hydrolysis of crystalline regions. Furthermore, when brown-rot fungi degrade timber, the plant cell wall of the S2 layer is degraded, but the S3 layer that is close to mycelia remains intact [106]. White pathogens are white-rot fungi. White-rot fungi work first on lignin and almost do not damage cellulosic polysaccharides. These fungi include *Poria subacida*, *Polyporus versicolor*, and *Pleurotus ostreatus*. They undermine the lignin to form a white substance, which is an oxidation process. They remove the methyl to form diphenol, which is decomposed by oxygenases.

(1) Degradation process of brown-rot fungi

In the early stages of the crystalline cellulose degraded by brown-rot fungi, the mechanical strength of cellulose is greatly reduced, but there is only a small weight loss and little reducing saccharide forms, which is significantly different from the filamentous fungi phenomenon mentioned in that the DP of the cellulose is reduced, but the reducing sugars are elevated in the enzymolysis process.

Initially, the cellulose degradation mechanism of the brown-rot fungi research mainly focused on looking for an unknown small molecule enzyme. It is envisioned that this enzyme is able to penetrate the plant cell walls, reducing the degree of cellulose polymerization. Although much research about extracellular substances of the brown-rot fungi has been performed, no small molecule enzyme has been found. In the 1960s, it was found that Fenton reagent H_2O_2/Fe^{2+} generated HO•, which had a strong ability to degrade cellulose, namely,

$$H_2O_2 + Fe^{2+} \xrightarrow{HO\cdot} +OH^- + Fe^{3+}$$

The reaction caused by the decline in the DP of cellulose is the same as the way that early brown-rot fungi degrade lignin. Further study detected the generation of HO•. The unique way brown-rot fungi degrade natural cellulose and the finding of HO• contributed to the proposition of the mechanism that brown-rot fungi HO• radicals oxidatively degrade cellulose. Gao et al. [107] detected the generation of HO• from the living body and the enzyme solution outside the cell of seven brown0-rot fungi, such as *Gloeophyllum trabeum*, *Lentinus edodes, Lentinus lepideus,* and so on. They found that the generation of HO• existed in certain correlation with the capacity of cellulose degradation. According to numerous studies, it is speculated that brown-rot fungi may produce an extracellular HO• oxidation system, and then HO• can pass through the cell wall, greatly reducing the DP in the early stage of cellulose degradation.

Although some work was performed recently, the degrading mechanism of brown-rot fungi to lignocellulose is not completely clear yet. Many conclusions are

based on the study of one fungus but were not tested on other fungi. In addition, the lack of a reliable, effective detection method has brought some problems, such as the detection of H_2O_2 and transient generated HO• and the detection of the vitality substance with a short-fiber-forming ability. Although there was some progress on the separation of the oxidation system, and the detection of small molecules and their oxidation-reduction characteristics have been reported, detection of the effect of small molecules on cellulose degradation and the formation of the HO• electron transfer process has not yet been achieved. Degradation mechanisms of brown-rot fungi have basically established the following conclusion: The brown-rot fungi may degrade cellulose via nonenzymes, with small molecules participating and involving the HO• oxidation mechanism. But, around the H_2O_2/Fe^{2+} reaction cycle, there are many hypotheses. Perhaps more than one small molecule active substance exists in extracellular brown-rot fungi, or the formation of the H_2O_2/Fe^{2+} system is by more than one mechanism. A degradation mechanism of brown-rot fungi is not suitable for other fungi [106].

(2) Degradation of white-rot fungi

Science first reported the degradation of the white-rot fungus *Phanerochaete chrysosporium* in the early 1980s, which aroused extensive concern in the environmental community. White-rot fungus is a peculiar kind of filamentous fungus. It can directly invade the cavity of wood cells and release enzymes for degrading lignin and other components (cellulose, hemicellulose, pectin, etc.), which results in wood rotting into a white spongy clump.

In the classification, most white-rot fungi are Basidiomycetes, and a few are Ascomycetes, including over 200 strains. *Phanerochaete chrysosporium* is a typical fungi, which is not *Aphyllophorales*. It has become the microorganism used to study lignin degradation. The most prominent feature of white-rot fungi is that they are by far the most effective, and the main, lignin-degrading microorganisms. They can completely degrade lignin to CO_2 and water, playing a key role in the natural carbon cycle [108].

The brown-rot fungi, soft-rot fungi, actinomycetes, and bacteria are generally believed to play a secondary role in lignin degradation [109, 110]. White-rot fungi secrete not only lignin degradation enzymes, but also cellulase, hemicellulase, and pectinase. So, they are more suitable to decompose lignocelluloses compared with *Trichoderma* spp., *Aspergillus* spp., and others [111]. Du et al. studied the degradation sequence of cellulose, hemicellulose, and lignin by white-rot fungus. The white-rot fungus first degraded hemicellulose and lignin and then the hemicellulose, cellulose, and lignin simultaneously. Lignin was soon degraded into small molecules of lignin and lignin monomer (guaiacyl monomer), and the monomer was oxidatively degraded. The degree of lignin degradation was deeper than that of cellulose and hemicelluloses. It had a strong advantage (about 45 %) and selectivity (about 0.5) to degrade lignin. From variation of the cellulose, hemicellulose, and lignin contents, the variation of carbohydrates (cellulose and hemicellulose) spectra and the relative intensity of the spectral fingerprint region, and compared with the xylan (hemicellulose) characteristic peaks and changes of the lignin-related peak, it could be inferred that white-rot fungus first used a small

amount of soluble sugars and other small molecules in wood chips, degrading a small amount of hemicelluloses to supply mycelial growth and to provide a carbon and energy source for lignin degradation. Then, it degraded cellulose, hemicellulose, and lignin. In the degradation process, the cellulose, hemicellulose, lignin, and other macromolecules were first oxidatively cleaved. Compounds of the cyclic structure such as a benzene ring were gradually oxidatively cleaved to a chain compound. The hydroxyl group, methylene group, and carboxyl group numbers increased. Hemicellulose and cellulose were degraded into small molecule acids (such as gluconic acid, lactic acid, etc.) or esters (glucose esters, etc.), which were used directly by the white-rot fungus [112].

The lignin degradation process by white-rot fungi can be divided into the following steps: (1) formation of a polyphenol structure through demethylation and hydroxylation; (2) production of hydrocarbon chain by adding oxygen to the cleavage polyphenol ring; (3) shortening of the aliphatic hydrocarbons by hydrolysis. Catalysis of lignin by degradation enzyme systems occurs as follows: (1H_2O_2 oxidase is generated, including intracellular glucose oxidase and extracellular GLOX. With the involvement of molecular oxygen, substrate can be oxidated correspondingly to form H_2O_2, activating over peroxidase and starting the enzymatic catalytic cycle. (2) LiP is an extracellular enzyme and needs H_2O_2. It contains heme. Its oxidation-reduction potential is high (oxidizable ionizing capacity of 1.5 V, the nonphenolic ring). It catalyzes nonphenolic aromatic substrate. (3) MnP is an extracellular enzyme and depends on Mn (II). It requires H_2O_2, and it contains heme. Its redox potential is lower than LiP. It catalyzes phenols, amines, and dyes, which rely on Mn for oxidation. (4) Regarding aerobic polyphenol oxidase-laccase (Laccase, EC 1.10.3.2), extracellular enzymes require O_2 as the oxidant. It is Cu-containing enzymes. Its redox potential is much lower than other lignin degrading enzymes. It catalyzes phenols related to lignin electron oxidation, especially syringyl lignin, forming phenoxide and leading to the fracture of group C_α by C_α oxidation as well as fracture of C_α-C_β on the side chain. (5) All or parts of reductase, methylation enzyme, protease, and cellulase enzymes construct the main body of the white-rot fungi degradation system. Mechanisms by which white-rot fungi degrade lignin are as follows: The mechanisms depend on the degradation system secreted by the cells, which is aerobic and activated by H_2O_2 formed by it. A series of radical chain reactions is initiated, which is triggered by the enzyme to achieve substrate nonspecificity of oxidative degradation, mainly including one-electron oxidation, lipid oxidation, cometabolism process and so on [113, 114].

3.7.3.3 Anaerobic Degradation of Cellulose

Some anaerobic cellulose-degrading microorganisms can produce cellulosome; these have cellulase, hemicellulase, and pectin enzyme activity. They can efficiently degrade a cellulose substance. Anaerobic cellulose-degrading microorganisms are extremely rich in nature, including anaerobic fungi and anaerobic bacteria. Anaerobic cellulose degradation fungi are mainly distributed in the *Neocallimastix,*

Piromyces, Orpinomyces, Ruminomyces, Caecomyces, and *Anaeromyces* genera, and anaerobic cellulose-degrading bacteria include 12 genera. Anaerobic bacteria and fungi are usually attached to the cellulose. Anaerobic bacteria lack the ability to effectively penetrate the cellulose, so if there is competition from other microbes and ATP limitation, they search selectively for other mechanisms of cellulose degradation to obtain cellulose hydrolysis products. Except for a few examples, anaerobic bacteria mainly produce cellulosome, which decomposes cellulose to obtain different fermentation end products, such as acetic acid, ethanol, and succinate [115]. For anaerobic bacteria, absorption on cellulose cells is common. Many raised organelle structures exist on the surface of *Clostridium thermocellum*, comprising multiple copies of cellulosome. When the cells contact the substrates, some claws elongate to make the cellulosome settle and arrange along the cellulose surface. The enzyme on the cellulosome begins to degrade the substrate, and a large amount of cellobiose is released to the environment. After a period of growth, the cells are separated from cellulose. The separated cellulosome remains on the surface of the residue cellulose to degrade continually [116].

The concept of the so-called cellulosome was first introduced by Israel Bayer and Lamed and colleagues after their in-depth study of the anaerobic cellulose-degrading bacterium *Clostridium thermocellum*. It is the extracellular enzyme complex that is able to degrade cellulose, hemicellulose, and pectin. It is produced by anaerobic bacteria such as *Clostridium* spp., *Acetivibrio* spp., *Bacteroides* spp., and *Ruminococcus* spp. Cellulosomes may be the largest extracellular enzyme complexes found in nature. It is reported that the molecular weight of cellulosome polymer is up to 100 MDa, but the molecular weight of the cellulosome monomer is only between 650 kDa and 2.5 MDa. A typical cellulosome is shown in Fig. 3.8.

Generally, there are two types of cellulase systems of anaerobic cellulose-degrading microorganisms. The first type is mainly composed of three separate extracellular enzymes, which are the same as cellulase referred to previously. These independent extracellular enzymes have their own binding domain and the CD, degrading cellulose synergistically. The second type uses a multienzyme complex cellulosome. Initially, it was thought that cellulosome was just a medium-connected structure between a microorganism and cellulose. Later, it was found that the cellulosome was a stable polymer multienzyme complex that connected a scaffold protein and multienzyme subunit through an anchored adhesion domain-domain interaction. It could effectively degrade fiber material [117].

The main catalytic components of the cellulosome are exoglucanases and endoglucanases, which have a different hydrolysis direction, playing an important role in hydrolysis. The different enzymes arrange on noncatalytic scaffold protein to ensure a high concentration in the local region. The correct arrangement of the respective components and the proper ratio make the cellulosome hydrolyze MCC efficiently. The cellulosome is incorporated in the cells and on the substrate so that the cells and cellulose are tightly joined together; thus, it is fit for the degradation of the substrate and rapid use of the sugar produced by the enzyme of the cellulosome. It was reported that the integrity of the cellulosome is important for ensuring

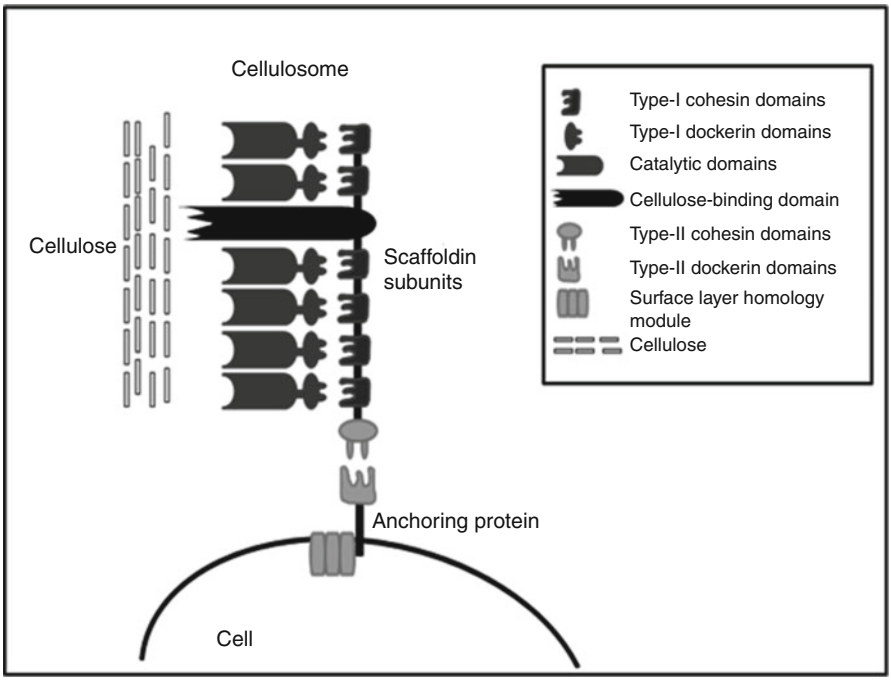

Fig. 3.8 Typical cellulosome diagram [115]

maximum enzyme activity. If the cellulase-binding domain of the cellulosome is removed, there is almost no effect on the catalytic activity of the soluble substrate, but reduces to a great extent binding to the insoluble substrate [118].

The rumen of ruminant animals is a natural fermentation tank in which there are a large number of anaerobic microorganisms, including bacteria, fungi, and protozoa. Lignocellulose is the main component of a ruminant's food. Rumen microorganisms digest lignocellulose by a synergistic effect, which is achieved by the adhesion of microorganisms and the hydrolysis of cellulose enzymes. In addition, the cellulose anaerobic degradation fungi also are present in the gastrointestinal tract and the droppings of other herbivores. However, in the natural state, cellulose degradation must be achieved under a variety of microorganisms in a microbial system after long interaction. Therefore, taking full advantage of the synergies of a variety of microorganisms in the natural world, artificial screening and construction of a microbial community have gradually attracted people's attention. In particular, the relationship between the anaerobic microorganisms and aerobic microorganisms has attracted increasing attention in recent years. On the one hand, aerobic microorganisms can provide a good anaerobic environment for anaerobic microorganisms. On the other hand, they can consume the metabolites of anaerobic microorganisms, thereby releasing the feedback inhibition of the product and adjusting the pH of the fermentation broth, which is more conducive to the growth of the microorganisms.

Furthermore, low molecular weight metabolites produced by aerobic bacteria might enhance the function of the degradation of cellulose by stimulating the growth of other bacteria.

3.8 Ecological Fundamentals of Cellulose Biotechnology

3.8.1 Development of Cellulose Microbial Ecology

Ecology plays an important role in solving contemporary major social problems. It has a great contribution to promote industrial and agricultural production, improve the ecological environment, and prevent environmental pollution. Ecology has become one of the fastest frontier disciplines. Microbial ecology, an important component of the ecology division, plays an important role in the ecosystem. As a discipline, it is the study of the relationship between microorganisms and the environment and its mechanism of action. In China, the research on microbial ecology was delayed.

Characteristics of microorganisms made the microbial ecology develop into an independent discipline in the 1960s. In the early 1960s, geological microbiology research was carried out, that focused on oil microorganisms, mineral microorganisms, and rot microorganisms. Beginning in the 1970s, there was stress on the concept of the microbial ecological system. In the mid-1980s, research on microbial ecology was carried out.

Since 2003, the penetration of genetics in microbial ecology, the infiltration of engineering mathematics, the popularity of computer technology, and the establishment of various new methods caused the research to branch out from different levels, such as the molecular, cellular, and community levels. A chess-type function was created to respectively clarify species ecology and its function and application in the macro- and microenvironments. Cellulose microbial ecology is an important part of microbial ecology; it gradually gained attention as a branch after the development of microbial ecology. Cellulose microorganisms play an important role in the nature of material and the energy cycle. Cellulose microbial ecology studies the structure of the microbial community and the law of interaction between the macro- and microenvironment systems and its applications. When looking at the flow of matter and energy in the ecosystem in terms of the carbon cycle, cellulose microorganisms play a role in macroecology not only as decomposers but also as producers and consumers. From the research areas, cellulose microbial ecology is a practical subject. There are strong relations between applied microbiology, soil science, environmental science, environmental microbiology, genetics, bioengineering, and other disciplines. It is an interdisciplinary and multidisciplinary field. The classic study of cellulose microbial ecology material cycle is also closely linked to the actual production. Cellulose microbial ecology is the key discipline of the carbon cycle in nature. Previous research always described the role of the microbial decomposition

of animal and plant remains, biotransformation, and material recycling. Now, the material circulation mechanism and its application is garnering increased attention. At the Changbai Mountain Forest Ecosystem Research Station, Inner Mongolia Grassland Ecosystem Research Station, and Xining plateau meadow ecosystem positioning station, research has been carried out on the material cycle, the types of microbial ecosystem, distribution, succession, substance transformation, and ecological factors on the decomposition rate of dried tree leaf litter. Research also included looking for ways to improve the forest, pasture, and meadow productivity in terms of cellulose microorganism ecology.

With the rapid development of economic construction, the microbial ecology studies will proceed more widely and deeply. The development of microbial ecology and cellulose microbial ecology engenders mutual promotion and constraints. With the progress of ecological engineering and biological engineering, cellulose microbial ecology will play an increasingly more important role in environmental information systems, the relationship in coordination with human resources and the environment, and development and utilization of rich cellulose sources.

In the research process of cellulose microbial ecology, it was discovered that cellulose microbes were extensive in nature. Bacteria, actinomycetes, fungi, and basidiomycetes all can produce cellulase. Each type of cellulose-decomposing microorganism shows different abilities to hydrolyze cellulose because of its different cellulase systems and different enzymatic hydrolysis efficiency. People noticed early on that microorganisms in the body of herbivorous animals have the unique ability to break down cellulose. During a long period of time, the focus has been on the study of anaerobic cellulose-decomposing bacteria. These bacteria generally do not secrete extracellular enzymes, and the products are always acetic acid, CO_2, H_2O, and so on. Later, study turned to aerobic cellulose-decomposing bacteria. In 1961, cellulase was first prepared from *Trichoderma* sp. *Trichoderma* cellulase has three components: 1,4-β-D-glucose hydrolase, 1,4-β-D-glucan cellobiase, and β-glucosidase. It has been the focus for a long time. Mary Mandels, a researcher from Natick Research Institute, made a great contribution to the genetics of *Trichoderma*. This researcher bred QM9414, MCG77, a series of cellulase superior strains. To commemorate the pioneer Elwyn T. Reese, a researcher on cellulase from the United States, a mutant was named *Enpenicillium javanicum*. Its CMC enzyme activity is as great as QM9414, while β-glucosidase activity is not too high. In 1981, the Hungarian Gyorgy Szakaes reported *Penicillium verrcubsum* WA30, a new screened strain, which had higher cotton enzyme activity. In the early 1980s, Gao and Qu from Shandong University of China isolated and screened *Penicillium paecilomyces* S28 and *Penicillium decumbens* JU1, which reached the international advanced level of cellulase in the early 1980s. Then, by further mutagenesis the JU-A10 mutant had higher cellulase activity, a relatively complete cellulase system, and a high level of β-glucosidase enzyme activity. In 1980, Garcia-Martinez et al. used the anaerobic bacterium *Clostridium thermocellum* to produce cellulase. In this, 95 % of its β-glucosidase was extracellular enzymes, which was relatively rare. Zhang, a researcher from the Chinese Academy of Forestry, Institute of Soil Science, studied

the decomposition of cellulose by anaerobic bacteria. The decomposition rate was 53 %, and the main product was glucose.

The number of cellulose-decomposing bacteria is low in alpine meadow soil. The number of cellulose-decomposing bacteria changes with the soil temperature, and it is highest between July and September. Compared with natural grassland, the number of cellulose-decomposing bacteria is reduced an average of 51 % compared to degraded pasture. Cellulose-decomposing bacteria are extremely rare in swamp meadow soils. Cellulose-decomposing bacteria show different decomposition degrees for cellulose, plant roots, and litter. A seasonal change of cellulose-decomposing bacteria is obvious. From the end of November to the following April, the cellulose decomposition rate is only 1.89 %, and the number of cellulose-decomposing bacteria gradually increases with increase of soil temperature, as does the decomposition rate of cellulose. The decomposition rate in August is 55.69 %. It reaches 16.98 % in September and only 0.67 % in November. The soil temperature is the primary factor affecting cellulose decomposition. The alpine meadow cellulose decomposition is carried out from June to September. The cellulose decomposition in these 4 months accounts for 91.5 % of the total annual amount of decomposition.

The decomposition rate of humilis grassroots and litter is the highest in the first month of the first year, with the roots decomposing 26.55–29.84 % and litter 20.34–22.95 %. Then, it quickly reduces during the next month. After 4 months, the monthly decomposition rates of root and litter drops to 2.16–6.47 % and 1.60 %–5.92 %, respectively. After 142 days, the plant root decomposition is 38.46–42.91 % of the original weight, and litter was 45.86–46.39 %. The decomposition rate of plant root decreases with soil depth.

3.8.2 Cellulose Microbes and Carbon Cycle of Earth's Ecosystems

Natural substances experience a reciprocating cycle between inorganic matter and organic matter. Photosynthetic organisms transfer inorganic matter into organic matter through photosynthesis to fulfill the needs of humans and animals. Then, microorganisms decompose the ecological debris and transform it into inorganic materials to fulfill the needs of green plants. Organisms, especially microorganisms, promote the carbon cycle, so that different forms of carbon can transform into each other, and the CO_2 in the atmosphere will not be depleted, maintaining life.

Microorganisms in nature play an important role in the material cycle. Microbial decomposition of organic matter and animal and plant respiration all can emit CO_2, but more than 80 % of it is generated by microbial activity. If there is no microbial decomposition of organic matter, there will be increasing organic matter on Earth. Not only will the limited CO_2 in the air soon be depleted by plant photosynthesis, but also the cycle of other inorganic elements in Earth's ecosystem will be interrupted.

In the carbon cycle of Earth's ecosystems, microorganisms play an important role. Carbon elements stored in organic matter are mineralized and released by microorganisms as soon as possible, making the biosphere an environment with a good balance of carbon. It is estimated that 90 % of carbon dioxide on Earth is formed by microbial decomposition. Most of the carbon dioxide is fixed by photosynthesis and accumulated in woody and herbaceous bodies in the form of glycans, especially on land; about 60 % of fixed carbon dioxide constitutes wood. In the wood, 75 % of the carbon is made up of polysaccharides (i.e., cellulose, hemicellulose, starch, pectin, and arabinose). More than an additional 20 % of carbon is made up of lignin and xylan; the protein content is only about 15 %. The polysaccharide contents in herbaceous plants and shrubs are even more than in timber. Such complex organic matter that has a huge yield is difficult to be broken down, and it is decomposed by some special microorganisms in the soil.

The decomposition of natural lignocellulosic feedstock produced by green plants through photosynthesis constitutes an extremely important component of matter and energy transformation in the natural ecological system. It is fulfilled by microbial degradation. Study of how the microorganisms in nature coordinate to complete the degradation of natural lignocellulosic feedstock lays the foundation for obtaining a high yield of bacteria and useful fermentable products. Although the microbial conversion function is an important part of the carbon cycle in the ecosystem, it is a huge waste in terms of the use of natural resources by human beings. Microorganisms play an important role in the natural carbon cycle. They participate not only in CO_2 fixation and photosynthesis but also in the decomposition of regenerated CO_2.

1. Photosynthesis: Photosynthetic microorganisms mainly include algae, cyanobacteria, and photosynthetic bacteria. They transform the CO_2 in the atmosphere and water bodies to organic carbide through photosynthesis. Especially in most of the aquatic environment, the main photosynthetic organisms are microorganisms. In the aerobic zone, cyanobacteria and algae dominate; in the anaerobic region, photosynthetic bacteria dominate.
2. Decomposition: The organic compounds in nature are mainly decomposed by microorganisms in the land and water. In aerobic conditions, they are completely oxidized to CO_2 by aerobic microbes; in anaerobic conditions, they are incompletely oxidized into organic acids, methane, hydrogen, and carbon dioxide by anaerobic fermentation.

Many microorganisms can decompose the organic compounds, such as fungi, actinomycetes, bacteria, and protozoa. Among them, the decomposition ability of fungus is especially strong, including some ascomycetes; imperfecti fungi and basidiomycetes, such as *Polyporus* spp., *Agaricales* spp., *Trichoderma* spp., and *Myrothecium* spp.; bacterial species of *Sporocytophaha myxococcoides*; actinomycetes species of *Streptomyces antibioticus*; and so on. Fungi are more active in the early stages of decomposition of hemicellulose. Then, later in decomposition they mainly rely on the role of actinomycetes. Fungi that can decompose

hemicellulose are widely distributed in the major groups of fungi. Major microbes that can decompose lignin include Basidiomycotina and Aphyllophorales, such as some species of *Fomes*, *Polyporus* spp., and *Polystictus* spp.

3.8.3 Formation of Industrial Ecology and Research Progress of Lignocellulose Biotechnology Ecoindustry

3.8.3.1 Formation of Industrial Ecology

In September 1989, Robert Frosch and Nicolas Gallopoulos published an article about sustainable industrial development strategy in the *Scientific American*. It first pointed out a view of a new mode of production used in industry and introduced the concept of industrial ecology, which is an industrial ecosystem that can run similar to a biological ecosystem. Plants absorb nutrients and synthesize foliage for herbivores. Herbivores are preyed by predators, and their excrement and dead bodies become food for other organisms. The basic principles of industrial ecology are the "4R" principles (reduce, reuse, recycle, replace), which are described in detail in Chap. 10.

3.8.3.2 Studies on the Foundation of Ecological Biochemical Engineering

The generation of ecobiochemical engineering is necessary for the development of science and technology. It is a new growth point of the biochemical engineering disciplines. The traditional biochemical engineering discipline is based on the theory of "three transfers and one reaction." The basis of ecological biochemical engineering not only emphasizes the theory of nonlinear science disciplines based on in vivo cell biological reaction but also creates the theoretical system of raw material component separation.

(1) The foundation of the theoretic system of raw material component separation [119]: According to the basic principles of industrial ecology 4R, lignocellulosic feedstock instead of foodstuff is the direction of development, but to make full use of natural lignocellulose, first it should establish the concept that raw material can be used for multiple components, by multiple ways, and by multistage conversion. It should improve the efficient and clean separation technology system of lignocellulosic feedstock, and then ecological biochemical engineering disciplines can be truly developed. It is considered that the purpose of the pretreatment of raw cellulose materials is the removal of the barriers of cellulose, such as lignin, and destroying the crystal structure of the cellulose macromolecules to improve the conversion rate of the enzymatic hydrolysis of cellulose. From that sense, the object of the pretreatment is to use the cellulose component of raw cellulosic materials, which is as same as the

current pulping process. However, the separation of components means refined cellulosic feedstock; those cellulosic feedstocks are not only treated as single-cellulose resources but also are regarded as multicomponents and multipurpose materials [120].

(2) Complete the theory of nonlinear science of in vivo cell biological reaction [121]: With the accumulation of biological knowledge, the deepening understanding of biological nature, especially the learning and thinking of self-organization theory, nonlinear chaos theory, modern information theory, and system theory, gradually defined the new concept of "four transfers and one reaction" using the normal force as the power source.

The so-called fourth transfer is extra "information transfer," adding to the conventional three-transfers concept. The theory of four transfers and one reaction in the chemical reaction engineering discipline is based on the potential and the fluid dynamics of chemical reactions as the power source. This three-transfers process fluid flow enhancement means, in addition to the flow direction of the three transfers, the heterogeneous reaction process is based on the interphase tangential force as a power source to achieve the purpose of enhancing mass transfer and heat transfer, which is called the boundary layer theory. This enhancement is effective for the multiphase particles of nonlife. A higher shearing force and greater transfer rate facilitate the reaction rate of particles. However, the situation will be different once multiphase particles are living cells. Cells, especially without cell walls, will be damaged and even die when the shearing force outside is too large. The cell membrane is formed by inlaid lipid and protein molecules, which are flowable multimolecular layers, similar to the liquid crystal and delicate. There are many cell secretions, such as polysaccharides, in the cell membrane, like the "guard." They not only search but also screen the transformation of nutrients and excreta out of the cell membrane. If the shear force captures the guard, cell metabolism will be affected. Because of the integrity of the biological information, the local damage can also cause overall disorders, the invalid of the biological activity, and even death. This phenomenon has been amply demonstrated in the mechanical agitation fermenter. However, the membrane is the door to exchange matter, energy, and information with the external environment. If the transfer rate of fluid within the boundary layer of the surrounding cells is too slow, it indicates the lack of oxygen supply and other nutrients from outside the cells. It makes the cells hungry or hypoxic, and the excrement cannot be removed in vitro. It also inhibits the metabolism, growth, and reproduction and even leads to death. So, it shows that, at least in the scope, strengthening the transport rate relying on fluid flow shear force is limited.

3.8.3.3 Ecological Industrialization Direction of Cellulose Biotechnology

Ecological biochemical engineering focuses on the main direction of the three aspects discussed next.

1. Component separation of raw materials is the premise of industrial ecology. Single-component utilization and development of a single technology plunders resources. The concept of a multicomponent, multipurpose raw resource must be established. If the component separation of raw material is not fulfilled, it will result in difficulty achieving environmental standards and economic efficiency, such as industrial alcohol fermentation and industrial enzyme production. To achieve the biological transformation of cellulosic resources, it is also necessary to separate the components. Therefore, the primary task of ecological biochemical engineering is to constitute the theory of component separation and multilevel conversion.
2. The core is to research and develop new processes, including clean, energy-saving, water-saving, and coupling processes. Liquid fermentation brings not only huge economic benefits to human beings, but also pollution and a waste of water resources. Therefore, sustainable development also needs to find new principles and theories of biological reaction engineering and to enhance the study of solid-state fermentation and fermentation coupling.
3. The transformation of raw materials is the only way to achieve the 4R principles. In the sustainable development strategy in today's world, economy, energy, food, environmental protection, and many other major issues are related to biomass renewable resource utilization. The biotransformation of lignocellulosic materials is the basic technology of the common issues. Once technical and economic breakthroughs are achieved, the scientific and social significance will be immeasurable. Lignocelluloses are the alternatives of the chemical and fermentation industries, but there is a huge potential and difficulty in the current cellulose resources utilization. The transformation of raw materials is one of the most challenging engineering issues in ecological biochemical engineering research [122].

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Chapter 4
Pretreatment and Primary Refining of Lignocelluloses

Abstract The efficient conversion of biomass resources is a worldwide scientific and technological issue, and it will take a long time to realize the large-scale economic utilization of biomass based on the investments and costs of existing technology owing to the lack of systematic theories and reorganization of their complexity. This chapter deeply researches the common problems that exist in the conversion and utilization process of lignocellulosic materials and explores the intrinsic reasons affecting them. On this basis, new systematic theories are proposed based on the structural characteristics of materials and the requirements of target products. Component separation of lignocellulose should be upgraded to the selectively structural fractionation process for the sake of fundamentally enhancing the utilization rate of biomass materials and ultimately achieving high-value utilization.

Keywords Pretreatment • Component separation • Selectively structural fractionation • Product engineering

4.1 Introduction

The efficient conversion of biomass resources is a worldwide scientific and technological issue, and it will take a long time to realize the large-scale economic utilization of biomass based on the investments and costs of existing technology owing to the lack of systematic theories and reorganization of their complexity. Therefore, there is an urgent need to research deeply the common problems that exist in the conversion and utilization of lignocellulosic materials and to explore the intrinsic reasons affecting them. On this basis, it is necessary to explore new systematic theories for the sake of fundamentally enhancing the utilization rate of biomass materials and ultimately achieving high-value utilization. Analyzing the economically unsatisfied reason for existing technologies, there are three common problems in the conversion and utilization of lignocellulosic materials [1]:

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1. The singular utilization of each component. A key reason for the difficulty in achieving industrial production of biomass feedstock is lack of an efficient component separation technique. It is impossible to control the characteristics and quality of the final product if the components of biomass feedstock cannot be effectively separated. The composition of lignocellulose is complex; thus, it is difficult to separate it into useful molecular components. Therefore, plants for the industrialization of biomass raw materials, such as furfural plants, paper mills, xylitol plants, and so on, just emphasize the use of a single component of lignocellulose; other components are discarded as waste, causing a serious waste of resources and environmental pollution. Therefore, insufficient utilization of lignocellulose did not play an important role in improving economic efficiency, but became the burden of the efficiency.
2. The singular utilization of technology. The use of a biological or thermochemical method in the process of biomass conversion was emphasized excessively, and the concept of multilevel use of the natural solid-phase organic materials was lacking.
3. Lack of systematic integration of technologies and research of ecological processes and engineering. At present, available technologies for treating biomass still are mostly the traditional technologies of starch fermentation and wood processing. For example, for raw material pretreatment, applying the traditional acid hydrolysis techniques in the paper industry caused the high cost of raw material pretreatment and environment pollution; for cellulase and ethanol fermentation, applying the fermentation processes and equipment of starch to ethanol resulted in high production costs because of the large investment of cellulase and low conversion of alcohol.

4.2 Connotation and Development History of Primary Refining of Lignocellulose

The three components (cellulose, lignin, and hemicellulose) of lignocellulose, especially straws, are cross-linked tightly. Because of the completely different chemical structure and properties, the utilization of each component is limited. Thus, the primary refining processes, such as appropriate pretreatment, fractionation and separation of components, and structural disconnection or partial change of structure, are necessary to achieve high-value utilization of lignocellulosic materials.

As early as 1890 or even earlier, some researchers in Germany began to use high-concentration chemical reagents to pretreat lignocellulose. These reagents, including acids, alkalis, and salt, can destroy the crystal structure of fiber in biomass and degrade cellulose. This can be considered the earliest form of primary refining of lignocellulosic materials. In the next 100 years, pretreating technologies were aimed at obtaining high yields of cellulose without concern for the utilization of other components. In the last half a century with increasingly in-depth research

Table 4.1 Comparison of the three stages of the primary refining of lignocelluloses

Development stage	Technologies	Product goals
Pretreatment	Single pretreatment	Single product
Component separation	Several technologies and their integration	Full utilization of each component and multiproducts
Selectively structural fractionation	Technologies with low energy consumption and high conversion based on the structural and functional properties of raw materials	The maximum value of product and cleaner production

on lignocellulose resources, it has been recognized that the pretreatment method focusing on a single component of lignocellulose not only was a waste of resources but also would cause serious environmental pollution. Therefore, the comprehensive utilization of lignocellulosic resources gradually attracted people's attention. This can be considered the second phase of the primary refining of lignocellulose materials. In the twenty-first century, an upsurge of exploitation and utilization of biomass resources began worldwide. How to break economic and technical barriers of the utilization of biomass required research to reexamine the utilization ideas and models of lignocellulose resources. Treating lignocellulose as functional macromolecules with complex chemical composition and structure, I proposed that the pretreatment–component separation process of lignocellulose should be upgraded to selectively structural fractionation according to the structural characteristics of materials and the requirements of target products. It would be beneficial for taking lignocellulose as the versatile raw material for a new generation of biological and chemical industry applications with minimum energy consumption, optimum efficiency, maximum value, and clean conversion. Table 4.1 gives the comparison of the technologies and product goals during the three stages of the primary refining of lignocellulose materials. The detailed connotations of the pretreatment, component fractionation and selectively structural fractionation are described next.

4.2.1 The Necessity of Pretreatment for Natural Lignocellulose

If natural lignocellulose is hydrolyzed directly with enzymes, the sugar yield is almost as low as 20 %, and most of the sugars are pentose and hexose, which are generated from the enzymatic hydrolysis of hemicellulose. Taking enzymatic hydrolysis of a crop with cellulase as an example, results (Table 4.2) indicated that, when excess cellulase was added (100 IU FPA g^{-1} substrate), the enzymatic hydrolysis rates of untreated raw materials were generally less than 10 %. Although the enzymatic hydrolysis rate of corncob powder and beet pulp were around 20 %, the reducing sugar from hemicellulose occupies a considerable proportion, giving 25 and 50 % of xylose and arabinose, respectively, in the hydrolyzed sugar.

Table 4.2 Enzymatic hydrolysis yields of different lignocelluloses with different enzymatic hydrolysis times [2]

Raw materials	Enzymatic hydrolysis yield (%)		Raw materials	Enzymatic hydrolysis yield (%)	
	24 h	48 h		24 h	48 h
Corn straw powder	6.5	9.4	Straw powder	9.5	10.4
Green corn straw powder	6.3	9.6	Wheat straw powder	4.5	5.6
Corncob powder	14.8	18.5	Sorghum cob powder	8.9	10.8
Beet pulp	23.7	25.7	Wheat straw ammonium sulfite pulp	35.7	48.1
Bagasse	0.7	1.4			

Natural lignocellulose is difficult to use as the growth substrate for microorganisms, but why can it be completely degraded in nature and be digested and absorbed by ruminants? In essence, the complete degradation of natural lignocelluloses in nature needs the synergistic action of several microorganisms, and decomposition and digestion also take a long time, even more than a decade. For ruminants, the digestion of natural lignocellulose also needs the synergistic action of various microorganisms, with shorter digesting time but incomplete digestion of natural lignocellulose. If fully simulating the natural degradation process, it is impossible to obtain the products needed.

Based on theoretical analysis, the average polymerization degree of natural cellulose is 8,500–9,500, with the basic unit (70 Å × 30 Å × 500 Å) of high crystallinity; the crystallinity degree is more than 70 % [3]. The crystallization of cellulose is arranged by the cellulose molecule, which folds tidily. The hydroxyls of the glucose molecules in the crystalline region combine with all of the intermolecular or extramolecular hydroxyl; that is, there is no free hydroxyl. The formation of crystal structure, not to mention the enzyme molecules, and even the water molecules are difficult to intrude internally. Studies have shown that the crystallinity has a negative correlation with enzymatic hydrolysis yields.

Natural lignocellulose has different pore sizes, which can be divided into macropores (2000 Å–10 μm) and micropores (below 200 Å). Pore size distribution of cellulose has significant effects on cellulase reaction efficiency. The average pore diameter of chemical pulp is 25 Å, 5–10 Å for cotton fibers; it is 59 Å for spherical cellulase molecules and 35 × 200 Å or 26 × 165 Å for elliptical cellulase. Studies have shown that the enzymatic hydrolysis rate of cellulose is related to enzymes with accessibility of 40 Å. The specific surface area can be calculated by the Brunauer-Emmett-Teller (BET) method based on the absorption of the N_2 molecule. Steam explosion pretreatment and dilute acid pretreatment can significantly increase the porosity of the cellulose, and the porosity generated depends primarily on the removal efficiency of the hemicellulose. Therefore, the specific surface area of cellulose mainly depends on the hemicellulose [4].

Natural lignocellulose contains hemicellulose and lignin. Lignin and hemicellulose form a solid bond layer to surround the cellulose. It can be inferred that hemicellulose mainly surrounds the amorphous region of cellulose, and lignin surrounds the crystalline region of cellulose. Hemicellulose is easier to remove than lignin. Thus, it is generally believed that the existence of lignin affects the enzymatic hydrolysis yields of cellulose. Therefore, lignin should be removed to improve the enzymatic hydrolysis yields of cellulose. But, there still are some issues under debate, such as whether lignin has inhibitory effects on the enzymatic hydrolysis process, whether there is a machinery space obstacle effect of lignin, whether the absorbed cellulase still has activity, and so on [5]. In addition, the residual rate of lignin has an obvious impact on the pretreatment cost of natural lignocellulose. There is a great difference in cost between the 100 % removal rate of lignin and 20–65 %. Increasing research has proved that it is not necessary to remove all lignin [6]. According to different sources of natural lignocellulose, removing 20–65 % of the lignin can significantly improve the enzymatic hydrolysis yields [2].

In summary, pretreatment of natural lignocellulose has significant effects not only on enhancing the enzymatic hydrolysis rate but also on decreasing the amount of cellulase (saturated, invalid adsorption).

4.2.2 The Proposal on the Component Separation Concept of Natural Lignocellulose

In general research reports or monographs, the purpose of pretreatment for lignocellulose materials was to improve the enzymatic conversion of cellulose by removing the lignin or destroying the crystal structure between cellulose macromolecules. So, the utilization of natural lignocellulose focused only on the cellulose. Various means were used to remove other components in lignocellulose and took advantage of the isolated cellulose, which is basically consistent with the pulp and papermaking industry process. Delignification treatment of natural lignocellulose follows the method of strong acid or strong alkali dissolving the lignin at a high temperature in the pulping process. It consumes plenty of energy and chemical raw materials and causes resource waste and environmental pollution. From the point of ecological and economic benefits, these methods are difficult to use in microbial transformation of natural lignocelluloses.

At present, the microbial transformation of natural lignocelluloses worldwide has achieved great progress but has not fully realized industrialization because of the high cost of pretreatment and cellulase, both directly related to the pretreating process. Taking the cost of cellulase as an example, to improve the enzymatic hydrolysis rate of cellulose, the enzymes and the availability of the substrate should both be considered. Considering cost reduction, the ideal pretreating process not only can improve substrate availability but also can reduce the invalid adsorption of cellulase and the inhibition of other components on cellulase, consequently reducing cellulase dosage. Therefore, pretreatment of natural lignocellulose materials is a key factor for the microbial transformation to fully access industrialization.

Pretreatment technologies in the present study presented the questions of high cost and discerning the reason for this situation. The current papermaking industry can well explain these questions. The reason can be attributed to the unitary technology, which only emphasizes the use of a single component, considering the other components as waste. The utilization of the lignin does not arouse sufficient attention, which not only causes environmental pollution but also wastes resources. Component separation of natural lignocellulosic materials means refining natural lignocelluloses. Natural lignocellulose is viewed not only as a cellulose resource but also as a multicomponent material. Natural lignocelluloses are refined as various individual components with certain purity, and these components are processed into valuable products, which involves new philosophical ideas and new demands proposed by the total utilization of natural lignocelluloses. The purpose of pretreatment for natural lignocellulose should completely abandon the traditional concept of singular utilization of cellulose and establish the new ideas of component separation and total utilization of biomass.

4.2.3 New Ideas of Biomass Feedstock Refining: Selectively Structural Fractionation

The compact spatial structure of lignocelluloses makes them difficult to use directly, so pretreatment is a necessary means to achieve highly efficient conversion. From the point of biomass total utilization, the existing raw material pretreatment technologies still aim at enzymatic hydrolysis and fermentation of cellulose, almost without considering the high-value utilization of hemicellulose and lignin. So, this is bound to seriously affect the healthy development of the utilization of lignocelluloses. In a search for how to break the economic and technical difficulties of the utilization of lignocelluloses, people have to reexamine the thoughts and models of the existing utilization of lignocellulose resources. Therefore, first pretreatment must be endowed with new meanings; second, a set of new comprehensive utilization technologies for several components must be established, namely, component separation technology. Component separation–directional conversion involve the further promotion of lignocellulose pretreatment. It is not only a pretreatment technology but also a resource allocation process for the macromolecules in lignocellulose. It can achieve the differentiated conversion of cellulose, hemicellulose, and lignin, which is the most important idea currently for the high-value utilization of biomass, and significant research progress has been made around the world [7].

However, there is still difficulty for the idea of component separation–directional conversion to break through the technical and economic problems of biomass as an industrial raw material to achieve a large-scale clean and efficient industry. Because lignocellulose is a high-energy macromolecule with complex structure and multifarious functions, the existing transformation routes of component separation–directional conversion need to consume some energy to destroy the structure of the biomass before the conversion. This method does not take into account the

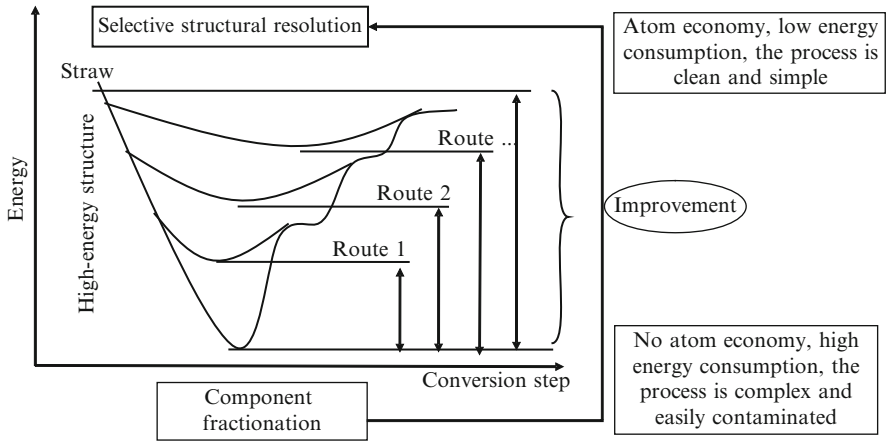

Fig. 4.1 Energy diagram of biomass conversion [8]

functional requirements of the product. This resolution, for some products, not only increases the energy consumption but also is not in accord with atom economy (Fig. 4.1). Therefore, to become the raw materials for the biological and chemical engineering industries, the biomass resources must develop refining processes and technologies that maintain the original structure of biomass to realize functional high-value utilization. I proposed that, based on the structural characteristics of materials and the requirements of the target products, pretreatment–component separation of lignocellulose should be upgraded to the selectively structural fractionation process [8]. The purpose of it is not only to obtain several kinds of products but also to realize minimum energy consumption, optimum efficiency, maximum value, and clean conversion of lignocellulose as the main raw material for a new generation of biological and chemical industries.

4.3 Pretreatment Technology of Natural Lignocelluloses

There are many kinds of pretreatment technologies for natural lignocelluloses; these can be divided into physical, chemical, and biological pretreatment.

4.3.1 Physical Pretreatment

Common physical pretreatment technologies include mechanical crushing, high-energy radiation, freeze grinding, γ-ray treatment, microwave treatment, pyrolysis, and so on. The main purpose of these technologies is to change the physical structure of the raw materials and increase the accessibility of cellulose on enzymes.

4.3.1.1 Mechanical Grinding

When lignocelluloses are pretreated by ball mill, vibrating mill, and roller, the lignin is still preserved, but the binding layer of lignin, hemicellulose, and cellulose is destroyed; polymerization of hemicellulose, cellulose, and lignin is decreased; and the crystal structure of cellulose is changed. The toughness of the cells in the plant tissue is determined by the amount of cellulose, hemicellulose, and lignin in the cell walls. Choong [9] found that toughness of *Castanopsis fissa* leaf can be predicted by the volume fraction of the cell wall and the neutral detergent fiber (NDF) content. Cellulose is a highly crystalline polymer that can effectively absorb the mechanical energy in the crush grinding process, resulting in changes of morphology and microsewing structure and decrease of crystallinity. Mechanical grinding can improve the response performance and enhance the hydrolysis and saccharification rate of lignocellulose.

Commonly used devices in mechanical grinding pretreatment include ball milling, compression ball milling, double-roll crushers, flow pattern momentum grinders, wet colloid milling, frozen grinding, and others; ball milling is the most effective pretreatment technology. Ball milling can loosen the structure of lignocelluloses and break the intermolecular hydrogen bond existing between microfiber and the crystalline region, consequently facilitating component separation of plant fibers. In the pulverization process, the morphology and fine structure of cellulose are changed, and the crystallinity decreases because of the absorption of mechanical energy. Ultrafine grinding technology is an interdisciplinary and cross-industry high-tech process that originated in the 1940s. In the ultrafine grinding process, because of the role of the mechanochemical effect, the physical structure and the chemical composition of materials will change, causing changes of physical and chemical properties. Compared with conventional grinding technology, the main features of the product after treatment by ultrafine grinding technology are small particle size, rapid increase of specific surface area, high material dispersibility, and improved adsorbability, solubility, chemical activity, and biological activity [10]. Jin and Chen studied the enzymatic efficiency, chemical composition, fiber morphology, and fiber composition of straw after ultrafine grinding, and the results showed that ultrafine grinding can separate pretreated straw easily [11]. The frozen grinding method pretreats the materials with liquefied gas under −100 °C and can destroy the combination of lignin and hemicellulose layer and retain lignin, thus increasing the affinity of enzyme to the cellulose.

Experiments showed that for Solka Floc (a pure cellulose substance without lignin), when ground for 36 h, the original fiber structure completely disappeared, and the hydrolysis rate increased by 2.12 times [12]. Waste newspaper after 7 days of grinding can be completely digested after 48 h by *Trichoderma viride* or *Trichoderma reesei*. Cotton pulp, after grinding for 60 min, can be completely degraded by the enzyme within 10 days. Enzymatic hydrolysis yields of Douglas fir and waste newspaper, after grinding for 120 h, almost reached the theoretical value. Cellulose degradation performance is directly related to the grinding time and grinding degree. Generally, when the materials are crushed into a particle size

of 10–30 μm, the enzymatic hydrolysis yields are almost up to 80 %. In addition, the ground powder of lignocellulose has small size and no swelling property, which can increase the substrate concentration and obtain high-concentration saccharified liquid. But, mechanical grinding technology also has some disadvantages, such as limited improvement of the saccharification rate, high energy consumption, and high cost of grinding. The grinding cost accounts for 50–60 % of the total energy consumption of this process. For example, if the material was reduced to a particle size of 200 mesh, the grinding costs reached as high as 53–110 dollars/t, applicability of which was not high, and the grinding treatment is not appropriate for some materials. If the material was decreased to particle size of 270 meshes, which required nearly 48 h of milling, the energy consumption accounted for 50–60 % of the total energy consumption of the saccharification process [13]. This method is also not suitable for a variety of materials processing. If particle size decreased to 200 meshes, the grinding cost was as high as 70–100 dollars/t raw material.

In recent years, many scholars found that the enzymatic hydrolysis rate of cellulose will increase exponentially when enzymatic hydrolysis is combined with wet grinding. In addition, some scholars also found that the yield of sugar will be greatly improved if there is some minor grinding in the process of solvent pretreatment of cellulose. The combination of multiform methods will be a development direction in the future for lignocellulosic material pretreatment because they will give full play to the advantages of each method.

4.3.1.2 Steam Explosion

A maiden attempt of steam explosion for the pretreatment of lignocelluloses was begun in the early 1980s. Raw materials were heated to 180–235 °C by steam and maintained for a certain time. Under high temperature and pressure, the acetyl of hemicellulose generates organic acids and then participates in the catalytic process of hemicellulose and lignin depolymerization. Hemicellulose is hydrolyzed into soluble polysaccharide, and lignin is softened because of the breakage of the α-propylene ether bond and parts of the β-propylene ether bond. As a physical and chemical method, steam explosion pretreatment makes the three main components of lignocellulosic substances be utilized respectively because it can effectively isolate the active fiber without chemicals or with slight use of chemicals. Therefore, steam explosion pretreatment technology has the advantages of no environmental pollution and low energy consumption, which makes it one of the most widely used methods for lignocellulose pretreatment.

Steam explosion utilizes high-temperature and high-pressure steam to treat the lignocelluloses, and component separation and structural change of lignocelluloses are realized through the process of instant decompression. Generally, raw materials are treated with high-pressure steam in the pressure resistance reactor, under conditions of 16–34 kg·cm^{-2}, 200–240 °C, holding for 30 s–20 min, and then decompressed rapidly, forcing the material released into the atmosphere. The earliest study of the Lotech Company indicated that, at any given pretreating

pressures, the pretreating time is different for obtaining the highest yields of xylose and glucose. Therefore, the company recommended that the best pretreatment conditions for holocellulose (including xylose and glucose) were 500–550 Pa for 40 s [14]. Since then, steam explosion technology has been widely used in the pretreatment of hardwood; softwood; agricultural waste (such as bagasse, wheat straw, rice straw, and cornstalks); and other noncellulose materials.

Chen [4] is committed to the idea that the steam explosion process imposes two effects on the structure of lignocellulose: a high-temperature cooking effect and the mechanical effect of instantaneous decompression. First, at the high-temperature cooking stage, hemicellulose is degraded into soluble sugar lignin in the intercellular layer is solubilized and partially degraded, which is beneficial for the selectively mechanical separation of lignocellulose by reducing the binding between cellulose. Second, under instantaneous decompression, superheated water flashes into steam, and the steam volume abruptly expands. The impact force generated by flashing and volume expansion destroys cell structure. In this stage, materials are torn into small pieces; fiber bundles are separated from each other, and their structures are loosened. So, cellulose is fully exposed, and lignin is redistributed. After using this treatment at 240–250 °C for 1 min, Schultz et al. [15, 16] found that the enzymatic hydrolysis yields of steam-exploded wood chips, rice husks, corncob, bagasse, and other lignocelluloses obviously were increased. The enzymatic hydrolysis yields of these pretreated materials were almost without decrease even after 8 months. Laser et al. gave results showing that the cellulose conversion rate of steam-exploded pretreated bagasse (216 °C for 4 min) could reach 67 % [17]. Under the steam explosion conditions of 230 °C for 1–2 min, Martinez et al. [18] pretreated broad-leaved wood chips, then the steam-exploded chips were used for enzymatic hydrolysis, for which the saccharification rate reached 90 %, and the enzymatic hydrolysis yields of steam-exploded eucalyptus and bagasse and the recovery yields of xylose both increased and generally reached 45–65 %.

Steam explosion pretreatment has several advantages. ① Pretreatment conditions can be easily optimized to produce high yields of specific chemicals from a wide variety of plant biomass. ② Hemicellulose, lignin, and cellulose can be separated during three different process units, consequently obtaining water-soluble, alkali-soluble, and water- and alkali-insoluble fractions, respectively. ③ Theoretical enzymatic hydrolysis conversion yields of cellulose can be achieved. ④ The lignin component is still suitable for chemical production even after steam treatment. ⑤ Sugars generated from hemicellulose can be fully utilized and converted into liquid fuels. ⑥ The release of fermentation inhibitors can be considerably reduced under optimal pretreatment conditions. And, the cost of the steam explosion pretreatment process is low; generally, a ton of material consumes 0.5–1.0 t steam. These advantages have made steam explosion become one of the most widely used pretreatment technologies for lignocellulosic biomass and shows increasingly prominent advantages.

In the steam explosion process, some chemical reagents usually are added as a catalyst, such as H_2SO_4 (or SO_2, CO_2, etc.). These reagents can also be added before steam explosion to improve the hydrolysis rate of hemicellulose and reduce the

inhibitor for enzymatic hydrolysis. The addition of this kind of reagent can increase the proportion of monosaccharides in the total hydrolysate and decompose some of the acid-soluble lignin. The removal of lignin and degradation of hemicellulose increase the pore size of cellulose, contributing to the enhancement of the hydrolysis efficiency of cellulose. However, the addition of chemical reagents causes environment pollution, and the equipment requirements also increase. Unpolluted steam explosion technology proposed by Chen [19] does not require adding any chemicals, just controlling the water content of the straw. It can isolate more than 80 % of the hemicellulose, and the enzymatic hydrolysis rate of straw is more than 90 %.

An intermittent steam explosion device in China was produced in 1989 by the Inner Mongolia Boiler Plant. However, rapid reaction and heating and uneven heating of materials of the intermittent steam explosion reaction still need to be solved. Currently, the most successful commercial application is the Staketech Biomass Conversion process in the Canada Staketech Company. This technology uses a continuous steam explosion process, and the heating time and temperature are strictly controlled.

The cost of steam explosion pretreatment is low; generally, a ton of material consumes 0.5–1.0 t steam. The enzymatic hydrolysis yields of steam-exploded lignocellulose would be improved markedly. But, in this process, lignin is still not removed, and the material is extruded at the same time, resulting in a low concentration of sugar even with a high substrate concentration; for example, 10 % of the substrate concentration can only obtain about 5 % sugar.

4.3.1.3 Microwave and Ultrasonic Pretreatment

A microwave is an electromagnetic wave ranging from 300 MHz to 300,000 MHz. The temperature of microwave treatment is closely related to the enzymatic hydrolysis yields of cellulose. Microwaves can make the internal molecular structure of material produce heat by collision, resulting in heating of the material. Generally, the appropriate processing temperature of microwave pretreatment is 210–220 °C; a temperature above this range will cause decomposition of material and decrease the enzymatic hydrolysis rate.

The mechanism of microwave processing is the temperature effect. The microwave processing temperature must be above 160–180 °C, which is just consistent with the thermal softening temperature of hemicellulose, lignin, and cellulose, 167–181, 127–193, and 231–253 °C, respectively. In addition, microwave pretreatment has advantages such as short treatment time, simple operation, and highly efficient saccharification. Jian et al. [20, 21] studied the supramolecular structure changes of cellulose and the reaction performance of microwave- and ultrasonic-pretreated materials, which indicated that microwaves and ultrasound can accelerate these two types of chemical reactions of cellulose. In particular, they can greatly improve the reaction conditions of cellulose highly selectively oxidized by periodic acid. Therefore, microwave pretreatment has significant meanings for improving the chemical reactivity of cellulose, opening

new reaction channels and the synthesis of new cellulose functional materials. To improve the enzymatic hydrolysis rate of straw and extracted xylose from the hydrolysates, Zhu et al. [22, 23] adopted three ways to deal with straw: microwave/alkali, microwave/acid/alkali, and microwave/acid/alkali/H_2O_2. Straw pretreated by microwave/acid/alkali/H_2O_2 had the highest weight loss, cellulose content, and enzymatic hydrolysis rate. In microwave/alkali pretreatment, xylose cannot be recycled, and in microwave/acid/alkali and microwave/acid/alkali/H_2O_2 pretreatment, xylose crystals can be obtained from hydrolysates. Tang et al. [24] pointed out that ultrasound pretreatment changes the morphological structure, accessibility, and oxidative activity of the lignocellulose, and the accessibility of cellulose increased with microwave processing time.

For now, this method is still in the laboratory stage. Because of higher processing costs, microwave pretreatment is difficult to realize for industrial applications.

4.3.1.4 Radiation Treatment

In the production process of viscose fiber and acetate fiber, high-energy rays such as electron beam and γ-rays are widely used to pretreat the lignocellulose raw materials to obtain the desired polymerization degree and increase the activity of cellulose, which would reduce wastewater and environmental pollution caused by the addition of chemicals. The role of ionizing radiation, on the one hand, is to depolymerize the cellulose, decrease the degree of polymerization (DP), change the molecular weight distribution, and make the molecular weight distribution more concentrated than for ordinary cellulose. On the other hand, ionizing radiation increases the activity and accessibility of cellulose by loosening the cellulose structure and affecting its crystal structure [25]. Therefore, in the production of viscose fibers, radiation treatment with the dissolution pulp can improve the reaction activity of the cellulose-generated viscose. The content of NDF cellulose, acid detergent fiber (ADF), acid-insoluble lignin (ADL), and reducing sugar in the cell wall would be reduced under high-degree radiation, thereby enhancing the digestibility of straw [26]. Stavtsov [27] studied the molecular weight distribution of irradiated cellulose and found it is more homogeneous than that without irradiation. Owing to the irregular cleavage of radiation, the electron beam can penetrate into not only the noncrystalline regions of cellulose but also the crystalline regions. Radiation (or electron beam) combined with chemical methods to pretreat straw and other agricultural waste can increase the degradation of cellulose, hemicellulose, and lignin better than one of the methods adopted separately. The same conclusion was obtained no matter whether the agricultural waste was pretreated with high radiation doses (up to 500 kG) and low concentrations of chemical reagents (up to 5 %) or a low radiation dose and high concentrations of chemical reagents. Siriwattana [28] used 10 kGy γ-ray to pretreat agricultural waste, which was dealt with by 20 % urea in advance; the degradation of NDF, ADF, ADL, cellulose, hemicellulose, lignin, and cutin were significantly higher than by urea pretreatment.

Because of the high cost and high energy, radiation pretreatment is still difficult to realize for a large-scale application. Generally, the radiation treatment cost is 138–156 dollars/t raw material, even higher than mechanical crushing [29, 30].

4.3.2 Chemical Pretreatment

In chemical pretreatment, acids, alkalis, or organic solvents were used to deal with the lignocellulose. Chemical pretreatment has been widely used in chemical agents dissolving the lignin and hemicellulose, reducing cellulose crystallinity, or dissolving cellulose. This method makes cellulose, hemicellulose, and lignin imbibitions and destroys the crystalline structure, so there are various forms of degradation products, such as fiber dextrin, cellobiose, glucose, dextran, and so on. Traditionally, in the pulp and paper industry, chemical agents were used to dissolve lignin for preparing paper products with high strength and a certain fiber length. However, chemical pretreatment requires corrosion-resistant equipment, needs to exclude a large number of chemicals, and causes difficulty in recycling lignin and hemicellulose resources, causing serious environmental pollution.

Chemical pretreatment includes inorganic acid pretreatment, alkali pretreatment, and organic solvent pretreatment.

4.3.2.1 Alkali Pretreatment

Swelling pretreatment of sodium hydroxide solution is one of the earliest and most widely used methods for lignocellulose pretreatment. The mechanism of alkaline pretreatment is to weaken the hydrogen bonding between cellulose and hemicellulose by the role of alkali and saponate the ester bonds between hemicellulose polysaccharides and other components of the internal molecular structure (such as lignin). With reducing the ester bond, the porosity of lignocellulose increases. The effect of alkaline pretreatment depends mainly on the lignin content in the raw materials. At low temperatures, 8–10 % [31] sodium hydroxide is the strongest swelling agent. An earlier study found that the accessibility of cellulose improved after alkali pretreatment, but it does not mean the entire reaction has been improved.

Reactivity of cellulose treated by alkali swelling is related to the conditions of mercerization, such as alkali concentration, the elution of the swelling agent, and the reaction temperature. Cellulose swelling in the alkali solution has an optimal concentration, for example, 18 % for cotton cellulose in sodium hydroxide solution. There is a generally accepted view that cellulose alkalization is an exothermic reaction. With the temperature increasing, the degree of cellulose swelling decreases; thus, the reaction activity of alkali cellulose is decreased. Therefore, the alkali-swelling pretreatment generally proceeds at low temperatures (such as 20 °C) [21, 26]. The high-temperature alkali pretreatment damages the original structure remarkably, and the new structure is formed quickly. After

high-temperature soaking, the content of pentosan, lignin, and inorganic impurities in cellulose is reduced, which not only is conducive to the penetration of the reagents but also provides the possibility of further changes of supramolecular structure of cellulose. Lu et al. have shown that NaOH pretreatment has a great influence on the chemical composition of lignocellulose. In pretreated material, cellulose swelled significantly, the cellulose crystallinity degree was reduced, the cellulose crystalline region was damaged, and the enzymatic hydrolysis became easier [32]. In addition, calcium hydroxide, ammonia, and the like can be used to remove lignin [33, 34]. In ammonia treatment, lignocellulose is soaked in 10 % ammonia solution (w/w) for 24–48 h to remove most of the lignin. Ammonia solution can remove the acetyl contained in raw materials, which is harmful to the fermentation. But, there will be some loss of hemicellulose in the high ammonia concentration. The Laboratory of Renewable Resources Engineering (LORRE) at Purdue University pretreated lignocellulose with ammonia and then transformed the xylose in hydrolysates with the breeding strains to xylitol, with a xylose conversion of 0.74 $g \cdot g^{-1}$ [30]. Kim and Lee [35] studied component separation of corn stover by hot water and ammonia two-step pretreatment; hot water pretreatment was used to degrade hemicellulose, and ammonia pretreatment was used to remove lignin. In this method, the enzymatic hydrolysis yields of xylan reached 92–95 %, the recovery yields reached 83–86 %, and lignin removal yields reached 75–81 %. After two-step processing, the cellulose content in the material was maintained at 78–85 %, and the enzymatic hydrolysis rate was significantly increased.

There are three types of alkali pretreatment: batch process, semicontinuous process, and continuous process [36]. In a batch process, materials and chemical agents are mixed with a solid-to-liquid ratio of 1:20 and pretreated at a temperature of 100–180 °C for about 20–45 min. In the semicontinuous process, materials and the chemical treatment agent are premixed for 30 min with a solid-to-liquid ratio of 1:1; then, the mixture is continuously added to the extruder and pretreated at 200 °C for a certain period of time. In the continuous process, dried materials are continuously filled in the extruder through the feeding device, and chemicals are injected into the extruder by an injection pump at an injection rate of 0.6 $L \cdot min^{-1}$. The pretreatment is conducted using the following conditions: solid-to-liquid ratio of about 1:4 and processing temperature of 100–200 °C. A batch process device is common equipment with a heating and stirring device. The semicontinuous process device is a PCM30-25G-type extrusion machine with a double-helix axis having the same rotation direction. The continuous process adopts the PCM65-30-type extruder, which is similar to the semicontinuous process device but with opposite rotation directions of the two axes. The batch process has the disadvantages of large consumption of chemicals and energy. The amount of chemical agent used in the semicontinuous process is 1/20 of the agent used in the batch process. The demand of grinding raw materials (2 mm) is slightly lower, and the heat of reaction cannot be fully utilized. In the continuous process, materials and chemical agents continuously mix, and reaction time is short, just 2 min. The reaction heat can be effectively utilized; raw material crushing requires a lower particle size of about 10 mm.

Although NaOH pretreatment has strong ability for delignification and reducing the degree of crystallinity, about 50 % of the hemicellulose is also dissolved, resulting in great losses. Generally, each gram of material consumes 0.1–0.15 g alkali; if processing 1 t of raw materials, the alkali cost is $22–23. In addition, the total cost for the grinding, mixing, and washing and reagent recycling is $60–90. Besides, the material swells with NaOH treatment, and its bulk density is low. The substrate concentration is lower (4–5 %), but it is too dense, and it is not conducive to mixing and transfer, so the enzymatic glucose concentration is low.

4.3.2.2 Cellulose Solvent Pretreatment

Part of the crystalline structure of cellulose would disintegrate when dissolved in solvent. When the cellulose precipitated out again, enzymatic hydrolysis of its structure is easier. In industrial production, the cellulose and its derivatives, such as viscose fiber, acetate fiber, cuprammonium fibers, and other cellulose products, often are used; they are prepared first through the dissolution of cellulose. The cellulose solvent can be divided into aqueous solvent and nonaqueous solvent.

Cellulose solvents for cellulose enzymatic hydrolysis include H_3PO_4 and cadoxen (cadmium oxide or cadmium hydroxide dissolved in ethylenediamine). The results show that the improvement of solvent for the cellulose enzymatic hydrolysis rate and hydrolysis rate is obvious, but solvent is expensive, and recovery costs increase. In the present study, natural cellulose materials mostly are cotton, grain, and so on; solvents are not suitable for processing of various raw materials. Wood pulp was pretreated by ethylenediamine and cadoxen and then was acid-catalyzed hydrolyzed; the average degree of polymerization (ADP) decreased from 160 to 26. The sample was pretreated by 70 % sulfuric acid; similar results were obtained. With application of an alkaline solution solvent containing sodium tartrate, ferric chloride, and sodium sulfite for pretreatment, results showed that the crystallization index of the sample was slightly higher than when treated by 60 % sulfuric acid; however, the hydrolysis speed of two samples was roughly the same [37]. According to the report, cadoxen pretreatment per ton of raw material cost $55–100.

There is a new cellulose solvent: ionic liquid. The ionic liquid is the liquid entirely composed of the ion and was a liquid salt at low temperature (<100 °C); it is also known as low-temperature molten salt and generally is composed of organic cations and inorganic anions. Compared with traditional organic solvents and electrolytes, ionic liquid has a range of prominent advantages: (1) It has almost no vapor pressure and is nonvolatile, eliminating the environmental pollution problems of volatile organic compounds, and it is colorless and odorless; (2) it has a large stable temperature range (from less than or close to room temperature to 300 °C) and good chemical stability and a wide electrochemical stability window; (3) the design of the ions can adjust the solubility of inorganic water, organic matter, and the polymer, and the acidity can be adjusted to super acid. Because of these special properties and performance of the ionic liquid, it and supercritical CO_2 and water constitute the three major green solvents. With the introduction of the

concept of green chemistry, a worldwide craze about ionic liquid has formed. In 1934, Graenacher first suggested molten chloride, N-ethyl-pyridine (nitrogenous bases), dissolving cellulose. It was innovative at the time, but there is a high melting point (118 °C) and high cost of this salt, so it lacked practicality. In 2002 and 2003, some researchers reported that the ionic liquid–chloro-1-butyl-methyl imidazole and 1-allyl-3-methyl imidazole can dissolve untreated cellulose [38–40], which opened the door for ion liquid application in the cellulose industry, especially in the field of the lignocellulose component separation and enzymatic hydrolysis. Liu and Chen used a newly synthesized ionic liquid chlorinated 1-butyl 3-methyl imidazole ([BMIM]Cl) to dissolve the steam-exploded wheat straw; results showed that the treated steam-exploded wheat straw was almost completely enzymatically hydrolyzed; the DP of cellulose and hemicellulose both decreased, and cellulose crystallinity and accessibility improved [41].

Generally, the effect of solvent pretreatment is obvious, but at the same time, there are issues such as corrosion and toxicity, resulting in environmental pollution problems, that have been the main reasons for the difficulty in the scale used in the pretreatment. If the solvent can be recycled or reused or can be replaced by poison analogues, this pretreatment method may achieve low cost and be promoted in the industry.

4.3.2.3 Organic Solvent Pretreatment

Organic solvent pretreatment is technology that separates lignin from natural cellulose materials; it can use a single solvent or a mixture solvent with several solvents. The solvents studied include alcohols (methanol, ethanol, butanol, etc.), ketones, phenols, dimethyl sulfoxide, and amines (n-butylamine). Some solvents are added with acetic acid, formic acid, hydrogen peroxide, sodium sulfite, sodium hydroxide, anthraquinone, and so on. Generally, the materials used at 1–2 h at 160–200 °C can remove about 80 % lignin, hemicellulose is almost completely dissolved, and the enzymatic hydrolysis rate of cellulose residue reaches 80 %. Awadel-Karim et al. [42] studied organic solvents such as acetone, which was a good cellulose-swelling agent. This is mainly because acetone not only can penetrate cellulosic materials internally but also has potential as a hydrogen bond acceptor. Its absorption of cellulose intramolecular and intermolecular hydrogen bonds can lead to tremendous changes of cellulose stereochemical properties, resulting in the weakening or destruction of the cellulose molecules of hydrogen bonds, which increases cellulose accessibility.

But, there are different effects on natural lignocellulose of the solvents mentioned, and recycling or reuse of some solvents is not economical. Organic solvent pretreatment has the following disadvantages: Almost all hemicellulose is dissoluted, including 50–100 % of the hemicellulose sugar; organic solvent pretreatment is under alkaline conditions, so an alkali recovery system is needed; solvent recovery of energy costs and security measures requires increased equipment costs; and processing conditions are more severe than for conventional chemical pulping.

4.3.2.4 Dilute Acid Pretreatment

In acid pretreatment, acid as a catalyst accelerates the autohydrolysis of lignocellulose raw materials. For example, using dilute sulfuric acid treatment of lignocellulosic raw materials to produce uronic, hemicellulose is hydrolyzed to xylose and other sugars by dilute sulfuric acid until the formation of uronic. The added sulfuric acid can remove hemicellulose and improve the digestion of cellulose in residual solids. In acid pretreatment, the quality of lignocellulosic raw material will certainly be lost, which is mainly caused by the hydrolysis of hemicellulose. The raw materials become porous because of hemicellulose hydrolysis, increasing the contact area of the cellulose and cellulase.

Hemicellulose can be dissolved in dilute acid (0.3–3 %) below 100 °C. In the process of dilute acid hydrolysis, most of the hemicellulose is dissolved in the acid solution; the reaction take places on the surface, and the reaction rate is fast. The cellulose is insoluble, and the contact area of acid and cellulose is small, so the reaction rate is slow. According to research, the speed of these two reactions differ by about 100 times. Because of hemicellulose dissolution, the cellulose enzymatic hydrolysis rate can be greatly improved. At the same time, hemicellulose sugar can be recovered.

Dilute acid hydrolysis has been successfully used in the pretreatment of lignocellulosic biomass. Dilute acid pretreatment and concentrated acid hydrolysis have been industrialized. However, because the parcel of cellulose polymer lignin in the acid treatment process has not been effectively removed, the matrix structure is still relatively close. The concentrated acid can be used to process lignocellulosic raw materials, but the concentrated acid is toxic and corrosive and requires acid-resistant equipment. After concentrated acid pretreatment, recycling the acid is required to minimize pollution of the environment, increasing the cost of production. The remaining acid must be neutralized for subsequent hydrolysis and fermentation after acid pretreatment, and then it will produce some inhibition for fermentation and the need to remove the salt, which both increase production costs.

4.3.2.5 Oxidation Pretreatment

The oxidation pretreatment process uses hydrogen peroxide, ozone, or oxygen under alkaline conditions to decompose and dissolute lignin, and cellulose can also be partially oxidized, enhancing the enzymatic hydrolysis rate. Ozone can be used to break down lignin and hemicellulose in the lignocellulosic material. In this method, lignin can have a large degree of degradation, with hemicellulose only attacked in a minor way; the cellulose is almost unaffected. In the ozone pretreatment process, after removing 60 % of the lignin, the enzymatic hydrolysis rate of the cellulose matrix increased by five times, the mass fraction of the lignin decreased from 29 to 8 %, and enzymatic hydrolysis yields increased by 57 % [43].

The wet oxidation method was proposed in the early 1980s; by heating and pressurizing, water and oxygen both participate in response to pretreatment of

corn stover. In the alkali condition, cellulose will swell, forming the alkalization cellulose, but will maintain the original skeleton to prevent the destruction of cellulose. Lignin is dissolved in alkali solution and is separated with cellulose so that the cellulose of high purity and has few by-products. Varga et al. [44] used the wet oxidation method to pretreat 60 g·L^{-1} corn stover at 2 g·L^{-1} Na_2CO_3 at 195 °C and 1.2×10^3 kPa for 15 min; the results showed that 60 % of hemicellulose and 30 % of cellulose were dissolved, 90 % of cellulose was separated, and the enzymatic conversion rate of cellulose was 85 %.

The method not only can separate lignin but also can oxidize cellulose. It still remains in the laboratory stage.

4.3.2.6 Supercritical Extraction Pretreatment

In supercritical conditions, the gaseous substance is in a particular state, with both some characteristics of the gas and some characteristics of the liquid, so that the substance in this state as a solvent for extraction shows many unique features. It has strong dissolving power that can dissolve silica, lignin, and so on, and the extraction separation and concentration separation can finish at the same time.

Supercritical processing enables cellulose to be isolated from natural lignocellulose material, improving the nature of cellulose and removing lignin. Because it completes the removal and recovery of lignin all at once, which creates conditions that utilize natural lignin and avoid the pollution of the environment, and the solvent can be recycled. Chemical reactivity of a methylamine–nitrous oxide mixture system is now reported; the solvent used a pressure range of 175–280 atm and a temperature of 170–185 °C. The other solvent system does not have a chemical reaction, such as CO_2-ethanol and CO_2-H_2O, isobutanol and isopropanol, at a high temperature of 400 °C.

Supercritical processing technology requires high temperature, high pressure, high-precision equipment, and large investment. At present, it remains in the laboratory stage.

4.4 Component Separation

Traditional pretreatments for natural lignocellulose raw material aim at the utilization of cellulose. However, the concept of component separation I first proposed satisfies the requirements of total biomass utilization of lignocellulose. This new component separation method makes all the components of natural lignocelluloses reasonably utilized.

4.4.1 Evaluation Criteria for Component Separation

Any science development is constantly improved and developed based on the original science and then to make qualitative progress. Pretreatment technologies for natural lignocellulose raw materials have been studied for decades and have both advantages and disadvantages. Some pretreatment methods can be made reference but should be given new meaning. New evaluation criteria should be established for component separation and can be summarized as follows: (1) Separation and recovery of components is the purpose, and it is not necessary to achieve complete component separation; (2) reduce the interaction between the various components and reduce enzyme invalid absorption and chemical usage; (3) all components should be effectively and economically recovered; (4) there should be clean production; and (5) low operating and investment costs are necessary [1].

4.4.2 Research Progress on Component Separation Technologies

A single technology cannot achieve the rapid separation of the three components at once. A combination of several technologies can develop their respective advantages. It is feasible for component separation. For example, steam explosion technology is a method used to realize the low-cost and effective recovery of hemicellulose, but lignin still remains in the residues. Ethanol extraction is an efficient method for lignin recovery but results in serious losses of hemicellulose because of severe conditions. Combined steam explosion pretreatment with ethanol extraction can make great full recovery of hemicellulose and lignin under mild conditions. First, hemicellulose is recovered by steam explosion; meanwhile, the X-propylene ether and some β-propylene ether in the lignin structure are broken, which is conducive for extracting lignin for ethanol extraction at mild conditions.

I have established a set of efficient and economic component separation technologies, based on the steam explosion technology, to achieve total biomass utilization of lignocelluloses.

4.4.2.1 Combination of Alkali Oxide and Steam Explosion

NaOH has a strong role in the delignification of raw materials. After removing the lignin, the cellulose content increases, but the cellulose structure becomes denser. To improve the enzymatic hydrolysis rate, it is necessary to increase the amount of enzyme and improve the solid-to-liquid ratio, which increases the cost of production

Table 4.3 Comparisons of ethanol extraction of wheat straw and steam-exploded wheat straw (extracted by 50 % ethanol at 180 °C for 1 h with a solid-to-liquid ratio of 1:50) [47]

	Wheat straw	Steam-exploded wheat straw
Yield of sugar (%)	0.00	25.22
Yield of sugar by enzymatic hydrolysis (%)	21.87	30.33
Total yield of sugar (%)	21.87	55.55
Sugar content of recovered lignin (%)	42.00	1.12
Delignification (%)	64.11	80.22

and is not feasible in actual production. Hydrogen peroxide has a strong ability for oxidation delignification and more obviously under alkaline conditions. Chen and Xu [45] invented a method of coupling steam explosion with alkaline hydrogen peroxide oxidation to pretreat straw. Steam-exploded straw and alkaline hydrogen peroxide solution were mixed in accordance with a weight ratio of 1:20–1:40 and oxidized for 12–36 h. After the treatment, the sugar concentration in the hydrolysate of steam-exploded straw increased to 100 $g \cdot L^{-1}$ [46].

4.4.2.2 Combination of Steam Explosion and Ethanol Extraction

In the steam explosion process, the hemicellulose can be effectively recovered. Under the same processing conditions, lignin in the steam-exploded wheat straw can be effectively isolated by ethanol extraction, and the recovered sugar yield is 2.5 times that of direct ethanol extracted from wheat straw. Meanwhile, pure xylose, glucose, and ethanol-extracted lignin can be obtained. The delignification effect of wheat straw treated by steam explosion pretreatment and ethanol extraction was higher than for the wheat straw treated by direct ethanol extraction. In the process of steam explosion, lignin has been softened and partially fractured, making the lignin easier to extract. The results of electron microscopy showed that, after ethanol extraction, fiber cells of the ethanol extract of steam-exploded wheat straw are dispersed, increasing the accessibility of cellulose and improving the performance of enzymatic hydrolysis and fermentation of raw materials. Comparisons of ethanol extraction from wheat straw and steam-exploded wheat straw are shown in Table 4.3.

4.4.2.3 Combination of Steam Explosion and Ionic Liquid Dissolution

Compared with traditional organic solvents and electrolytes, ionic liquids have advantages in that they are colorless and odorless, produce less pollution, and have stable chemical properties. The design of the ions can adjust the solubility

of the inorganic substance, water, organic substance, and polymer, and the acidity of ionic liquid can be adjusted to the superacid condition. Chen et al. explored the dissolution performance of water-washed steam-exploded wheat straw in ionic liquid [BMIM]Cl to achieve component separation of raw materials.

After steam explosion, more than 80 % of hemicellulose in wheat straw was degraded into soluble sugar present in the hydrolysates; hence, the residue of steam-exploded wheat straw is mainly cellulose and lignin. Liu and Chen proposed that the dissolution of water-washed steam-exploded wheat straw in [BMIM]Cl was markedly increased. They also found that [BMIM]Cl could dissolve low molecular weight lignin, but lignin with high molecular weight was not dissolved [48]. Based on this result, high-purity lignin can be isolated from raw materials. The removal of lignin and hemicellulose not only weakened the invalid adsorption role of lignin on cellulase but also increased the accessibility of cellulose, thus improving the consequent enzymatic hydrolysis and fermentation performance of steam-exploded wheat straw. Wheat straw and steam-exploded wheat straw were treated with [BMIM]Cl, and the enzymatic hydrolysis and fermentation performances of the [BMIM]Cl-treated materials were compared. This indicated that, after the ionic liquid [BMIM]Cl treatment, the enzymatic hydrolysis rate of wheat straw and steam-exploded wheat straw increased substantially. The cellulase fermentation ability of [BMIM]Cl-treated steam-exploded wheat straw was enhanced. The highest enzyme production of [BMIM]Cl-treated steam-exploded wheat straw was 118.64 $FPU \cdot g^{-1}$ dry koji after 5 days fermentation and was only 99.20 $FPU \cdot g^{-1}$ dry koji for the untreated group. Therefore, raw materials processed by the ionic liquid [BMIM]Cl combined with steam explosion can achieve separation of hemicellulose, cellulose, and lignin.

4.4.2.4 Combination of Steam Explosion and Glycerol Microwave Pretreatment

Relative to ethanol and other low-boiling-point organic solvent, glycerol is a new type of pretreatment solvent. Because of its high boiling point (290 °C), it can achieve low-pressure and high-temperature pretreatment. Thermosensitivity and good temperature control make glycerol able to achieve rapid heating and cooling. As a triatomic alcohol, it can change the chemical structure of lignocellulose by oxidation and alcoholysis, remove lignin from cellulose and hemicellulose, and achieve separation of the three chemical components [49]. Sun and Chen combined steam explosion with glycerol microwave pretreatment to achieve component separation of lignocellulose. First, lignocellulose was steam exploded and then washed by water, and hemicellulose was obtained from the washing solution after standing precipitation. Then, water-washed steam-exploded residue was dried to a moisture content of 3–5 ml/g and intermittent microwave treated with glycerol. The reacted residue was dissolved in hot water. The filter residue was crude cellulose. Last, the filtrate was precipitated statically to obtain lignin. A combination of steam explosion pretreatment and glycerol microwave pretreatment can isolate about 70 %

of crude cellulose (dry weight) from the lignocellulose; the content of isolated hemicellulose and lignin accounted for 80 % and 85 % of the content of raw materials, respectively [50].

4.4.2.5 Combination of Steam Explosion and Wet Ultrafine Grinding Pretreatment

Particles can be crushed to the micron level by ultrafine grinding; thus, the surface area can be greatly improved. Based on the view of increasing the reaction surface area, energy consumption for ultrafine grinding is not higher than for traditional mechanical crushing [51, 52], but the application of this technology is limited in the field of lignocellulose pretreatment [53]. Chen achieved fractionation of lignocellulose at the cellular level by the combination of steam explosion and wet ultrafine grinding. The results showed that after treatment by steam explosion and superfine grinding, rice straw can be quickly and efficiently divided into powder parts (and easily be enzymatically hydrolyzed) and residue parts (which are difficult to enzymatically hydrolyzed) on the premise of not destroying the main chemical components of rice straw and not producing inhibitory substances for the downstream process. But, it was also found that the steam explosion combined with superfine grinding was destructive to the cellulose itself.

Because of the heterogeneous structure of rice straw, micronized properties differ from tissues; especially when there is a high water content, the water-holding capacity and tenacity of fibrous tissue and nonfibrous tissue, including parenchyma, epidermal tissue, and vessels, have significant differences. To investigate the effect of superfine grinding by high-speed airflow on the fractionation of the nonfibrous fraction from the fibrous fraction, a fluidized bed opposed jet mill (FJM-200, power 1.5 kW) was used to treat steam-exploded rice straw with a high moisture content and of relative low steam explosion severity to remove the nonfibrous tissues. Contrary to the general superfine grinding process, the material with a high moisture content retained fibrous tissue flexibility and avoided grinding. The nonfibrous tissue, with a relatively lower water retention property, was ground and fractionated from fibrous tissue and then was collected from the discharge opening [51].

The fibrous tissue part is mainly composed of fibrous tissue, with a high proportion of fiber cells, high cellulose content, and low ash content; the nonfibrous tissue part is mainly composed of the parenchyma, epidermal tissue, and catheter, with a low cellulose content and high ash content. The content of the fiber cell in fibrous tissue was 63.1 %, which was 37.8 % higher than in the original straw. In the enzymatic hydrolysis process of steam-exploded straw, the relative content of fiber cells in the enzymatic residue decreased to 51.1 % from 74.5 % in the steam-exploded straw before the enzymatic hydrolysis; the relative content of the parenchyma cells decreased by 65.8 %, and the relative content of the epidermal cells rose to 40.1 % from 5.1 %. This showed that fiber cells and thin-walled cells

are easier to enzymatically hydrolyze, and epidermal cells are more difficult. It further proved that the combination of steam explosion technology and wet ultrafine grinding technology was able to achieve the separation of raw materials in the tissue and at the cellular level and to achieve the high value of transformed straw and integrated utilization.

4.4.2.6 Combination of Steam Explosion and Mechanical Combing Technology

Chen et al. [54] researched steam explosion pretreatment technology coupled with mechanical combing to pretreat cornstalks and achieved the separation of the fiber bundles and miscellaneous tissue at the tissue level. Fiber of corn straw was fully exposed by steam explosion pretreatment; miscellaneous cells were disrupted or deformed, and hemicellulose was partially degraded; mechanical combing can remove the hybrid cells that adhered to the surface of the fiber bundles and achieved the separation of fiber tissue and miscellaneous tissue. The tests found that the conditions of steam explosion used to separate fiber in different parts of the corn straw and other tissues were inconsistent. The skin required the highest-intensity conditions, followed by leaves and core. Under suitable steam explosion conditions and water content (30 %), fiber bundles and miscellaneous tissue can achieve separation by mechanical carding. After separation, the weight ratio of fiber tissues and miscellaneous tissues was about 3:2. The miscellaneous tissue of corn straw steam exploded coupled with combing had good fermentation performance, and cellulase production by miscellaneous tissue in the leaf fermentation reached 194.18 FPU g^{-1} dry koji. The isolated fibrous tissue had excellent pulping performance. Under the conditions of 50 % ethanol concentration, 180 °C temperature, and 2 h reaction time, the pulp yield was up to 45 %, and the lignin content in the pulp was less than 3.5 %.

4.4.3 Developing Prospects for Component Separation Technology

The purpose of component separation is to separate each component of natural lignocellulosic feedstocks with maximum yields and as much as possible retain the integrity of the molecule and achieve the optimized utilization and greatest value of lignocellulose. The existing pretreatment technologies have some disadvantages. It is believed that under the concept of component separation, in compliance with the new evaluation criteria for component separation, the breakthrough of component separation technology will lead the comprehensive development of total biomass utilization of natural lignocelluloses and finally achieve the strategic shift of the chemical industry and fermentation industry.

4.5 Selectively Structural Fractionation

4.5.1 Development History of Selectively Structural Fractionation

4.5.1.1 Functionally Whole Conversion

There is a long history of biomass utilization by human beings. Papermaking and charcoal manufacturing techniques in ancient Egypt are successful examples of biomass utilization. But, after the discovery of oil and coal, the development of new energy and a chemical industry based on them greatly facilitated the development of civilization and made biomass utilization slowly fade. The first global oil crisis in 1973 made the study of biomass regain attention. At first, research of biomass mainly focused on the use of biomass to obtain energy heat, electricity, or fuels such as ethanol and now focus on developing various conversion technologies.

China is a large agricultural country with a long history of recycling straw-based biomass resources, including compost, returning it to the field, forage, fuel, and so on. However, because of the limitations of the times and technology, conversion of biomass has some problems, such as the low-level utilization and low utilization efficiency. With the development of technology, biomass conversion technologies continue to improve, and its conversion rate also gradually increases. In the twentieth century, with the development of technology and the aggravation of the energy crisis, the research of biomass utilization rapidly developed; biomass gasification and biomass liquefaction have achieved certain results.

The direct combustion, gasification, pyrolysis, liquefaction, and other biomass utilization technologies focus on the overall function conversion of biomass feedstock, but biomass is a structurally complex, functional supramolecular structure; using overall function conversion to achieve the primary refining of biomass feedstock, it is impossible to achieve the maximum value of the biomass materials.

4.5.1.2 Fully Functional Conversion

The complex composition of biomass determines that the biomass feedstock is a functional macromolecule body, and the different components are able to generate different functional products. Thus, in addition to the direct combustion and other fully functional conversions, based on the three main components of cellulose, hemicellulose, and lignin utilization, a series of studies has been carried out and has made some progress.

The paper industry mainly uses the cellulose component; therefore, it needs to adopt various means to remove the hemicellulose and lignin. Thus, the other

components are not used, resulting in the waste of resources. The paper industry mainly uses a strong acid or alkali to dissolve lignin at high temperatures, which will consume much energy and many chemical raw materials, resulting in the waste of resources and environmental pollution.

Similarly, in the fields of biomass fuels such as ethanol, to obtain fermentable sugar the primary refining of biomass materials includes physical, chemical, and biological methods. However, the end result of these methods is still aimed at the enzymatic hydrolysis and fermentation of cellulose; the high-value use of hemicellulose and lignin needs a few considerations. Therefore, these pretreatment technologies are primary refining of biomass materials; they can only achieve the utilization of one or two major components in biomass, and the other components are damaged or wasted.

Based on these problems, to achieve total utilization of biomass a new set of comprehensive utilization technologies should be established. Components fractionation- oriented conversion technology is the primary refining technology to achieve the comprehensive utilization of multiple components of biomass. Components fractionation-oriented conversion technology is a further improvement of primary refining of biomass. It is not only a pretreatment method but also an allocation process of macromolecular components in biomass feedstock, which can achieve the transformation of cellulose, hemicellulose, and lignin separately. It is the most important idea regarding high-value utilization of biomass at present.

4.5.1.3 Fractionation and Partially Functional Conversion

Existing process routes of component separation-orientation conversion usually consume energy to destroy the structure of the biomass, then to convert it. This method does not take the functional requirements of the product into account; total demolition not only increases energy consumption but also lowers the atom economy for some products. Thus, it is still difficult for the idea of biomass component separation–directional conversion to break through the economic problems of biomass as industrial raw materials to achieve large-scale clean and efficient industrialization.

Because of the findings of the 973 Project of China, to upgrade the pretreatment–component separation of lignocellulose to the selectively structural fractionation process based on functional requirements of products and to make the biomass materials become common industrial materials for biomass-based energy, materials, and chemical products, Chen et al. proposed the use of the structural characteristics of materials and the requirements of the target products.

Product engineering aimed at product demands develops products to meet end-user performance. At present, product engineering has become an important area of research. Biomass product engineering is based on renewable biomass resources as raw materials and oriented to the needs of people for production and living for the

design, processing, and production of biomass products. The production process for biomass product engineering refers to the biomass feedstock refining process that aims at the desired product function and utilizes one or more functional molecules in the biomass feedstock to design the corresponding product, which is the selectively structural fractionation process of biomass feedstock.

4.5.2 Status Analysis of Selectively Structural Fractionation Technology

4.5.2.1 Product Engineering of Biomass

Lignocellulosic raw materials are functional supramolecular structures interwoven by the cellulose, hemicellulose, and lignin. The multicomponent structure determines the diversification of the biomass-based products throughout bio-based energy, materials, and chemicals and other fields.

First, biomass energy products such as fuel ethanol, biodiesel, and pyrolysis bio-oil are booming. Ethanol mixed with a certain percentage of gasoline can be used as a gasoline engine fuel, and the engine need not be changed if less than 25 % ethanol content is used. The ethanol has a larger octane value than gasoline and contains oxygen; it is both antiknock and an accelerant. Raw materials for fuel ethanol include starch (such as corn), sugar feedstock (such as sucrose), and lignocellulose (such as straw) and are currently dominated by the starch and sugar feedstock. Lignocellulose to produce fuel ethanol is technically feasible but has not broken through the economic costs. In the long term, by the limitations of starch and sugar feedstock production, the use of lignocellulose materials to produce fuel ethanol is the ultimate goal [55]. Biodiesel is the fatty acid methyl esters derived from animal, plant, or microbial oil and methanol by a methyl esterification reaction; it can completely replace fossil diesel oil directly used as diesel fuel. With the advantages of low sulfur content, biodegradability, and environmental friendliness, it has received much attention. At present, biodiesel production technology is mature and has achieved industrialization, but the problem is the lack of an abundant lipid source and high production costs. The price makes it difficult to compete with fossil diesel fuel. Hence, finding new sources of oil and the development of efficient low-cost lipase and clean and efficient catalyst are the focus for current study [56–58]. Pyrolysis bio-oil is a liquid fuel from biomass using the thermochemical conversion method. The thermochemical process is rapid and efficient, but the pyrolysis bio-oil composition is complex, low quality, and unstable, so there are many issues that need to be solved [59]. The use of biomass energy products not only can reduce the dependence on fossil fuels, but also, because of the characteristics of zero emissions of carbon dioxide in its life cycle, can slow the “greenhouse effect” caused by the increase of atmospheric carbon dioxide.

Second, with the development of pharmaceutical and aerospace technology and the need for environmental protection, the products of biomass functional materials emerge endlessly. Environmentally friendly materials prepared from lignocellulose have multiple functions, such as water absorption, adsorption, flame resistance, corrosion resistance, capture, high strength, photolysis, environmental protection, and so on. Cellulose acetate is the most widely used fiber plastic. Depending on the requirements, selection of different formulations of cellulose acetate and additives can result in preparation of different products. So far, cellulose acetate has a wide range of uses in various fields (e.g., automobiles, aircraft, building supplies, cosmetics, photography, printing, films, paint, etc.) [60–62]. Polyhydroxyalkanoate (PHA) is a kind of polyester that widely exists in the cells of microorganisms, especially in bacterial cells. Plastics such as polyethylene and polypropylene (PP) synthesized by PHA and oil raw materials have similar material properties [63]. PHA can be prepared by renewable resources as raw materials and can be completely degraded to carbon dioxide and water then released into the natural ecological circulatory system [64]. Therefore, PHA is considered a "green plastic." It can replace traditional plastic, which is difficult to degrade, causing extensive attention in the world of academia and industry. In addition, PHA is biocompatible, resulting in its great potential in tissue engineering. PHA can be used as implant material and stent in vivo cell growth, such as for heart valves, cardiovascular repair material, and graft material. In addition, the study of polylactic acid and silk protein material is also in full swing; the biological function of silk protein is expected to have deeper and wider development and application in the military, aerospace, medicine, environmental protection, and other fields [63, 65].

Biomass organic fertilizer is another important part of biomass product engineering. The current new technology used in agricultural production brings a breakthrough for the production of agricultural products. After the 1950s and 1960s, fertilizer has been widely used, resulting in the rapid development of agricultural production, and the degree of commercialization of agricultural products has increased. At the same time, the extensive application of chemical fertilizers brought some side effects, such as severe environmental pollution, declining soil fertility, soil structure, compaction, and so on. Therefore, modern agriculture must change the model of relying solely on fertilizer and turn to the use of organic fertilizers instead of chemical fertilizers to achieve sustainable development. Biomass organic fertilizer is characterized as safe and with rich nutrients, containing both a large number of elements crops need, trace elements, and growth-stimulating substances; it contains much organic matter, which can improve soil properties and fertility and eliminate soil pollution. It also has a slow, steady, and lasting function as a fertilizer [66]. Organic fertilizer resources are rich in China. Straw, animal manure, and urban living garbage all can be used to produce organic fertilizer. At present, because of improper handling, these substances often become the source of pollution in a local area; therefore, producing high-quality organic fertilizer not only has economic significance but also important ecological significance.

Biomass feed [67] is also an important part of biomass product engineering. The development of feed resources is a necessary condition for the development

of animal husbandry. Many countries in the world use the characteristics of their own resources to select different resources as feed sources. The feed source of New Zealand, Australia, and other countries is mainly forage; the United States and other developed countries with sufficient food resources develop grain-based, high-input, high-output animal husbandry. China is a densely populated country. With population growth and the reduction of arable land, the increase of food cannot catch up with the rapid growth of the livestock industry. Thus, no matter forage or grain will be able to support the rapid development of China's animal husbandry [68]. Straw is an important raw material resource; its production is extremely rich in our country. Grain output in China has been nearly 470 million tons in 2004; at the same time, this was accompanied by about 650 million tons of various types of straw. Developing feed resources based on the straw will provide a strong guarantee for the development of animal husbandry in China.

Converting biomass resources into a series of chemical products is another important area of biomass product engineering. At present, the world's chemical industry is still based on the oil and natural gas industry. Crude oil is the main raw material for the production of resins, fibers, and pigments and other chemicals. Approximately 10 % natural gas, 21 % natural liquefied gas, and 4 % crude oil are used to produce chemical products. With the advent of the oil crisis, people have had to develop new resources to replace increasingly expensive and depleting petroleum resources. Many C_1-C_4 chemical products are obtained through starch, cellulose, and other carbohydrates. In the 1970s, an oil crisis made these chemical products change reliance on fossil fuels to biomass, and the United States and Brazil developed an appropriate policy to use renewable resources to partially replace fossil fuels. But, with the decline in oil prices in the 1980s, much work performed to replace the petrochemical industry was given up. Today, the world faces an oil crisis again, which makes it again time to focus increasingly on renewable resources. At the same time, the use of renewable resources from plants (the carbon from photosynthesis, carbon dioxide fixation) can achieve a closed carbon cycle and reduce carbon dioxide emissions. The chemical industry based on renewable resources also has important ecological significance.

4.5.2.2 Primary Refining of Biomass Materials: Selectively Structural Fractionation Technology

(1) Bio-based energy

Stove burning is the original way to utilize biomass [69], and its efficiency is only about 15–20 %. With the development of technology, biomass conversion technologies continue to improve, and biomass conversion utilization efficiency gradually increases, such as boiler combustion, which uses the modern boiler technology, which not only is efficient but also can realize industrial production; currently, garbage incineration uses boiler technology to dispose of rubbish. As early as 1979, the United States began to burn garbage to generate electricity; the

total installed capacity of power generation was over 10,000 MW, with a single-unit capacity up to l0–25 MW; pressure molding of fuel curing molds the biomass and then burns it by traditional coal-fired equipment, which can shrink the volume of biomass feedstock, greatly increasing the energy density of the fuel, to improve utilization efficiency. In recent years, molding technology for biomass has seen certain developments in China; for example, Zhejiang University, Liaoning Energy Research Institute, and Northwestern Agricultural University all have researched and developed the technology and equipment for biomass molding technology.

Biomass gasification technology is a popular direction of the technology of biomass energy utilization. This method changes the shape of the biomass feedstock, and energy conversion has greatly improved compared with the direct combustion of solid biomass [70].

Biomass pyrolysis technology is considered one of the most potent for developing biomass utilization [71]. Biomass liquid fuels with the advantages of easy processing, storage, transport, and easy of utilization for generating heat and electricity have drawn international attention and recognition. In developed countries, biodiesel grew to form an industry. In 2011, the biodiesel production in the United States and The European Union reached about 2.8 million tons and 8.1 million tons, respectively. The European Commission plans to make the biodiesel market share reach 12 % in 2020.

In the past few years, based on lignocellulose, the application of industrial biotechnology in the preparation of high-purity biofuel has achieved significant progress. Fuel ethanol, because of its high octane and antiknock ability, has become the most widely used biofuel; it is also the ideal gasoline substitute and has been widely used in some countries and regions. Biotransformation of cellulosic feedstocks to fuel ethanol has become a hot area of investigation for high-tech research and industrialization. However, the most controversial point for the development of cellulosic ethanol is its high production cost compared with gasoline or grain-based ethanol. Compared with grain ethanol production, the cost of straw ethanol is high in pretreatment and the costs of cellulose, ethanol fermentation, and distillation. In straw ethanol production, the cost of enzyme preparation accounts for 30–50 % (for grain ethanol, it is just 8 %). Meanwhile, the low ethanol concentration causes the cost of ethanol distillation to account for about 20 % (14 % for grain ethanol) of costs. Therefore, how to reduce the production cost of cellulosic ethanol becomes the hot topic and difficult problem at home and abroad [71].

To reduce the production costs of straw biotransformation to fuel ethanol, many research institutions and companies have performed much research. Novozymes Company of the United States and the U.S. National Renewable Energy Laboratory (NREL) have performed years of research, reducing the cost of cellulase to produce 1 gal of fuel-grade ethanol from initially more than $5 to less than 50 cents currently, which greatly advanced the progress of the commercial operation of bio-based ethanol fuel. Novozymes and NREL continue efforts to reduce production costs, and the goal is to reduce the cost of cellulose for production of 1 gal of fuel-grade ethanol to 10 cents. An international biomass fuel ethanol project is the U.S. NREL process and Canada's Iogen process. Iogen Corporation is a major producer of

Canadian industrial cellulase and hemicellulase. In 2003, Iogen Corporation built a cellulose fuel ethanol device of 40 $t \cdot d^{-1}$ (and combined with its enzyme production). Since then, Iogen cooperated with Petro-Canada Corporation to build the world's largest fuel ethanol plant with lignocellulosic wastes as raw materials, expecting the production costs to be reduced to $\$0.24 \cdot L^{-1}$ after adopting new technologies. In northern Sweden, a new ETEK Corporation (Ethanol Teknik) was built. For this company, a total investment for the full set of industrial equipment was €16 million; the production capacity was to deal with 2 t of dry biomass materials per day, producing 400–500 L of alcohol.

In cellulase preparation, Genencor Corporation developed a new enzyme, Accellerase 1000. It contains a variety of effective enzymes for catalyzing lignocellulose into fermentable sugars. Novozymes Corporation identified several new enzymes and configured a new cellulose enzyme complex to improve the degradation of enzymes. In the cellulose saccharification and fermentation process, the simultaneous saccharification and fermentation, simultaneous saccharification and cofermentation process, and the integration of biological processes have been research hot topics in recent years. The best result for a simultaneous saccharification and fermentation process for NREL was more than 80 % of the cellulose converted into ethanol at 38 °C. In product separation, the international focus is not only to develop pervaporation membrane separation technology for the low concentration of ethanol but also to develop coupling of fermentation and separation. In the building engineering, cellulosic ethanol technology began to enter the demonstration phase of industrialization, and the international community has built dozens of sets of pilot production lines or industrial pilot production lines. For example, in 2004 Canadian Iogen built a cellulose ethanol demonstration plant to handle 40 t of wheat straw per day, with an annual capacity of 260,000 gal of ethanol, which is considered to be the largest device currently.

In China, the biomass fuel ethanol project is also developing rapidly. Chen et al. had a unique advantage in various unit operations in the entire technology chain of straw biotransformation to fuel ethanol and made enzymatic hydrolysis of straw and fermentation to fuel ethanol successfully realize industrialization. In 2006, Hongzhang et al. and Shandong Zesheng Corporation built a 3,000-t demonstration project of straw biotransformation to fuel ethanol. In terms of cellulase production, the new solid-state fermentation reaction devices allow the average activity of cellulase using steam-exploded straw as a substrate reached 120 $FPA \cdot g^{-1}$ dry koji, and the highest activity can be up to 210 $FPA \cdot g^{-1}$ dry koji. In terms of the cellulose saccharification and fermentation process, a synchronous system of enzymatic hydrolysis and fermentation coupled with a hollow cellulose membrane was established; in terms of product separation, an ethanol fermentation and separation synchronous system was established. In addition, the straw solid-phase enzymatic saccharification–liquid fermentation of ethanol–adsorption and separation of the triple coupling system was built. The pilot production line achieved stable operation [55].

(2) Bio-based Materials

The use of renewable plant raw materials, such as crops, trees, plants, and their debris and inclusions, through biology, chemistry, and physics methods, to produce bio-based materials and chemicals has become a research hot topic in recent years. The frontiers of materials includes study based on the C_2, C_3, C_4 platform compounds and their derivatives, in particular the study of polymer materials based on these compounds as a monomer; biomass functional polymer materials design, characterization, and compositing; green catalyst preparation and performance evaluation; biological material application study; development of a natural epoxy resin, water-based epoxy resin, and phenolic resin; midstream and downstream process validation of bio-based platform chemicals and biological materials; and so on.

① Steam-exploded straw to produce ecoplate

Use of agricultural residues to produce plate can be traced to the early twentieth century. In 1905, Germany had made plate by mixing straw and adhesive, followed by the United States using wheat straw to produce insulation board. In the 1970s, the feasibility of manufacturing structural panels of straw was studied. Sugarcane bagasse, the earliest material for plant-based plate production, was considered the only agricultural residue used to produce high-quality composite plate. In 1921 in the United States, the first sugarcane plate mill was built. Since the late 1940s, the production of plates with bagasse and stalk crop straw as raw materials has had a different degree of development. The fiber structure of cotton stalk is similar to hardwood, but the content of polysaccharides and extractives is high; thus, performance related to waterproofing and mold of the plate is poor. At present, research most widely deals with wheat straw and rice straw plant-based plate. With the application of isocyanate resin into the straw plate, the glue problem has obtained a good solution. Plate performance has been greatly improved without the release of formaldehyde. In terms of fiber separation, the following studies have been performed: double-screw extruder method, high-pressure hot mill method, extruder coupling with hot mill method, and so on. All are based on exerting strong shear on straw scrap to destroy the surface wax and the silicon compound layer. A light wall plate produced by wheat [72] straw has excellent performance related to light mass, sound insulation, thermal insulation, and so on; thus, it can be used for quick housing and temporary construction. At present, the production line has been established in over 20 countries. But, there are problems in raw material preparation, glue systems, slab transport system, as well as the mold release system.

The Natural Forest Protection Project does not allow the consumption of a large number of natural forests, which also provides an opportunity for the application of straw ecoplate. Producing ecoplates and building materials by biomass is an effective high-value conversion and can maintain the ecological balance while meet the needs of plate.

Existing technologies using straw to produce new materials in domestically and internationally generally do not do any processing on natural straw, only a physical smashing processing; some chemical thermosetting resins (such as formaldehyde)

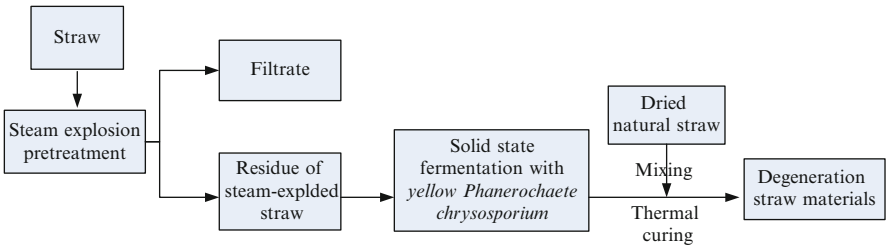

Fig. 4.2 Processing route of steam-exploded straw preparing denatured straws [73]

and other additives are needed to control the dimensional stability of the materials and to produce the plant fiberboard, plant fiber packing materials, disposable tableware boxes, and so on. The use of a large number of chemical adhesives and additives not only results in the increase of material costs but also mainly leads to a pollution problem. In terms of taking crop straw without adding adhesive to produce building materials, a current report in the literature was that straw received simple heat treatment first, in a thermal curing process; only using the hydrolysis sugar but not the adhesive role of lignin, the cellulose performance did not improve. Thus, the product cost was high, performance was unstable, and there was no industrial production.

The development direction for the eco-material is for it to be "completely biodegradable and low cost." Although straw is an abundant renewable and low-cost resource, there are many problems for straw preparation of eco-materials at home and abroad. According to the issues of adhesive contamination, nonuniform sizing, and performance instability in the plate industry, it must be improved from the root and the use of environmentally friendly green adhesive promoted and new technology for straw plate production (such as steam explosion technology) developed to meet industrial demand. Based on the similarities and differences of the chemical composition and structure of straw and wood, Chen and others [73] selectively fractionated cellulose, hemicellulose, and lignin, proposed the new concept for denatured straw, developed a set of straw degeneration technologies, and proposed clean production eco-materials methods for denatured straw. Straw denatured technology has solved the difficult machining of natural straw; also, the thermal curing process is without addition of adhesive, only controlling a certain moisture content to make the hydrogen bond rearrange in the cellulose. The process not only can improve the mechanical strength of the eco-materials but also solve the problem of secondary pollution. The process route is shown in Fig. 4.2.

Denatured straw is natural straw that has changed its structure and properties by physical, chemical, or biological methods. Cellulose, hemicellulose, and lignin are degraded effectively to generate thermal cured bioresin plastic to make straw fiber self-adhesive to meet the molding needs of construction plate. In steam-exploded straw, the active groups in lignin increase, have properties similar to the

polyphenols, and can be used as a natural adhesive. Hemicellulose is degraded into soluble sugar, dehydration sugar, and furfural; it can replace formaldehyde to react with lignin. In the process of steam explosion, cellulose is not degraded, but the degree of crystallinity increases [74].

② Biomass fiber/plastic composites

In recent years, with the increasing requirements for material performance, it has been difficult for a single material to meet the requirements. Therefore, preparing the coexistence of good comprehensive performance of multicomponent composite materials is an important direction of research and development in the domestic and foreign materials field. At present, research on natural fiber composite processing technology mainly focuses on the following areas: (a) biodegradable blend composites based on lignocellulose and its derivatives; (b) polymer materials with the lignocellulose component as the main raw material; and (c) biomass material processing technology.

Compound modification, because of its richness in raw materials and simple means and methods, has achieved good progress. The materials blended with cellulose include both natural polymers and synthetic polymer materials. Natural polymers for blending include protein, chitosan, and starch. In addition, the study of cellulose and the synthetic polymer interpenetrating polymer network (IPN) has been reported; polyurethane/benzyl konjac glucomannan (PU/B-KGM), polyurethane/nitrated lignin, and polyurethane/nitrocellulose can be prepared to a series of biodegradable films with excellent performance as materials that are waterproof and have good mechanical properties and optical transmittance. In terms of biodegradable materials, polyethylene glycol (PEG), polycaprolactone (PCL), and polylactic acid blend with cellulose to prepare multifunctional materials.

Biomass fiber/plastic composite materials, namely, WPC (wood-plastic composite), are a new type of environmentally friendly composite materials based on wood fiber or plant fiber as reinforcements or fillers, blended with thermoplastic resin (PP, polyvinyl chloride [PVC], etc.) or other composite materials. Because the advantages of both wood materials and plastics are combined, industrialization began in the United States in the 1980s. The main application areas are in construction products, infrastructure, the transportation industry, and daily necessities. The key for manufacturing high-strength and low-density biomass fiber/plastic composite material is to reduce the density of the material while maintaining the physical and mechanical properties of the material [51] and reaching the light requirements of polymer materials. This has become a new topic in the field of biomass fiber/plastic composite materials. After foaming, biomass fiber/plastic composites not only have reduced density, but also have improvement in some physical and mechanical properties; these unique advantages provide great potential for applications in many fields. It can be used for lightweight and high-strength parts, sports equipment, and insulation materials in the packaging and construction industries.

In the 1990s, people began to study the natural fiber-reinforced composite materials at home and abroad. Foreign research of natural hemp fiber composite

materials and their products started early, and there is a wide range of industrial application examples. In China, the National University of Defense Technology, Sun Yat-sen University, Northwest Textile Institute, and so on have performed research on hemp fiber-reinforced polyurethane and unsaturated polyester resin but have not seen industrial applications. The heterogeneity of natural fibers and their incompatibility with organic synthetic polymer limit their application in the field of composite materials. Natural fiber can be effectively modified by surface treatment, graft copolymerization, and the interface coupling methods to improve combination with the polymer resin. In recent years, India and China's Sun Yat-sen University studied the impact of alkalization, acetylation, and cyanoethylated modification of mechanical properties and electrical properties of sisal and epoxy composites. The National University of Defense Technology researched the mechanical properties of epoxy composites reinforced with jute fiber (cloth) compared with glass fiber-reinforced resin composites and explored the application of hemp fiber-reinforced resin composites. Natural fiber-reinforced amino resin is a thermosetting plastic with excellent electrical insulating properties, heat resistance, and flame resistance and good mechanical strength; thus, it has been considered a superior alternative for synthetic plastics in a certain range.

Processing technologies for polymer plastics include molding, extrusion, blow molding, injection molding, and so on. Usually, synthetic polymer material has a relatively regular molecular structure and morphology. But, the structures of lignocellulose and other natural polymers are often affected by the source of raw material, season, geography, and so on, leading to complex and changeable processing performance. Lignocellulose polymer materials must be modified before universal molding, extrusion, blow molding, injection molding, and other processing to improve plasticity, rheology, and liquidity.

Microcellular foam technology in recent years has developed from the single-polymer foam to composite microcellular foam, but research on biomass fiber/plastic composite foam started relatively late. At present, the majority of foaming biomass fiber/plastic composite is PVC-based WPC materials, mainly utilizing the extrusion foaming process. The foaming process for biomass fiber/plastic composites is basically divided into two categories according to foaming method: physical foaming and chemical foaming. Development of biomass fiber/plastic composites is the result of increasing environmental issues. The wide range of sources of raw materials, cheap production costs, increasingly improved performance, and environmental friendliness make these the preferred alternatives to wood and plastic, and they will play a broader role in more areas.

The overall structural modification (fractionation) of the straw can improve the plastic nature of the straw materials and the compatibility of straw-modified materials and the plastic compound, which is expected to improve the dosage of straw and reduce the cost of WPC production.

(3) Bio-based fertilizers

① Biofertilizer technology

In agriculture, since long ago, farmers have known that feces and urea, waste garbage, and discarded leaves and other materials can be converted into fertilizer through composting. These fertilizers can provide plenty of soil humus and nutrients. Its main roles are the following:

(a) It improves the physical properties of the soil, making the soil soft and porous and increasing its water retention properties.
(b) The cation exchange capacity of humus is much higher than fore clay; thus, N, P, and K elements and other nutrients can be adsorbed by humus, enhancing the ability of nutrients to be maintained in the soil.
(c) The chelation of some ingredients in humus can reduce the hazards of heavy metals on crops.
(d) Compost is a slow-released fertilizer. Nitrogen in the compost almost exists in the form of protein. When it is released to the field, the protein slowly breaks down into ammonia or other forms of salts available to the crops.
(e) Compost is rich in microorganisms. Compost can increase the microbial content of soil. A variety of active ingredients produced by microorganisms can be directly or indirectly available to plants and play a useful role.

Although composting has many advantages, since the 1960s, with increasing demands for food caused by the growth of China's population, chemical fertilizer has played a key role in agriculture. Large-scale use of chemical fertilizers not only caused environmental pollution but also brought about serious soil compaction and soil fertility decline. Therefore, converting garbage, agricultural and forestry wastes, a variety of organic sludge, as well as human and livestock feces into compost-based materials and preparing highly efficient organic compound fertilizers through various means have significance in both agriculture and environmental benefits.

Meanwhile, in rural areas, a large number of crop stalks, such as wheat straw, rice straw, cornstalks, and other fiber crops, are stacked in the fields or combusted in situ combustion, which not only induces fires and seriously pollutes the environment but also wastes resources. Therefore, in recent years, the comprehensive utilization of crop straw has become a hot topic for scientists around the world. In recent years, to solve problems of soil compaction and nutrient depletion of the land caused by excessive use of fertilizers and pesticides, many countries adopted bioorganic fertilizer, such as yeast organic fertilizer, EM microbial organic fertilizer, HM microbial organic fertilizer, and so on. According to the determination of the National Collection of Type Cultures, various types of microbial organic fertilizers in parts of China actually are a variety of microorganisms, and multiple microorganisms combined with organic matter (such as feces, weeds, straw, etc.) can be fermented to generate organic ecological fertilizer. The main miocroorganisms in various types of composite microflora are roughly the same, about 80 % of the yeast group and lactic acid bacteria, actinomycetes, bacillus, fungi, and photosynthetic microorganisms, with more than 80 kinds of microorganisms.

② Solid-state fermentation of composite microflora to prepare sand-improved fertilizer

Land desertification is one of the most serious ecological and environmental problems in China. Although desertified land management and improvement efforts have increased in recent years, a stalemate of management and desertification remains. During the 1990s, China began using straw to manage the desertified land. Straw is buried to form a sand prevention barrier or covered to prevent sand. It has not fundamentally solved the problems; thus, this application is a limited supporting measure.

Drought and water shortage, damage of vegetation, and poor soil texture are the main driving force for land desertification, therefore, scholars recognized that improving soil texture, increasing the ability to conserve water, and vegetation construction are the fundamental measures to combat it. The application of chemical materials has become a hot topic; however, the cost is too high, and absorptivity of plants for chemical improvers is limited. Long-term application not only will pollute the groundwater but also will cause soil compaction. In this situation, the research for highly absorbent materials that enhance soil fertility and are biodegradable becomes increasingly urgent.

Straw is the world's most abundant renewable resource. According to statistics, annual straw yield is over 2.9 billion tons worldwide; in China, crop straw yields over 700 million tons per year. Straw is rich in elements; generally, carbon accounts for 50 %, hydrogen 6 %, oxygen 44 %, and nitrogen 0.05–0.4 %. Therefore, the role of straw in controlling and improving land desertification has gradually drawn attention again. From existing research, the test result for straw as the main raw material for improved materials for desertified land was not weaker than chemical materials, and compared with simple chemical materials, this kind of improved material has the advantages of easily obtained raw materials, low cost, long duration, and so on.

The existing pretreatment process of using straw to prepare improved material for desertified land is not ideal. It causes environmental pollution and demands additional inorganic fertilizer, humic acid, nutrients, and other ingredients in the process, resulting in a high application cost. Based on these issues, Chen et al. [75] developed a new method to prepare desertified land improvement materials by solid-state fermentation of the steam-exploded straw. By this new method, the straw texture and characteristics were improved, the effectiveness of biofertilizers increased, and technology costs of land desertification prevention and control were reduced the process route is shown as Fig. 4.3.

According to detection, compared with purely mechanically crushed straw, steam-exploded straw had higher water absorption (32–40 % more than reference substance), and the water retention rate was 80–100 % higher than the reference. After solid-state fermentation, materials had the efficacy of biofertilizer and resistance to plant diseases. After fermentation, in the desertified land improved materials, the organic matter content was higher than 60 %; humic acid content was

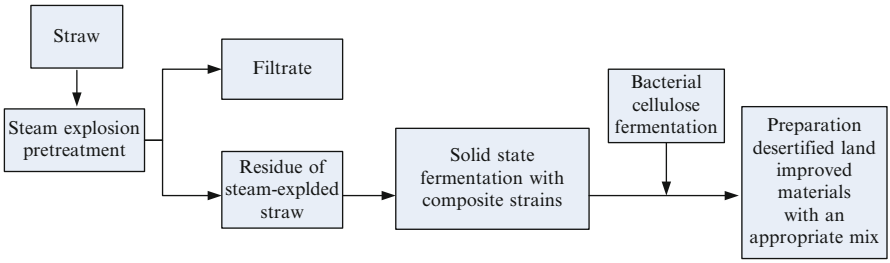

Fig. 4.3 Process route for preparation of improved straw materials of desertification land

higher than 15 %; the total number of effective viable microorganisms was higher than 300 million per gram; and the nitrogen, phosphorus, and potassium content was higher than 5 %.

(4) Bionic feed

Bionic feed is also called artificial rumen feed. The principle is to mimic the environment of cattle and sheep rumen to use an artificial method to degrade cellulose and increase the amount of crude protein by beneficial microbial fermentation. The nutrients of crop stalks through bionic treatment can be improved greatly. The texture becomes soft and sticky, and the feed has a sour flavor; thus, animals like to eat it. For fermented straw, 15–20 % (up to 35 %) of crude fiber is broken down, true protein is increased by 50 % or more, and it contains 18 amino acids. Crude fat is increased more than 60 %, and volatile fatty acids are also significantly increased. The straw bionic feed for cattle, pigs, chickens, ducks, and fish can replace 50–80 % of concentrated feed, and the feeding effects are not lower than normal feeding levels [76]. At present, there are mechanized and semimechanized large-scale fermentation facilities in domestic use. They can prepare 1,500 kg bionic feed at once. Stirring, feed control, and feed mixing in the fermentation process are all controlled by the machinery [77].

(5) Bio-based chemicals

In 2004, the U.S. Department of Energy ranked polyols such as glycerol and sorbitol as one of the most important 12 kinds of "building block" molecules in the development process of biomass. Traditional bio-based polyols are first to hydrolyze cellulose to glucose by chemical or biological methods, further converting it into energy or organic chemicals. At present, research on lignocellulosic feedstocks converted to the polyol substances has mainly been based on cellulose as a raw material. Chemical catalytic hydrogenation of straw can create a targeted product containing hydroxyl, which is the process with high atom economy, and has been given attention by international and domestic research in recent years. However, because straw has low carbon content, low energy density, and heterogeneity, the process of the straw conversion to polyol is much more complex than that of

pure cellulose. The traditional process of biomass hydrolysis to glucose and then the conversion is long, conversion efficiency is low, and environment pollution is a result. However, gasification, pyrolysis, and liquefaction tend to provide whole transformation of raw materials. In this process, all the sugars in the raw materials are reduced to the corresponding sugar alcohol, resulting in separation difficulties of the target product.

Therefore, future research will primarily focus on the following aspects:

① In cellulose, there are a large number of hydroxyl groups and strong hydrogen bonds, easily generating a supramolecular structure and resulting in difficult solubility of raw materials in most solvents. How to effectively destroy or weaken the hydrogen bonds in the reaction system is a common problem in the biomass-based conversion process.

② The catalysts of cellulose to prepare sugar alcohols are mainly inorganic acids, but the pollution by such catalysts is large scale and has a dramatic effect on the environment. There is also a precious metal catalyst, but it increases raw material costs. Therefore, based on the C–O–C bond catalytic breaking mechanism, comparing the reactivity of the glycosidic bond in neutral medium and acidic medium and designing and preparing highly efficient, highly selective, nonpolluting catalytic system may achieve the rapid depolymerization of cellulose under mild conditions.

③ Selective breakage of the C–C bond and the C–O bond. The process of cellulose conversion to ethylene glycol and propylene glycol includes a hydrocracking process of the C–C bond and C–O bond. These bond energies are large, and the requirements for product formation for the cracking type and location of bonds are high; thus, a highly active catalyst system must be designed and prepared. However, the high catalytic activity will generate methane, methanol, and other by-products, resulting in decreasing selectivity. Therefore, the design and development of a catalyst system to make the C–C bond and C–O bond effectively crack, with selectivity of region and location, are the core and keys for this direction of research.

4.5.3 Development Tendency of Selectively Structural Fractionation Technology

China is a major importer and consumer of fossil resources, but it is also a big country of biomass resources. Exploiting the theory and technology of conversion of biomass resources and developing the next-generation refining process for the biological and chemical industries with lignocellulosic biomass resources as the raw materials are the important tasks of the biorefining industry in China. These also are the forefront and focus of international academia and industry concerns.

Creating a new industrial model based on biomass as the common raw material must break through the key scientific problems that constrain biomass from

becoming a new generation of raw materials for the biological and chemical industry based on the engineering point of view. Based on basic science research and the ecological concept of a circular economy, developing a refining technology for the original structure and function for the biomass retained and establishing the process of the lowest atom utilization and energy consumption can make biomass become the common raw material for bio-based energy, materials, and chemicals. To achieve this aim, the following three key scientific problems should be addressed:

(1) Relationships of structure fractionation and function utilization of biomass feedstock
Biomass is function of a macromolecule's body with low carbon content, low energy density, and a high degree of heterogeneity. The different structure fractionation modes will produce different functional components. Therefore, clarifying the relationships between the structural fractionation and functional utilization of biomass feedstock and improving the level of high-value utilization of biomass become the first of the key scientific issues for biomass as a common industrial raw material.

(2) Relationships between structural fractionation of raw materials and heterogeneous catalytic reactions
Lignocellulosic biomass has a dense and impermeable high-level structure, so direct catalytic conversion is difficult and affects the performance of the transformation products. Selectively structural fractionation can avoid the high cost and pollution problems in the separation and conversion process of existing components. However, people must recognize the relationships of structural fractionation and selectively catalytic reactions, characteristics of catalytic conversion reaction rules based on structural fractionation, which is the second key scientific issue for developing the new-generation refining system for raw material for the biological and chemical industries.

(3) Optimization principles of refining based on efficient low-cost raw materials
Because of the complex structure and high degree of heterogeneity of raw materials, the utilization of straw must be a process of multistep, overall processing and respective utilization belonging to the cluster reaction system. The refining process for biomass feedstock must be based on minimum energy consumption, the best efficiency, and the maximum clean conversion. Selectively structural fractionation and product functional conversion are the new approaches to achieve the most economical and most effective targets. It is also important to recognize the cluster reaction mechanism in the biomass refining system and analyze the refining system for complex solid material and the matching principle for each system; these are the third of the key scientific issues in efficient refining of biomass feedstock.

Cellulose, hemicellulose, and lignin in the lignocellulose cross-link into a complex three-dimensional network structure. The existing research for single-component or pure cellulose utilization has the essential differences between the reactions of biomass solid-phase complex components. Selective deconstruction, interaction, and control of the target product of biomass components is a new field of

chemical research. Therefore, understanding the structural properties of the biomass and biotransformation structure-activity relationship is the critical foundation for a new-generation refining process for raw materials in the biological and chemical industries.

The functional high value of biomass belongs to the raw material refining cluster reaction system, based on the most economical and most effective procedures and methods to achieve the transformation of raw materials. Therefore, recognizing the cluster reaction mechanism in each process of the biomass multistage directional conversion refining system and analyzing the refining system based on the full utilization of biomass solid materials and the matching coupling principle of each process are the keys to achieve efficient refining of biomass feedstock. The major scientific issues involved include process interrelation of the large-scale, multilevel, multiscale, and high-value conversion and exploring the energy conversion and transfer rules in the biomass process industry based on systems engineering principles and methods. On this basis, the major scientific issues also include analyzing the economic and technical feasibility of the functional process and summarizing the biomass cluster reaction mechanism and the universal law to achieve the integrated optimization of biorefining.

Taking bioethanol as a model product, the research focus is on the coupling effects and optimization principles of hydrolysis and the biodegradation process of refined biomass feedstock and analysis of the heat and mass transfer laws and the reaction mechanism in the bioreactor to establish the matter and energy transfer law and coupling process optimization principle of the continuous process that integrates straw hydrolysis, fermentation, and product in situ adsorption and separation.

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Chapter 5
Applications of Lignocellulose Biotechnology in Ecological Agriculture

Abstract When people are enjoying the convenience brought by sustained economic development, they are also faced with the enormous pressure from population, resources, and the environment. Agricultural waste is an important biomass resource whose main component is lignocellulose. As a by-product of agricultural production and renewable resources, utilizing the numerous resources legitimately not only can meet the needs of agricultural production but also can effectively reduce the production of environmental pollutants and damage to ecology. With the improvement of the level of economic development and science and technology, lignocellulose will play an increasingly important role in the improvement of the agricultural ecological environment.

Keywords Ecological agriculture • Single-cell protein • Biofertilizers • Biological pesticides

5.1 Bio-feed

5.1.1 Single-Cell Protein

Single-cell protein (SCP), also known as microbial protein, is microbial thallus cultivated artificially from industrial and agricultural waste and oil waste. Thus, the SCP is not pure protein, but the cytoplasm consists of protein, fat, carbohydrates, nucleic acids, nonprotein nitrogen compounds, vitamins, and inorganic compounds.

5.1.1.1 Significance and Research Advances of SCP Production from Lignocellulosic Materials

With the development of the aquaculture industry, the shortage of feed protein becomes increasingly acute. To alleviate the contradiction between supply and

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demand of feed protein, since the 1960s many countries and regions have been committed to research and application of SCP and have made gratifying progress. In recent years, with the increasing demand of SCP, there has been a shortage of raw material for SCP. Since the 1990s, the production and research of SCP revealed some new trends: The key point of the SCP production and research focus is on the search for cheap raw materials to reduce economic costs. The raw materials for SCP fermentation and production are abundant and mainly in the following four categories: The first category is industrial and agricultural waste, including wastewater from pulping and paper, glutamate production, tofu making, the sugar industry, beer production, and organic chemistry industry. The second category is industrial residue, including residue of orange, potato, cassava, grape, and sugarbeet; and the residuals of sugar making and vinegar and cornstarch production. The third category is petroleum resources. Fourth is cellulose raw material, which is available in a great amount, including agricultural and forestry wastes [1, 2].

China is a big agricultural country and ranks first around the world for average annual output of crop straw. The average annual output of crop straw, which is the most easily obtained biomass resource, is about 700 million tons in China. However, as the main component of biomass, cellulose is difficult to degrade; its development and use are limited for a long time, and most of its fate is burning or returning to the field. To obtain fully reasonable and effective use of the natural cellulose resources, many countries have been dedicated to the study of the various processing methods for resources. Among them, production of SCP has become a potential orientation [3]. To produce SCP from cellulosic raw materials not only can save many foods, relieve protein shortage, reduce feed costs, and promote revolution of the feed industry, but also promote the development of aquaculture to increase the output of livestock and poultry products, improve people's diet structure, and finally enhance people's living standards. Meanwhile, the use of cellulosic biomass can curb the burning of straw and other agricultural waste, reduce environmental pollution, protect the ecological environment to form a virtuous cycle, and ultimately achieve the sustainable development of agriculture and society. Therefore, the use of agricultural waste and other renewable resources to produce protein feed is imperative.

The history of human production of food by microorganisms has existed for thousands of years, and the modern culture of microorganisms as food was begun in Germany. As early as 1890, the Germans first found the possibility of microorganisms as a direct human and animal nutrition protein resources, and in 1915, the industrial production of edible yeast using molasses as the raw material was realized. After World War I, many countries began to study the production of feed yeast. After World War II, to ease the food deficit, edible yeast production played an important role in relieving postwar food shortages in Germany and the former Soviet Union, and SCP entered rapid development. In 1967, an international conference on SCP was held at the Massachusetts Institute of Technology, and SCP was verified there as one of the important ways to solve food and feed problems [4]. The research institute from the U.S. Department of Agriculture selected a microbial strain that could not only fix the nitrogen in the air but also use the crop straw fibers

as the sole carbon source, and the protein content of the straw after fermentation increased three to four times. Researchers from the University of Aston in the United Kingdom screened a white-rot fungus, which made straw digestibility increase from 9.63 to 41.13 %; researchers from the United States cloned both xylanase and cellulase gene into the yeast cell to construct engineering bacteria that could directly use the straw and other crop stalks to produce SCP feed [5].

For the selection of a microbial strain to produce SCP feed from crop straw fermentation, researchers prefer to adopt a mixed-microorganism fermentation system [6]. Chen et al. used a solid-state mixed-microorganism fermentation system to allow crop residues such as cornstalk and wheat straw transform into animal feed directly [7]. Zhang et al. prepared SCP by grain fermentation with a mixed-microorganism system consisting of *Geotrichum, Candida tropicalis,* and *Trichoderma,* and the crude protein content in the fermentation products was up to 32.1 % [8]. Li et al. [9] studied the biological conversion of grains into high-protein feed by a two-strain fermentation system using the primary strain JZ-1 and the auxiliary strain JZ-2.

5.1.1.2 Strains for SCP Production

Microbial strains for SCP production are currently divided into four major categories: nonpathogenic and non-toxin-producing yeast, bacteria, fungi, and algae. The selection of strains should be changed according to the different raw materials and give priority to the following conditions: high yield of bacterial cells, protein, and other nutrient content; simple culture conditions for rapid growth and reproduction; product that is nontoxic, tasty, and digestible for feeding animals.

5.1.1.3 SCP Production from Cellulose

From the perspective of technology, SCP production is achieved by cultivating microorganisms using lignocellulose as a substrate and obtaining a large number of bacterial cells. Because of the complexity of the lignocellulose structure, the SCP production process using lignocellulose as raw materials generally includes the pretreatment of raw materials, fermentation, bacterial cell recovery, and protein extraction. The pretreatment process has been mentioned, and the microbial cells can be obtained by liquid fermentation or solid-state fermentation. The bacterial cells obtained from the fermentation go through a follow-up process, such as cleaning, cell disruption, protein extraction and washing, and the final SCP production [10].

There are three ways to produce SCP using lignocellulose: The first is to saccharify the raw materials by cellulase and hemicellulase and then to produce SCP by fermentation of the yeast and other microbes. The second is simultaneous saccharification and fermentation to produce SCP using cellulose- and hemicellulose-degrading bacteria and yeast. The third is to produce SCP using a hydrolysate of cellulose and hemicellulose [11]. In actual industrial production,

the choice of fermentation often depends on the strain characteristics, material characteristics, product characteristics, equipment status, technical feasibility, and cost accounting. Currently, production of SCP feed using lignocellulose as raw material employs solid-state fermentation of mixed bacteria according to the characteristics of the materials, which means simultaneous saccharification and fermentation in the same container by the fermentation of yeast and microorganisms that can produce cellulase. Strains are generally divided into two categories: one is various genera of mold, such as *Aspergillus* spp. and *Trichoderma* spp. Individual bacteria and actinomycetes, which can decompose the lignocellulosic materials, also show potential value for application. The other category is various genera of yeast, which mainly feeding yeast, *Candida utilis*, *Candida tropicalis,* and *Candida parapsilosis*. In the process, the sugars from the enzymatic hydrolysis of cellulose can be used for fermentation immediately without accumulation and eliminate feedback inhibition to the saccharification response and finally improve saccharification and fermentation efficiency. Increasing attention has been paid to the process because of its advantages of simple operation, low investment, and energy consumption [2, 12].

5.1.1.4 Hemicellulose and SCP

Hemicelluloses are main chemical components of raw materials from plant fiber. They can be not only homogeneous glycan and heterologous glycan but also various glycan consisting of different monosaccharides with different linkages among the monosaccharides [13]. According to the different proportions and different substituents, hemicelluloses are composed of the different sugar units, which include D-xylose, L-arabinose, glucose, galactose, D-mannose, glucuronic acid, 4-O-methyl-glucuronic acid, galacturonic acid, traces of rhamnose, fucose, and O-methylation neutral sugars. These structural units prefer to form heterogeneous glycans by 2-4 linkage of various structural units rather than forming homogeneous glycans by identical structural units when they constitute the hemicelluloses [14].

The content of hemicelluloses is a fourth to a third of the total amount of plant resources; for example, the content of hemicelluloses is about 28 % in cornstalks, 34.9 % in barley grass, and 35.8 % in rice straw [13]. Therefore, hemicellulose is a rich and inexhaustible source from renewable plant resources.

Hemicellulose is an important component of cellulosic materials. Steam explosion technology, which not only can improve enzymatic hydrolysis of cellulose but also recover the hemicelluloses, can be used to extract the water-soluble hydrolysis materials of hemicelluloses from the steam-exploded materials and then produce SCP, alcohol, and other products that are urgent for human beings [15]. Making full use of the hemicellulose is one of the keys to fully utilize biomass [16]. Currently, fermentation production of xylitol [17], 2,3-butanediol [18], SCP [15], and ethanol [19] uses pure xylose as a substrate at home and abroad. Submerged liquid fermentation for SCP production emits large quantities of organic wastewater

and seriously pollutes the environment. For this reason, Chen et al. from the Institute of Process Engineering (IPE), Chinese Academy of Sciences (CAS), studied high-density fermentation using hemicellulose hydrolysate from steam explosion for SCP production to achieve the lowest energy consumption and wastewater emitted in the cell separation process and finally achieve cleaner production [1]. In the experiments, *Trichosporon cutaneum* 851 strains were used as the fermentation strain; the aqueous extract from the steam explosion of wheat straw (1.5 MPa and 10 min) was used for the medium with the addition of an appropriate amount of N sources, S source, and other trace elements to produce SCP. The repeated fed-batch fermentation experiment was carried out in a 2-L automatic fermentor with mechanical mixing and a cell dry weight of 45 $g{\cdot}L^{-1}$; a production rate of 4.4 $g{\cdot}(L{\cdot}h)^{-1}$ was obtained in the experiments.

5.1.1.5 Production Instance of SCP Fermentation

(1) Yeast production using hydrolyzate of plant fiber
This technique is an indirect conversion process. The cellulose and hemicellulose are first hydrolyzed into sugars by acid or enzyme; after that, the hydrolyzate is used to produce SCP by yeast fermentation. Zhou et al. used sorghum straw as raw materials that have been hydrolyzed by cellulase to produce SCP by *Saccharomyces cerevisiae* fermentation. The fermentation process includes raw material smashing, burdening, sterilization, enzymatic hydrolysis, and fermentation [20]. Although SCP produced by fermentation of straw hydrolyzate has a higher nutritional value, its production is difficult to industrialize because of the high cost of the process.

(2) Feed protein production from liquid fermentation using straw as the substrate
Cellulose-degrading microorganisms not only produce cellulase and hemicellulase to degrade cellulose and hemicellulose in the growth process but also utilize inorganic nitrogen to synthesize bacterial proteins. Therefore, the fermentation processes of cellulose-degrading microorganisms can transform the straw into protein feed. Wu et al. studied the production of SCP by mixed liquid culture technology using bagasse as the only carbon resource. The results showed that hybrid strains of *Rhizopus nigricans* and *Trichoderma* sp. showed better decomposing activities in the mixed fermentation of *Cellulomonas* sp., *Trichoderma* sp., *Rhizopus nigricans,* and *Saccharomyces* sp. The crude protein content of the dry fermentation product was 260 $g{\cdot}kg^{-1}$ under cultivation at 32 °C for 108 h using bagasse without any treatment [11, 21].

It can be found from the fermentation process discussed that when adopting liquid fermentation, by directly using the straw as substrate and inoculating cellulose-degrading bacteria to produce protein feed, the fermentation conditions are easier to control and are suitable for microbial growth, and the product has a higher nutritional value. However, liquid fermentation produces large amounts of wastewater after harvest of the fermentation product, and the specific fermentation

method requires strict pH, temperature, ventilation, and mixing control. Therefore, the high cost of liquid fermentation for SCP production leads to a lack of competition in the economy.

(3) SCP production by solid-state fermentation
Solid-state fermentation has advantages of less investment in equipment, ease of operation, applicability to small- and medium-size enterprises, and suitability for fermentation of various residues from the food fermentation industry. Wang et al. used steam-exploded cornstalk to produce SCP by double-strain mixed solid-state fermentation of *Trichoderma reesei* 96-32 and *Candida tropicalis* Y-19; the protein content of the product was 31.82 %, the cellulase activity reached 105 $U \cdot g^{-1}$, and the cellulose enzymatic hydrolysis rate was 56.88 % [3].

Because of the difficulty of mass transfer and heat transfer in the scale-up process of traditional solid-state fermentation, Chen and Li developed a solid-state fermentation reactor with two dynamic changes of air, which controlled the gas phase by cyclical changes of sterile air pressure to strengthen the transfer effect of gas and heat and realized pure culture of solid-state fermentation. The original fermentation equipment can achieve the scale-up cultivation with a little improvement, and the culture methods are basically the same as the traditional training methods.

5.1.2 Artificial Rumen Fermentation Feed

1. Preparation mechanism
The preparation mechanism for artificial rumen fermentation feed is based on the principles that the microbial flora in the rumen of ruminants can absorb and digest roughage such as straw, hay, and vines and artificially simulating the ecological conditions of the rumen for microbes to convert roughage with a high content of crude fiber into absorbable feed for monogastric animals. After crushing, alkalization, inoculation with rumen microorganisms, addition of a nitrogen source and other nutrients, and an easy fermentation process, the crude fiber is degraded into small molecules to synthesize protein, fat, and vitamins and other nutrients that are easily absorbed. This feed is soft, thick, and dark brown or dark reddish brown and provides better breeding effects because of the palatability to livestock and strong adaptability [22].

2. Raw materials and additives for artificial rumen fermentation
Various raw materials suitable for preparing roughage can be fermented to artificial rumen feed by grinding. Certain external conditions are required for the growth and reproduction of normal rumen microbes, such as favorable nutrients and a stable pH. Necessary carbon sources are provided by the raw materials, and the

remaining deficiencies must be removed by addition of an appropriate amount. Urea or ammonium sulfates are commonly used as nitrogen sources, alkaline chemicals are used as the alkali sources, and alkaline and acidic phosphate are used as buffers [23].

3. Strain preparation
Rumen contents and rumen fluid are the microbial sources for artificial rumen fermentation. There are three kinds of methods to obtain the rumen contents: ① Suck 1.5–2.5 kg inoculum from the rumen by a stomach tube according to the siphon principle. Feed the right amount of fine material and high-quality legume forage 3–5 days before sucking the inoculum from the rumen. ② Obtain rumen contents or rumen fluid directly from healthy cattle and sheep butchered in the slaughterhouse. ③ Obtain contents from a rumen fitted with a permanent gastric fistula.

The inoculum should be prepared in the solid state to make it easy for preservation, transportation, and manufacturing, which are conducive to promotion and application. The processes involve removing the grassroots and drying the inoculum in a 760-mmHg vacuum oven at 40 °C and crushing [23].

4. Production process
Currently, there are many reports on the artificial rumen fermentation feed process. The fermentation process and a simple fermentation tank for a farmer's homemade rumen feed are introduced as follows:

① Simple fermentation pool for rumen feed
Generally, the simple fermentation pool is made of cement, sand, and brick as well as a large barrel, drum, and stone cylinder. There are ridges on the wall of the pool and a cover on the pool to seal it. The fermentation pool should be solid and firm, and there should be no leakage of water and air for the bottom and wall of the pool. A simple stirrer and a pH controller should be set in the fermentation pool and the pH adjusted using alkaline buffer solution such as ammonia or lime supernatant. More than two fermentation tanks can be built for each household to expand subculture fermentation by turns [22].

② Process for farmer's homemade rumen feed
The simple process is as follows: Raw materials → Pulverization → Alkalization → Fermentation → End products.

5. Identification of quality
Sensory identifications such as seeing, smelling, and touching are employed generally to identify the quality. The fermented feed has a denser rumen content smell after opening the cylinder and is dark yellow or dark reddish brown. The dry feed material is floating in the upper cylinder; the thinner material is in the lower part, and the fiber softens when touched by hand [22, 23].

5.1.3 Silage Feed

The preservation technology that makes silage maintains the quality of green feed stably for a long time by fermentation in a sealed condition [24]. The raw materials of silage can be the whole plant or a part of the plant. This feed can effectively reserve the nutrients of the green plant to ensure freshness, palatability, and high digestibility, which is a comparatively good feed, particularly for ruminants such as cattle and sheep.

The principle of silage preparation is to convert the sugars in the raw materials into lactic acid to form an acid environment by anaerobic fermentation of lactic acid bacteria in the raw materials and the air. The acid environment will decrease the pH of silage materials to below 4.2 and inhibit and kill various harmful microorganisms to save the feed for long-term usage. To meet the requirements of lactic acid bacteria fermentation, certain soluble sugars are needed in addition to the guarantee of anaerobic and water conditions [25].

In the process of creating silage, because of the effects of raw material, weather, and other factors, it is difficult to ensure the quality of silage and the successful silage process. To accelerate the silage rate and improve the quality and utilization of silage, the addition of appropriate additives is an extensive and effective method for modulation of silage feed [26].

Types of silage additives are divided into four categories: fermentation inhibitors, nutritional additives, fermentation accelerants, and inhibitors of corruption: ① Fermentation inhibitors, such as formic acid, formaldehyde, and so on, partly or fully inhibit the growth of microorganisms to reduce the loss of nutrition in the fermentation process when the silage fermentation is carried out to a certain extent. ② Nutritional additives, such as urea and minerals, mainly improve the nutritional value of silage and generally do not show any promoting function for fermentation. ③ Fermentation accelerants, such as lactic acid bacteria, sugars (soluble carbohydrates), and enzyme preparation, produce more lactic acid to speed up the silage process by enhancing the activities of the lactic acid bacteria. ④ Inhibitors of corruption, such as propionic acid and formate, prevent silage corruption by secondary fermentation (bacteria refermentation) [13, 26, 27].

5.1.4 Microbial Silage Feed

Silage, which belongs to natural fermentation using microorganisms in nature, has strict demands on time and environment as the operating season is short and cannot effectively control the microorganisms in nature. Therefore, there are certain restrictions to popularize silage. Compared with silage, microbial fermentation is directional fermentation. The active microbial strains are added in the fermentation process to become the dominant strains in the early stage of fermentation and inhibit the growth of harmful microorganisms to stabilize feed quality [28].

Microbial silage feed production technology is easier to learn and popularize compared with traditional feed silage. The specific steps generally include the following: ① Straw for microbial silage is cut into 3–5 cm pieces for sheep feed and 5–8 cm pieces for cattle feed. This process makes straw compress easily and improves the utilization of the microbial silage cellar to ensure the quality of microbial silage feed production. ② Resurrection of microbes for straw fermentation occurs by dissolution in sugar water. ③ The microbial agents are mixed with straw simultaneously to install the mixture into the cellar, spraying some risen bacteria on every layer of straw and, according to the specific circumstances, adding some corn flour, wheat bran, and other accessories. Each layer of straw should be compacted to exhaust the air in the straw and the gap to provide an anaerobic environment for the growth of fermentative bacteria. ④ Salt powder is sprinkled on the top layer after cellar installation, and the silage feed is covered with plastic film after compaction [29].

The silage feed is generally stored for 21 days in the summer and autumn when temperatures are higher. When the temperature decreases, the storage time can be extended, and the silage feed should be sealed by a plastic cover after each use [13].

5.2 Biofertilizers

Fertilizer is an important agricultural production resource. Modern agriculture is based on petroleum because the fertilizer is mainly chemical fertilizer, which requires high energy consumption to produce. For example, producing 1 kg of nitrogen requires about 15,000 kcal. The productivity of nitrogen per day around the world consumes about about 320,000 t of fossil oil [30]. Currently, China has become the first country in the world for fertilizer production and consumption; the total fertilizer consumption in 2002 reached 43.39 million tons, accounting for 30 % of the total consumption in the world [31]. The long-term immoderate use of fertilizers and pesticides in the vast rural districts of China reduced soil permeability and loosening properties greatly, resulting in serious soil compaction and soil nutrient imbalance. From the perspectives of the future, the numerous soil environment damages for crop growth will seriously affect sustainable agricultural development [32]. Therefore, more organic fertilizer and less chemical fertilizer should be vigorously promoted in agricultural production to avoid repeating the mode of treatment after pollution from developed countries and to develop sustainable agriculture.

5.2.1 Straw Ecological Fertilizers

Straw not only has a high calorific value and crude fiber content but also contains abundant organic matter, nitrogen, phosphorus, potassium, magnesium, calcium,

Table 5.1 Comparison of straw-returning technology [33]

	Method	Advantages	Limits
Direct returning	Crushing returning Whole-straw returning Cover returning	Easy operation; time and labor saving; high efficiency; soil and water conservation; releases the damage from temperature catastrophe; promotes crop growth	High energy consumption; high cost; limited mechanical application in hilly region; cannot control the spread of disease; straw limitation in nonharvest season
Indirect returning	Compost returning Incineration returning Pass rumen returning Biogas residue returning	Low cost; easy operation; high content of K_2CO_3; high utilization efficiency; reduces usage of chemical fertilizers; high quality of organic fertilizers	Time consuming; high labor intensity; low output; air pollution; loses much energy and C, N, and P
Quick returning by biochemical rot	Catalytic rotting Rapid rotting Microbial rotting	High degree of mechanical automation; easy to industrialize Short cycle; high yield; less environmental pollution using aerobic fermentation; high and stable fertilizer efficiency	Difficult to screen excellent microbial mixing strains and chemical reagents; strict control of operation conditions; pretreatment of straws; high costs of equipment and operation

sulfur, and other important trace elements, which will continue being released for crop use in the straw-rotting process. Therefore, the use of various forms of straw returning can increase the content of soil organic matter and available nutrient content; improve soil fertility; mitigate the imbalance contradiction of nitrogen, phosphorus, and potash; adjust soil physical properties; reform the low-yielding fields; and form soil covering by organic matter to keep the soil moist; it also increases crop yields and optimizes the ecological environment of farmland [33]. The forms of straw-returning technology (Table 5.1) are various and mainly divided into three categories: direct returning, indirect returning, and quick returning by biochemical rot.

5.2.1.1 Direct Returning

There are various straw-returning technologies, such as direct returning by mechanical grinding, returning by mulching, and returning by retaining the stubble. Straw returning by mechanical grinding means to smash the postharvest straw and return it to the fields. The technique is convenient, fast, and low cost and can process a

large amount of straw in a timely manner to avoid environmental pollution brought by rot and burning of straw. Straw returning by mulching is divided two ways: One is to blanket the straw on the field surface; the other is stubble mulching, which means retaining 15–20 cm of stubble after crop harvest and flattening the stubble one or two times to spread it on the soil surface. This not only reduces the volume of transport, accelerates the threshing speed, and saves labor and energy but also obtains better fertilizer because of the rapid rotting of straw. High stubble returning keeps the stubble 20–30 cm after harvest of rice or wheat and directly returns it to the field using the rotavator [34].

5.2.1.2 Straw Fertilizer by Rumen Pass Returning

In rumen pass returning, the straw is first used as livestock feed and returned to field in the form of excreta by digestion of livestock. Currently, the pass rumen-returning technique of ammoniated silage is widely used and achieves a benign circle of straw-feed-livestock-fertilizer-food [35].

5.2.1.3 Straw Returning by Compost

Compost is created by a process that converts organic materials into fertilizer by microorganism fermentation under manual control conditions. In the process of microbial decomposition of organic matter, not only are many nitrogen, phosphorus, and potassium compounds that can be absorbed by plants generated but also new humus is synthesized, which constitutes an important active substance for soil fertility. The factors that affect the compost mainly include moisture, temperature, C/N ratio, and inoculant; they are briefly described next [36, 37].

Moisture: Proper moisture content is one of the key factors of compost. A large number of studies have shown that the suitable moisture content for compost is generally 60–80 %. With this condition, the CO_2 generation rate, the bacterial growth, and oxygen intake reach their maximum.

Temperature: Temperature mainly affects the growth of microorganisms. The compost temperature is generally controlled between 45 and 65 °C. Once the temperature exceeds 65 °C, it will inhibit the activities of microbial growth. Composting is an exothermic process, and timely cooling by ventilation methods should be used in the composting process.

C/N ratio: The appropriate C/N ratio of compost is from 20:1 to 30:1. When the C/N ratio is too low, the compost will affect the growth of crops; otherwise, it is not conducive for the growth of microorganisms.

Inoculant: Generally, adding inoculant to the material will accelerate the fermentation rate of the composting. For example, adding 10–20 % compost into the heap material can accelerate the fermentation rate. Meanwhile, artificial flora can be established by isolated active microorganisms from compost by strain options.

There are still many problems using straw and other lignocellulosic resources to produce biofertilizer, such as the low degree of mechanization and the low substrate conversion rate, which seriously affect the effective use of straw organic fertilizer. Therefore, vigorous development of the combination of mechanization and biotechnology will resolve the problem of straw returning effectively.

5.2.1.4 Other Fertilizer

(1) Humic acid fertilizer
Humic acid fertilizer is a new type of fertilizer containing humic acid substances. The main categories of humic acid fertilizer are humic acid, nitro–humic acid, and purified humic acid products. The first two are usually mixed with nitrogen, phosphorus, potassium, and trace elements to prepare humic acid compound fertilizer, mainly used as base fertilizer. Purification of humic acid is mainly used for irrigation or spraying of crops as a growth regulator and is also used for soaking of seed to improve germination and encourage a strong sprout. Humic acids show a wider application in the fields of industry, agriculture, animal husbandry, and medicine in China. The traditional humic acids are prepared using coal resources as raw materials, which means humic acids are obtained by consuming nonrenewable coal resources. Other individual manufacturers produce humic acids by microorganism fermentation using agricultural by-products as raw materials. However, the cycle of microbial fermentation production is long, and the products are not stable. Chen et al. invented a method to produce humic acid by steam-exploded straw using straw as raw materials. The technique was to load the straw into a steam explosion tank and then explode the raw materials with steam pressure to obtain steam-exploded straw containing humic substances. After recovery, fulvic acid and humic acid are mixed by soaking the steam-exploded straw with alkaline solutions. The principle of this method is that, in the cooking process, the reactivity of lignin increases because of the cleavage of part the ether bond of lignin and leads to the formation of humic acid substances by the reaction of lignin and the nitrogen-containing compounds, ash, and sugars from hydrolysis of hemicellulose [38].

(2) Ecological fertilizer from-steam exploded straw
The traditional process for prepare fertilizer, including explosion of straw at high temperature and high pressure, composting under natural conditions, and mixing with inorganic fertilizers shows some disadvantages: The sugars hydrolyzed by the treatment with explosion under high temperature and high pressure are wasted and pollute the environment; the organic matter conversion rate is still low; this fertilizer is from inorganic fertilizer, and the straw just plays the role of carrier. Because of the shortage of existing technology using straw to produce organic fertilizer, Chen et al. provided a method to prepare ecological fertilizer using steam-exploded straw as raw materials for solid-state fermentation to achieve ecological regulation of soil quality and beneficial microbial populations and prevent fertilizer pollution and waste [39].

5.2.2 Lignin Fertilizer

Lignin is a kind of naturally renewable, abundant organic macromolecular resource that widely exists in various higher plants in nature. However, the large amount of natural resource has not been utilized well yet because of its complex structure, heterology of physicochemical properties, and difficulty to isolate. Lignin has a comparatively large surface area and can be used as a carrier of fertilizer by adsorbing and enfolding the fertilizer to achieve controlled release. Moreover, lignin, as a natural polymer, can be converted to humic acid by the degradation of microorganisms to improve the physicochemical properties of soil and enhance soil permeability to proven soil compaction [40].

5.2.2.1 Controlled-Released Nitrogen Fertilizer

A nitrogen source is one of the three elements required for plant growth; nitrogen exists naturally in two forms: inorganic and organic. The nitrogen source of inorganic forms can be directly absorbed and used by plants. Because of the high carbon/nitrogen ratio of lignin, the degradation rate of lignin by microorganisms in the soil is slow. Therefore, the nitrogen resources in nitrogen-containing lignin prepared by chemical addition of ammonia can be released until the lignin is degraded by soil microbes; then, the released inorganic nitrogen resource can be absorbed by plants. So, the fertilizer of nitrogen-containing lignin is long acting and slow releasing and can delay the dissolution rate to improve utilization efficiency [41]. The ammonia-oxidized lignin is obtained by the oxidation degradation reaction of lignin dissolved in dilute ammonia under certain conditions of temperature and oxygen pressure and the existence of catalyst. The nitrogen content of the lignin after chemical modification is up to 15.47 % [42].

In addition, there are lignin sulfonate and alkali lignin nitrogen fertilizers. Lignosulfonate shows certain inhibition to urease activity as well as nitrification and denitrification to reduce the losses of ammonia volatilization. Lignin-modified urea also inhibits soil urease activity, can slow the ammonia volatilization loss, and can significantly improve the content of available nitrogen in the soil [43].

5.2.2.2 Lignin Phosphorus Fertilizer

Phosphorus fertilizer generally exists in an ion state after fertilization and is easily fixed by Fe, Al, Ca, and other elements in soil colloids, which suppress the crop to absorb phosphate and leads to a huge loss. Phosphate fertilizer by the modified lignin can reduce the chemical precipitation and fixing of phosphate and improve the utilization efficiency. According to a study by Mu and Zeng, the content of available phosphorus in the soil improved 10–20 % using modified lignin fertilizer [44].

In addition, activated phosphate fertilizer prepared by activation of lignin to rock phosphate and oxidized lignin provides the basis for the application of improved phosphate fertilizer.

5.2.2.3 Lignin Compound Fertilizer

Organic and inorganic compound fertilizer is a major trend in the development of the fertilizer industry. The lignin organic-inorganic fertilizer mixed by lignin with conventional fertilizer not only can decrease the hydrolysis rate of inorganic salts to achieve sustained release but also can inhibit urease activity and improve soil quality and fertility. Chen et al. found that, in the same field capacity conditions, lignin compound fertilizer decreased ammonia volatilization loss by 27 and 7.8 % compared with urea ammonia and inorganic compound fertilizer for 8 days, respectively. In flooded conditions, lignin compound fertilizer decreased ammonia volatilization loss by 14.5 % compared with urea ammonia; the content of available phosphorus in the cumulative leachate of lignin compound fertilizer increased 34 % compared with that of inorganic compound fertilizer after percolation experiments for 14 days [45].

5.2.2.4 Compound Fertilizer of Lignin and Trace Elements

Lignin can form trace element fertilizer by complexation with Fe, Cu, and Zn because of its reactive groups, such as hydroxyl, carboxyl, and carbonyl groups. Chelated zinc fertilizer prepared by oxidized lignin obtained from oxidation of dilute nitric acid showed higher fertilizer efficiency compared with $ZnSO_4$ fertilizer [46]. Ma and Wang prepared lignin-chelated zinc fertilizer using alkali lignin as a substitute for the relatively expensive Zn-EDTA (ethylenediaminetetraacetic acid). And, the experiments showed that the biomass of samples treated with lignin-chelated zinc fertilizer was higher than that treated with inorganic zinc fertilizer. The biomass of corn treated with 2 mg/kg lignin-chelated zinc fertilizer was almost equivalent to that treated with 20 mg/kg inorganic zinc fertilizer and showed better economic efficiency [47].

5.3 Biological Pesticides

With the development of society, there is a growing recognition that the use of chemical pesticides is at the cost of sacrificing the ecological environment and human health. A high usage of chemical pesticides not only causes environmental pollution but also severely destroys soil ecology flora. Minimizing the use of chemical pesticides and producing pollution-free agricultural products are the requirements of the majority of consumers of agricultural products, the needs for

the protection of the living environment, and the development of the agricultural economy. Therefore, biological control for sustainable development of agriculture plays an increasingly important role in agricultural production, and the development of biopesticides has become a main area of modern pesticides research and development. Biological pesticides are a class of pesticide formulations that use living organisms or their metabolites to control harmful creatures, such as pests, pathogens, weeds, nematodes, and rodents, or to synthesize pesticide formulations with specific functions by bionics design. Biopesticides can be divided into four parts according to their compositions and sources: microbial pesticides, microbial metabolite pesticides, botanical pesticides, and animal pesticides. According to the control object, the biopesticides can be divided into insecticides, fungicides, herbicides, acaricides, rodenticides, and plant growth-regulating agents.

Among the various biological pesticides, there are some that have several close relationships with natural cellulosic raw materials, such as the microorganism or the enzyme of cellulose degradation, their degradation products, and the medium. These types of biological pesticides are reviewed next.

5.3.1 Biological Pesticides from Trichoderma *spp.*

Trichoderma spp., widely existing in nature, are one of the main lignocellulose-degrading microorganisms. They are a kind of bacteria commonly found in soil, rotting wood, and other plant organisms. Common strains include *T. viride*, *T. harzianum*, *T. koningii,* and *T. polysporum. Trichoderma* spp. grow rapidly and can produce large amounts of spores, which are mainly green and pale yellow and yellow-green in some cases; some mycelia have thick-walled spores [48, 49].

Since Weindling first found the parasitic effects of *Trichoderma* spp. on several soil fungi in 1932, researchers have found *Trichoderma* spp. can prevent and control many other plant diseases. Because of the advantages of wide adaptability, multimechanisms of biocontrol, nontoxicity to humans and animals, lack of pesticide residue, and lack of pollution to the environment, *Trichoderma* spp. have received increasing attention in the biological control of plant diseases. More than 50 *Trichoderma* spp. formulations have been registered and commercialized [50].

5.3.1.1 Control Effects

Since the first time that Weindling found *Trichoderma* spp. had an antagonistic role in regard to plant pathogenic bacteria, experts and scholars around the world have performed many trials and in-depth research on the development of biological agents of Trichoderma. A variety of commercial biological agents of *Trichoderma* spp. has been produced abroad, such as Topshield (*Trichoderma harz* T-22 strains) in America and Trichodex (*Trichoderma harz* T-39 strains) in Israel. These agents show an efficient control effect in the prevention of plant diseases and obviously increase product yield.

Currently, the most widely used agents are *T. viride* and *T. harzianum*, mainly for the prevention and treatment of gray mold of vegetables, wheat sheath blight, red bean root rot, and cotton damping off.

5.3.1.2 Biocontrol Applications

Trichoderma spp. can generate three kinds of propagules in their growth cycle: mycelia, conidia, and chlamydospores; these all play a certain role in biocontrol preparations.

(1) Mycelia preparation
The advantages of mycelia preparation are that biocontrol microorganisms grow rapidly in the soil, and the storage and activation process does not have to maintain sterile conditions. The disadvantages are that it is inconvenient to use and has a short storage period.

(2) Conidia preparations
At the present, conidia are mostly commercially prepared.

(3) Chlamydospore preparation
Chlamydospores are produced by *Trichoderma* spp. under resistance to stress conditions. Their advantages are resistance to dry and low-temperature conditions, but relevant studies of this preparation are few because of the relatively harsh artificial culture conditions [50].

Now, *Trichoderma* preparations for production are mostly spore preparations. The spore preparations made from *Trichoderma* spp. fermentation go though the seed coating or soil treatment to prevent and cure diseases effectively in the seeding period. The main applications are as follows: ① soil treatment; ② seedling and corm processing; ③ aboveground application to prevent and control disease; ④ fine strains and strain combination screening; ⑤ the mixed applications of a small amount of fungicide; and ⑥ antagonistic substance extraction, purification, and activity assay.

5.3.1.3 Mechanisms

The antagonism of *Trichoderma* spp. to plant pathogens is the result of multiple mechanisms, which are generally believed to occur as discussed next.

(1) Competitive effects
The competitive effects of *Trichoderma* spp. concern the competition for nutrients and for space. Because of the strong saprophytic features, high adaptability, rapid growth, and reproduction of *Trichoderma* spp., nutrition and space can be used quickly, which plays an important role in the inhibition of pathogenic fungi.

(2) Hyperparasitism
Hyperparasitism refers to a series of complex processes, including identification, contact, twining, penetration, and parasitism. After the identification of *Trichoderma* spp. to host pathogen, its hyphae can directly invade or wrap around the host hyphae cell, penetrate the mycelia of the pathogen, and absorb nutrients by secreting extracellular enzymes to dissolve the cell walls [51], which causes the enlargement, deformation, or shrinkage of the pathogen cell, the contraction of protoplasm, and the rupture of the cell wall.

(3) Antibiosis
Trichoderma spp. can produce antagonistic chemicals against plant pathogens in their metabolic processes, including antibiotics and enzymes, such as trichodermin, neomycin, green trichodermin, and antimicrobial peptides.

(4) Collaborative antagonism
The collaborative antagonism of *Trichoderma* spp. may be the result of integration of simultaneous or sequential action of two or three mechanisms. Baker's research showed that the functions of *Trichoderma* to *Pythium* included the two mechanisms of antibiotic production and hyperparasitism. *Trichoderma* spp. not only can produce antibiotics but also can secret a variety of extracellular enzymes to degrade the cell wall and inhibit mycelial growth and spore germination of soil-borne plant pathogens. Corella et al. proposed the synergistic effect model of mycin and the cell-wall-degrading enzymes and believed that the function of cell-wall-degrading enzymes was to make toxins quickly transmit and interact with the specific sites of plasma. Moreover, there is a synergistic role among the different cell-wall-degrading enzymes [51].

(5) Induced resistance
Previous studies of the mechanism of biocontrol focused on the interaction between microorganisms and ignored the role of the host plants. Researches recently found that biocontrol agents can induce each part of the organization of the host plant to form resistance to pathogens. As reported, when the hyphae of biocontrol microorganisms penetrate the epidermis cortex of cucumber roots, the peroxidase content increased in the plant, and the chitinase activity of the plant increased at the same time [48].

5.3.1.4 Fermentation of *Trichoderma* spp.

The first problem to solve is obtaining a large amount of *Trichoderma* cells if *Trichoderma* is widely used to control plant diseases. Because of the special nutritional and environmental conditions needed for *Trichoderma* growth, it is not easy to obtain a great quantity of biomass. Because the biocontrol effect of *Trichoderma* is widely accepted, there are numerous reports of *Trichoderma* culture matrix and fermentation production, including liquid culture, solid-state culture, and solid-liquid biphasic fermentation.

(1) Liquid fermentation

The medium for liquid culture of *Trichoderma* spp. can be low-cost syrup, yeast extract, and other agricultural fertilizers. Papavizas, Dunn, and coworkers imitated the industrialized production conditions using a medium that consisted of molasses and yeast extract to study the growth of *Trichoderma* sp. in a 20-L fermentor. They found that this medium could obtain an ideal outcome, and the spore quantity was up to 10^9/g dry weight. Cottonseed flour or corn syrup instead of beer yeast can also obtain the desired effect [52].

The literature on *Trichoderma* spp. liquid culture to obtain high biomass in China is a relatively small body. Zhao and Wang [53] made some attempts in the field of liquid fermentation. Chen et al. [54] used 13 kinds of culture media for cultivation of *T. viride* and determined the inhibition of filtrate to *Sclerotinia sclerotiorum*. The results showed that the culture medium with 20 g glucose, 2 g tartaric acid, and trace inorganic salts achieved the best effect.

(2) Solid-state fermentation

Currently, the substrate materials for solid-state fermentation mainly include nutritional matrix, such as rice bran, cornstalk, corncobs, wheat straw, rice straw, vinasse, sugarcane bagasse, and grains; and other matrix without nutrition, such as vermiculite and sponge [50, 55]. Lewis and Papavizas [56, 57] studied the sporulation of *Trichoderma* in a variety of solid media, including quartz sand with sawdust, cocoa shell powder, coffee husk powder, peanut shell powder, corncobs, and wheat bran. The results showed that wheat bran had the most obvious effects, and the stimulating effects of corn flour and peanut shell powder on the sporulation were similar. Zhu et al. cultivated *Trichoderma* sp. using the medium of city garbage plus 30 % wheat bran with 30 % of inoculum for 8–10 days; the spore amount was up to 10^9/g [58]. Compared with liquid fermentation, the advantages of solid-state fermentation are less investment, easy operation, low energy consumption. The disadvantages are labor intensity because of the imperfection of solid-state fermentation equipment, susceptibility to bacterial contamination, and low and unstable yields. The key lies in the solid-state fermentation reactor. Chen et al. from the IPE, CAS developed a pressure pulsation solid-state fermentation reactor for *Trichoderma* spp. fermentation, which showed obvious advantages.

(3) Solid-liquid biphasic fermentation

Solid-liquid biphasic fermentation collects a large number of mycelia or blastospores rapidly generated by liquid fermentation and then transfers to them to a solid nutrition matrix or inert carrier matrix to produce abundant conidia. The process combines dual advantages of the fast growth of liquid fermentation and conidia preparation of solid-state fermentation, which has been widely used in current research and development of fungicide products [50].

In short, the use of *Trichoderma* spp. as a biocontrol has many advantages: a strong saprophytic effect, the wide range of available use, use of cheap natural lignocellulosic materials as substrate, large sporulation, and ease of industrialized production.

5.3.2 *Oligosaccharins*

Oligosaccharins are a class of active molecules that show physiological regulation functions. They mainly refer to the degradation products of natural lignocellulosic materials and chitin, which contain bioactive oligosaccharide chains such as xyloglucan oligosaccharide, pectin oligosaccharide, β-xylan oligosaccharides, fungal β-dextran oligosaccharides, chitin oligosaccharides, some glycoprotein oligosaccharide chains, and oligosaccharide peptides. A small amount of oligosaccharides can stimulate a strong disease-resistant response in plant cells and generate and accumulate disease-resistant substances, such as phytoalexin, phenylalanine ammonia lyase, chitinase, and lignin [59]. Recent studies found that the oligosaccharins not only can stimulate a variety of defense reaction systems, including phytoalexin synthesis, but also can effectively regulate plant growth and developmental processes.

Microbial diseases are crucial causes of crop failures because of the wide range of plant pathogens and mutations. Chemical pesticides, although quick and efficient, always lead to a variety of side effects if they are applied for long term and without limitation. Actually, the plant has a natural resistance to some microbial infection and can start self-defense in response to a pathogen attack after microbial infection. Studies have shown that this self-defense capability can be induced by exogenous oligosaccharins, and the use of oligosaccharins as biological pesticides not only can simplify disease prevention and control measures but also can replace the use of chemical pesticides to reduce drug residues and environmental damage [60].

In the early 1980s, it was found that oligosaccharins involved in plant growth and development, as signaling molecules participating in the regulation of plant growth and development, such as cell division, differentiation, formation of new organs, and embryogenesis. Tran Thanl Van et al. also proposed that some plant morphogenesis regulatory factors, such as pH and ionic concentration, play a regulatory role by activation of specific hydrolases on the cell wall to release oligosaccharins [61].

Much research into oligosaccharide application in agricultural practice has been performed. Yu et al. [62] studied the effect of amino oligosaccharin on the prevention and control of tobacco virus disease; the results showed that amino oligosaccharides could effectively promote the growth and development of tobacco plants and showed a significant role in the prevention and control of tobacco virus diseases. In particular for cucumber mosaic virus, the control efficiency of indoor inoculation was up to 34.92 %. Biochemical parameters revealed that spraying the amino oligosaccharin and inoculating virus can effectively promote the accumulation of proline in the tobacco plant, reduce the alondialdehyde content in the plant, and adjust resistance to external stress. The studies from Mei et al. [63] showed that pectin oligosaccharides could promote the growth of callus of *Euptelea pleiospermum*, argotaenia, and rice and affect suspension cell growth of *Berberis vulgaris* and jatrorrhizine biosynthesis. Liu et al. [64] studied the physiological effect of chitosan on wheat seeds; the results showed that chitosan could promote the beginning of the wheat seed embryo cell cycle and increase the number of

wheat roots, which meant that chitosan could promote the division of wheat seed embryo cells. The chitosan pretreatment of wheat seeds could relieve the inhibition of deoxynivalenol (DON) on the growth of etiolated wheat seedlings and the start of embryonic cells, which meant that oligosaccharides improved the resistance of plants to pathogenic toxins.

Oligosaccharins have a prominent role in many aspects, and their mechanism of action was also studied in some research. With deepened study, there will be a more profound understanding of molecular structure and the mechanism of action, qualitative and quantitative analysis, and particularly practical applications.

5.3.3 Pectinase

Pectinase is one of the world's most applied enzyme preparations and the general name of the enzymes for pectin decomposition. The pectinase is usually divided into three types: protopectinase, esterase, and depolymerase [65]. Pectinase plays an important role in the current field of bioengineering, which is widely used in the food, textile, pharmaceutical, paper, environment, biotechnology, and feed industries. For example, pectinase can be used as a biological pesticide because it can induce plant disease resistance. Pectinase can also be used for the production of green food to reduce the amount of chemical pesticide and to some extent reduce the hazards of chemical pesticides on the environment and human beings. Bai et al. [66] achieved good results when they studied the application of *Aspergillus niger* pectinase to induce disease resistance of cucumber and tomato. Peng et al. [67] studied the role of *Penicillium oxalicun* pectinase on induced resistance of cucumber against scab, and the effect was significant. Chitosan with molecular weights from 20 to 50 kDa obtained from enzymolysis by pectinase from *Aspergillus niger* under pH 3.0 and 37 ° C can induce resistant effects on plant disease [68].

5.3.3.1 Microorganisms for Pectinase Fermentation

Natural pectinase is widely present in animals, plants, and microorganisms; the pectinase yields from animals and plants are too low to extract in large scale; microorganisms are an excellent biological resource for the production of pectinase [66]. At present, microbial fermentation methods are mostly used for the industrialized preparation of pectinase. The microorganisms of pectinase production are shown in Table 5.2. The production microbes for commercial enzyme preparation are mainly some fungi, including *Aspergillus niger, Rhizopus oryzae,* and *Rhizomucor meihei,* and plant pathogens such as *Endothia parasitica* and *Candida spp. Saccharomyces* spp. can also produce a wide range of pectin-degrading enzymes.

Extensive research on pectinase strain screening has been performed by Chinese scholars since the 1980s. Cui et al. [70] obtained a mutant cp-85211with a high yield

Table 5.2 Common strains for pectin enzyme production [69]

Microbes	Genus
Mycetes	*Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Geotrichum* spp.
Bacteria	*Erwinia* spp., *Pseudomonas* spp., *Bacillus* spp., *Clostridium* spp., *Xanthomonas* spp.
Yeast	*Trichosporm* spp., *Kluyveromyces* spp., *Saccharomyces* spp., *Aureobasidium* spp.

of pectinase by treatment of *Aspergillus niger* cp-831with 0.03 % nitrosoguanidine; the strain cultivation was scaled up by the Wuxi Enzyme Factory. Chen and Zhao [71, 72] reported on studies about pectinase production by liquid and solid-state fermentation of *Aspergillus oryzae* C491.

5.3.3.2 Pectinase Production by Microbial Fermentation

Pectinase is prepared by microbial fermentation mainly by three methods: liquid fermentation, solid-state fermentation, and immobilized cell cultivation. Solid-state fermentation is widely used for pectinase production in China because it requires less investment, has a fast start, and has other advantages. Most of the pectin substrates for liquid fermentation are from different sources of pectin products, and for solid-state fermentation, lignocellulosic agricultural waste or agroprocessing by-products are used as carbon sources and pectinase inducer [73]. Sharma and Satyanarayana [74] used liquid fermentation of *Bacillus pumilus* to produce pectinase that was stable in high alkaline and thermal conditions. Botella et al. [75] used grape pomace as a substrate and *Aspergillus awamori* as the inoculum to produce circumscribed polygalacturonase by solid-state fermentation. Studies from Almeida et al. [76] showed that a bioreactor with a circular flow packed bed favored immobilization of *Kluyveromyces marxianus* CCT 3172 cells to produce pectinase.

Different culture methods have different effects on the pectinase production. Maldonado and Strasser de Saad [77] compared the characteristics of pectinase synthesized by two culture methods of *A. niger*. They claimed that the regulation of pectinase synthesis is different under different culture conditions. The yields of pectinesterase and polygalacturonase in solid-state fermentation using pectin as the sole carbon source were four and six times greater compared with that of submerged fermentation, respectively. The extra glucose added under solid-state fermentation conditions was conducive for improving the production of pectinesterase and polygalacturonase, but the glucose added under submerged fermentation conditions significantly affected the synthesis of pectinesterase and polygalacturonase. Viviana [78] also pointed out that, for *A. niger*, there were distinct physiological responses to different culture methods. The fermentation conditions have a great influence on the composition of fungal pectinase and the kinetic and chemical characteristics of the enzyme. The production of pectinesterase under submerged fermentation conditions was five times greater compared with that under solid-state fermentation conditions; the yield of pectinesterase increased 30 %. However, the production of pectinolytic

activities under submerged fermentation was only a third of that under solid-state fermentation. The pectinase fermented under solid-state fermentation conditions showed it was more stable to extreme pH and temperature, and the fermentation period was shorter [69].

The studies, beginning from the finding of pectinase as a biodegradable pectin enzyme to the wide application of the enzyme, have covered more than half a century, and the prospects for pectinase development are still extremely broad because of the versatility of applications. In particular, pectinase production using agricultural waste such as lignocellulose enables recycling of the natural resources and is conducive to environmental protection and human health with good economic and social benefits.

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Chapter 6
Applications of Lignocellulose Biotechnology in Bioenergy

Abstract A shortage of fossil energy is considered a serious problem. Bioenergy, derived from renewable lignocellulosic resources, has attracted much interest from governments around the world owing to its desirable features: a secure source of supply, limited conflict with land use for food and feed production, and low pollution. Considerable progress has been achieved in bioenergy production, including new methods proposed, new processes developed, new projects established, and so on. However, there are still challenges that need further investigation. The most controversial problem is technical and economical feasibility. This chapter introduces the background, status, and development of bioethanol, biohydrogen, and biogas production and points out problems that exist in relation to them.

Keywords Bioenergy • Fuel ethanol • Biohydrogen • Biogas fermentation

6.1 Introduction

Along with the twenty-first century came two bottlenecks that are becoming increasingly serious; one is environment, and the other is a shortage of fossil energy. In recent years, with the high-level economic growth of China, the demand for fossil fuels such as petroleum, coal, and natural gas is increasing substantially. Accordingly, environmental pollution is increasing greatly. Because of the shortage of petroleum, there is serious pollution from coal utilization coupled with a large population base and per capita consumption of energy and resources in China that is far below the world average. To remove the overreliance on traditional energy sources and reduce environmental pollution, many countries are looking for alternatives to traditional energy to develop new energy without delay.

Among many kinds of new energy, including solar energy, nuclear energy, wind energy, hydroenergy, tidal energy, geothermal energy, and biomass energy, biomass is emerging as a source. It is not only a kind of energy but also a raw material for making chemical products. Not only can it ease energy and environmental

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problems, but also it is renewable. Therefore, its development is a current focus. Biomass refers to renewable organic materials, including agricultural products and agricultural wastes, wood and its wastes, animal wastes, urban wastes, aquatic plants, and so on [1]. A variety of organisms, including all plants, animals, and microorganisms, are formed through photosynthesis. Biomass is no exception. It is also formed through photosynthesis directly or indirectly. Most important, biomass can be transformed into conventional solid, liquid, and gaseous fuels. At present in China, total lignocellulosic biomass resources are more than 3 billion tons of dry weight per year, which are equal to over 1 billion tons of petroleum and about three times the current petroleum consumption. In China, the traditional use of crop straw generally is to send it back to farmland. But, accounting for only 15 % of the total straw stubble, a field can reach a balance of soil organic matter. Coupled with changes in the structure of rural energy and intensive production, the large amount of straw gradually becomes a kind of useless burden. What is worse, the phenomenon of burning straw in situ becomes increasingly serious. The resulting flue gas has become a social nuisance. Despite injunctions by all levels of governments, the phenomenon of burning continues [2]. Therefore, making full use of lignocellulosic resources, that is, transforming them into energy, chemicals, food, feed, new materials, fertilizers, and so on, is an effective method to solve the problems human face today and is important to economic development and environmental protection. This chapter mainly describes the status of and trends for the variety of clean energy products made from lignocellulose.

6.2 Lignocellulose Fermentation of Fuel Ethanol

Fuel ethanol is a kind of high-quality liquid fuel. It can replace gasoline, diesel, and other petroleum-based fuels. Thus, it is considered one of the alternatives with the most potential to replace petroleum fuels. Fuel ethanol can be made from biomass in two ways: thermochemical conversion and biological conversion [3].

At present, bioethanol feedstocks can be divided into three major groups: (1) sucrose-containing feedstocks (e.g., sugarcane, sugar beet, sweet sorghum [4], and fruits); (2) starchy materials (e.g., corn, milo, wheat, rice, potatoes, cassava, sweet potatoes [5, 6], barley, and puerarin [7–10]); and (3) lignocellulosic biomass (e.g., wood, straw [11], grasses [12], and fermentation residue [13]). In the short term, the production of bioethanol as a vehicular fuel is almost entirely dependent on starch and sugars from existing food crops. However, with the expansion of the bioethanol industry, the price of raw material will become a bottleneck. Lignocellulosic biomass is envisaged to provide a significant portion of the raw materials for bioethanol production in the medium and long terms because of its low cost and high availability [14].

In bioethanol production, according to their advantages in resources, different countries use these resources in different ways. For example, in the United States, the materials are mainly corn (starchy), but in Brazil, the materials are mainly

sugarcane (sugars). In China, fuel ethanol production reached 1.7 million tons in 2009, mostly using food crops such as corn, wheat, and rice as raw materials. However, according to China's national conditions, on the one hand, vigorous development of the fuel ethanol industry is necessary. On the other hand, fuel ethanol should not compete for food with people and compete for fields with food. Above all, increased efforts to develop lignocellulosic resources for fuel ethanol production are of great value.

6.2.1 Use of Lignocellulose to Produce Bioethanol

Use of lignocellulose as a raw material to produce bioethanol can be completed by both biological conversion and thermochemical conversion methods. Among these, biological conversion contains a hydrolysis stage of substrates and a subsequent fermentation stage; thermochemical conversion contains gasification of substrates and subsequent fermentation or a catalytic reaction. They are respectively described next.

6.2.1.1 Biological Conversion

Lignocellulose is composed of three main components: cellulose, hemicellulose, and lignin. They tightly twist around each other. Thus, the structure of lignocellulose is complex and must be changed into small molecules of sugar so it can be utilized by microorganisms. Biological conversion of lignocellulose to bioethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/distillation.

To increase the yield of hydrolysis, a pretreatment step is needed that softens the biomass and breaks down cell structures to a large extent. A successful pretreatment must meet the following requirements: (1) improve formation of sugars or the ability to subsequently form sugars by hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost effective. There are many types of processes to pretreat biomass. They are physical methods, physical-chemical methods, chemical methods, and biological methods [14].

Acid hydrolysis and enzymatic hydrolysis are two types of processes to hydrolyze cellulosic biomass. Acid hydrolysis is also divided into concentrated acid hydrolysis and dilute acid hydrolysis. The principle of concentrated acid hydrolysis is that crystalline cellulose can be completely dissolved in 72 % sulfuric acid, 42 % hydrochloric acid, and 77–83 % phosphoric acid at a low temperature, resulting in the homogeneous hydrolysis of cellulose. Among them, concentrated sulfuric acid is used most commonly. The main advantage of concentrated acid hydrolysis is the high yields (e.g., 90 % of theoretical glucose yield). The principle of dilute acid hydrolysis is that dilute acid can cause the change of cellulose fine structure (such

as degree of polymerization, fiber density, crystallinity, etc.). The disadvantages of dilute acid hydrolysis are the low yields (about 50–60 %) and that sugar degradation products can also cause inhibition in the subsequent fermentation stage [1]. In all, acid hydrolysis needs a large amount of acids and causes problems associated with equipment corrosion and energy-demanding acid recovery. Compared with acid hydrolysis, enzymatic hydrolysis has many advantages, such as mild hydrolysis conditions, high yield, low equipment requirements, and low energy consumption. But, there are also disadvantages: slow reaction rate, high cost, and so on.

Straw hydrolysate is a mixture of many kinds of sugar, with glucose having the highest content, then xylose. In addition, there is a small amount of other kinds of pentose and hexose and some small-molecule oligosaccharides. These sugars can be used by microorganisms to produce a variety of products. Actually, in nature, some fungi and bacteria can ferment the sugars to produce ethanol under anaerobic or anoxic conditions. In human society, alcoholic fermentation has also several thousand years of history. In alcohol and other traditional brewing fermentation industry and modern industry, the yeast *Saccharomyces cerevisiae* has been widely used. As early as 1810, Gay-Lussac had already established the reaction of yeast fermentation of glucose to produce alcohol:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

In this case, 100 g glucose can be fermented to produce 51.4 g ethanol and 48.8 g CO_2. In this reaction, 1 molecule of glucose generates 2 molecules of ATP. As part of the sugars is used for yeast growth, ethanol yield is slightly lower than the theoretical value. In the fermentation process, 91 % of energy contained in glucose is converted into ethanol to be preserved. Therefore, using lignocellulose to produce ethanol is an excellent energy conversion technology [15].

Compared with glucose metabolism, xylose metabolism is more complex. Part of xylose is converted into other by-products in the metabolic process. The ethanol theoretical yield is 0.46 g/g xylose, which is slightly lower than glucose. If the xylose could be used to produce ethanol, the ethanol production would increase by 25 %. Therefore, the breakthrough of xylose fermentation to produce ethanol is the key to determine the economic viability of cellulosic ethanol [16].

The traditional recovery process of alcohol from the fermentation broth is generally distillation. However, alcohol and water can form azeotrope (95 % ethanol). Thus, extractive distillation and azeotropic distillation need to be further used to produce anhydrous ethanol. There are several methods using 95 % ethanol to produce anhydrous ethanol: dehydration of a water absorbent (such as molecular sieve adsorption dehydration), azeotropic dehydration, organic adsorption dehydration, pervaporation membrane adsorption dehydration, ion exchange dehydration, salt dehydration, and so on [17].

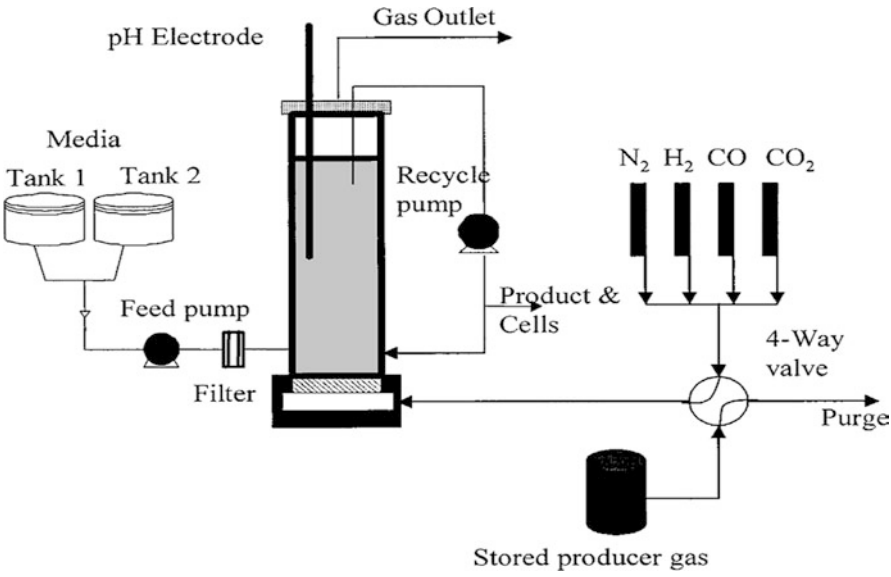

Fig. 6.1 Continuous fermentation device for biomass synthesis gas to generate alcohol [3]

6.2.1.2 Thermochemical Conversion

In thermochemical conversion, lignocellulose is first thermochemically gasified to generate synthesis gas, and then synthesis gas is changed into ethanol by a biological or chemical catalytic method.

There are two ethanol production processes that currently employ thermochemical reactions. The first system is actually a hybrid thermochemical and biological system. Lignocellulosic biomass materials are first thermochemically gasified, and the synthesis gas (a mixture of hydrogen and carbon monoxide) is bubbled through specially designed fermentors. A microorganism that is capable of converting the synthesis gas is introduced into the fermentors under specific process conditions to cause fermentation to bioethanol at last [14, 18]. Datar used switchgrass as a raw material. First, the fluidized bed gasifier generated gas with a composition of 56.8 % N_2, 14.7 % CO, 16.5 % CO_2, 4.4 % H_2, and 4.2 % CH_4 under 750–800 °C. Then, the producer gas was utilized in a bioreactor to generate ethanol and other products via fermentation using P7 clostridial bacterium under 37 °C and absolute anaerobic conditions [3, 19]. The process is presented in Fig. 6.1.

The second thermochemical ethanol production process does not use any microorganisms. In this process, biomass materials are first thermochemically gasified, and the synthesis gas is passed through a reactor containing catalysts, which causes the gas to be converted into ethanol [14, 20]. Phillips used the second method to produce ethanol: First, biomass was treated in the absence of oxygen at a high temperature (600–1,000 °C) to produce mixed cracked gas, of which the

main components were CO, CO_2, H_2, CH_4, and N_2. Then, the mixed cracked gas was changed into ethanol and mixed C3+ alcohols. Thermochemical conversion is a simple technology; one of its benefits is the ability to convert a wide variety of biomass feedstocks, including agriculture and forestry residues, low-quality coal, black liquor, urban organic solid waste, and so on [3, 21].

6.2.2 Lignocellulose Fermentation to Produce Bioethanol

After pretreatment, the process that follows contains four typical biological processes: cellulase production, enzymatic cellulose hydrolysis, and fermentation of hexose and pentose. According to the degree of integration of the different steps, the process can be divided into separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation, simultaneous saccharification and cofermentation (SSCF), and direct microbial conversion (DMC).

6.2.2.1 Separate Hydrolysis and Fermentation

In SHF, lignocellulose feedstock is first pretreated and hydrolyzed into liquid hydrolysate containing fermentable sugars, such as glucose and xylose; then, the hydrolysate is fermented into ethanol. The advantage of SHF is that both enzymatic hydrolysis and fermentation can be conducted at their respective optimal conditions. The optimum temperature and pH of enzymatic hydrolysis are, respectively, 45–50 °C and 4.5–5.5, and the optimum temperature and pH of fermentation are 30–35 °C and neutral, respectively. But, in the process of cellulose enzymolysis, cellobiose accumulation will inhibit endoglucanase and exocellobiohydrolase; glucose accumulation will also inhibit the β-glucosidase enzyme. Therefore, the concentration of cellulose cannot be too high. This has reduced the efficiency of enzymatic saccharification, thus affecting ethanol yield [22, 23].

6.2.2.2 Simultaneous Saccharification and Fermentation

To reduce the cost of bioethanol, simultaneous saccharification and fermentation was developed in the 1970s, as shown in Fig. 6.2. That is, cellulose hydrolysis and hexose fermentation occur in the same reactor. The glucose generated in the cellulose hydrolysis process is utilized by microorganisms quickly. This method will eliminate the feedback inhibition of glucose, improve enzymatic hydrolysis efficiency, reduce the amount of cellulase and the required response equipment, and decrease the possibility of pollution. However, because of the inconsistency of optimal conditions for enzymatic hydrolysis and ethanol fermentation as a combination, the temperature of simultaneous saccharification and fermentation is generally 37–38 °C.

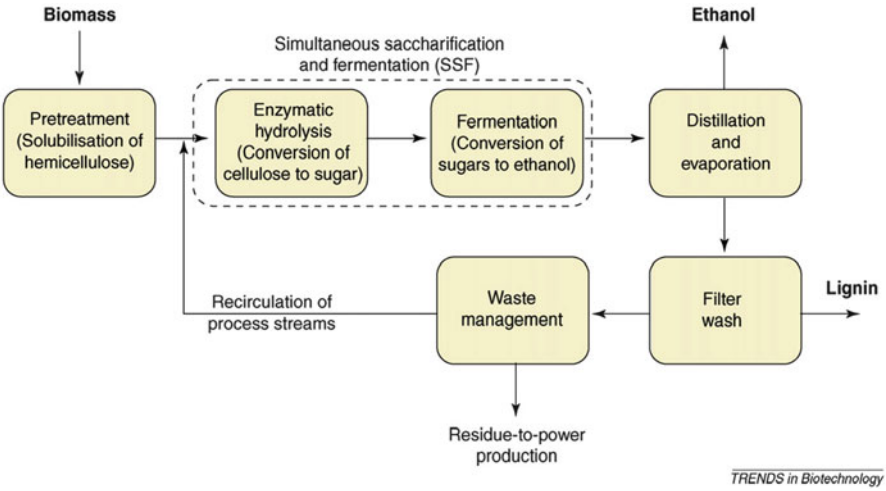

Fig. 6.2 The system of simultaneous saccharification and fermentation [24]

To solve this problem, the process and breeding were respectively researched. For example, using heat-resistant yeast and bacteria to replace the traditional *Saccharomyces cerevisiae* could improve the fermentation temperature so it is close to the optimum temperature of cellulase. Thus, to improve overall efficiency, Szczodrak et al. selected 58 yeast strains from 12 genera to test their ability to grow and ferment carbohydrates in a standard Durham tube test at 40, 43, and 46 °C. The results showed that the fermentation capacity of the strain *Fabospora fragilis* CCY51-1-1 reached about 56.0 $g{\cdot}L^{-1}$ from 140 $g{\cdot}L^{-1}$ of ethanol at 43 °C, which equaled 74 % of the theoretical conversion rate. However, when the temperature was raised to 46 °C, the fermentation capacity of *F. fragilis* CCY51-1-1 was significantly decreased, and the conversion rate was reduced to 46 % of the theoretical conversion rate [25, 26]. Xiao and Li [27] put forward a dispersion, coupled, parallel system of biotransformation from cellulose to ethanol (Fig. 6.3).

In this system, saccharification, fermentation, and separation of alcohol are conducted separately. In saccharification, enzymatic hydrolysis can be carried out at a higher temperature. Then, enzymes and enzymatic hydrolyzate are separated through a nuclear pore membrane. Enzymes are returned to saccharification for enzymatic hydrolysis again; enzymatic hydrolyzate went into the fermentation segment. This would not only solve the inconsistency of the hydrolysis and fermentation temperatures, but also eliminate the sugar inhibition on enzymatic hydrolysis. Similarly, in fermentation, yeast cells can be separated from fermentation broth through a membrane and then returned to the fermentation section to continue fermenting. However, the residual fermentation broth is distilled to produce alcohol. Thus, the inhibition of yeast from alcohol is eliminated. Digested by this system, the cellulose conversion rate reached 81 %, while the conversion rate of the general cellulose enzymatic process was 66 %. The efficiency of the former was 3.9 times

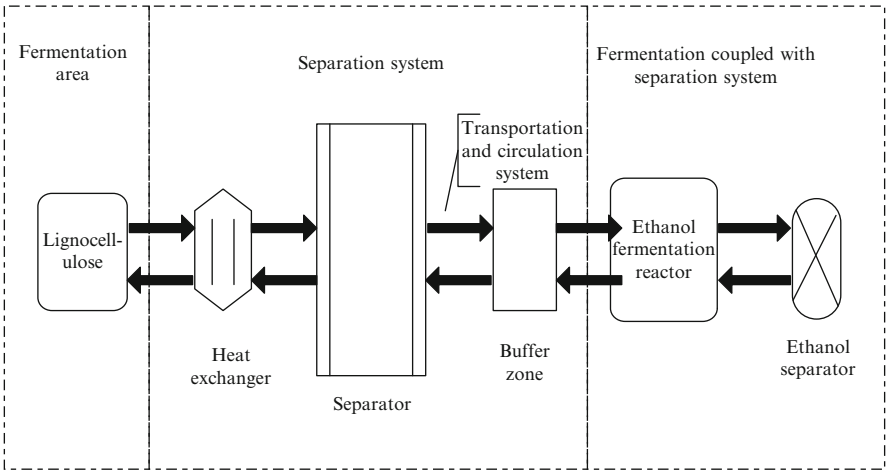

Fig. 6.3 The dispersion, coupled, parallel system of biotransformation of lignocellulose to ethanol [27]

that of the latter. Alcohol concentration, fermentation rate, and conversion rate in this system were 8.14 %, 0.66 $g \cdot L^{-1} \cdot h^{-1}$, and 80.1 %, respectively, which were respectively 1.8, 1.3, and 1.7 times of simultaneous saccharification and fermentation.

As the structure of lignocellulose is complex, its utilization process is accordingly complex. Chen et al. [28] researched simultaneous saccharification and fermentation as well as fed-batch simultaneous saccharification and fermentation of different compositions of lignocellulose material. They found that lignin hindered cellulose simultaneous saccharification and fermentation, while hemicellulose had a dual role. On one hand, the presence of hemicellulose reduced the crystallinity of cellulose, which is conducive to fermentation; on the other hand, xylose and xylooligosaccharides, enzymatic hydrolysis products of hemicellulose, would inhibit enzymatic hydrolysis of hemicellulose and further impede the enzymatic hydrolysis of cellulose to reduce cellulose the simultaneous saccharification fermentation rate. In these, the promotion effect is the main one; the inhibitory effect only exists in fed-batch simultaneous saccharification and fermentation and late stage of batch simultaneous saccharification. Therefore, removing lignin and reserving hemicellulose are good for improving the cellulose simultaneous saccharification and fermentation rate.

In general simultaneous saccharification and fermentation processes, the liquid, which is rich in five-carbon sugars, produced in the pretreatment stage is fermented separately. With the development of novel microorganisms to ferment glucose and xylose simultaneously, SSCF appeared. That is, sugar juice and cellulose obtained after pretreatment are treated in the same reactor, which further simplifies the process.

6.2.2.3 Simultaneous Saccharification and Cofermentation

SSCF carries out enzymatic hydrolysis and xylose and glucose fermentation in a single bioreactor using mixed bacteria or bacteria with engineered xylose metabolism. It has several advantages, such as lower cost, shorter process time, lower contamination risk, and fewer inhibitory effects during enzymatic hydrolysis. Thus, it attracts increasing research.

Koskinen et al. obtained two thermophilic bacteria (strains AK15 and AK17) and used them to ferment lignocellulose. They could use both glucose and xylose to produce ethanol and hydrogen. The strain AK17 was tolerated exogenously and added ethanol up to 4 % (v/v) [3, 29]. Ryabova et al. obtained a mutant strain of *Hansenula polymorpha* that was vitamin B_2 deficient from the yeast *Pichia stipitis*. This strain could ferment both glucose and xylose up to 45 °C [3, 30]. Kim et al. used barley hull pretreated using aqueous ammonia to produce ethanol. The addition of xylanase along with cellulase resulted in a synergetic effect on ethanol production in SSCF using treated barley hull and recombinant *E. coli* (KO11). With 3 % (w/v) glucan loading and 4 mL xylanase enzyme loading, the SSCF of the treated barley hull resulted in a 24.1 $g{\cdot}L^{-1}$ ethanol concentration at 15 FPU cellulase/gram glucan loading, which corresponds to 89.4 % of the maximum theoretical yield based on glucan and xylan [23, 31]. Zhang et al. developed a kinetic model to predict batch SSCF by the xylose-utilizing yeast *Saccharomyces cerevisiae* RWB222 and the commercial cellulase preparation Spezyme CP. The model accounted for cellulose and xylan enzymatic hydrolysis and competitive uptake of glucose and xylose [23, 32].

6.2.2.4 Direct Microbial Conversion

DMC combines cellulase production, cellulose enzymatic saccharification, and fermentation of hexose and pentose into a single step, thus reducing the reaction vessel and saving costs. But, in this process, the resistant ethanol concentration of the strain is low, and a variety of by-products is generated. Thus, the final ethanol yield is low. In DMC, *Clostridium thermocellum*, *Clostridium thermohydrosulphaircum,* and *Thermoanaerobacter ethanolicus* are the focus of studies [33]. Christakopoulos et al. found that the cellulase hyperproducing strain F3 of *Fusarium oxysporum* fermented glucose, xylose, cellobiose, and cellulose directly to ethanol in 1989. Conversion of cellulose to ethanol was markedly affected by the pH of both aerated preculture and anaerobic fermentation. Optimum values of cellulose conversion to ethanol were obtained when aerated and anaerobic processes were carried out at pH 5.5 and 6, respectively. Maximum ethanol concentrations of 9.6 and 14.5 $g{\cdot}L^{-1}$ corresponded to 89.2 and 53.2 % of the theoretical yield [23, 34]. Balusu et al. used *Clostridium cellulolyticum* SS19 to produce ethanol from cellulose in anaerobic submerged fermentation. The concentrations of filter paper, corn steep liquor, cysteine hydrochloride, and ferrous sulfate in the medium that were optimal for

ethanol production were 45, 8.0, 0.25, and 0.01 $g \cdot L^{-1}$, respectively. The organism produced 0.41 g of ethanol/gram of the substrate consumed (81 % yield efficiency) in the nutritionally optimized medium [23, 35].

6.2.3 Key Technology of Lignocellulose Fermentation to Produce Bioethanol

6.2.3.1 Pentose Fermentation

Lignocellulose is composed of three main fractions: cellulose, hemicellulose, and lignin. By hydrolysis, cellulose is changed into a six-carbon sugar (mainly glucose), which is easier for conventional industrial microorganisms to handle to convert to ethanol. However, hemicellulose is changed into five-carbon sugar (mainly xylose), which cannot be utilized by conventional industrial microorganisms generally. However, hemicellulose is a large proportion of lignocellulose. Therefore, the breed of microbes for fermenting pentose to ethanol is a key technology consideration [36].

(1) Microorganisms for xylose fermentation
Species of bacteria, yeasts, and filamentous fungi are capable of fermenting xylose to ethanol. The advantages of bacteria are the many available species, high fermentation rate, and efficiency of not only monosaccharides but also cellulose and bio-high molecular glycans. But, it is easily inhibited in lower concentrations of sugar or alcohol. In addition, there are many by-products generated in the bacteria fermentation process, resulting in a lower final ethanol yield.

Regarding yeast for xylose fermentation, they can be broadly divided into two categories. One class can ferment under anaerobic conditions, such as *P. tannophilus*. Another class needs some oxygen when fermenting, such as *Candida utilis* [37]. Compared with ethanol fermentation by bacteria, yeast have the advantages of high alcohol conversion, high yield, high tolerance, fewer by-products, and less possibility of contamination. Tian et al. used *P. tannophilus* As 21585 to ferment xylose to produce ethanol. When the concentration of hydrolysis xylose was 30 $g \cdot L^{-1}$, the maximum ethanol yield rate was 0.39 $g \cdot g^{-1}$ (85 % of the theoretical yield) [38]. Tang et al. obtained a strain, *Candida shehatae* TZ8-13, that can ferment both xylose and glucose efficiently. The ethanol yields were 21.6 and 24.2 $g \cdot L^{-1}$ respectively, when the concentration of both xylose and glucose was 60 $g \cdot L^{-1}$ [36, 39].

There are few fungi for pentose fermentation, which limits the related research. Current research mainly focuses on *Fusarium oxysporum* and *Neurospora crassa*, which can autoproduce cellulase and hemicellulase and ferment pentose and hexose to ethanol, thus directly producing ethanol and simplifying the process and reducing the cost. Therefore, this is an important direction of lignocellulose utilization that cannot be ignored.

Besides the strains mentioned from nature, now many recombinant strains have been obtained by genetic engineering techniques. The two main ideas are as below: One is to introduce pentose metabolic pathways to efficient hexose-utilizing strains, and the other is to introduce key genes of high ethanol yield to strains that can utilize mixed sugar but the yield is low. The commonly used host strains contain *Saccharomyces cerevisiae*, *Zymomonas mobilis,* and *E. coli*. Sun et al. amplified genes *pdc* and *adhB* encoding essential ethanologenic enzymes in *Z. mobilis* using the polymerase chain reaction (PCR) technique. Recombinant plasmids pKK-PA and pEtac-PA were constructed in which genes *pdc* and *adhB* were placed under the control of the *lac* promoter, respectively. The results indicated that by introducing both *pdc* and *adhB* the ethanologenic pathway was successfully constructed in *E. coli* [40, 41]. Bao et al. cloned *xylA* gene from *Thermus thermophilus* and transformed it into *S. cerevisiae* after treatment. The recombinant xylose isomerase showed activity. Recombinant *S. cerevisiae* strain that can simultaneous express *xylA* and overexpress *TK11* and *TA11* can grow in medium in which xylose as the sole carbon source. By flask fermentation, the strain fermented xylose to ethanol, and the yield was 1.3 $g \cdot L^{-1}$ [41, 42].

(2) Mechanism of pentose fermentation

Currently, the study of the mechanism of pentose fermentation has mainly concentrated on the microbial metabolism of xylose. Xylose isomerization is the initial biochemical reaction. First, xylose is converted to xylulose catalyzed by xylose isomerase in microbial cells and to phosphate xylulose catalyzed by xylulose kinase. Then, it enters the pentose phosphate cycle. After a series of biochemical reactions, it is finally changed into ethanol and other metabolites.

In xylose isomerization, metabolic pathways of bacteria, filamentous fungi, and yeasts are different. To most bacteria and actinomycetes, it is only a one-step reaction catalyzed by xylose isomerase. To yeast and filamentous fungi, first xylose is reduced to xylitol by a NADPH-dependent xylose reductase (XR), and then xylitol is oxidized to xylulose by NAD-dependent xylitol dehydrogenase (XDH). Take yeast as an example: The yeast xylose fermentation is conducted under facultative anaerobic conditions, and the overall reaction formula is as follows:

$$6C_5H_{10}O_5 \rightarrow 9C_2H_5OH + 12CO_2$$

Therefore, the theoretical yield of xylose fermentation to ethanol is 0.46 g ethanol$\cdot g^{-1}$ (in terms of glucose mass), which is lower than the yield of glucose fermentation to ethanol, 0.51 g ethanol$\cdot g^{-1}$ (in terms of glucose mass) [41].

6.2.3.2 Immobilized Cell Fermentation

There are several advantages of immobilized cell fermentation. For example, cell concentration is increased, and the cell can be used continuously. Thus, the final alcohol concentration in the fermentation broth can be improved. Currently,

immobilized yeast and *Zymomonas* spp. bacteria are studied most. Commonly used carriers include calcium alginate, carrageenan, porous glass, and the like. It has been reported recently that microorganisms are immobilized in a gas-liquid interface to ferment, which is more active than immobilization on a solid medium. In addition, immobilized mixed-cell fermentation can improve efficiency; for example, immobilized yeast and cellobiose enzyme together could convert cellobiose into alcohol directly [1].

6.2.4 Demonstration Project

Lignocellulose is the most abundant renewable resource on the planet; it can be derived from industrial and agricultural waste, forestry waste, and municipal waste. The conversion of lignocellulose to bioethanol has attracted much interest from governments around the world. In the United States, Verenium Corporation has the first demonstration cellulosic ethanol plant, with an annual output of 5,299,000 L of cellulosic ethanol; the plant was put into operation in May 2008. Besides that, a cellulosic ethanol industrialization demonstration project jointly supported by the U.S. Department of Agriculture and the Department of Energy also includes the companies Abengoa, Broinusing, and Iogen, which use corn straw, whole corn (including straw), and wheat straw as raw materials, respectively [43].

Bioethanol in China started late but developed rapidly. At present, China has already become the world's third-largest producer of bioethanol after the United States and Brazil. A number of breakthroughs in key technologies have been made in the cellulosic ethanol industry of China, and several sets of pilot plants and demonstration projects have been constructed. The National Key Laboratory of Biochemical Engineering, Institute of Process Engineering (IPE), Chinese Academy of Science (CAS), has been committed to the study of cellulose conversion since more than 10 years ago and achieved improvements in solid-state fermentation technology industrialization and fractionation utilization of whole-straw biomass. Based on these techniques, a demonstration project with annual straw ethanol outputs of 3,000 t has been successfully established at Zesheng Bioengineering Technology Company, Limited, in Shandong Province, China. This demonstration achieved the high-efficiency and environmentally friendly production of ethanol by integrating the following technologies: 5-m^3 steam explosion system, 100-m^3 cellulase solid-state fermentation system, and 110-m^3 straw solid-phase enzymolysis; and synchronization fermentation-adsorption-separation triple coupled-reaction device, as well as equipment [44]. Another project with annual straw ethanol outputs of 5,000 t has been successfully established and started trial operation in 2008 at Tianguan Group in Henan Province, China. This project includes the construction for an annual output of 10,000 t cellulase and 5,000 t straw ethanol plant sets and related public works. East China University of Science and Technology started to study the technology of agricultural and forestry waste to produce bioethanol in 1990–1995. A pilot plant with annual straw ethanol outputs of 600 t using acid

hydrolysis has been established and passed identification by the Ministry of Science and Technology, China. The project uses sawdust and rice husk as raw materials, and allegedly the cost is about 6,000 RMB for each ton of ethanol.

6.3 Biohydrogen

6.3.1 Introduction of Hydrogen Energy

Cleanliness, efficiency, and renewability are the focus of new energy. Hydrogen satisfies these conditions. Hydrogen is a secondary energy, compared with other kinds of energy; it has the following significant advantages:

(1) Hydrogen itself is nontoxic. When burned, its only by-products are water and a little nitride hydrogen, not like fossil fuels, which generate large amounts of soot agent, CO, CO_2, hydrocarbons, lead compounds, and so on. The small amount of nitride hydrogen would not pollute the environment after treatment, so hydrogen provides the cleanest energy.

(2) The combustion property of hydrogen is good. It is easy to light and burns fast. It has a wide combustible range mixed with air. Except for nuclear fuel, the heating value of hydrogen is the highest of all fossil fuels, chemical fuels, and biofuels. The combustion heats of hydrogen and gasoline are as follows:

$$\text{Combustion heat of hydrogen}: 2H_2 + O_2 \rightarrow 2H_2O + 115.60 \text{ kcal}$$

$$\text{Combustion heat of gasoline}: C_5H_{12} + 8O_2 \rightarrow 5CO_2 + 5H_2O + 781 \text{ kcal}$$

Converted into combustion heat per kilogram, hydrogen is 28,900 kcal·kg^{-1}, while gasoline is 10,848 kcal·kg^{-1}. Therefore, the combustion heat of hydrogen is nearly three times the combustion heat of gasoline, which is about 4.5 times the combustion heat of coke.

(3) The thermal conductivity of hydrogen is the best of all gases—11 times the majority of the others. Therefore, hydrogen is an excellent heat transfer carrier in the energy industry.

(4) Hydrogen can be used in many cases. For example, it can be used as engine fuel directly, chemical raw materials, fuel cell fuel [45, 46], structural materials (solid-state hydrogen), and more. It does not require major transformation of the existing technology and equipment to use hydrogen instead of coal and oil. The existing internal combustion engine needs only a little modification so it can be used.

(5) Hydrogen can exist as metal hydrides in the gas, liquid, or solid state. It can satisfy storage, transportation, and various different application environment requirements.

(6) Development of hydrogen energy has promoted the development of other related studies, such as for fuel cells, and the research and development of new hydrogen storage materials. Fuel cells are using hydrogen as fuel. Compared with ordinary batteries, fuel cells transfer chemical energy stored in the batteries instead of storing chemical energy. The reverse reaction of water electrolysis is carried out within them. It is not necessary to charge the battery; only external fuel and oxidizer need to be provided to generate electricity continuously. In the reaction process, the machine parts also do not necessarily move. Thus, the whole process is quiet. And, the efficiency is 2.5 times that of the internal combustion engine. Besides that, a fuel cell leaves only electricity, heat, and pure distilled water, so is without pollution. The hydrogen fuel cell would produce enough power to meet humankind's distant future needs.

In view of these advantages, the development of hydrogen energy has aroused great interest. As early as the 1990s, the United States, Japan, Germany, and other developed countries initiated a research and development plan for hydrogen energy. Its short-term goal is the commercialization of hydrogen fuel cell vehicles and, using this as the lead, to promote hydrogen energy to account for a considerable share of the overall energy system in about 20 years. The long-term goal is that hydrogen becomes the main energy source instead of fossil energy when fossil energy is running out. The demand for hydrogen is rapidly expanding. Hydrogen production in the twenty-first century must be environmentally friendly and must not rely on fossil fuels. Therefore, under the premise of no pollution or less pollution, the raw materials for hydrogen production need to be effective and renewable. Therefore, using lignocellulose to produce hydrogen by biotechnology is an important direction, particularly for agricultural countries, such as China.

With the continuous progress of science and technology, hydrogen energy applications are no longer unrealistic. It is proposed that the future economy will become a "hydrogen economy." Hydrogen energy is transferred into power, with electricity sent to millions of households, becoming a kind of general fuel; with electricity, they will be the two pillars of the energy system in twenty-first century.

6.3.2 Research Status of Hydrogen Production Technology

There are several methods to produce hydrogen, such as compound re-forming, decomposition, photolysis or hydrolysis, electrolysis of water, and microbial fermentation or photosynthesis [47]. At present, the commonly used methods are mainly three types: (1) chemical methods, (2) electrolysis of water, (3) biologic methods. The first two methods have been studied for a long time, and the biologic methods are new.

6.3.2.1 Chemical Methods

Use of fossil fuels such as coal, oil, or natural gas to produce hydrogen is common worldwide currently. It has mature technology and industrial installations, accounting for more than 90 % of the global hydrogen production [48, 49]. Its methods mainly include heavy oil partial oxidation, natural gas transformation with steam, and coal gasification [50].

However, there are many disadvantages when fossil fuels produce hydrogen. First, this process requires much primary energy, such as oil, natural gas, and coal, which cannot fundamentally solve the energy problem. Second, the process is not advanced, economic efficiency is low, and pollution is serious. The emissions contain a large amount of carbon monoxide, sulfur dioxide, and oxides of nitrogen; these harmful gases cause significant environmental pollution. Because of the complex composition of the gas product, the cost of subsequent separation is also high.

6.3.2.2 Hydrogen Production by Water Electrolysis

Water electrolysis for hydrogen production is a completely clean method; it can be used to adjust peaking and store energy in power plants. That is, when demand for electricity is low, the redundant power is used in water electrolysis to produce hydrogen; when the demand for electricity is high, the chemical energy stored in hydrogen is converted to electricity by a chemical or electrochemical method. However, the main problems with this approach are high energy consumption, low efficiency, and restriction in site hydrogen production. Currently, an efficient hydropower solution is being developed by scientists all over the world. For example, high-temperature pressurized water electrolysis has been developed by Japanese scientists. Its efficiency has reached 75 %. A solid polymer electrolyte (SPE) method has been developed by General Electric Company in the United States. Its efficiency has reached 90 % [47, 50].

6.3.2.3 Biomass Hydrogen Production

Because of so many shortcomings of the traditional methods of hydrogen production, people began to turn their attention to find renewable and cheap raw materials and clean and efficient methods of hydrogen production. Biomass, as storage for solar energy, has advantages of renewability, low sulfur content, wide distributed, and large amount. Therefore, it is a sustainable clean and strategic energy. Using biomass for hydrogen production not only can reduce pollution but also can save nonrenewable energy. Now, it is becoming a research focus.

There are two main routes for biomass-based hydrogen production. One is the thermochemical route, such as gasification. The other is the biochemical route. These two routes are introduced next.

(1) Biomass gasification hydrogen production

Biomass gasification means thermochemical conversion of biomass, resulting in production of combustible gases consisting of carbon monoxide (CO), hydrogen (H_2), and traces of methane (CH_4). This mixture is called producer gas [51–53]. Producer gas can be used to run turbines and engines directly [54]. Biomass gasification is the earliest development process and the nearest to scale production technology of all biomass thermochemical processes. This method requires the moisture content of biomass materials to be below 35 %. Compared with coal, biomass materials generally contain a low ash content and almost no sulfur, while coal contains about 1 % of sulfur and about 10 % of ash, so there is no need to worry about sulfur contamination and ash treatment with biomass gasification. In addition, the ash content of biomass can be used as a fertilizer. Therefore, biomass is more suitable for gasification processing than coal.

The main reaction steps of biomass gasification are as follows [54, 55]: (1) Biomass is transferred into gas, coke, and first tar via thermal decomposition. (2) First tar is converted into gas via pyrolysis, and secondary and tertiary tar are meanwhile generated. (3) Pyrolysis of secondary and tertiary tar occurs. The chemical reactions include heterogeneous and homogeneous gasification reactions for generating coke during pyrolysis, combustion of coke, and an oxidation reaction of combustible gases generated during pyrolysis.

The main process of biomass gasification is shown in Fig. 6.4. To provide the thermodynamic conditions demanded by the reaction, air, oxygen, or steam is needed during the gasification process to realize the partial combustion of raw materials. The main difference between gasification and combustion is that in combustion sufficient oxygen is supplied to make the raw materials burn fully. The goal of combustion is to obtain energy, and the final products are carbon dioxide, steam, and other flue gases that cannot be recombusted. In gasification, only part of the oxygen demanded by the thermal chemical reaction is supplied to retain as much energy as possible in the combustible gas obtained after the reaction. The gasification medium can affect fuel gas composition and the ease of tar treatment, which is generally air (oxygen), steam, or the mixture gas of oxygen and steam. Corella et al. [57] agreed that cracking of tar produced in gasification catalyzed by dolomite using steam or a mixture of steam and pure oxygen as the gasification medium is easier than using air as the gasification medium. The former main products are H_2, CO, and a little CO_2, with these noncondensable gases and macromolecular hydrocarbons condensing at room temperature. Then, pyrolysis oil is converted into hydrogen and other flammable gases. Finally, noncondensable hydrogen-enriched gas is obtained after a re-forming reaction [58]. Because of the composition of gases generated, gasification is complicated. It is difficult to purify hydrogen gas. In addition, biomass gasification requires high-quality equipment, and the efficiency of hydrogen production needs to be improved.

Biomass gasification reactors can be classified primarily as updraft fixed-bed gasifiers, downdraft fixed-bed gasifiers, and circulating fluid beds. For the updraft gasifier, the structure is simple, and it is easy to conduct gasification. However, when the wet materials fall from the top, part of the water is taken away by the rising hot

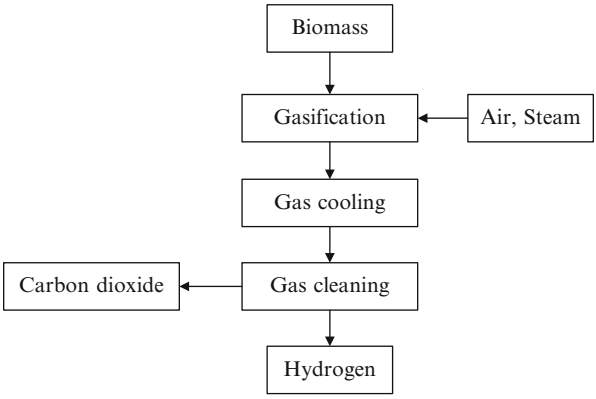

Fig. 6.4 The main process of biomass gasification to produce hydrogen [56]

air, resulting in the decrease of the H_2 content in the product gas. The downdraft gasifier improves the H_2 content of the product gas, but its structure is complex and difficult to operate. In recent years, the fluidized bed gasifier has been a study focus. It is able to handle fuels with different shapes and sizes and transfer heat and mass quickly. Therefore, it is more suitable for continuous large-scale production [59].

At present, although biomass gasification technology has been maturing, there are still many problems, such as high tar content. Unneeded tar would form tar aerosols and polymerize into a more complex structure, which is not conducive for steam re-forming to hydrogen. Three methods are generally used to reduce tar generation. They are appropriate setting of the gasifier, appropriate control and operation, and addition of catalyst. The operating parameters, such as temperature, gasifying agent, and residence time, play major roles in tar formation and decomposition. In addition, the added catalyst not only can reduce tar content but also can improve the product gas quality and conversion efficiency. Wei et al. [60] have studied the effects of different naturally occurring catalysts, limestone, olivine, and dolomite in the same unit, and found that dolomite reveals a comparably good performance in terms of catalytic activity of tar destruction and the consequential increase in the production of gases [54].

Generally, biomass-catalyzed gasification is still in the stage of laboratory research worldwide. In addition, both gasification and catalytic processes need to consume a great deal of energy, which is an important aspect that needs improvement.

(2) Microbial metabolism for hydrogen production

Microbial metabolism to produce hydrogen utilizing organics is a new method of hydrogen production. This method not only is environmentally friendly, clean, and energy saving but also can take advantage of wastewater, solid waste, urban waste, and lignocellulose, combining waste utilization with energy recovery. Therefore, it has a broad application prospect. Next, the status quo and the existing problems of microbial metabolism for hydrogen production are analyzed.

6.3.3 Development, Status, and Problems of Biological Hydrogen Production

As early as the nineteenth century, people recognized that bacteria and algae could produce molecular hydrogen. The oil crisis in the 1970s prompted awareness of governments and scientists regarding the urgency of seeking alternative energy, with biological hydrogen production first considered practically possible. Since then, various biological hydrogen sources and hydrogen production technologies have started to be researched. Currently, the dual pressure of energy and environment resulted in the reemergence of biological hydrogen production research. A variety of modern biotechnologies applied in biohydrogen production has greatly promoted the development of biohydrogen production technology. At present, it is reported that the hydrogen-producing taxa include photosynthetic organisms (photosynthetic bacteria, cyanobacteria, and green algae); nonphotosynthetic organisms (obligate anaerobic bacteria, facultative anaerobic bacteria); and archaeas. Special hydrogen metabolism systems exist in these microorganisms. In general, different kinds of microbial mechanisms and processes of hydrogen production are mainly the ones discussed next.

6.3.3.1 Dark Fermentation Hydrogen Production

Nonphotosynthetic organisms that can produce hydrogen by dark fermentation include obligate anaerobes, facultative anaerobes, and archaeas (e.g., *Clostridium butyricum*, *Clostridium acetobutylicm*, *Escherichia coli*, *Enterobacter aerogenes*, *Azotobacter chroococcum*, *Ruminococcus albus*, *Rhizobium*, etc.). Compared with photosynthetic hydrogen production, dark fermentation has more advantages, such as strong hydrogen-producing ability and rapid growth rate of the strains; wide range of low-cost substrates; lack of need for alight source; and simple and convenient reactor design, operation, and management. Compared with photosynthetic hydrogen-producing strains, which mostly belong to the medium-temperature (about 35 °C) type, the anaerobic fermentation hydrogen-producing strains belong to the high-temperature type. They can produce hydrogen at about 55 °C, and at high temperature, it is not easy to infect bacteria. Thus, the reaction conditions are relatively easy to implement, and anaerobic fermentation hydrogen production is easy to realize industrialization [61].

(1) Anaerobic fermentation hydrogen production
Fermentative hydrogen-producing strains mainly include four categories: *Enterobacter*, *Clostridium*, *Escherichia,* and *Bacillus* spp. Among them, *Enterobacter* spp. and *Clostridium* spp. are studied most. Fermentative hydrogen-producing strains can utilize a variety of substrates to produce hydrogen catalyzed by nitrogenase or hydrogenase. These substrates include formic acid, lactic acid, pyruvic acid, short-chain fatty acids, glucose, starch, xylose, cellobiose, sulfide, and others.

Industrial wastewater, sewage, municipal solid waste, sludge, and agricultural waste contain a large amount of available cellulose, hemicellulose, and carbohydrates. Using them to produce hydrogen not only can reduce the cost of substrates but also can purify the environment. Li et al. [62–64], using steam-exploded straw as a substrate, used *Clostridium butyricum* AS1.209 to produce hydrogen through simultaneous saccharification and solid-state fermentation. The maximum hydrogen production reached 68 $mL \cdot g^{-1}$ steam-exploded straw. Noike et al. [65] used bean curd manufacturing waste as a substrate to conduct a continuous hydrogen production experiment at pH 5.5 and 35 °C and achieved initial success. Taguchi et al. [66] used *Clostridium* sp. to ferment cellulose hydrolyzate. After 81 h of static culture, the substrate degradation rate was 0.92 mmol glucose$\cdot h^{-1}$, and the hydrogen production rate was 4.1 $mmol \cdot h^{-1}$. Van Ginkel et al. [67] studied the potential for biological hydrogen production from apple-processing wastewater, potato-processing wastewater, and confectioners-processing wastewater, and found that potato-processing wastewater obtained the largest hydrogen yield, 0.21 $L \cdot COD^{-1}$ (Chemical Oxygen Demand). Wang et al. [68] examined the effects of different pretreatments on the hydrogen production of sludge and found that freezing and thawing and sterilization increased the specific hydrogen yield to 1.5–2.5 times that of untreated sludge.

The traditional fermentation hydrogen production mainly includes the butyric acid type and propionic acid type. For example, glucose is utilized by *Clostridium acetobutylicm* and *Clostridium butyricum* to conduct acetone–butyric acid fermentation associated with hydrogen production [69]. Li et al. [70], using carbohydrate as the hydrogen donor, discovered a new type of fermentive hydrogen production: ethanol-type fermentation. Its products include ethanol, acetic acid, hydrogen, CO_2, and a small amount of propionic acid and butyric acid. Production of hydrogen by methanogens has also been reported. Methanogens convert the previous product, methane, into hydrogen catalyzed by hydrogenase. Valentine et al. [71], using four species of methanogens (*Methanothermobacter marburgensis*, *Methanosaeta thermophila*, *Methanosarcina barkeri*, and *Methanosaeta concilii*) obtained a large amount of hydrogen.

(2) Facultative anaerobic bacteria fermentation to produce hydrogen
Facultative anaerobic bacteria, including *E. coli* and *Enterobacter* spp., contain a cytochrome system. Hydrogen can be produced by the metabolic pathway of formic acid decomposition [72]. In the process, pyruvate is converted into formic acid by decarboxylation catalyzed by pyruvate formate lyase when a suitable electron acceptor or oxygen is lacking. Then, the generated formic acid is decomposed to hydrogen by hydrogenase.

6.3.3.2 Archaeas Hydrogen Production

The extreme thermophile is a special kind of bacteria. Fardeau et al. [73] screened a new thermophilic strain, *Thermotoga hypogea,* from an oil-producing well. The

strain can grow at 90 °C. Through improvement and training, it can achieve a higher level of hydrogen production. The VanNiel research team from the Netherlands has been committed to research in this area and filtered out two extreme thermophiles, *Caldicellulosiruptor saccharolyticus* (grown on sucrose) and *Thermotoga elfii* (grown on glucose). The hydrogen production is 3.3 mol H_2/mol C_6 sugar units, which is about 83 % of the theoretical maximum. In addition, these two bacteria have a wide range of substrates, and they have already been used to produce hydrogen utilizing wastepaper pulp and lignocellulose [74–79]. Ma and Adams [80] found that the cytoplasmic hydrogenase of *Pyroeoccus furiosus*, which is responsible for H_2 production with ferredoxin as the electron donor, has been shown also to catalyze the reduction of polysulfide to H_2S. This enzyme is named sulfide dehydrogenase (SuDH) and catalyzes the reduction of polysulfide to H_2S with NADPH as the electron donor. The disposal of excess reducing power produced during fermentation is carried out through coupling with the ferredoxin oxidoreductase system. The oxidoreductase system also contains NADP–ferredoxin oxidoreductase, sulfide dehydrogenase, and more [69].

6.3.3.3 Mixed Strains to Produce Hydrogen

Strain is one of the key factors in biological hydrogen production technology. Pure strains have higher hydrogen productivity, but the fermentation conditions are strict, and the cost is high. For lignocellulose and other organic waste, because of the complexity of composition, the coordination among bacteria is important. Analyzed in the view of microbial ecology, the coordination among bacteria is actually an alternate or symbiotic relationship between the strains. The effect of mixed stains in biodegradation, sewage treatment, and improvement of bioproduct production, heavy metal tolerance, and methanogenesis is obvious. Compared with a pure culture of strains, specific advantages of mixed strains include (1) no sterilization requirements; (2) less secondary pollution because of that the intermediate procedures, such as repeated adapter and preservation, are reduced by using mixed strains, and therefore the chance of secondary pollution is greatly reduced; (3) adaptive capacity owing to microbial diversity, and the capacity to use mixed substrates; and (4) the possibility of a continuous process [81]. Therefore, in recent years, mixed strains for producing hydrogen have received increased attention.

(1) Mixed strain dark fermentation to produce hydrogen
Mixed strain dark fermentation to produce hydrogen is studied currently. The strains include not only natural unknown strains, such as anaerobic activated sludge and cow dung, but also artificial strains built on known strains. Li et al. [82] used anaerobic fermentation sludge to treat high-concentration wastewater directly and compared it with the use of pure hydrogen-producing bacteria selected from anaerobic fermentation sludge. It was found that the bio-producing hydrogen ability of the mixed bacteria was higher than that of pure bacteria because of the coordination among bacteria. By using refined sugar as a substrate, the largest capacity H_2 production was 76.4 $mL \cdot g^{-1} \cdot h^{-1}$. Experiments showed that the

coordination among bacteria can stabilize the ecological system so that the value of bio-producing hydrogen would be increased. Liu et al. [83] studied the ability of hydrogen production of *Clostridium butyricum* D2, *Enterobacter aerogenes* C3, and *Candida maltosa* M4. With a mixed culture of these three strains at 36 °C for 48 h, the volume of hydrogen production was 22.2 mL, and the average rate of hydrogen production was 15.42 $mL{\cdot}H_2{\cdot}h^{-1}{\cdot}L^{-1}$, which showed that a mixed culture of three strains had a synergistic effect on hydrogen production. Zhang and Xing [84] analyzed a mixed culture consisting of gfp-harbored *Enterobacter aerogenes* and *Clostridium paraputrificum* for hydrogen production. The concentrations of the respective strains in the mixed culture were calculated using aerobic fluorescence recovery (AFR) technology. Fan et al. [85] treated the simulated organic wastewater containing sucrose and starch with natural anaerobic microorganism bacteria from cow dung compost. The biohydrogen was produced through anaerobic hydrogen fermentation with synchronous purification of the wastewater.

(2) Mixed strain phototrophic hydrogen production

Mixed strain phototrophic hydrogen production has been little studied at present. Han et al. [86] reported the most suitable conditions based on experiments of three kinds of mixed strains of *Rhodopseudomonas* growing at different combinations of carbon source, nitrogen source, pH value, and growth temperature. Miura et al. [87] achieved stably sustained continuous production of hydrogen with high molar yield through a combination of dark fermentive hydrogen evolution by *Chlamydomonas* sp. strain MGA161 and hydrogen photoevolution by a marine photosynthetic bacterium W-1S in an alternating light-dark cycle as a model of the day-night cycle. Zhang et al. [82, 88] studied the main conditions of hydrogen production by mixed cultivation of *Rhodobacter sphaeroides*. The result showed that the anaerobic and illumination atmospheres were necessary for hydrogen production, and suitable conditions were a temperature range from 32 to 40 °C, pH from 5 to 8, and inoculation from 5 to 15 %. The highest hydrogen production with 1 % glucose was 1.62 $L{\cdot}L^{-1}$ in the best condition.

(3) Mixed photosynthetic and dark fermentation hydrogen production

The substrates utilized by different kinds of microorganisms vary greatly. Dark fermentation strains can utilize a variety of organic compounds, even some large molecules, such as cellulose. The by-products include short-chain organic acids generally, which cannot be further used by dark fermentation strains. These organic acids decrease the pH value and reduce system stability. At the same time, these short-chain organic acids happen to be suitable substrates for photosynthetic hydrogen-producing microorganisms. Therefore, coupling these two methods together not only can increase hydrogen production but also can improve the utilization of organic matter. The process has become a new direction in the development for the biohydrogen production process.

Miyake et al. [89] immobilized *Clostridium butyricum* and *Rhodopseudomonas* sp. to produce hydrogen through glucose degradation; the yield was 7 mol $H_2{\cdot}mol^{-1}$ glucose. Yokoi et al. [90] used a mixed culture of *C. butyricum, Enterobacter aerogenes,* and *Rhodobacter sphaeroides* to produce hydrogen from a sweet potato

starch residue. Stable hydrogen production was observed for more than 30 days, and the average hydrogen yield was about 4.6 mol $H_2 \cdot mol^{-1}$ glucose. Hydrogen production using *C. butyricum* singly was only 2.4 mol $H_2 \cdot mol^{-1}$ glucose, and nitrogen sources had to be added to the medium. The reason was that *C. butyricum* can produce hydrogen from the starch because it has the ability to produce amylases, while *E. aerogenes* cannot utilize the starch directly and must utilize sugars such as glucose and maltose, which were generated in the degradation of the starch by amylases from *C. butyricum.* In addition, in this process, not only hydrogen was produced in glucose degradation but also small molecular organic acids were produced that cannot be utilized by both *C. butyricum* and *Enterobacter aerogenes*, which decreased the pH value of the medium and changed the growth conditions of the microorganisms, resulting in the decrease of hydrogen production. While *C. butyricum, E. aerogenes,* and *R. sphaeroides* were cultured together, the organic acids can be degraded by *R. sphaeroides* so that the pH value of the medium is kept constant; thus, glucose can be fully utilized and hydrogen production greatly increased [91].

Mixed photosynthetic and dark fermentation can increase hydrogen production greatly. However, to maintain the stability and high yield of a microecological system, modern biological technology must be used. Because of the application of mixed-culture technology and new biotechnology, green energy production technology for biohydrogen production has great potential and superiority.

6.3.4 Hydrogen Production by the Fermentation of Lignocellulose

From the development trend of biohydrogen production, using low price and abundance source materials is an efficient way to lower the high cost. A new development direction in this field is the utilization of biomass, especially cellulose. The complex structure of lignocellulose is the main obstacle. At present, converting lignocellulose into energy mainly involves the following three stages: The first is pretreatment to destroy the complex lignocellulose structure. Second is transforming pretreated lignocellulose into fermentable sugars. The third stage is converting fermentable sugars by microbial fermentation into different products, such as hydrogen and so on.

At present, there have been few reports of hydrogen production by the fermentation of lignocellulose. Lay [92] transformed microcrystalline cellulose into hydrogen using extreme thermophilic anaerobic bacteria. Taguchi et al. [66] transformed cellulose hydrolyzate into hydrogen using *Clostridium* sp. These studies have achieved good experimental results, but what they used is cellulose, not natural lignocellulose.

Li et al. [62] produced hydrogen by simultaneous saccharification and fermentation from steam-exploded corn straw (SECS) using *C. butyricum* AS 1.209. After steam explosion pretreatment, straw was loose and soft, and the accessibility to cellulase was increased. Many monosaccharides, such as glucose, xylose, lactose, and so on, produced in the process of steam explosion could be used as feedstock by *C. butyricum* AS 1.209 for fermentation initiation. For straw that shows relatively low density, the maximal hydrogen yield reached 68 $mL \cdot g^{-1}$ SECS using solid-state fermentation.

Although the study of hydrogen production from lignocellulose has made progress, the cost of each craft, mainly the cellulase cost, is still high. In addition, the hydrogen production efficiency is still needs to be further improved. So, great efforts are needed for actual utilization of lignocellulose resources.

6.4 Biogas Fermentation

Biogas typically refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen. It is primarily CH_4 and CO_2 and may have small amounts of hydrogen sulfide (H_2S), moisture, CO, and so on. The composition of biogas varies depending on the materials and conditions. For example, biogas fermented from human and livestock manure sometimes has methane concentrations above 75 %, while using straw as a substrate can generally produce biogas with about 55 % methane.

Biogas, fermented in biogas digesters, has been widely used for household energy in China's rural areas. However, with the increasing severity of energy and environmental issues, the requirements for traditional biogas are increasing. Its use would include a large-scale centralized gas supply, gas-fired power generation, transportation, and conversion of chemical products. In this sense, biogas would become a new energy that uses various biomasses as raw materials and is produced by large-scale automation of the modern industrial fermentation process in the future [93]. Therefore, great efforts to develop biogas not only can solve the current fuel shortage but also can be the way to produce alternative energy in the future. Thus, it is an event of great strategic significance.

6.4.1 Biogas Utilization Around the World

Currently, biogas utilization development is in the large-scale, intensive direction. Europe, the United States, and other developed countries commonly use livestock manure as the anaerobic fermentation substrate to produce biogas. In Germany, the annual power production from biogas reaches 6×10^{10} kWh, equal to 11 % of the

total electricity output [94]. In Sweden, natural gas is partially replaced by biogas and then used as vehicle fuel gas, in which the biogas proportion is about 45 %. There are more than 4,000 biogas-powered cars now, and the first biogas-powered train has already been developed [95]. In Britain, biogas can replace 25 % of the country's gas consumption. The United States established a large number of methane automated factories utilizing livestock manure after the energy crisis in 1973. There are also 31 biogas plants running successfully, such as the biogas project in Iowa State, which can treat about 1,500 livestock's waste per day [96].

China has been testing applications of biogas since the 1950s. Rural household biogas digesters are common in China. The volume of these digesters is 5–12 m^3, and the rate of gas yield per unit volume of digester reaches 0.1–0.4 $m^3 \cdot m^{-3} \cdot day^{-1}$. There are also about 2,000 medium-size biogas projects, which can produce 5.5–10^9 m^3 biogas annually. Since 2000, considering the situations and experiences in different places, the Ministry of Agriculture has developed several projects throughout the country [97, 98].

6.4.2 Physiological and Biochemical Processes of Biogas Fermentation

Biogas fermentation is a complex process. It can be artificially divided into three basic stages. Initially, the hydrolytic and fermenting bacteria convert organic materials to small molecule water-soluble compounds, such as monosaccharides, amino acids, glycerin and fat. This stage, hydrolysis, is usually slow and is considered to be the rate-limiting stage of solid organic matter degradation and anaerobic fermentation of suspended solids in wastewater. Second, the hydrogen-producing acetogenic bacteria and the homoacetogenic bacteria convert the products of the first stage to acetic acid, hydrogen, carbon dioxide, and so on. Finally, the methanogenic bacteria convert acetic acid, CO_2, and H_2 into methane. More than 70 kinds of methanogenic bacteria have been studied; they can take advantage of small molecules, such as formic acid, acetic acid, hydrogen and carbon dioxide, generated by the other stages to produce methane gas.

The reaction rate is different depending on the substrate. For example, for lignocellulose, hydrolysis is usually the rate-limiting stage. To sugars, starch, proteins, and other soluble substances, because they can be rapidly decomposed by microorganisms, the third stage is often the rate-limiting step. The anaerobic digestion process can be divided into three stages, which are carried out simultaneously in the anaerobic reactor system and maintain a dynamic equilibrium. If the dynamic equilibrium is broken by the external factors, such as pH value, temperature, or organic load, the fragile methanogenic bacteria tend to be affected first. Thus, fatty acids accumulate gradually and finally lead to acidification and stagnation of the system.

6.4.3 Biogas Fermentation Process

6.4.3.1 Impact Factors

Temperature and pH are two important factors regulating fermentation flora and strengthening gas production. According to the optimum temperature, the biogas production process can be divided into mesophilic (35 °C) digestion and thermophilic (55 °C) digestion. Methanogenic bacteria are sensitive to changes in temperature. For example, if the system temperature drops 5 °C in a short period of time, biogas production would decrease significantly. On the other hand, the pH value could influence the end products of hydrolysis acidification. Because propionic acid cannot be used as the substrate by methanogenic bacteria, in a two-phase fermentation process, it is possibly to reduce propionic acid enrichment and try to produce more butyric acid and ethanol by regulating pH, thus avoiding the loss of the carbon source and improving the gas production rate.

Stirring is also important to biogas production. It not only can enhance mass transfer and increase the contact of materials with microorganisms but also can reduce the heterogeneity of temperature and concentration in the device. At present, the methods commonly used include gas stirring, hydraulic stirring, mechanical stirring, and others. On the other hand, mass transfer can also be enhanced by improving the process, such as sludge return, water circulation, and leachate circulation [93].

In the biogas fermentation process, the carbon-to-nitrogen ratio (C/N) of the feedstock has been found to be a useful parameter in adjusting acid production in the hydrolysis acidification stage. Its effects mainly include two categories: It affects biological oxidation of organic matter in the microorganism body and further leads to the change of NADH/$NADH^+$ ratio in anaerobic microorganism cells and the amount of fermentation products. In the other, it can decide which kind of bacteria will be enriched, thus regulating the acid fermentation type [99]. In the anaerobic fermentation system, the C/N is best at 25:1–30:1 generally. Too high a C/N would reduce the conversion rate of carbon, while too low a ratio would increase the accumulation of ammonium salts in the system. Regarding the raw materials that have different carbon and nitrogen contents, the C/N is often regulated through mixed dosing to achieve a suitable level. China's straw production is large. If straw, livestock manure, municipal solid waste, excess sludge, and other organic waste could be used widely in mixed dosing, not only the shortage of raw materials could be eased but also the environment in both cities and villages could be improved.

For lignocellulose, which is difficult to degrade, pretreatment is an efficient method to improve the biogas production rate. The pretreatment methods are various, including physical, physicochemical, chemical, biological, and other methods. These above methods can be used either alone or in combination.

6.4.3.2 Process

(1) Single-phase digestion
Hydrolysis acidification and methane production are conducted in a reactor, namely, as single-phase digestion. The process has many advantages, such as a simple process, small investment, easy operation and management, and relatively mature engineering technology; thus, it is widely applied. According to statistics, about 90 % of the existing processes are single phase, such as the Dranco process, Kompogas process, Valorga process, and so on. The main substrates of single-phase digestion are municipal solid waste and livestock manure, which are readily biodegradable organic waste. The studies of single-phase digestion that utilized lignocellulose such as straw are few, and all were small scale and at the pilot stage [100]. There are mainly two shortcomings of the single-phase process. One is that, because of the optimal conditions required by hydrolysis acidification and gas production are different, the efficiency of the entire process is decreased. The other is that, compared with methanogenic bacteria, the metabolism of acid-producing bacteria is faster; thus, it is easy for them to cause the system to become acidified, eventually leading to the collapse of the entire aerobic digestion.

(2) Two-phase digestion
To avoid the disadvantages of single-phase digestion, two-phase digestion has been developed. In two-phase digestion, hydrolysis acidification and methane production are conducted and optimized separately. In an acidogenic reactor, hydrolysis acidification is enhanced while methane production is inhibited at conditions of low pH and hydraulic retention time. Then, the volatile organic acids and alcohols generated are exported to a methanogenic reactor, in which they are converted into methane. The main advantage of two-phase digestion is that hydrolysis acidification and methane production are both optimized, thus leading to a larger overall reaction rate and biogas yield. In recent years, based on two-phase digestion, improvements have been constantly proposed to further strengthen acid production and final gas production. For example, by integrating conditions, both acid and hydrogen are produced in the first stage, then the acid is exported to the second stage and fermented to methane, thus achieving the coproduction of hydrogen and methane. Currently, coproduction of hydrogen and methane is a hot issue in the study of anaerobic digestion [101, 102].

However, two-stage digestion has not been widely applied as it adds to complexity and as a consequence increases investment and operational costs. At present, most biogas plants still rely on single-phase digestion. However, because of the advantages in efficiency and stability, two-stage digestion has broad prospects.

(3) Dry anaerobic fermentation
According to the content of total solids (TS) in substrates, anaerobic fermentation is divided into wet anaerobic fermentation and dry anaerobic fermentation. In wet anaerobic fermentation, the TS content is generally below 10 %. With advantages of a quick start, mature technology and management, and easy access to materials, wet anaerobic fermentation is the mainstream technology of biogas production. But,

the technology also has significant disadvantages, such as the large reactor volume, difficult separation of biogas and residue, and so on. [103]. In dry anaerobic fermentation, the TS content is 20–30 %. Compared with wet anaerobic fermentation, dry anaerobic fermentation has the following advantages: (1) utilization of a variety of sources of solid organic waste, such as municipal garbage, crop straw, manure, and so on; (2) low operating costs and high capacity per volume unit; (3) saving of water; (4) relatively easy follow-up treatment; (5) stable running process, no scum or precipitation; and (6) relatively low odor emissions [104].

Although dry anaerobic fermentation has such advantages, its application is still a problem. The reasons are as follows: On one hand, mass transfer is not efficient. Accumulation of intermediate metabolites causes feedback inhibition, and low moisture content influences diffusion of cells or enzyme, further affecting the contact of cells or enzyme with substrate. Thus, the substrate conversion rate and gas production rate are reduced. To overcome these difficulties, methods of increasing the quantity of inoculation and strengthening mass transfer can be used to achieve the rapid startup and stability of the system, but the running cost would be increased. On the other hand, the structure, composition, and particle size of substrates are not uniform; thus, it is difficult to control the system, and continuous operation is unstable [104]. Based on these technical difficulties, studies are being carried out around the world.

Study on dry fermentation of household biogas was started in the 1980s in China. Although some achievements have been made, the scale is still small. Ye and Wei began to study an automatic discharging dry fermentation device and the corresponding semicontinuous process in 1986 and passed identification chaired by the Ministry of Agriculture in August 1988 [105]. Ma et al. [106] developed a dry fermentation tank that can maintain constant voltage. Its gas production can satisfy lighting (May to November) and cooking (June to October) for a family of four. Gan [107] studied dry anaerobic fermentation stirring reactors utilizing livestock manure and designed the horizontal stirring reactor. The influence of operating parameters was studied through tests, and the optimal operating parameters have been obtained.

The study of dry fermentation abroad began earlier and is more advanced than in China. At present, biogas dry fermentation technology has been mature abroad. For example, the garbage dry fermentation system, airbag dry fermentation system, wet and dry combined fermentation system, leachate storage bucket dry fermentation system, and other large-scale biogas dry fermentation systems have already been put into production.

6.4.4 Problems in Biogas Fermentation

(1) Low temperature

As previously mentioned, biogas fermentation is affected significantly by temperature. The temperature changes according to season. Especially in winter, the low temperature leads to low or even no gas production. For example, in northern China,

the gas supply is often not stable for as long as several months. Worse, some digesters are damaged by frost. This situation has affected farmers' enthusiasm for biogas seriously and is bad for the development of biogas. Currently, the technology of biogas production in winter mainly involves heat preservation measures, heating methods, the fermentation process, and bacterial cluster optimization [108].

(2) Diversity of raw materials
Currently, raw materials for biogas production are mainly human and animal manure. The utilization of lignocellulose is relatively less. Therefore, on one hand, improving the digestibility of lignocellulosic materials to increase gas production is a method. On the other hand, based on local raw material characteristics, regulating the substrate ratio, such as municipal solid waste, sludge, agricultural waste material, and human and animal manure, and carrying out codigestion is another method to alleviate the shortage of raw materials [93].

(3) Industrialization
Anaerobic fermentation technology for industrial organic wastewater treatment is developing fast and is approaching the industrialization level, but the industrialization for treating solid organic waste for energy production is still not mature. Compared with other countries, anaerobic fermentation technology for treating solid organic waste in China still falls behind. For example, the rate of gas production is low, the maintenance and management levels are low, the technology and equipment are not mature, the process of industrialization is slow, and so on [96].

6.4.5 Study of Lignocellulose Biogas Production

At present, substrates utilized in household biogas digesters in China are mainly livestock manure. Thus, the shortage of raw material is a problem. On the other hand, crop straw production in China reaches about 700 million tons annually, of which rice straw, wheat straw, and cornstalks account for more than 70 %. If these agricultural wastes can be utilized to produce biogas, not only the ecological environment could be improved but also the energy shortage could be eased. Utilizing lignocellulose to produce biogas has been studied, and the factors concerning the rate of gas production, such as the pretreatment, enzymolysis, vaccination rate, temperature, pH, size of particle, stirring style and rate, heavy metals, process, and so on have been discussed. There are still some problems to be solved for industrialization using lignocellulose to produce biogas, such as low gas production, high cost of pretreatment, and ease of secondary pollution production. If these problems could be solved, lignocellulose fermentation to biogas would have broader prospects [109]. From the points of pretreatment and solid-state fermentation using periodical stimulation, Chen and Li [110] proposed a new method using solid-state fermentation to solve the difficulty in the treatment of fermentation residue, to enhance the utilization of raw materials, and to increase gas production by coupling with pretreatment. The main advantages of this method are as follows:

Steam explosion pretreatment destroys the impact structure of raw materials, which is conducive to microbial degradation, thus improving the utilization rate of straw. In the steam explosion process, the solid wastes can be effectively sterilized, which is beneficial for subsequent operation. In addition, periodic stimulation can strengthen mass and heat transfer and improve uniformity of temperature and humidity, thus eliminating the stirring apparatus and increasing the yield of biogas.

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Chapter 7
Applications of Lignocellulose Biotechnology in the Chemical Industry

Abstract The complex composition of biomass determines that lignocellulose is a functional macromolecule body. The method of pretreatment and biological conversion for a single component not only causes the waste of raw materials but also brings the problem of environmental pollution. Different components are able to generate different functional products. This chapter introduces some representative chemicals converted from lignocellulose, including acetone-butanol, lactic acid and polylactic acid (PLA), levulinic acid, 2,3-butanediol, xylitol, furfural, xanthan gum, bacterial cellulose (BC), and so on, with the purpose of providing guidance for the clean, cheap, and highly efficient production of chemicals using lignocellulose as the raw material.

Keywords Chemical industry • Acetone-butanol fermentation • Organic acid • Furfural • Lignin-based chemicals

7.1 Introduction

Biotechnology used in the cellulose chemical industry has great potential because it can change the traditional production process to reduce costs, improve quality, or produce products difficult to synthesize by a chemical method. It also can open new sources of cellulose chemical raw materials to save oil resources. Cellulose and hemicellulose are polysaccharide polymers that can be hydrolyzed to glucose, xylose, and other sugars. These sugars can be changed into ethanol, acetone, butanol, acetic acid, butanediol, and other liquid fuels and chemical raw materials by microbial fermentation. These sugars also can be used as raw materials for organic acid fermentation. The monomer of lignin is phenylpropanoid derivatives, and these substances can be further transformed into other chemical products and used as basic organic chemical raw materials [1]. The chemical raw materials converted from cellulosic feedstock are shown in Fig. 7.1.

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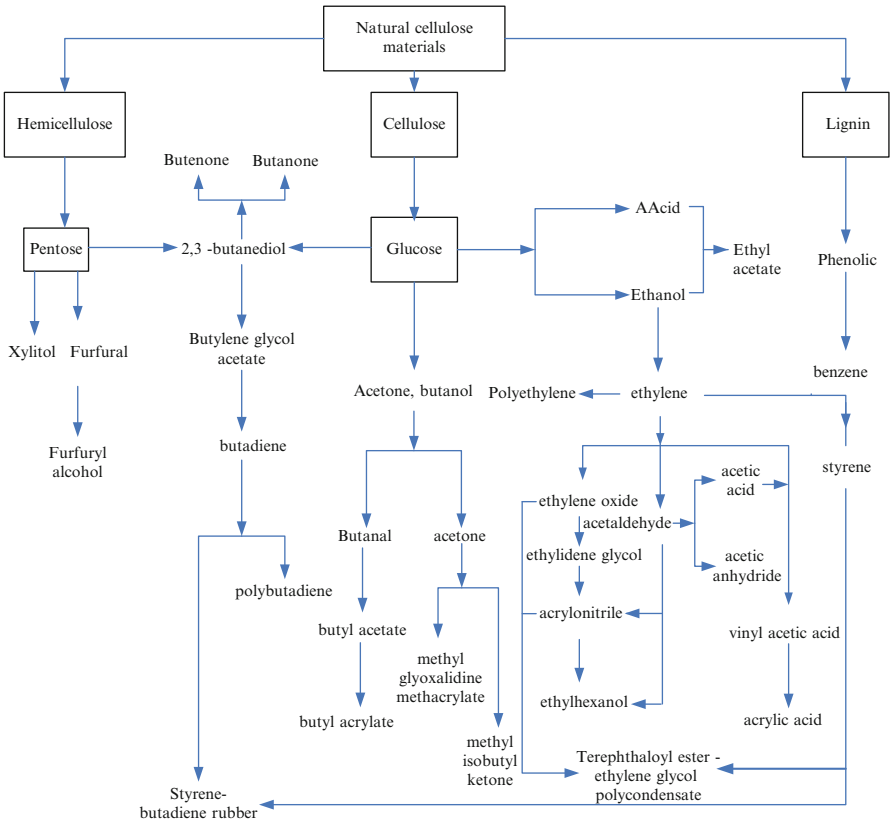

Fig. 7.1 The schematic diagram of natural cellulosic feedstock changed into chemical raw materials [1]

It should be noted that the major route of biotransformation of natural lignocellulosic materials is to isolate lignocellulosic materials into three components—cellulose, hemicellulose, and lignin—by certain means; then, these components are further biotransformed (or fermented) into each kind of small molecule for direct used, are synthesized into polymer materials, or are further converted into cellulosic materials without separation. The complex composition of biomass determines that lignocellulose is a functional body of macromolecules. Different components are able to generate different functional products. The method of pretreatment and biological conversion for a single component not only causes a waste of raw materials but also brings the problem of environmental pollution. So, the idea of component separation-orientation conversion and selectively structural fractionation of biomass will play an important role in the development and utilization of natural lignocellulose [2, 3].

7.2 Fermentation of Acetone-Butanol

Acetone and butanol are important chemicals and organic solvents with many applications in spray paint, explosives, plastics, pharmaceuticals, plant extract, plexiglass, synthetic rubber, and other industrial products. In practice, butanol has the advantages of relatively high calorific value in comparison with ethanol, low vapor pressure, low miscibility with water, and nearly the same energy value as gasoline and can be used in the existing fuel delivery infrastructure and in existing automobile engines without modification. All these advantages mean butanol has been considered more promising for third-generation biofuels than ethanol.

The high cost of traditional raw materials (starch and molasses), accounting for 60 % of the total cost of acetone-butanol fermentation, becomes an important limiting factor of the acetone-butanol fermentation industry. Meanwhile, with the rapid expansion of the population on Earth and the rapidly increasing standard of living, there is a serious shortage of grain for food and industrial use. Lignocellulose resources are the most widely distributed and provide the largest amount of renewable resources. In Asia, rice straw is the main agricultural by-product, 0.561 billion tons annually. In China, there are about 100 million tons of agriculture and forestry plant waste each year. If this type of resource can be used for the raw materials of the fermentation industry, it will ease the food shortage and at the same time create enormous wealth for humans.

7.2.1 Cultivation of Strains

Acetone and butanol fermentation is conducted by *Clostridium* spp., whose typical representatives are *C. butyricum* and *C. acetobutylicum*. This is a class of gram-positive strict anaerobes; oxygen is toxic to them because oxygen can form peroxides by flavoprotein and release O_2^- from the peroxy group by flavin oxidase. The bacteria, which are capable of producing acetone-butanol, do not have catalase or superoxide dismutase. Therefore, they cannot decompose the material to make it nontoxic. The growth of many strains of *Clostridium* is inhibited by oxygen.

Acetone-butanol fermentation is a fermentation process with the conversion of sugar into butanol and acetone. At the same time, acetic acid, butyric acid, and ethanol are also produced, and CO_2 and H_2 are released in the fermentation process. At present, strains that produce acetone-butanol are *C. acetobutylicum* and *C. beijerinckii,* using corn as a substrate, and *C. acetonicum,* using molasses as a substrate [4]. *Clostridium acetobutylicum* produces butanol in the stationary phase, while *C. beijerinckii* produces it in the log phase. The picture shows the molecular formula of conversion from glucose into acetone and butanol.

$$\underset{\text{Glucose}}{12C_6H_{12}O_6} \rightarrow \underset{\text{n-Butanol}}{6CH_3CH_2CH_2CH_2OH} + \underset{\text{Acetone}}{4CH_3COCH_3} + \underset{\text{Ethanol}}{2CH_3CH_2OH} + 18H_2 + 28CO_2 + 2H_2O$$

In the preservation of high-yield strains, it is necessary to rejuvenate to avoid loss of activity. Experiments showed that the highly heat-resistant strains have high yield and strong fermentation capacity, so liquid cultures should be heated to 100 °C for about 1–2 min in the rejuvenation, then cooled, so that the cells that have lower resistance are killed, and the most suitable spore can be collected. Repeating the whole process is advised to obtain better strains. Because of invasion by phages, earlier, especially in the 1920s, even a whole production batch was often destroyed. Some phages are exclusively parasitic in certain strains of *Clostridium* spp., but now phage-resistant *Clostridium* spp. strains are able to be cultivated. In modern industry, phage-resistant *Clostridium* spp. strains are commonly used.

Some of the strong chemical mutagenic factors, such as methyl sulfonic acid ethyl ester (EMS), nitrosoguanidine (NTG), allyl alcohol, and so on, as well as ultraviolet and physical mutagenic conditions are usually used to screen *Clostridium* strains with high yield of solvent production. Annous and Blaschek [5] reported that through NTG mutagenesis and 2-deoxyglucose screening, a high-yield solvent mutant BA 101 was obtained from *C. beijerinckii* NCIMB 8052. Studies by Formanek et al. [6] showed that the bacteria could obtain 27.5 g L^{-1} total solvents from P2 medium containing 6 % glucose. Chen and Blaschek [7] used this mutant strain to ferment on the MP2 medium added to 60 mM acetic acid and 8 % glucose fermentation and found that it could produce up to 32.6 g·L^{-1} of total solvents, including a butanol content of 20.9 g·L^{-1}, which was the highest level of international batch fermentation of acetone-butanol. Subsequently, Qureshi et al. [8–10] studied the industrial applications of this strain combined with the solvent extraction process. Zhang et al. [11] obtained *Clostridium acetobutylicum* EA 2018 and EA2019 strains by soil sample separation, chemical mutagenesis, and resistance screening. The solvent yield fermented by the two mutants in 8 % of the corn mash was 20 g·L^{-1}, in which the percentage of butanol was up to 70 %, which was 10 % higher than the original strain.

With the development of molecular biology techniques, metabolic engineering transformation of *Clostridium* spp. is possible. With the introduction of exogenous genes and regulatory factors, metabolic engineering is different from strain transformation in the traditional sense. The use of recombinant technology can change the enzymatic reactions, and the transform and regulation functions can increase the target composition in the cell. The first attempt at metabolic engineering transformation of *Clostridium* spp. for solvent production was to overexpress *adhE*; however, the solvent production did not increase significantly [12]. Harris et al. knocked down *solR* gene, which suppresses the solvent produced in *C. acetobutylicum* ATCC 824, and then added the *adhE* gene. In cell growth medium (CGM) containing 8 % glucose and with a pH of 5, they obtained 27.94 g·L^{-1} total solvents with 17.6 g·L^{-1} of butanol, which currently the highest solvent yield from genetically engineered bacteria [13].

7.2.2 Substrate Utilization

Clostridium acetobutylicum is able to use polymeric substrates such as starch and xylan, but not cellulose [14]. The degradation of starch is mediated by α-amylase, and genetic data indicates that the presence of α-amylase is evident in at least two α-amylase genes. Larch wood xylan is hydrolyzed by the action of endoxylanases and β-D-xylosidase. *Clostridium beijerinckii* also grows on starch and employs the catalytic activities of glucoamylase and α-amylase. However, direct butanol fermentation using lignocellulosic biomass as the raw material is a consolidated bioprocess by a cellulolytic, solventogenic bacteria that remains a future task. Only fairly active minicellulosomes were found in *C. acetobutylicum* ATCC 824, and attempts to correct reading frame errors and improvement of promoters did not lead to substantial cellulase production. Sabathé et al. [15] tried to modify a *C. acetobutylicum* strain to utilize cellulose directly. However, from the results, the achievement of this objective also will need a long process.

At present, one of the major bottlenecks hampering economic viability is the cost of substrates, accounting for up to 60 % of the total production costs. The high cost of substrates, including molasses, whey permeate, corn, and starchy roots, is a major factor affecting the economic viability of butanol production by fermentation. Lignocellulose is the most abundant renewable resource on the planet and has great potential as a substrate for fermentation [16]. For the use of lignocellulosic substrates, several strategies have been studied, such as the use of hydrolysates, coculture with true cellulolytic organisms, or the addition of cellulases to the fermentation medium.

Many researchers have been carrying out lignocellulose degradation to sugars for butanol fermentation since the 1980s. Yu et al. [17] reported that *C. acetobutylicum* was grown in acid hydrolysates of steam-exploded aspen wood chip with final butanol yields of 9.0 $g{\cdot}L^{-1}$ (0.26 g of butanol/g sugar consumed). However, it should be noted that pretreatment of agricultural residues, such as corn fiber, with acid/alkali results in generation of inhibitors that inhibit fermentation. Marchal et al. [18] described a one-step hydrolysis and fermentation process involving the use of cellulase from *Trichoderma reesei* and conversion of alkali-pretreated wheat straw into butanol and acetone by *C. acetobutylicum*. The results obtained for solvent concentration (17.3 $g{\cdot}L^{-1}$) and overall conversion time (36 h) demonstrated an improved performance over the separate hydrolysis and fermentation operation. Querishi et al. [19] studied butanol production from wheat straw hydrolysate in batch cultures using *C. beijerinckii* P260. When wheat straw hydrolysate was supplemented with 60 $g{\cdot}L^{-1}$ glucose, the resulting medium containing 128.3 $g{\cdot}L^{-1}$ sugars was successfully fermented (because of product removal) to produce 47.6 $g{\cdot}L^{-1}$ solvent, and the culture utilized all the sugars.

In China, research on substrate substitution for butanol fermentation also has attracted increasing attention. Chen et al. [20] reported that 12.8 $g{\cdot}L^{-1}$ total solvent concentration and 29.9 % yield were obtained in batch broth by *C. acetobutylicum* grown on rice straw enzymatic hydrolysate. Li and Chen [21, 22] investigated

enzymatic hydrolysis of steam-exploded cornstalk in a membrane reactor coupled with fermentation of acetone and butanol by *C. acetobutylicum* AS 1.132. A butanol yield of 0.14 $g \cdot g^{-1}$ (cellulose plus hemicellulose) and maximum butanol productivity of 0.31 $g \cdot (L \cdot h)^{-1}$ were obtained with a dilution rate of 0.075·h^{-1}. The conversion rates of cellulose and hemicellulose were 72 and 80 %, respectively.

7.2.3 Continuous Fermentation and Immobilized Cell Fermentation

The continuous cultivation technique can be used to improve reactor productivity and to study the physiology of the culture in a steady state. Reports have indicated that continuous cultivation can be utilized with the same efficiency as batch cultivation for solvent concentration and yield but with improved efficiency regarding productivity. However, as a precaution of single-stage continuous processes for the industrial scales, solvent production is not stable and declines with time, with a concomitant increase in acid formation, although high solvent yields can be achieved for various time periods. Fick et al. [23] maintained the stable continuous cultivation of *Clostridium acetobutylicum* on a complex medium containing 40 $g \cdot L^{-1}$ glucose for 2 months. The solvent yield was 13 $g \cdot L^{-1}$, but there was less-efficient use of the substrate. Two- or multistage continuous fermentation systems have been investigated in an attempt to separate the propagation phase from the production stage. A laboratory-scale two-stage system was reported by Bahl et al. [24]. A solvent concentration of 18.2 $g \cdot L^{-1}$ (12.78 $g \cdot L^{-1}$ butanol) with a yield of 0.34 $g \cdot g^{-1}$ and a productivity of 0.55 $g \cdot L^{-1}$ per hour was obtained from *Clostridium acetobutylicum* DSM 1731 in a two-stage phosphate-limited chemostate. The former Soviet Union in the 1960s [25] conducted multilevel continuous fermentation (7–11 fermentation tanks) for solvent production. China in the same period also carried out relevant research. The Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Science [26], started the studies on acetone butanol multilevel continuous fermentation, of which the solvent yield was 2.3 times higher than for batch fermentation.

Generally, the materials for immobilization of acetone butanol *Clostridium* cells are absorbents such as clay brick, porcelain, and the like. Qureshi et al. [27, 28] immobilized cells of *C. beijerinckii* onto clay brick particles by adsorption and achieved a productivity of 15.8 $g\,(L\ h)^{-1}$. The use of immobilized cells combined with advanced solvent recovery technologies for fed-batch or continuous fermentation studies has also been carried out in recent years.

7.2.4 Solvent Recovery Process

Recently, some new online solvent recovery technologies have become available, such as membrane-based systems, liquid-to-liquid extraction, adsorption, and gas

stripping. Membrane-based systems show a high selectivity for solvents but might suffer from clogging and fouling and seem to be more suitable for use with immobilized cells. Liquid-to-liquid extraction also has high selectivity, but emulsions might form to render the process less suitable. All these procedures can be designed to allow online product recovery so that butanol toxicity and inhibition to the fermentation is reduced. High concentrations of substrates and cells result in high productivity. However, a sugar concentration of more than 60 $g \cdot L^{-1}$ in a nonintegrated process without product removal is not used, which leads to a low concentration of cells because of butanol inhibition. The usual maximum concentration of total solvents in the fermentation broth is 20 $g \cdot L^{-1}$, of which butanol is only about 13 $g \cdot L^{-1}$. Ezeji et al. [29] devised the integration of a fed-batch and gas-stripping system to recover solvent in acetone-butanol-ethanol fermentation; a total of 500 g glucose was used to produce 232.8 $g \cdot L^{-1}$ solvents. Qureshi et al. [30] discussed the separation of butanol from aqueous solutions and fermentation broth by adsorption of silicalite, resins, bone charcoal, activated charcoal, bonopore, and polyvinylpyridine. Silicalite appeared to be more attractive to concentrate butanol from dilute solutions (5 to 790–810 $g \cdot L^{-1}$) and required less energy for butanol recovery (1,948 kcal kg^{-1} butanol). To improve efficiency and reduce adsorbent costs, the method of biomass absorption to produce anhydrous ethanol appeared domestically and abroad [31]. Hassaballah and Hills [32] reported the use of cornmeal as adsorbent to condense 85 % (w/w) alcohol vapor, and the product yield was up to 99.8 % (w/w). Westgate et al. [33] indicated that starch was a well-established adsorbent for drying ethanol, and the purity of ethanol and the energy consumption were both promising.

7.2.5 Present Situation and Problems of Cellulosic Butanol Fermentation Technology

The traditional acetone-butanol fermentation substrates used are corn and molasses; the production of butanol is generally 9–13 $g \cdot L^{-1}$, and the raw material cost accounts for 60–70 % of the total cost, which is an important reason to limit the development of the butanol fermentation industry. Another factor that cannot be ignored is the toxicity of solvent on the microbial cells; especially, when the concentration of butanol production reaches 13 $g \cdot L^{-1}$, the fermentation will stop. It is also a limiting factor affecting solvent production. In addition, in the acetone-butanol fermentation process, energy consumption for traditional distillation recovery of butanol is high. Steam consumption accounts for 70 % of the cost of the entire power, and too much wastewater is produced by distillation recovery of butanol, which increases environmental protection costs.

Lignocellulose has the most potential as a fermentation substrate, but there are also many problems in the process. Now, *Clostridium* as a major industrial strain cannot effectively hydrolyze lignocellulose. Therefore, it is necessary to carry out

pretreatment of lignocellulose to convert cellulose and hemicellulose into monosaccharides so that they can be used for butanol fermentation by *Clostridium*. At the same time, at the pretreatment stage of lignocellulose, many complex products that inhibit acetone-butanol fermentation will be produced. Moreover, in the process of lignocellulose utilization, people tend to use a single technology, emphasize the utilization of one component, and ignore the use of other components, resulting in waste of raw materials and environmental pollution, which are main reasons the economics of the biomass industry cannot compete with the petrochemical industry.

Compared with ethanol fermentation by yeast, in butanol fermentation, not only glucose can be used, but also xylose generated from hemicellulose degradation process is able to be used. Thus, it is possible that the straw can be fermented into butanol instead of traditional starch, and it is also an effective way to use hemicellulose.

Lignocellulose mainly is composed of cellulose, hemicellulose, and lignin, which are widely used as raw materials for chemical production. Cellulose and hemicellulose can be directly converted to produce furfural, organic acids, and other chemical products and papermaking raw materials. In addition, cellulose and hemicellulose can be converted into glucose, xylose, and other fermentable sugars through hydrolysis. After straw is pretreated to remove soluble hemicelluloses, the remaining solid material consists mainly of lignin and cellulose. Then, lignin and cellulose with high purity can be obtained by alkali treatment. Cellulose can be hydrolyzed to glucose as the raw material of the fermentation process and can produce a variety of cellulose derivatives through various conversion technologies to achieve the comprehensive utilization of biomass resources and provide good economic and social benefits.

Because of the high costs and low efficiency of enzymolysis and difficulty of cellulose degradation in butanol fermentation using straw as substrate, Chen et al. from the Institute of Process Engineering (IPE), Chinese Academy of Sciences (CAS), chose hemicellulose hydrolyzate from straw to directly ferment into acetone and butanol, leaving lignin and cellulose in the straw to realize comprehensive utilization. In cooperation with Jilin Ji'an Biochemical Company, Limited, they hydrolyzed straw hemicellulose by acid catalysis and then used the hydrolysate to ferment butanol. In addition, they established a demonstration project with an annual output of 600 t butanol and the matching industrial system by independent processing, providing a totally new production line first at home and abroad.

7.3 Production of Organic Acids

Organic acids containing one or more carboxyl groups are widely distributed in nature, and they have been found in animals, plants, and microorganisms in vivo. Organic acids such as lactic acid, citric acid, gluconic acid, malic acid, clothing heptanoic acid, tartaric acid, succinic acid, and acetic acid are widely used in

industry. Except for tartaric acid, which is a by-product of wine fermentation from grapes, most of the other organic acids are produced by fermentation. Although lactic acid and malic acid have a large ratio of chemical synthesis production, the final product is a racemic type that the body cannot absorb. So, the production of L-type organic acids in the food and pharmaceutical industries is the inevitable trend of future development.

The common view is that the metabolic pathways for the fermentation production of organic acids enter the citric acid cycle by the Embden-Meyerhof Parnas (EMP) pathway. Most of the industrial versatile use organic acids mentioned can find the corresponding location on the metabolic pathways. So, we can achieve the accumulation of organic acid by metabolic regulation. In addition, the original precursors of organic acids by microbial fermentation are glucose and sucrose. Then, the hydrolysis of sucrose or glucose is likely to become raw materials for production of these organic acids. In fact, the traditional fermentation industry did use such substances as the fermentation raw materials, such as raw sugar, refined sugar, or glucose solution; some starch-containing material, such as corn, potato, and rice; and beet molasses or cane molasses, the by-products of the sugar industry rich in sucrose.

With the deepening of research on cellulose, cellulose hydrolysates as raw materials for organic acid fermentation have received increasing attention.

7.3.1 Lactic Acid and Polylactic Acid

Lactic acid is an important organic acid. The amount of lactic acid worldwide already exceeded 1.2×10^5 t in 1994 and is increasing yearly at the rate of 14 %. Early lactic acid was produced by a chemical synthesis method. In 1863, Wilcenus invented the method to produce lactic acid by hydrolysis of lactonitrile made from acetonitrile and hydrogen cyanide. Later, there were some companies using this method for the commercial production of lactic acid. As the technology advanced in recent years, lactic acid is produced mainly by fermentation. The raw materials are carbohydrates, such as starch, cellulose, and so on. Industrial production can even use some of the waste, such as molasses from the sugar industry, whey from the dairy industry, and sulfite pulp waste from the paper industry.

Lactic acid is an important organic acid. It is widely used in the food, chemical, and pharmaceutical sectors. In recent years, lactic acid has been used as a raw material to manufacture new biodegradable packaging materials. This material not only has the same barrier property as polyester, but also has the same gloss, transparency, and processing ability as polystyrene. Using it instead of the current widely used plastic packaging materials can be a fundamental solution to the problem of plaguesome white pollution, a kind of pollution that made by the undegradability of waste plastic and toxicity of additives. This provides a huge market for the development of the lactic acid industry.

Polylactic acid does not exist in nature. It is generally synthesized from lactic acid. PLA is a synthetic straight-chain aliphatic polyester. The high molecular weight of PLA can be obtained by polymerization of lactic acid cyclization or direct polymerization of lactic acid. PLA has good biodegradability and good biocompatibility and bioabsorbability; thus, it will not leave any environmental problems when degraded. In the medical field, it has been considered to be the most promising biodegradable polymer for making surgical suture materials, such as for wound healing. Thus, its research and development are extremely active.

7.3.1.1 Microorganisms Producing Lactic Acid

Many microbes can produce lactic acid, and the most widely used in industry are the strains of lactobacilli, which have a high lactic acid synthesis rate and yield. They are not easily contaminated in the desired fermentation conditions (temperature higher than 40 °C, low pH, low oxygen concentration, and high concentration of lactic acid). The *Lactobacillus* spp. include those with obligatory homofermentation, facultative heterofermentation, and obligatory homofermentation. The first two have value for industrial production because these bacteria ferment on hexose, and the main product is lactic acid. Benchmarks for breeding these two types of bacteria are production of a stereoisomer of lactic acid; the type of carbohydrates utilized; and complex nutrients (such as vitamins and amino acids). Fermentation by-products and the tolerance level of lactic acid should also be considered. In short, the best strains are able to take advantage of cheap raw materials, ferment quickly and fully, and need fewer other nutrients. The best strains should also have such features as production of a mass of specific three-dimensional lactic acid, small cell yield, and almost no by-products.

7.3.1.2 Microbial Transformation of Lactic Acid

Conventional lactic acid fermentation is mainly based on starch as the raw material. In recent years, lignocellulosic biomass has been used as raw material for lactic fermentation, which saves food and reduces feedstock cost. So, it became the focus of the study of lactic acid fermentation. Cellulose from straw as a raw material for lactic acid fermentation is described in this section. Figure 7.2 shows the mechanism of lactic acid production from cellulose materials.

Before lactic acid fermentation, cellulose raw materials should be pretreated by physical or chemical methods, then the right amount of cellulase is added, and hydrolyzation occurs at a certain temperature (usually 50 °C) for a certain time. After that, the saccharification liquid can be obtained. Then, a certain amount (typically 10 % v/v) of bevel seed is added after activation to the saccharification liquid, and culture occurs under the optimum growth temperature of the fermentation bacteria.

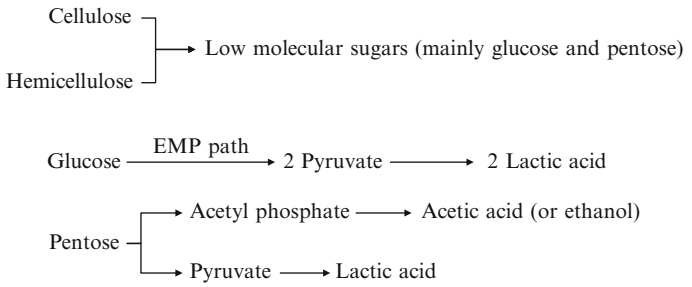

Fig. 7.2 Mechanism of lactic acid production from lignocellulosic materials

In lactic acid fermentation, the sugar content of the medium is closely related to the production of lactic acid; the best sugar content should be determined based on the characteristics of the selected strains. Zhuang [34] used treated straw as the raw material to ferment lactic acid with *Lactobacillus*. The results showed that, as the sugar content of the straw cellulose liquid increased from 100 to 140 g L^{-1}, lactic acid production was in an upward trend, but the changes of the conversion rate were small. When the sugar content was increased to 150 g·L^{-1}, the lactic acid yield and the conversion rate were highest. They were 132 g·L^{-1} and 88 %, respectively. When the sugar content was greater than 150 g·L^{-1}, although lactic acid production had a small increase, the conversion rate was in a downward trend. It actually caused the waste of sugars.

As lactic acid fermentation is not aerobic fermentation, the microbial cells do not proliferate in the fermentation process, so the amount of seed inoculation should be large to ensure that there are always enough microbial cells in the fermentation process. Chen et al. [35] used screened lactobacillus, ZJU-1 (*Lactobacillus* sp.), to ferment on the enzymatic hydrolyzate of steam-exploded fiber materials. The result showed that 10 % of inoculum was appropriate, which was consistent with the report of Zhuang et al. [34].

In addition, the temperature and time of fermentation have an important impact on the lactic acid fermentation process, so in the experiment it is necessary to explore the optimum fermentation conditions based on the selected strains as well as the raw material.

7.3.1.3 Synthesis and Production of Polylactic Acid

Preparing monomers of lactic acid by biological fermentation is the first step of PLA synthesis, and then the PLA with high molecular weight is synthesized by chemical methods. Currently, there are two synthesis methods; one is direct polycondensation. Another is the indirect synthesis method, in which lactide is synthesized from lactic acid first and further synthesized into PLA with catalyst [36, 37]. The former is simple and an important way to reduce the cost of PLA

production, but the intensity is low, and the usability is not strong because the molecular weight is less than 4,000. Indirect polymerization preparation of high molecular weight PLA is most applied and studied by far because a large amount of catalyst is available, and the method has less demand on the reaction conditions. This method lies in the preparation, purification, and polymerization processes of lactide [38–41].

Studies of the synthesis of PLA mainly focused on two aspects. One is in terms of products, blending and modifying the PLA polymer to achieve different features and functionality of the product to meet the needs of all aspects of social life. Another is the synthesis process, with catalyst preparation and process optimization to improve product quality, reduce production costs, and expand production scale, at the same time trying to reduce the environmental impact of chemical production and develop a green synthesis process.

Worldwide, a representative of research institutes and manufacturers of PLA is Cargill Dow Company in the United States, who began to developed and produce PLA products in 1997; the commodity was named NatureWorks™ ploy actide (PLA) [42]. At that time, the annual production capacity of this company was only 16,000 tons. In November 2001, the company invested $300 million to build a factory in Minnesota with an annual output of 140,000 tons of PLA, using two-step polymerization technology, which is by far the largest PLA-producing factory in the world. Nowadays, the annual production capacity of Cargin Dow Company in America has reached 500,000 tons. And it is estimated that the gross annual value will reach 1,000,000 tons within ten years. At the same time, the company is active in license transfer, including a PLA production plant in Asia. Many scientific research institutions and companies in China in recent years have also been actively involved in the development and research of PLA, such as Tongji University, Chengdu Institute of Organic Chemistry, Changchun Institute of Applied Chemistry, CAS, Beijing University, and so on. They carried out the research in PLA chemical synthesis and made a series of findings; several PLA production lines have also been built in Zhejiang Province and Harbin Province of China. In short, PLA synthetic production has commenced worldwide, and the product types gradually become richer and production cost is decreasing, resulting in the strength to compete with existing olefin compounds.

7.3.2 Levulinic Acid

Levulinic acid is an important chemical product. In 1997, American Biofine successfully developed a cost-effective technology using biomass to produce levulinic acid and gained the Presidential Green Chemistry Challenge Award. The application of this technology can greatly reduce the levulinic acid price and settle the foundation of levulinic acid as a new green platform chemical. In recent years, the use of biomass to prepare levulinic acid has attracted the attention of many researchers worldwide. Cellulose and hemicellulose first are hydrolyzed into monosaccharides (glucose and fructose) by mineral acid (typically sulfuric acid and

hydrochloric acid); then, monosaccharides are dehydrated by heating to generate 5-hydroxymethylfurfural and further decarboxylated to levulinic acid. Research materials include furfural residue, xylose residue, cottonseed hulls, waste paper, sorghum, starch, and so on [43]. In addition, levulinic acid also can generated from furfuryl alcohol in acid condition through ring-opening and rearrangement reactions [44].

Currently, there are mainly two methods of levulinic acid preparation: furfuryl alcohol–catalyzed hydrolysis and direct biomass hydrolysis. In the former method, furfuryl alcohol as the raw material is acid catalyzed into levulinic acid. Representative processes for the furfuryl alcohol catalytic hydrolysis process are those of Japan Chemicals Company, Japan's Ube Industries, French organic synthesis companies, and the Goodrich Company method in the United States [43]. Although the method has advantages such as a simple process and uniform product quality, the raw material furfuryl alcohol is from furfural hydrogenation. It is costly, and its sources are limited, so it has been gradually phased out. Direct hydrolysis uses biomass containing cellulose or starch hydrolyzed under acidic conditions. The dilute acid hydrolysis of the biomass is a multiphase hydrolysis that occurs between the solid phase of lignocellulose and acid solution at high temperature; cellulose and hemicellulose are fully hydrolyzed into sugars.

Cellulose is hydrolyzed into glucose, while hemicellulose is hydrolyzed into pentose. The soluble components obtained by hydrolysis are mainly saccharides (such as xylose, glucose, cellobiose, and sugars); sugar aldehyde (such as furfural, hydroxymethyl furfural); and organic acid (such as levulinic acid, formic acid, acetic acid). Hexose from cellulose degradation is dehydrated to form 5-hydroxymethyl furfural and then further decarboxylated into levulinic acid; the reaction process forms a large number of intermediate products and relates to a large number of side reactions. Pentose from hemicellulose degradation arises from the occurrence of intermolecular dehydration and cyclization; three molecules of water are removed, then generate furfural, and the furfural is oxidized into furfuryl alcohol, thereby generating a small amount of the levulinic acid in a further degradation process.

The existing process of direct biomass hydrolysis contains intermittent dilute acid hydrolysis and continuous dilute acid hydrolysis.

1. Intermittent catalyzed hydrolysis method: Biomass raw materials are added to the reactor at one time, and the catalyst is added to carry out hydrolysis until the reaction is completed, finally separating and purifying levulinic acid. The process of this method is relatively simple; just let the biomass raw material react with an inorganic acid (hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid, etc.) solution at a high temperature and then separate and purify levulinic acid. However, because of the complexity of the biomass feedstock, the yield and purity of the product from different types of biomass would be different [45]. The highest yields of the product reached 59.8 %, while the lowest yield was 16.4 %.
2. Continuous catalytic biomass hydrolysis: Representative processes are the twin-screw extruder method from the University of Nebraska, Lincoln, in the United States, which uses starch as the raw material and dilute sulfuric acid as a catalyst; and the method of the Biofine Company, which uses waste cellulose as the raw

material and dilute sulfuric acid as a catalyst, and adopts two continuous reactors for catalytic hydrolysis. The former method obtains a levulinic acid yield of about 70 % and has the advantages of process continuity, fewer reaction steps, and shorter reaction time. Thus, it is suitable for commercial production [45]. The latter obtains a 70 % yield and has less by-product, easier separation procedures, and the highest yield reported. Currently, Biofine is trying to use different biomass materials, such as municipal solid waste, paper mill waste, waste wood, agricultural waste, and other biomass, as raw material in test production [46].

Because of the difficulty of levulinic acid recovery by liquid inorganic acid catalysis, Chen and Jin [47] used steam-exploded straw as the raw material and the solid superacid as a catalyst to study the possibility of solid superacid catalytic straw to prepare levulinic acid in the microwave field and reactor kettle. The conclusions were as follows:

① Solid superacid can catalyze straw to prepare levulinic acid. The yield in the reactor kettle was higher than in the microwave field. Judging from the yield changes of the main sugars generated in the reaction process and the intermediate product furfural accompanied by the variation in chemical composition of the reaction residues, cellulose was more likely to be decomposed by solid superacid than hemicellulose.

② In the reaction kettle, using the steam explosion and supergrinding straw as the raw material, when the weight ratio of solid superacid and straw was 2:1 and there was a reaction temperature from room temperature to 200 °C for 40 min and maintained at 200 °C for 5 min, the yield of levulinic acid was 71.7 %, accounting for nearly 100 % of the theoretical yield, 1.69 times the yield of steam-exploded straw and 2.36 times the yield of original straw under the same conditions. Use of steam explosion–wet supergrinding straw fibrous tissue as raw materials and catalysis by solid superacid can achieve a yield of 65.9 %, equaling 1.55 times that of steam-exploded straw and 2.17 times that of original straw as raw materials. This indicated that the steam explosion–supergrinding straw can effectively improve levulinic acid yield.

③ In this process, the loss of catalyst activity is serious. The yield was 38.6 % using reusable old catalyst, which was 71.3 % lower than with the new catalysts. The mechanism of deactivation and resurrection processing needs further research.

④ Using rice straw after cellulase predigesting, the yield by catalysis of solid super acid decreased by 5.5 times using the enzymatic rice straw, while the yield increased by 48.3 % by inorganic acid catalysis. This indicates that there are differences between the mechanism of solid superacid catalysis and that of inorganic acid catalysis, which needs further study.

⑤ Developing new solid superacid catalyst and pretreatment method suitable for straw structure and composition and studying the process and mechanism are keys for using solid superacid to catalyze lignocellulose to prepare levulinic acid in a large scale.

7.3.3 Citric Acid

Citric acid, as one of the major metabolites in almost all the microorganisms used in the industry production, is the largest producer of organic acids, and the annual output is more than 3×10^5 t. It is widely used in the food, drug, chemical, and other fields. Its main raw material is a solution of pure sugar, starch and its hydrolyzate, and sugar beet or sugarcane molasses. Using a cellulase solution as the material to produce citric acid is rarely reported.

7.3.3.1 Strains for Citric Acid Fermentation

There are many microbial species that can produce citric acid, such as *Mucor piriformis*, *Penicillium lufeum*, *Penicillium citrinum*, *Paecilomyces divaricatum*, *Aspergillus niger*, *Aspergillus clavatus*, *Aspergillus wentii*, *Aspergillus awamori*, *Aspergillus fenicis*, *Aspergillus fumaricus*, *Aspergillus saitoi, Aspergillus usamii*, *Trichoderma virid,* and *Ustilago vulgaris* [48]. But, the strains that really have industrial production value, that is, a higher rate of acid production and the ability to take advantage of a variety of sugars as a carbon source, are *A. niger*, *A. awamori,* and *Saito aspergillus*. The strains of citric acid produced in China are *A. niger*; they are treated through mutation breeding and are not the wild type. They not only have acid-producing ability but also are able to adapt to extensive raw materials and fermentation conditions, which helps reduce costs.

7.3.3.2 Microbial Conversion of Citric Acid

"Microbial conversion of citric acid" means converting other substance into citric acid with microbial method. According to the idea of lignocellulose fractionation and full use of biomass, Chen et al. [49] separated cellulose from wheat straw by delignification using alkali-oxygen-anthraquinone cooking. The obtained cellulose with a low lignin content is conducive to the subsequent use of cellulose. Then, cellulase with 30 IU·g^{-1} substrate was added, and the hydrolyzate was obtained after enzymolysis for 30 h at 100 rpm and 50 °C. This enzymatic solution can be fermented to produce citric acid by *A. niger* . The fermentation process can be conducted by thermostat fermentation and cycle-variable temperature fermentation, described next.

(1) Constant-temperature fermentation
Enzymatic hydrolyzate and the fermentation medium are added to the fermentor, and the pH is adjusted to 5.6 with aqueous ammonia. The cultivation conditions are 200 rpm at 30 °C for 100 h. The fermentation broth was separated by filtration.

(2) Periodic variable-temperature fermentation
Enzymatic hydrolyzate is added simultaneously with the fermentation medium to the fermentor; the pH is adjusted to 5.6 with aqueous ammonia. The fermentation is carried out at 30 °C for 2 h and then at 40 °C for 2 h to complete a cycle of variable-temperature fermentation. Samples from certain times in this process are needed to analyze the citric acid content and determine the optimum fermentation time.

Since the discovery that pyrolysis of cellulose resources could produce monosaccharides (mainly 1,6-β-D glucopyranosyl, LG), various microorganisms that are able to use LG have been found in nature, but their utilization efficiency is not high. Zhuang et al. [50] reported the study of direct use of LG for citric acid production, but this needed anhydrous ethanol to crystallize LG from the pyrolysis liquid, which does not help reduce production costs. Glucose from the acid hydrolysis of LG pyrolysis liquid for citric acid fermentation can solve the problems of loss in the crystallization process and high cost and improve the utilization efficiency. Yu and Zhang [51] successfully conducted this process. Xie et al. [52] also explored citric acid fermentation by *A. niger* using glucose, LG, and cellulose pyrolysis liquid.

7.4 2,3-Butanediol Production

Among bio-based chemicals, some of the key platform compounds are widespread [53], such as PLA, 1,3-propanediol, 3-hydroxy acid, citric acid, succinic acid, itaconic acid, levulinic acid, and so on. However, the cost for chemical synthesis of 2,3-butanediol is high because of its special structure. Although the 2,3-butanediol fermentation process has internationally reached the level of the alcohol industry, industrial production has not been realized because of the high overall cost. Thus, its use has not been fully developed. In recent years, with the vigorous development of industrial production, the demand for 2,3-butanediol has increased yearly, but 2,3-butanediol is researched and reported little domestically. Therefore, 2,3-butanediol as a potentially valuable compound again calls for concern. Its main purpose and related microorganisms and the fermentation process status are comprehensively reviewed next.

2,3-Butanediol is a chiral compound that is colorless and odorless and has three stereoisomers: dextro-, levo-, and meso-isomers. The molecular weight of 2,3-butanediol is 90.12 kDa with a high boiling point (180–184 °C) and low freezing point (−60 °C). As a valuable liquid fuel, its combustion value is 27,198 J g^{-1}, which is comparable to methanol (22,081 J g^{-1}) and ethanol (29,005 J g^{-1}). The reaction of dehydration, dehydrogenation, and hydration can be used to generate methyl ethyl ketone, diacetyl, butene, and butadiene.

As a chemical intermediate, 2,3-butanediol can be used to prepare an important industrial organic solvent, methyl ethyl ketone; it also can be converted to high combustion value diacetyl by dehydration, which has a wide range of applications

as fuel additives as well as generating rubber monomer such as 2-butene and 1,3-butadiene. Esterified forms of 2,3-butanediol are precursors in the synthesis of polyimide, which can be applied to drugs, cosmetics, lotions, and the like. Diacetyl forms of 2,3-butanediol generated by catalytic dehydrogenation can be used as a kind of valuable food additive. 2,3-Butanediol can be used as monomers to synthesize polymer compound, and levorotatory forms of 2,3-butanediol can be used as antifreeze agents because of its low freezing point. Other compounds can also be generated, such as styrene, octane, and 2,3-butanediol by a condensation and polymerization reaction. It can be widely used in inks, cosmetics, lotions, antifreeze, fumigants, softeners, plasticizers, explosives, drug chiral carriers, and so on as an additive. It can also be used for the production of polybutylene terephthalate resin, γ-butyrolactone, and spandex fibers in materials and textile production and processing industries. The demand for 2,3-butanediol in the international market is soaring because of its wide range of uses. It has attracted worldwide attention as a liquid fuel additive, especially from the point of view of environmental considerations [54–56].

7.4.1 Microorganisms Used to Produce 2,3-Butanediol

Currently, the microorganisms used to produce 2,3-butanediol are mainly bacteria. Widely reported strains include *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus licheniformis*, *Aerobacter aerogenes*, *Enterobacter aerogenes*, *Serratia marcescens*, *Aeromonas hydrophila*, *Enterobacter cloacae*, *Serratia* spp., and *Pseudomonas* spp. These bacteria are used to generate the target product of 2,3-butanediol in the fermentation of glucose as well as by-products such as acetoin, ethanol, acetic acid, lactic acid, and succinic acid; the acetoin is also called acetyl methylcarbinol or 3-hydroxy butanone, which is the precursor compound of 2,3-butanediol. The acetoin could be converted to 2,3-butanediol by acetoin reductase. The optical purity of 2,3-butanediol is different with different strains used in fermentation. For example, *Bacillus polymyxa* generates D-(−)-2,3-butanediol, and *B. subtilis* and *Aeromonas hydrophila* can generate the D-(−)-2,3-butanediol and meso-2,3-butanediol; *Serratia marcescens* may generate L-(+)-2,3-butanediol and meso-2,3-butylene glycol. Usually, different microorganisms can produce two stereoisomeric forms.

The ability of strains to produce 2,3-butanediol is different. *Klebsiella pneumoniae* and *B. polymyxa* are studied more widely for their ability and potential in the production of 2,3-butanediol. Especially, *K. pneumoniae* is studied more because of its wide variety of substrates. Both glucose and xylose can be used by *K. pneumoniae* as substrate, and it has a strong ability to adapt to the environment [56].

7.4.2 2,3-Butanediol Metabolic Pathway of Strains

The metabolic pathways of producing 2,3-butanediol in bacteria have been studied clearly, mainly related to acetolactate synthase, acetolactate decarboxylase, 3-acetoin reductase, and 3-acetoin oxidase enzymeskey enzymes.

Glucose fermented by *Polymyxa paenibacillus* to produce 2,3-butanediol may be described as the following metabolic reactions [57]:

$$\text{Glucose} \rightarrow 6\ CO_2 + 4\ ATP + 10\ NADH_2;$$
$$\text{Glucose} + 2\ H_2O \rightarrow 2\ \text{acetate} + 2\ CO_2 + 4\ ATP + 2\ NADH_2 + H_2;$$
$$\text{Glucose} \rightarrow \text{acetoin} + 2\ CO_2 + 2\ ATP + 2\ NADH_2;$$
$$\text{Glucose} \rightarrow 2,3\text{-butanediol} + 2\ CO_2 + 2\ ATP + 1\ NADH_2;$$
$$\text{Glucose} + 2\ NADH_2 \rightarrow 2\ \text{ethanol} + 2\ CO_2 + 2\ ATP + 2\ H_2;$$
$$\text{Glucose} \rightarrow 2\ \text{lactate} + 2\ ATP;$$

Thus, the 2,3-butanediol metabolic reactions are available in a total reaction relation expressed as

$$6\ \text{Glucose} + 2\ H_2O \rightarrow 2,3\text{-butanediol} + \text{acetoin} + 2\ \text{acetate} + 2\ \text{ethanol} + 2\ \text{lactate} + 14\ CO_2 + 16\ ATP + 13\ NADH_2 + 3\ H_2$$

All of these provide a good theoretical basis in microbial fermentation to produce 2,3-butanediol.

7.4.3 Microbial Transformation of 2,3-Butanediol

As early as 1906, Harden and Walpole carried out research on biotransformation to produce 2,3-butanediol by *K. pneumoniae*. In 1926, Ponker used *B. polymyxa* to produce 2,3-butanediol with fermentation. In 1933, Fulmer et al. improved the fermentation level of *Klebsiella oxytoca* and pointed out the potential for industrial production. During World War II, 2,3-butanediol production drew much more attention; *Klebsiella oxytoca* and *B. polymyxa* strains had been used for the production of 2,3-butanediol to compensate for the 1,3-butadiene scarcity and reached the pilot level. However, with the rapid development of the oil industry, as 1,3-butadiene can be cheaply obtained in oil production, the 2,3-butanediol potential market was replaced by petrochemical products. In recent years, with the vigorous development of industrial production, 2,3-butanediol demand has increased yearly, and the lack of oil resources and the rise of oil prices has led to wide attention for 2,3-butanediol both at home and abroad as a compound with potential value.

7.4.3.1 Research Advances for Fermentation Substrate

The hot spots of the researchers' attentions on 2,3-butanediol nowadays can be divided into four parts: identification of the key genes and enzymes involved in the 2,3-butanediol metabolic pathway, development of new strains and metabolic engineered strains for high yield or chiral 2,3-butanediol production, strategies for fermentation optimization such as simultaneous saccharification and fermentation, and technology improvement for combining process involved in 2,3-butanediol recovering processing. Future research should focus on new cheap and renewable resources for substrate, and new technology for fermentation and separation.

Many types of carbohydrates can be used for microorganism (especially bacteria) fermentation to produce 2,3-butanediol. The most commonly used substrates are glucose and D-xylose, L-xylose, D-ribose, D-arabinose, and various five-carbon sugars.

Abroad, Olson and Johnson [58] utilized *Aerobacter aerogenes* to convert 266 $g{\cdot}L^{-1}$ of glucose into 97 $g{\cdot}L^{-1}$ 2,3-butanediol and 14 $g{\cdot}L^{-1}$ 3-acetoin. Yu and Saddler [59] obtained 113 $g{\cdot}L^{-1}$ 2,3-butanediol and 3-acetoin from 226 $g{\cdot}L^{-1}$ glucose by *Klebsiella pneumoniae* fermentation. Zeng et al. [60] obtained a final concentration of 110 g L^{-1} of 2,3-butanediol and 3-hydroxy-butanone in the fermentation of *A. aerogenes*. De Mas et al. [57] compared 2,3-butanediol yield of *B. polymyxa* ATCC 842 and ATCC 12321 using glucose, xylose, lactose, arabinose, galactose, and fructose as substrates. Lee and Maddox [61] used lactose as substrate to produce 2,3-butanediol, and Perego et al. [62] studied the effects of temperature, inoculation volume, and substrate concentration on the target product using starch hydrolyzate as a substrate. When using glycerol as a substrate, the ratio of these two kinds of products of 2,3-butanediol and 1,3-propanediol varies with the fermentation conditions, such as pH and oxygen flow [63], in *Klebsiella pneumoniae* fermentation.

Although there are many reports about 2,3-butanediol fermentation abroad, domestic researchers in this area are just beginning, and few studies have been carried out. Qin et al. [64] used industrial glucoses as a carbon source and cheap diammonium phosphate as a nitrogen source and fermented them by the fed-batch method with *Klebsiella* sp. LN145 to produce 2,3-butanediol. The maximum yield of 2,3-butanediol and 3-acetoin was 84.0 $g{\cdot}L^{-1}$ and 10.5 $g{\cdot}L^{-1}$, respectively. The molar conversion rate of 2,3-butanediol reached 91 % of the theoretical level, and the conversion rate reached 1.8 $g{\cdot}L^{-1}{\cdot}h^{-1}$. Li et al. [65] used a central composite design and response surface analysis to optimize the culture medium of *Serratia marcescens*, making the 2,3-butanediol yield increase from 14.03 to 39.27 $g{\cdot}L^{-1}$. Ji et al. [66] and Ma and Xiy [67] studied the metabolic flux analysis and metabolic flux through the metabolic process in which the substrate was converted to 2,3-butanediol and tried to study the fermentation metabolism laws for 2,3-butanediol.

In summary, the studies to ferment 2,3-butanediol using glucose, xylose, lactose, and fructose as substrates have been developed to a level close to that of ethanol

to meet industrialization requirements. However, one of the important factors to cause the high cost of 2,3-butanediol production is the high price of these sugars. Therefore, one of the important factors in reducing the cost of 2,3-butanediol production is searching for low-cost carbohydrates as a fermentation substrate.

Because lignocellulose is the most abundant organic matter in nature, if lignocellulosic biomass resources were used as renewable raw materials to convert carbohydrates into 2,3-butanediol with bacteria, the cost is expected to be further reduced [68]. Thus, many researchers used various lignocellulosic hydrolyzates as substrates to carry out the work of microbial fermentation to produce 2,3-butanediol. Strains that are able to use lignocellulose degradation for biotransformation must have some important properties, such as a wide range of substrate utilization, higher yield, fewer by-products and strong anti-inhibition. [69]. According to reports, *Klebsiella pneumoniae* is the ideal strain that can make use of all the major sugars (hexose, pentasaccharide, and some disaccharides) and uronic acid degraded from cellulose and hemicellulose as a carbon source [70, 71]. The sugars contained in the hemicellulose in trees can also be efficiently converted after acid hydrolysis [70] or enzymatic hydrolysis [71]. On this basis, Yu et al. [72] used the cellulose and hemicellulose as substrates to coculture *Trichoderma harzianum* E58 and *Klebsiella pneumoniae* in the continuous fermentation process, in which the cellulose degradation ability of *Trichoderma harzianum* E58 was used to obtain glucose and xylose first, and then *K. pneumoniae* was used to transform them to produce 2,3-butanediol. The study of pretreatment, hydrolysis and fermentation of pulverized corncob to produce 2,3-butanediol by Cao et al. [73] showed that, in the pretreatment process, the lignin and the alkaline extract can be separated with cellulose and hemicellulose by dissolving, and hemicellulose can be hydrolyzed. Then, the 2,3-butanediol simultaneous saccharification and fermentation can eventually be realized by *Klebsiella oxytoca* ATCC 8724 in the yeast extract-malt extract-peptone (YMP) using pretreated corncob as a carbon source. Chen and Zhang [74] used *Paenibacillus polymyxa* S-07 in the appropriate medium (glycerol pretreatment of wheat straw 70 g L^{-1}, an initial pH of 5.5, 1.5 % yeast extract) and fermentation conditions (incubation temperature of 35 °C, speed 120 rpm) to obtain a 2,3-butanediol conversion rate of 19.4 % with glucose as the carbon source. When atmospheric glycerol autocatalytic pretreatment of wheat straw was used as a carbon source, the maximum cellulase activity could reach 0.946 U·mL^{-1} after 96 h of fermentation without cellulase added. The fermentation was ended after 120 h, and strains were able to secrete cellulase and a small amount of xylanase. But, activity of lignocellulosic hydrolysis enzyme was not high (cellulose and hemicellulose degradation rates reached 56.7 % and 32.2 %, respectively), and the 2,3-butanediol conversion rate was also not high (7.73 %). However, the exploration achieved the microbial conversion of lignocellulosic biomass to bio-based chemicals and provided a new approach for the synthesis of other bio-based products by integrating cellulase preparation, enzymatic hydrolysis, and saccharification into a system.

7.4.3.2 Research Advances of Fermentation Process Control

An important role is played by pH in the regulation of the metabolism of bacteria, especially in fermentation containing a large number of composite products. The fermentation process of 2,3-butanediol contains many by-products, such as 3-hydroxy-butanone, ethanol, acetic acid, and lactic acid. The optimum pH of the related enzyme in the route of 2,3-butanediol generation and those of by-products is not consistent; thus, the pH of the fermentation process will affect not only the growth of bacteria but also the metabolic processes of bacteria [75]. Generally, alkaline conditions are beneficial to the generation of organic acids, resulting in low yields of 2,3-butanediol. Under acidic conditions, the yield of organic acids is decreased to a tenth of that under basic conditions, while the yield of the main product, 2,3-butanediol, is high [56]. Raspoet et al. [76] found that for *B. licheniformis* using glucose as the carbon source, the highest yield of 2,3-butanediol could be obtained at pH 6.0. Jansen and Tsao [68] found that when *K. oxytoca* used xylose as a carbon source, the cells grew fastest at pH 5.2, and 2, 3-butanediol yield was also the highest under anaerobic conditions. Thus, the optimal pH values in the fermentation process to produce 2,3-butanediol are closely related to the fermentation strain types and characteristics and the type of substrates and the concentration.

Dissolved oxygen (DO) is also important in the fermentation process. 2,3-Butanediol is the typical product of anaerobic metabolism, but when *K. pneumoniae* is used as a starting strain, adequate ventilation can increase the yield of 2,3-butanediol. The explanation for this phenomenon from Long and Patrick is that the substrate and the product can be uniformly dispersed in the fermentation broth during ventilation by stirring, which can improve the efficiency of the fermentation. Laube and Martin [77] found that, compared with shake flask culture, *B. polymyxa* consumed less xylose substrate and produced less 2,3-butanediol in static culture. Their initial explanation was that CO_2 produced during fermentation in shake flask cultures can be released in a timely manner, resulting in speeding up the xylose metabolic rate and increasing 2,3-butanediol production. However, further study showed that 2,3-butanediol production was higher at a speed of 125 rpm compared to 300 rpm.

Too much ventilation is unfavorable for 2,3-butanediol formation. Jansen et al. [68] found that, in *K. oxytoca* fermentation of 2,3-butanediol with xylose as the carbon source, the most important factor that affected 2,3-butanediol production was the oxygen transfer rate (OTR), which was directly affected by the ventilation parameters. The study found that DO has a significant effect on the conversion of 3-hydroxybutanone, which is a precursor substance of 2,3-butanediol in the fermentation process. Moes et al. [78] found that, in *B. subtilis* fermentation of 2,3-butanediol, different DO levels in the process would affect the composition ratio of the end products 3-hydroxyacetone and 2,3-butanediol. Further study showed that when the DO concentration was more than 100 $\mu g\ kg^{-1}$, the major product was

3-hydroxyacetone, and when the DO concentration was below 100 μg kg^{-1}, the major product was 2,3-butanediol. Therefore, by controlling the DO concentration, 3-hydroxyacetone can convert into 2,3-butanediol or 2,3-butanediol converts into 3-hydroxyacetone in a reversible manner.

Beronio and Tsao [79] further studied the effect of oxygen transmission rate on *K. oxytoca* batch fermentation to produce 2,3-butanediol. The study showed that the fermentation process can be divided into two stages: the energy-coupling growth stage and energy-uncoupling growth stage. In the energy-coupling growth stage, the growth of bacterial cells is coupled with the amount of oxygen; the maximum yield of 2,3-butanediol could be obtained at this stage, and the energy growth only occurred when the DO concentration reached a certain limit. By constantly increasing the partial pressure of oxygen in the gas pass to control the oxygen mass transfer coefficient, the DO level could be maintained constant, so the growth of *K. oxytoca* could be maintained in an equilibrium state and the major by-product of ethanol in 2,3-butanediol production could be suppressed. In addition, the ventilation quantity not only affected the yield of 2,3-butanediol but also affected the optical activity of the final product 2,3-butanediol in some certain strains.

Nakashimada et al. [80] found that increasing the supply amount of oxygen reduced the meso-type 2,3-butanediol ratio and thus significantly increased the ratio of D-type 2,3-butanediol (2R, 3R) in *P. polymyxa* culture. Therefore, the yield of 2,3-butanediol can be controlled by adjusting the amount of oxygen supply in the fermentation process. The amount of oxygen in the bacterial cells that can be used in the fermentation process depends on the DO level of the fermentation liquid, while the DO level can be regulated by the oxygen pressure in the gas phase (oxygen ratio), so the partial pressure of oxygen can be used as the control parameters in the fermentation process. Syu and Hou [81] predicted the kinetic parameters in the fermentation process of *K. oxytoca* with the neural network and studied how to effectively use the neural network to control the fermentation process under different oxygen pressure conditions.

In short, the discovery and related research of 2,3-butanediol has been conducted for nearly 100 years around the world. Mainly because of the economic problems of production costs, most productions remain at the laboratory or pilot stage in spite of significant progress in all aspects of the production process.

7.4.3.3 Status of Butanediol Fermentation

There are many strains used for 2,3-butanediol fermentation in nature, and that provides a larger choice of strains. But, because 2,3-butanediol belongs to the primary product of the bacterial metabolism, its production is limited, and the maximum theoretical yield is 0.50 $g \cdot g^{-1}$ glucose. As shown in Table 7.1, the transformation the fermentation of many strains has reached a high level, and the 2,3-butanediol fermentation process basically has reached the level of the alcohol industry internationally.

Table 7.1 2,3-Butanediol-producing strain levels [82]

Strains	Carbon source	Maximum yield (g L^{-1})	Yield (g g^{-1})
Aerobacter aerogenes	Glucose	36.5	0.30
Bacillus amyloliquefaciens	Glucose	30	0.33
Bacillus licheniformis	Glucose, sucrose, and starch	5.16	0.44
Bacillus polymyxa	Glucose	13	0.29
Bacillus polymyxa	Starch	38	0.28
Paenibacillus polymyxa	Glucose	–	–
Enterobacter aerogenes	Glucose	110	0.48
Enterobacter cloacae	Glucose, xylose, and arabinose (corn fiber)	34.4	0.43
Klebsiella oxytoca	Xylose	49	0.33
Klebsiella oxytoca	Molasses	98.6	0.48
Klebsiella pneumoniae	Whey	13.7	0.39
Klebsiella pneumoniae	Glucose	39.6	0.43
Serratia marcescens	Sucrose	–	–

However, the main problem limiting the industrialization of bio-based 2,3-butanediol is the high economic cost. Future works remain to be carried out, including the following aspects [55, 56]:

1. *Strains*. An emphasis is on strains, focusing on strains that are able to directly use the hydrolyzate of lignocellulose, are suitable for high-density culture, and produce less by-product.
2. *Substrate*. For a natural renewable resource, lignocellulose as a carbon source instead of the glucose currently used has strong economic feasibility, but the lack of an effective technology to separate different components and the high cost of cellulase both result in difficulty of 2,3-butanediol industrialization using lignocellulose.
3. *Stereo-specific mechanism*. There are three kinds of stereoisomers of 2,3-butanediol, and its corresponding metabolic enzymes also are highly stereo-specific. It will also be a research focus in the future to clarify the stereo-specific mechanism in the catalytic process and improve optical activity and the purity of the target product.
4. *Separation and extraction*. The cost-effective separation of 2,3-butanediol has been an important reason to limit its large-scale industrial production. 2,3-Butanediol has some properties, such as a high boiling point, high hydrophobicity, and other impurities in the fermentation broth, that make the purification process difficult. Therefore, the development of a high-efficiency, low-cost separation and extraction process is the key to reduce production costs and expand the production scale.
5. *Process*. New technology and optimization of the fermentation process should be further explored. To minimize the middle process, the emphasis is on effectively reducing the cost while improving the target product yield.

7.5 Xylitol Production

Xylitol is a five-carbon sugar alcohol. Its sweetness equals that of sucrose, and the heat value equals that of glucose. Xylitol metabolically is not involved with insulin and can adjust abnormal glucose metabolism in vivo, with use as a nutritional and therapeutic agent for diabetes. The xylitol has strong antiketone ability and is available to rescue patients with ketoacidosis. Xylitol can slow the speed of fatty acid production in plasma but not cause a rise of blood sugar. Thus, it is also used as a hepatoprotective drug for patients with hepatitis. Xylitol has good thermal stability and does not react with amino acids in heat. It can be made into a variety of preparations, as nutritional medicines, with amino acids. Xylitol also has an exceptional anticaries function as food. Xylitol has excellent characteristics as glycerin and other polyhydric alcohols do. Thus, it has broad application prospects in the pharmaceutical and chemical industries.

Currently, the method of industrial production of xylitol can be summarized as follows: First, to hemicellulose rich in xylan is hydrolyzed to obtain purified xylose, and then catalytic hydrogenation, column chromatography, and recrystallization are used to obtain xylitol. The entire process consists of a series of complicated purification steps; from xylose to xylitol, the yield is only about 50–60 %, with production costs that are about ten times that of sucrose. The relatively high costs limit the scope of xylitol use.

Biological conversion processes to produce xylitol are likely to reduce the production costs of the process route, for which the fermentation does not need to purify xylose, and the separation step can also be simplified. The enzymatic synthesis is likely to achieve continuous and efficient production.

7.5.1 Microorganisms Used to Produce Xylitol

According to previous studies, only a small part of bacteria can generate xylitol in nature and the efficiency of filamentous fungi is also not high, but the yeast relatively easily changes xylose into xylitol. A study showed that the superior performance of the yeast strains for producing xylitol was mainly concentrated in the genus *Candida*, such as *Candida guilliermondii*, *Candida tropicalis*, *Candida mogii*, *Candida parasilosis*; and partly in *Debaryomyces*, such as *Debaryomyces hansenii*, and in *Pachysolen,* such as *Pachysolen cannophilus*.

7.5.2 Regulation of Microbial Xylitol Synthesis In Vivo

Xylose can be converted into 5-phosphorylation xylulose, which is the intermediate of the hexose monophosphate pathway (HMP), in two paths. One is that xylose is changed to xylulose by xylose isomers and then phosphorylated by xylulose

kinase to form 5-phosphatexylulose. Another path is that xylose is reduced to xylitol by NADPH-dependent xylose reductase and then oxidated to form xylulose by nicotinamide adenine dinucleotide (NADH)-dependent xylulose enzyme. Then, xylulose is phosphorylated into 5-phosphate xylulose; the oxidation-reduction path is the main path that yeast uptake and utilize xylose. 5-Phosphate xylulose is eventually converted into triose phosphate by HMP and then enters into the glycolytic pathway [83].

According to the research results of Barbosa et al. [84], the total metabolic equation of yeast transforming xylose to xylitol under conditions of nutritional balance and NADH as the coenzyme is as follows:

$$126 \text{ xylose} + 3\ O_2 + 6 \text{ ADP} + 6 \text{ Pi} + 48\ H_2O \rightarrow 114 \text{ xylitol} + 6 \text{ ATP} + 6\ CO_2$$

According to this equation, the theoretical yield of xylitol is 0.905 mol xylitol/mol xylose and 0.917 g xylitol/g xylose.

Under the same assumptions, Barbosa et al. reported that the total metabolic equation under anaerobic conditions is as follows:

$$48 \text{ xylose} + 15\ H_2O \rightarrow 42 \text{ xylitol} + 3 \text{ ethanol} + 24\ CO_2$$

Accordingly, theoretical xylitol yield under anaerobic conditions is 0.875 mol xylitol/mol xylose, which is 0.887 g xylitol/g xylose.

7.5.3 Xylitol Microbial Conversion

The materials most used in xylitol fermentation are plant hemicellulose hydrolyzates, such as hydrolyzates of corncobs, hardwood, straw, cottonseed hulls, and sugarcane bagasse. In addition, xylose and glucose can also be used as a raw material of the fermentation, but the cost of the latter is higher.

7.5.3.1 Preparation and Detoxification of Hemicellulose Hydrolyzate

Corncobs, cottonseed hulls, and bagasse are excellent raw materials for preparation of hydrolyzate rich in xylose monosaccharide. Dilute sulfuric acid and hydrochloric acid are the most commonly used catalysts. Hydrolysis of plant materials under the conditions of high temperature and strong acid produces a large number of substances toxic to the yeast. Although dilute acid (0.7 %) may detoxify the poison, the hydrolyzate often contains a large number of nonfermentable oligosaccharides.

Toxic substances in hemicellulose hydrolyzate of yeast include furfural (degradation by-products), acetic acid (from acetylated xylan released), some of the lignin derivatives (such as phenolic compounds), heavy metal ions, and so on. The sensitivity of yeasts to these toxicants is different. Overall, furfural toxicity is greater

than acetate. Lignin degradation products, which are a class of compounds with –COOH, –OH, –CHO, and –CH=CH– groups connected to the benzene ring, are more toxic than furfural. Generally, the toxicity of the aromatic acid is lower than the corresponding aldehyde.

Detoxification means a purification process to remove the toxic substances in hemicellulose hydrolyzate. Vacuum evaporating can improve the xylose concentration of the hydrolyzate and at the same time remove most of the furfural, acetic acid, and certain volatile noxious components. The activated carbon can also remove most of the acetic acid and some of the acid-soluble lignin derivatives. Calcium can remove some important inhibitors, which can be removed by the activated carbon. An excess of lime (pH 10) can remove toxic substances and phenolic substances effectively. Solvent extraction, an ion exchange resin, and steam treatment can remove some of the noxious components.

Because of the complex toxic composition, only by combining a variety of treatment methods can better detoxification effects be achieved. The excess lime treatment can significantly improve cell growth, but only after extraction with ether can significantly improve the yield of xylitol. With combined use with an ion exchange resin, the activated carbon is superior to the excess lime method.

When timber is treated with alkali (NaOH) and acid hydrolysis successively, most of the acetic acid can be removed. But this will increase the concentration of the lignin derivatives in the hydrolyzate to reduce the fermentation performance. The hydrolyzate of corncob and wood raw material can obtain good xylitol fermentation performance after treatment by aqueous ammonia, neutralization, and concentration.

7.5.3.2 Influencing Factors of the Xylitol Fermentation Process

At present, optimization of process parameters using pure xylose to ferment xylitol focuses on the following several aspects: ventilation, nitrogen source, xylose concentration, other sugars and auxiliary matrix, strain domestication, inoculated volume and seed age, pH and temperature, and fermentation type.

(1) Ventilation

Oxygen is the essential factor in xylose assimilation. However, excess oxygen would cause the oxidation of NADH into NAD+ and reduce the activity ratio of xylose reductase (XR) to xylitol dehydrogenase (XDH), which is not conducive for xylitol accumulation. Generally, the growth of cells was positively correlated with ventilation, and the xylitol conversion rate was negatively correlated with ventilation. Under aerobic conditions, the consumption of sugar is mainly used for cell growth; under microaerobic conditions, most of the xylose is changed into xylitol, and thus the yield of alcohol is small. In xylose metabolism, xylose reduction by reductase needs NADPH, while converting oxide xylose into xylulose by xylitol dehydrogenate requires NAD^+. Under microaerobic conditions, excessive production of NADH impedes xylitol oxidation and promotes the accumulation of xylitol in the fermentation broth.

In hydrolyzate fermentation, with the increase of the ventilation volume, the cell amount increases. But, the excessively large amount of bacterial cells would consume a large amount of xylose. On the other hand, excess ventilation is conducive to the consumption of the inhibitory substances (e.g., glucose and acetic acid). Acetic acid not only will interfere with the cell membrane of xylose absorption but also will inhibit the activity or synthesis of the xylose reductase. The anaerobic fermentation of glucose also impedes xylose fermentation to xylitol. Vandeska et al. [85] studied the effect of ventilation on the activity of *Candida boidinii* dehydrogenase and found that as OTR increased from 10 to 30 mmol·L^{-1}·h^{-1}, the cell biomass increased sixfold, XR/XDH was reduced from 2.8 to 0.98, and the yield of xylitol was reduced from 0.38 to 0.23 g·g^{-1}.

Horitsu et al. [86] pointed out that, for efficient production of xylitol, a high level of DO should be maintained in the early period of culture, then reduce the DO level to inhibit microbial respiration. The two-stage aeration is effective for improving the rate of xylitol formation. In the later stage of low levels of DO, the redox potential of the medium is a sensitive indicator for monitoring the DO level. Kim et al. [87] reduced ventilation to reduce the redox potential of the medium to the optimum value of 140 mV in the second stage when they cultured *C. tropicalis*. After fermentation for 66 h, 300 g·L^{-1} xylose were changed into 250 g·L^{-1} xylitol, with a generation rate of 3.79 g·L^{-1}·h^{-1}.

(2) Nitrogen source

Xylitol accumulation is significantly affected by nitrogen source. The suitable nitrogen source and concentration are different between the yeast strains in the different media at different pH conditions. An organic nitrogen source can reduce the level of *Candida shehatate* XDH. Yeast extract is the best organic nitrogen source. Using straw hemicellulose hydrolyzate to culture *Candida guilliermoneii*, urea used as a nitrogen source was better than ammonium sulfate at pH 4.5, and xylitol yield increased by 25 %; there was no significant difference when the pH was 5.3 or 6.0. Some scholars mixed organic and inorganic nitrogen sources and achieved good results.

(3) Xylose concentration

Enhancing the initial xylose concentration can improve both the concentration of xylitol and the yield and formation rate of xylitol. However, the sugar tolerance of different strains is significantly different.

When the xylose concentration was increased from 50 to 100 g·L^{-1}, there was no significant change in the maximum growth speed (μ_{max}) of *C. parasilosis, C. guilliermondii,* and *C. tropicalis*, but the μ_{max} of *C. mogii* fell by almost half. It was found that the XR activity of two kinds of yeast, *C. guilliermondii* and *C. parasilosis,* was significantly affected by xylose concentration. The optimal xylose concentration for production is located near the concentration when XR activity reaches the maximum. In shake flask culture of *C. tropicalis* KFCC-10960, as the xylose concentration was increased from 50 to 250 g·L^{-1}, xylitol yield increased from 0.76 to 0.90 g·g^{-1}. When the xylose concentration was 150 g·L^{-1}, the fermentation rate of xylitol reached the maximum (2.9l g·L^{-1}·h^{-1}). Then, it

would decrease greatly, and when the xylose concentration was 250 $g \cdot L^{-1}$, the xylitol formation rate was only 1.44 $g \cdot L^{-1} \cdot h^{-1}$.

According to the existing data, the generation rate and the concentration of xylitol of several glucose-tolerant yeast in a fermentor are, respectively, as follows: 1.04 $g \cdot L^{-1} \cdot h^{-1}$ and 221 $g \cdot L^{-1}$ for *C. guilliermondii*; 3.18 $g \cdot L^{-1} \cdot h^{-1}$ and 210 $g \cdot L^{-1}$ for *C. parapsilosis*; 4.56 $g \cdot L^{-1} \cdot h^{-1}$ and 251 $g \cdot L^{-1}$ for *C. tropicalis*; and 4.67 $g \cdot L^{-1} \cdot h^{-1}$ and 221 $g \cdot L^{-1}$ for *D. hansenii*. Their xylitol yield is generally from 70 to 82 %, and only the yield of *C. tropicalis* on glucose/xylose medium was more than 90 %.

In terms of hemicellulose hydrolyzate as a fermentation substrate, the xylose concentration is closely related to raw materials, hydrolysis method, and detoxification extent. The toxicant is concentrated in the xylose concentration process. When the toxicant concentration exceeds a certain value, the yeast cannot grow.

The toxic tolerance of yeast to different raw material hydrolyzates is different. Unless it has been specially refined, *Candida* sp. 11-2 can hardly utilize xylose in bagasse hemicellulose hydrolyzate. This strain can utilize corncob hydrolyzate after ammonia treatment, in which the xylose concentration is up to 130 $g \cdot L^{-1}$, and the xylitol formation rate is up to 1.94 $g \cdot L^{-1} \cdot h^{-1}$. The xylitol formation rate and yield of this strain utilizing ammonia-treated wood hydrolyzate were 5.38 $g \cdot L^{-1} \cdot h^{-1}$ and 0.74 $g \cdot g^{-1}$, respectively.

(4) Other sugars and auxiliary matrix

The utilization rates of glucose, mannose, and galactose by *C. guilliermondii* and *C. tropicalis* are 2.2, 2.0, and 3.8 times that of xylose, respectively. These hexoses are mainly used for the growth of yeast cells and generally do not accumulate the corresponding polyhydric alcohol, and most of the arabinose consumed by the yeast only generates a small amount of arabinitol.

If sugars easier to use than xylose were added as a carbon source, xylose consumption for cell growth and NADPH regeneration would be inhibited. Then, actual yield of xylitol may reach or exceed the theoretical yield. Culturing *C. tropicalis* KFCC-10960 under conditions of a glucose/xylose ratio of 10–20 %, the xylitol yield was over 0.9 $g \cdot g^{-1}$. When the ratio of glucose exceeded 20 %, a large amount of ethanol was generated, severely reducing the generating rate and conversion of xylitol.

(5) Strain domestication

Domesticated strains can directly ferment hydrolyzate after a simple detoxification treatment (only after the calcium carbonate and hydrochloric callback). For example, *Debaryomyces hansenii* NRRI Y-7426, after domestication six times, fermented the hydrolyzate with a xylose concentration of 1.5 %. The final xylitol concentration increased from 0.1 to 0.45 %. If more domestication and selection were completed, the strain's adaptability to harmful substances in the hydrolyzate and fermentation capacity would be further improved, which should be an effective way to increase yield and reduce costs.

(6) Inoculated volume and seed age

Increasing the inoculation amount by centrifugation collection can effectively shorten the training time and improve the xylitol formation rate. Microaerobic culturing of *D. hansenii* in 140 $g \cdot L^{-1}$ xylose medium for 72 h, when the inoculum volume was 0.3 $g \cdot L^{-1}$ cells (equivalent to stem cells), the xylitol production was 60 $g \cdot L^{-1}$; when the inoculum volume was increased to 3.0 $g \cdot L^{-1}$ cells, the xylitol production was 105.8 $g \cdot L^{-1}$. The high density of cells inoculated apparently alleviated the toxic inhibitory effect in hemicellulose hydrolyzate to improve fermentation performance. Using wood hydrolyzate containing 58–78 $g \cdot L^{-1}$ xylose to culture *D. hansenii*, when the inoculum volume was 16 $g \cdot L^{-1}$ cells (fresh cells), the fermentation was almost impossible; when the inoculum volume was increased to 50–80 $g \cdot L^{-1}$ cells, the xylitol yield was 39–41 $g \cdot L^{-1}$.

(7) pH and temperature

Because only molecular acetic acid causes inhibition, thus inhibition of acetic acid has a close relationship with pH value. In *Candida guilliermondii* fermentation using bagasse hydrolyzate, when pH was 6.0 and the OTR was 10 or 22.5 h^{-1}, acetic acid was not used up until the end of fermentation. Under the same pH, when the OTR was 35 h^{-1}, acetic acid was rapidly depleted, and its inhibition effect was negligible. When the initial pH was 4.0 and ORT was 22.5 or 35 h^{-1}, acetic acid was rapidly depleted; even the OTR was controlled at 10 h^{-1} during the whole process, acetic acid would also rapidly depleted. Regarding the effect of the fermentation, the concentration of the xylitol, and the yield of xylitol and xylose consumption rate reached their highest when the pH was 6.0 and the highest xylitol yield was 79 %.

The pH and temperature could affect the vitality of two key enzymes: xylose reductase and xylitol dehydrogenase. It was found that, for *C. guilliermondii* fermentation using bagasse hydrolyzate, the activity of xylose reductase was highest from pH 4.0 to pH 6.0, the activity of xylitol oxidase increased with the rise of pH and temperature, and the activity of xylitol dehydrogenase was highest at pH 6.5 and 35 °C. The xylose conversion rate and xylitol production rate reached a maximum at pH 6.0 and 35 °C. The presence of xylitol had no effect on activity of xylitol dehydrogenase of this strain, which showed that the optimum condition of xylitol production and conditions at which the activity of xylitol dehydrogenase were not consistent with the optimum condition of xylose activity.

The pH would change in a fermentation process with the decomposition of nitrogen sources. Therefore, the optical pH is different when using different nitrogen sources.

For example, in fermentation with *C. guilliermondii* utilizing straw hemicellulose hydrolyzate, if ammonium sulfate is used as a nitrogen source, the optical pH is 5.3 for xylitol production, and if urea is used as the nitrogen source, the optical pH is 4.5. This is because, in the fermentation process, the urea will be decomposed to generate ammonia and carbon dioxide, so the pH of the fermentation broth increases. Therefore, when considering the optimum pH in fermentation, the nitrogen source should also be considered.

(8) Fermentation type

The previous study of the author's lab showed that among batch; fed-batch (hydrolyzate containing 79 $g{\cdot}L^{-1}$ xylose was added at a speed of 28 $mL{\cdot}h^{-1}$ after culture for 65 h); semicontinuous culture (two thirds of the culture were removed when cultured to 63 h, then supplied with hydrolyzate), the maximum xylitol concentration and production rate of semicontinuous culture (34 $g{\cdot}L^{-1}$ and 79 %, respectively) were significantly higher than for batch culture and fed-batch culture. This is because in semicontinuous culture, the cells are further adapted to the hydrolyzate, thereby improving the conversion rate and production efficiency. If the xylose concentration exceeded a certain value, it would limit the growth of yeast cells, resulting in extension of the fermentation time and a great decrease of the product formation rate. Many scholars immobilized yeast cells to produce xylitol. Silva et al. used porous glass to culture *C. tropicalis* in a fluidized bed reactor; 155 $g{\cdot}L^{-1}$ xylose was converted into 90–95 $g{\cdot}L^{-1}$ xylitol, and the formation rate was 1.35 $g{\cdot}L^{-1}{\cdot}h^{-1}$. According to the research of Yahashi et al. [88], the effect of using nonwoven fiber to immobilize *C. tropicalis* was best.

Oh and Kim [89] reported that if the xylose concentrations exceeded 150 $g{\cdot}L^{-1}$, an apparent lag phase would appear in the growth of *C. tropicalis* KFCC-10960 cells. They controlled the initial xylose concentration of 150 $g{\cdot}L^{-1}$ and added a high concentration of glucose/xylose at a constant speed when the cell density was up to 15 $g{\cdot}L^{-1}$ and the total xylose addition was 270 $g{\cdot}L^{-1}$. After 55 h of fermentation, the xylitol concentration was 251 $g{\cdot}L^{-1}$, the product formation rate was 4.56 $g{\cdot}L^{-1}{\cdot}h^{-1}$, and the conversion rate was 0.93 $g{\cdot}g^{-1}$.

Enzymatic synthesis of xylitol is a delicate and promising process route, but it does not yet have the competitiveness with the current fermentation process. According to current data, *C. guillietmiodii*, *C. tropicalis*, *C. parapsilosis*, and *D. hansenii* microorganisms have the potential for xylitol fermentation because they can exhibit a high production rate or high conversion rate under high xylose concentrations. *Saccharomyces cerevisiae* strains expressing the XYL1 gene must be imported to the xylose permease system and utilize both xylose and hexose, then xylitol formation rate may be improved. Batch constant-speed feeding culture is an effective method of eliminating the inhibitory effect of a high concentration of xylose and increasing the rate of product formation. This batch culture technology may become another feasible way to handle the toxic effect of hemicellulose hydrolyzate.

Glucose addition can effectively improve xylitol yield. However, excess glucose in media will generate a higher concentration of ethanol, which would seriously affect the formation rate of xylitol. This effect prompts that an excessively high proportion of glucose or other hexoses should not be contained in the cellulose material hydrolyzate. Hemicellulose hydrolyzate contains many components toxic to microbial metabolism and may also have some substances that inhibit cells from secreting xylitol. Research on more cost-effective detoxification technology is an urgent issue for a large-scale fermentation process using hemicellulose hydrolyzate to produce xylitol.

7.6 Furfural Production

Furfural is an important heterocyclic organic compound, which is a kind of liquid with colorless to yellow color, almond kind of smell, melting point at −36.5 °C, and boiling point at 161.1 °C. The molecular weight of this substance is 96.06, and the molecular formula can be denoted as $C_5H_4O_2$. It can be slightly dissolved in water but is soluble in hot water, ethanol, ethyl ether, and benzene [90, 91]. With the increasing demand for furfural, its production has grown from 2.5×10^4 tons in 1955 to more than 3×10^5 tons in the most recent 30 years worldwide. Manufacturing plants were first founded in 1954 in China and have developed to more than 140 factories. At the same time, the annual production in China ranks fourth in the world, with about 5×10^4 tons, just after the United States (1.5×10^5 t), the former Soviet Union (8×10^4 t), and the Dominican Republic (6×10^4 t). The export volume ranks first in the world because about 80 % of its products are exported [92]. The production of furfural is based on forestry and crops that are inexhaustible raw materials, so this is significant in developing the furfural industry and its downstream products and broadening their applications worldwide as the oil, coal, natural gas, and other energy shortages are increasingly serious [93, 94].

The molecular structure of furfural consists of a furan ring and an aldehyde group. These functional groups equip furfural with specific properties, such as hydrogenation, oxidation, chlorination, nitration, and condensation, just like aldehyde, ethers, diene, and aromatics. These reactive chemical properties mean furfural is used as a raw material for a large number of derivative products. Furfural is obtained from xylose dehydration cyclization and is mainly used in the fields of synthetic fibers, synthetic resins, perfumes, paint, synthetic drugs, and so on [95].

7.6.1 Raw Materials for Furfural Preparation

Furfural is obtained by dehydration of pentose, which is a by-product of agriculture. It still cannot be obtained through the chemical synthesis.

$$(C_5H_8O_4)\,n + nH_2O \rightarrow C_5H_{10}O_5$$
$$C_5H_{10}O_5 \rightarrow C_5H_4O_2 + 3H_2O$$

The production of furfural mainly uses pentosan in plants, so raw material with a higher pentosan content is better. The degree of polymerization of pentosan in plants is generally 80–150, while the degree of polymerization in natural cellulose is about 1,500–3,000. Therefore, pentosan is much easier to hydrolyze compared with natural cellulose. Plant fiber pentosan can be almost completely converted to pentose cellulose, while the hydrolysis of cellulose would not occur under the same conditions if suitable conditions were selected. Currently, the raw materials used for the production of furfural are agriculture and forestry by-products, including

Table 7.2 Principal component analysis of several furfural feedstocks

Raw materials	Hemicellulose (%)	Cellulose (%)	Lignin (%)
Corncob	39.3	36.9	9.2
Corn stover	30.1	33.4	6.8
Bagasse	20.6	35.4	18.6
Rice husk	19.8	36.1	30.8

corncobs, sunflower shells, cottonseed hulls, rice husks, bagasse, and cotton rod, among which corncob contains the highest pentosan content (up to 38 %), and the furfural latent content is about 25 %. The component analysis of these materials is shown in Table 7.2. Besides xylose, arabinose (may be obtained from gums or degradation oxidation of left-handed calcium gluconate and hydrogen peroxide), D-ribose, and D-lysose, these five-carbon sugars can also be used to generate furfural with different abilities. In addition, ascorbic acid and some uronic acids, such as glucuronic acid, which is a kind of hexuronic acids, may also be used to generate furfural. The furfural residue can be further decarboxylated to generate the new green platform chemical levulinic acid [43].

7.6.2 Methods to Prepare Furfural

One-step and two-step methods are used to produce furfural. The one-step method is widely used, but it has disadvantages of low yield, increased energy consumption, and large amounts of waste residue [96]. The two-step method is better than the one-step method, with up to 70 % conversion, and the raw materials can be used comprehensively for the waste residue produced after hydrolysis can still be used to produce glucose. The two-step method is becoming the trend for furfural production [97].

7.6.2.1 One-Step Method

The one-step method is widely used in most enterprises. That is, the hydrolysis of pentosan and pentose dehydration cyclization to produce furfural occurs in a hydrolysis reactor at the same time. The furfural production processes and technologies have been greatly improved, first from the single pot to multipot tandem and continuous production processes after development for decades. The application processes used widely include the Quaker Oats, Agrifuran, Petrolechimie, Escher Wyss, Rosenlew, and RRL-J processes. All the processes use steam from water to strip the furfural generated in the reaction. The one-step method consumes a large amount of steam. Usually, a ton of furfural consumes 18–25 t steam, with a low yield of furfural of about 60 %, and produces a large amount of waste. These residues are mainly composed of cellulose, lignin, unreacted

hemicellulose, and residual catalyst. The way to dispose of these waste residues is mainly using them to generate steam.

Ma and Feng [98] used rice husk to replace the traditional material corncob for furfural production and researched the optical process. The results showed that the best condition for furfural production using rice husk was as follows: The sulfuric acid concentration was 10 %, reaction time was 4 h, reaction temperature was 170 °C, and the liquid-to-solid ratio was 6:1. The yield was up to 62 %, which was 12 % higher than that of corncob.

Zhuang and Liu [99] used corncobs as raw materials to optimize furfural conditions. To 80 g corncob, 55 g NaCl and 12 mL acid were added, and then the hydrolyzate was heated to 102–105 °C for 5 h with electric heating after 11.5 h soaking and 12 h hydrolysis. The distillate was set in the beaker, and 10 % sodium carbonate solution was added to adjust the solution to slightly acidic (pH about 6; the amount of soda ash used was about 5 % of the distillate) and then retained for about 20 min after stirring. The yellow, oily liquid obtained was furfural, and the isolated yield was 6.3 g after separation using a separator.

7.6.2.2 Two-Step Method

Pentosan hydrolysis and pentose dehydration cyclization are carried out separately in the two-step method. The two-step process is more complex the one-step process, equipment is expensive, and the dehydration process is not mature, so no practical application was performed in industrial production. However, with the development of furfural production technology, as well as the increased requirements for raw material utilization, the two-step method with its high yield and cellulose and hemicellulose in the raw materials effectively separated and used is becoming an inevitable trend for industrial furfural production in the future.

In the 1940s, Dunning and Lathrop [100] studied the two-step method. They used sulfuric acid as the catalyst and corncob as the raw material in a set of continuous equipment for production. The first-step hydrolysis reaction occurred at 98 °C with a concentration of 5.8 % sulfuric acid; the pentose yield was up to 95 % or more after 129 min. The residue generated by hydrolysis was treated with 8 % sulfuric acid at 120 °C for 8 min to obtain about 90 % glucose, which could be used to produce industrial alcohol. The pentose solution was catalyzed by sulfuric acid to dehydrate to obtain 69 % yield of furfural.

Moreau et al. [101] used mordenite and faujasite as catalysts to dehydrate xylose to produce furfural. The dehydration reaction was carried out in a 300-mL autoclave equipped with a stirrer. In their study, 3.75 g xylose, 1 g catalyst, and 50 mL water were added to the reactor; at the same time, 150 mL methyl isopropyl ketone or toluene were also added as the reactive extraction agent. The reaction was carried out when the temperature was increased to 170 °C with a stirring rate of 700 rpm under a nitrogen atmosphere. The results showed that furfural selectivity could be reached 96 % when the xylose conversion rate was lower, and the best catalyst was zeolite with fewer mesoporous.

Dias et al. [93] studied sulfonic acid loaded on the microporous-mesoporous silica as the catalyst to dehydrate xylose to obtain furfural. The reaction was carried out in a microreactor and used dimethyl sulfoxide, water, and methyl isopropyl ketone or toluene as the solvent. In the reactor, 30 mg xylose, 20 mg catalyst, and 1 mL solvent were added, and then the reaction temperature was increased to 140 °C and kept for 24 h. The conversion rate of xylose was 90 % with 82 % selectivity for furfural. The results proved that the sulfonic acid loaded on microporous-mesoporous silica materials was an efficient catalyst, but the surface of these silicate catalysts were prone to coking results in decreased yield.

Kim and Lee [102] also performed a study of the supercritical CO_2 extraction process, in which sulfation titanium oxide and sulfation zirconium oxide were used as solid catalysts to prepare furfural. In this work, 10 g catalyst and 400 g water were added to the reactor, CO_2 was aerated to bring the pressure of the system to 8 MPa, and then the temperature was raised to 180 °C. Supercritical CO_2 was pumped to a pressure of 20 MPa when the temperature was steady, then 100 g of 10 % xylose aqueous solution were added. Supercritical CO_2 was pumped from the bottom of the reactor continuously, and samples were obtained from the liquid for analysis during the reaction. However, the yield of furfural was 60 % using sulfated titania oxide as a catalyst; the yield was about 50 % using sulfated zirconia as a catalyst. There was no sintering phenomenon in these two catalysts.

7.6.3 Preparation and Separation of Furfural Using Hemicellulose from Steam-Exploded Corn Stover

Because of the problems of a limited amount of corncobs, large acid consumption, and difficulty in comprehensive utilization of furfural slag in current furfural production, Chen et al. [103, 104] explored a new process using water extraction from steam-exploded corn straw to prepare furfural (Fig. 7.3). First, they used the steam explosion technology to pretreat cornstalks, in which most of the hemicellulose was degraded and separated. Xylose from the degraded hemicellulose of steam-exploded corn straw was extracted by water, and then the xylose is used to produce furfural under high-temperature and high-pressure conditions. The optical conditions of steam explosion, water extraction, and furfural production and the phenomenon of coking were studied.

The results showed that the effect of steam explosion was better when the pressure was 1.4 MPa and kept for 4 min. The structure of materials obtained under such conditions was loose, and the xylose dissolution rate could be up to 3.25 % in water extract. Several components were greatly changed in cornstalks; among them, the hemicellulose content dropped from 23.7 to 5.55 %. Conditions for water extraction were also investigated, and the effect of water extraction was better with a solid-liquid ratio of 1:6 and 80 °C hot water. The water extraction time did not show

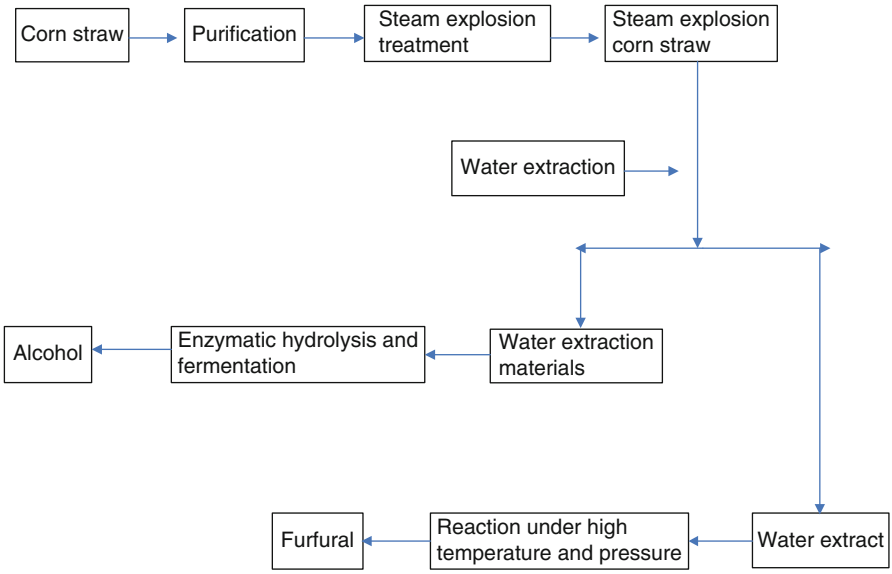

Fig. 7.3 The process of preparation and separation of furfural using hemicelluloses from steam-exploded corn stover

any significant effect on the xylose concentration. Taking the next step into account, they used 80 °C hot water and a solid-liquid ratio of 1:4 to extract for 15 min, and the water extract tended to be acidic, containing much sugar and acids. The optical conditions of the steam explosion corn straw and water extraction process to produce furfural were 2 % hydrochloric acid at 180 °C for 2 h. The reasons for the coking phenomenon in furfural preparation by steam explosion and the water extraction process were further studied by Fourier transform infrared (FTIR) spectroscopy. The advantage of this method is that the corn straw feedstock has a low price, has a wide variety of sources, and has a high furfural yield (76.6 %), which is helpful for the comprehensive utilization of materials.

7.7 Xanthan Gum Production

Xanthan gum is a kind of microbial extracellular heteropolysaccharide composed of glucose, mannose, and glucuronic acid in a molar ratio of 2.8:2:2. The main chain of xanthan gum is a repeating unit of β-1,4-glycosidic bonds formed by the two D-glucose molecules. Its skeleton structure is similar to cellulose; that is, the C3 unit between the two glucoses has a trisaccharide side chain composed of two glucomannans and a glucuronic acid [105, 106]. The unique molecular structure

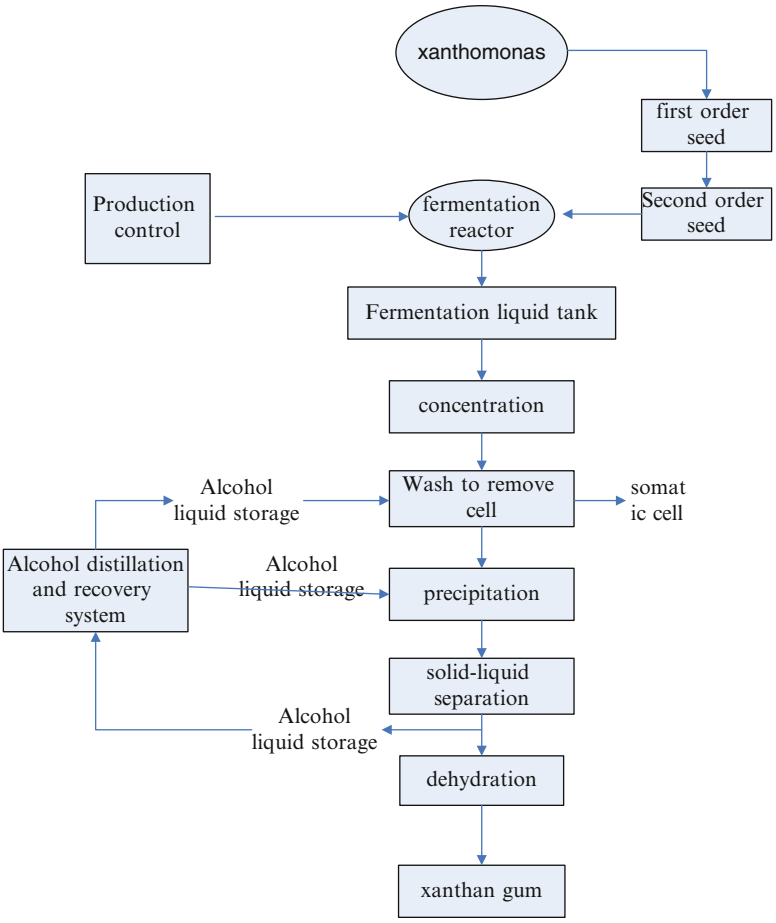

Fig. 7.4 Xanthan gum production process [106, 108]

of xanthan gum gives it unique physical and chemical properties, such as good thickening, thixotropy, emulsifying properties, and false plasticity. Xanthan gum is a green, nontoxic, safe substance. Therefore, it is widely used in the food industry. In addition, it is one of the most superior biological glues.

Since the 1950s, production of a variety of microbial polysaccharides by fermentation technology has been a hot topic, and several microbial polysaccharides have already been made at the industrial scale. Xanthan gum is one of the most representative of those with the highest commercialization degree. The production process for xanthan gum is shown in Fig. 7.4. The key parts are selection and transformation of xanthan gum production strains, the development and control of the new fermentation process, and separation and purification of the xanthan gum product.

7.7.1 Production Strains and Their Transformation

Xanthomonas spp. are used in xanthan gum production. *Xanthomonas campestr* is a natural bacteria originally isolated from turnip. This bacterium generates polysaccharide with the same chemical composition in the medium of cabbage extract and artificially made medium. Therefore, *X. campestr* is an excellent strain for industrial production of xanthan gum. In addition, *X. phaseoli*, *X. malvacearum,* and *X. carotae* can be used as fermentation strains [107]. The xanthan gum production process is shown in Fig. 7.4.

The strain has critical effects on the yield and quality of products. It was reported that gene recombinant technology was used in the sequencing, separation, screening, and expression of gum-producing genes; however, recombinant strains for industrialization have not yet been reported. If the gum-producing genes can be isolated by genetic engineering and then imported into complete new strains to make the strain show double or more production genes and be stably inherited, xanthan gum production would be increased exponentially.

Xanthan gum can be produced in batch fermentation, semibatch fermentation, or continuous fermentation [109–111]. In industrial production, aeration-agitation fermentors by deep fermentation in batch culture are usually applied. The actual processes include seed fermentation, seed expansion culture, and fermentation. The type that should be selected for a specific fermentation process is determined by the metabolic characteristics of the microorganism used and the optimum conditions. In batch fermentation, the concentration of substrate will decrease gradually. In continuous fermentation, the concentration of substrate throughout the fermentation process remains substantially unchanged, and lack of substrate does not appear under normal circumstances, which is beneficial to cell growth. On the other hand, the substrate may lead to inhibition, leading to decreased final yield. In semibatch fermentation, substances can be added according to the characteristics of different growth stages of cell growth and to create an optimal physiological environment for cell growth. Many studies have shown that semibatch fermentation is better for xanthan gum fermentation [111].

Because of the viscosity of the xanthan gum solution and the large amount of oxygen needed in the fermentation process, some reactors are not suitable for production. An air-lift reactor can effectively solve the problem of heat caused by agitation and aeration in xanthan gum fermentation and can reduce energy consumption; thus, it is suitable for continuous fermentation. A tower reactor equipped with a fluidized bed was applied in batch fermentation for its high bubble transfer coefficient. A pump-type statically mixing and recycling reactor is able to increase the transfer rate of the oxygen in the fermentation broth with high viscosity, which can improve the production of xanthan gum. Because of the special characteristics of the xanthan gum fermentation broth, it is difficult for ordinary mixers for pulps such as a disk turbine agitator and a blade turbine-type impeller to meet the requirements, although their energy consumption is smaller. In recent years, to meet the demands of the high viscosity and high ventilation rate of the

fermentation process, grid-type and window-type stirring blades were invented. The so-called grid type is actually the stirring shaft equipped with a gate-shaped frame in the middle and then separately or simultaneously accompanied by one or more disk turbine agitators. The window type is similar to an evolution of the marine impeller.

7.7.2 Improvements of Xanthan Gum Production Technology

7.7.2.1 Alternative for Fermentation of Raw Material

In the process of xanthan gum fermentation, the high viscosity of the fermentation broth caused by the polysaccharide accumulation brings the problem of DO limitation, so the normal fermentation feedstock concentration is not high (common carbon source concentration of 40 g L^{-1} [112]). If the carbon source concentration of the fermentation medium is high, the yield will be reduced. As mentioned, straw is a type of raw material with complex composition and relatively low density. Its hydrolyzate concentration is often not high. Thus, it is difficult to produce alcohol, lactic acid, and other products with a high concentration [113], but it is able to meet the requirements of xanthan gum fermentation. Therefore, from the perspective of process, using straw to replace starch and other food raw materials is theoretically feasible. In addition, although the xanthan gum is a bulk product, generally its production is thousands of tons per year, and in this scale, raw material collection would not be a problem.

Chen and Zhang [114, 115] studied the use of straw enzymatic liquid for xanthan gum fermentation and established the fermentation process. At first, the sensitivity of *X. campestris* to steam explosion suppression components as well as the removal of these substances were studied. The results showed that weak acids, furan derivatives, and phenolic substances inhibited xanthan gum fermentation. In steam explosion, using the combination of low pressure and a long maintenance time can effectively reduce the generation amount of the inhibitor. Furfural, 5-(hydroxymethyl) furfural, and phenols were generated 37.1 %, 37.8 %, and 20.6 % less, respectively, under the conditions ($R_0 = 7{,}552$) of 1.3 Mpa, 8 min in steam explosion, then 1.4 MPa for 6 min ($R_0 = 7{,}787$). Calcium phosphate flocculation can remove 30 % of the phenolic substances, steam-exploded straw air-dry processing can remove 78.3 % of formic acid, 94.5 % of furfural, and 88.8 % of hydroxymethyl-furfural. The medium was optimized based on removal of the ingredient for inhibiting; then, the production of xanthan gum reached 20.7 g/L, and the carbon source conversion rate reached 0.62 g g^{-1}. Finally, the influence of xylose on fermentation was studied. The results showed that the ability of *X. campestris* to utilize xylose is weak (consumption rate of 0.053–0.060 g L^{-1} h^{-1}), and a xylose proportion higher than 50 % even inhibited growth, but the presence of a small amount of xylose (total sugar 10 %) can increase the molecular weight, acetyl group content, and acetone acyl group content of xanthan gum and thus improve product quality.

7.7.2.2 Solid-State Fermentation on Inert Adsorption Carrier

The strengthening method commonly used in the liquid fermentation process is improving the stirring device, for example, by increasing the stirrer diameter or using slurry mixing [116]. This way, although oxygen transfer is improved, higher agitation energy consumption is difficult to avoid. As energy saving becomes the consensus today, looking for a new alternative with low energy is worth actively studying. In solid-state fermentation on an inert adsorption carrier, the carrier provides a stable interface for oxygen to transfer through the gas and liquid, which makes it possible to solve the DO limitation in xanthan gum fermentation and therefore improve the fermentation product concentration.

To solve the problem of high viscosity and difficulty in high-concentration fermentation of liquid fermentation producing xanthan gum, Chen et al. [117, 118] used an inert adsorption carrier to produce xanthan gum and studied the dynamics characterization of its fermentation process. The results showed that, under conditions of polyurethane resin carrier particles of 0.5 cm^3, a solid-liquid ratio of 1:15 (w:w), and 4.5-cm bed depth, the concentration of xanthan gum came to 38.65 g L^{-1}, which amounts to 1.8 times that of liquid fermentation. The impact of static solid-state fermentation and pressure pulsation solid-state fermentation were further analyzed, and it was found that pressure pulsation can improve the xanthan gum fermentation level, and its product concentration was 42.62 g L^{-1}.

7.7.2.3 Extraction and Separation of Xanthan Gum

Extraction of xanthan gum aims to obtain the finished product by separation, purification, drying or other means according to product quality requirements. Cells, carbohydrates, inorganic salts, and other impurities and a large amount of water in the xanthan gum fermentation broth need to be removed. Separation of xanthan gum is a high-cost step, which accounts for 50 % of the overall production costs. The main steps are as follows: precipitation of the cells and xanthan gum, dehydration, drying, and grinding. The xanthan gum fermentation broth has a high viscosity, and it is difficult to manage it. In the separation, cells are the major obstacle.

Although a variety of methods exists that can inactivate cells in fermentation broth, there still are some shortcomings. The enzymatic method has the problem of high costs. Chemical reagent changes pH easily, thus reducing the pyruvate content of the product. Therefore, pasteurization is generally used. Because of the high temperature of this method, the solubility of xanthan gum can also be improved, and the viscosity of the solution is reduced to a certain extent, which is conducive to the subsequent centrifugation or filtration. In light of the high viscosity of the fermentation broth of xanthan gum, it needs to be diluted before filtration (diluent is generally water, alcohol, or alcohol-containing low concentrations of salts) to facilitate the removal of the bacterial cells and impurities and the xanthan gum precipitation. The methods commonly used are centrifugation, filtration, enzyme

degradation, hypochlorite oxidation, ultrafiltration, and so on, and precipitation by ethanol or isopropanol [119, 120] is usually used for preparation of xanthan gum, for which the principle is xanthan gum agglomerating in certain solvents.

7.8 Bacterial Cellulose Production

Cellulose is a kind of chain polymer made from D-glucose linked by β-1,4 glycosidic, which is the richest resource on the planet and the biosynthetic material most closely related to human life. Cellulose is widely spread in trees, cotton, and other plants, and small amounts are present in some individual bacteria and lower animals. It is the main component of the vascular plants, lichens, and part of the algae cell wall. Cellulose can be divided into plant cellulose, animal cellulose, and BC according to different sources. At present, most of the industrially applied cellulose is obtained from plants, such as trees, bamboo, rice straw, cotton, flax, jute, ramie, and marijuana. Animal cellulose, also known as chitin, is a kind of polysaccharide biopolymer that can be widely found in the shell of lower organisms, fungi, algae, arthropods, crabs, and insects and in the viscera and cartilage of mollusks such as squid and cuttlefish in nature. It has a wide range of applications in the pharmaceutical, engineering, food, cosmetics, agriculture, environmental protection, and enzyme immobilization carrier industries and so on [121].

The synthesis of cellulose is not just a function of the plant. Some microbes can also be efficient in synthesizing cellulose, of which Ax (short for *Acetobacter xylinum* X-2) is an outstanding representative. Discovery and chemical characterization of BC synthesized by *A. xylinum* was first reported by Brown in 1886 [122]. But, the characteristics and industrial applications of it were not investigated until 100 years later. Compared with traditional plant cellulose, BC has many excellent properties, such as high purity, high degree of polymerization, high degree of crystallization, high hydrophobicity, high Young's modulus, high strength, and nanofineness [123, 124]. Because of these characteristics, BC has become a potential new biomaterial in the food industry [125–127], biomedical materials [128, 129], composite materials [130, 131], the paper industry [122], and other fields.

7.8.1 Strains for Bacterial Cellulose Production

The microorganisms capable of synthesizing cellulose include *Acetobacter* spp., *Agrobacterium* spp., *Pseudomonas* spp., *Achromobacter* spp., *Alcaligenes* spp., *Aerobacter* spp., *Azotobacter* spp., *Rhizobium* spp., and *Sarcina* spp. Among these nine species, *Acetobacter xylinum*, *Acetobacter aceti*, *Acetobacter acotigenum*, and *Acetobacter pasteurianus* have realized the industrialization of acetic acid bacteria cellulose. *Acetobacter xylinum* has the best ability to synthesize cellulose [132].

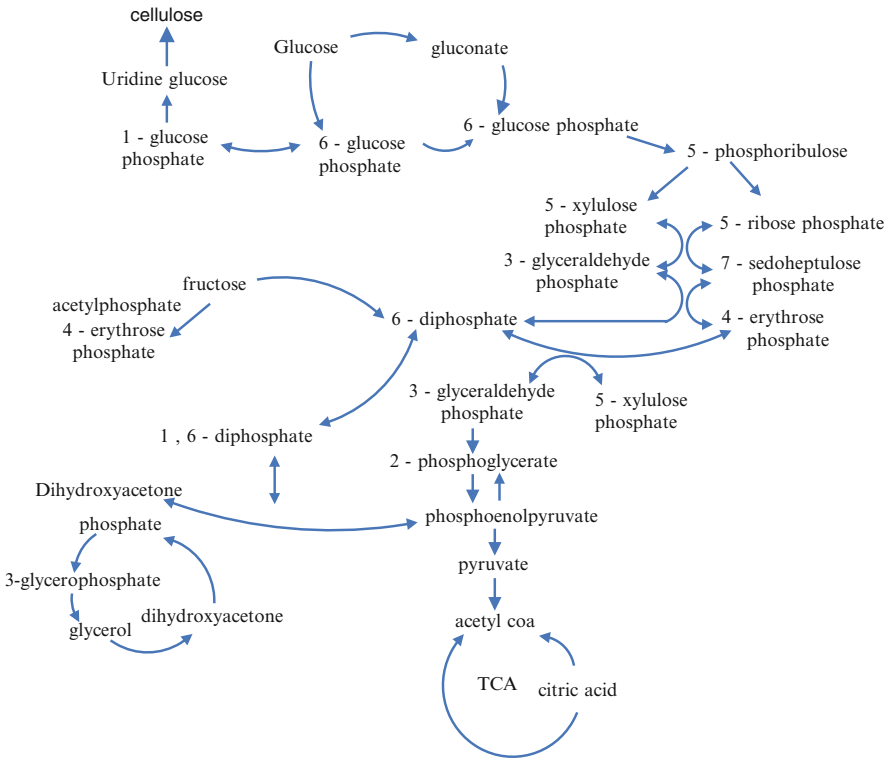

Fig. 7.5 Biosynthetic pathway of bacterial cellulose in *A. xylinum* [133]

7.8.2 *Biosynthetic Pathway of Bacterial Cellulose*

Cellulose biosynthesis is a complex process, as shown in Fig. 7.5. Bacterial cellulose is a molecular chain polymerized with glucose molecules linked by β-1,4-glycosidic bonds, and hydrogen bonds intermolecularly and intermolecularly form the network structure. Its biosynthesis can be divided into four major processes: polymerization, secretion, assembly, and crystallization. These four processes are highly coupled and are also closely related to specific sites on the cell membranes. The four processes are roughly described as follows. (1) Glucose is converted into 6-phosphoric acid glucose by glucose kinase. (2) 6-Phosphate-glucose is converted into 1-phosphoric acid glucose by isomerase. (3) UDPG (uridinediphosphate-D-glucose) is generated from 1-phosphoric acid glucose catalyzed by UDPG pyrophosphorylase. (4) The β-1,4-glucoside chain is produced from UDPG catalyzed by cellulose synthase and then reassembled to form cellulose [133]. In the process of cellulose synthesis, uridine glucose is the direct precursor, while glucose-1-phosphate, as a branch point, can be either further used to synthesize cellulose or continue to be oxidized and decomposed in the pentose phosphate cycle or the citric acid cycle and

then generate glucose to be further converted to cellulose via pentose cycle and the gluconeogenesis pathway. Thus, in the BC fermention, the proper methods can be used to inhibit or block the formation of pentose and make the carbon source used for cellulose synthesis as much as possible to improve the utilization coversion of raw materials and obtain higher BC yield.

7.8.3 Microbial Transformation of Bacterial Cellulose

7.8.3.1 Static Culture of Bacterial Cellulose

Bacterial cellulose can be cultivated statically or dynamically. In the static method, the cellulose film is produced on the surface of the fermentation broth. Its production is affected by the ratio of area and volume. Because the surface area of the culture liquid container is certain, with the increase of liquid layer thickness, the DO is reduced, which inhibits the production of BC. Bacterial cellulose synthesized by the static method can be achieved through a one-step or two-step process. The one-step process means the culture medium is inoculated and then cultured statically; in the two-step method, before static culture, strains with massive growth and high vitality should be obtained first through aerobic incubation [134]. This process will improve cellulose yield and shorten the fermentation cycle.

7.8.3.2 Static Culture Reactor for Bacterial Cellulose

Cellulose presents as filamentous, stellate, irregular flocculent, or lumpy in the fermentation broth when using shake culture, but because the agglomeration of cellulose bacteria does not produce cellulose under aeration and agitation culture conditions, it results in the decline of final cellulose production. In this respect, to improve cellulose yield in the mechanical agitation fermentor, Kouda et al. [135] used five stirrers to perform the test and found the impeller stirrer was more suitable for BC because of a better mixing effect and higher oxygen transfer coefficient. Chao et al. [136] performed a study in which *Acetobacter* sp. BPR2001 was batch cultured using circulation gas in a 50-L reactor; it was found that 3.8 $g{\cdot}L^{-1}$ of BC could be obtained when ventilation fermentation lasted for 67 h. When the oxygen-enriched air was passed into the reactor, the yield of BC production doubled ($0.116\ g{\cdot}L^{-1}{\cdot}h^{-1}$), which was twice that in the fermentor with mechanical stirring. Cellulose presents as single oval-shaped micelles in the air-lift reactor, unlike the formation of filamentous and cellulose microslug in a mechanically stirred tank. The results showed that the BC slug suspension had a higher volumetric oxygen transfer coefficient in a 50-L circumfluent air-lift reactor than in filamentous suspensions. Later, Cheng et al. [137] improved the air-lift reactor with rectangular wire mesh to improve the oxygen transfer coefficient and mixing time to make BC 10-mm

diameter spherical particles. A 7.7 $g \cdot L^{-1}$ yield would be reached within 72 h, which is three times that of the conventional bubble column.

The unique design of the reaction equipment is important for the industrial production of BC when considering the pros and cons of static culture and dynamic culture. Sakairi et al. [138] designed a platter without stress that can be used for continuous culture in static culture to cultivate gel film. One end of the platter is equipped with the roll and the cleaning disk for the sodium lauryl sulfate solution; the cellulose film can be collected directly, showing its potential applications in industrial processes. Krystynowicz et al. [139] designed a horizontal bioreactor equipped with a different turntable or shaft based on fully integrating the advantages of static culture and ventilation mixing culture as well as more efficient use of the nutrient medium. They found that low speed and high surface area were more suitable to the growth of *Acetobacter xylinum* E25 and the synthesis of BC. Bungay and Sarafica [140] and Sarafica et al. [141] designed a rotating disk reactor to realize shorter fermentation time than with conventional stationary culture, and some of the different particle sizes of the solid particles can be added in the production of the cellulose film to form a new composite material.

Bacterial cellulose film produced through static culture and dispersive BC produced through dynamic culture are identical in chemical properties but somewhat different in microstructure. The differences are the low polymerization degree and crystallization degree of cellulose produced by the dynamic culture and the lower cellulose content. Bacterial cellulose produced by the dynamic method is lower in Young's modulus and has a higher water-holding capacity relative to the cellulose produced by the static method, which has a higher viscosity in the dispersed suspension.

When ventilated stirring is used for the cultivation of aerobic acetic acid bacteria, cellulose generated results in the increase of the viscosity of the fermentation broth and decrease in OTR and affects material transfer as well as the yield of cellulose. Kouda et al. [135] studied the rheological properties of the fermentation broth through the process of generating BC under the conditions of aeration agitation culture. 1 % bacterial cellulose and 2 % carboxymethyl cellulose (CMC) solution were compared, it is found that CMC is mixed uniformly, while the BC was not dissolved very well when the rotational speed was lower than 15 rpm. The non-Newtonian behavior of BC suspension increased the low shear rate viscosity of the fermentation broth. They also studied the effects of the oxygen and carbon dioxide pressure change on cellulose synthesis. Some approaches can be taken to improve the OTR by providing oxygen-enriched air and improving the working pressure (when the stirring rate is low). The results showed that the yield of the cellulose was not increased with the increase of the concentration of DO, but it decreased with the increase in working pressure. The reason for the decline was that the carbon dioxide concentration increased when the working pressure increased, and the high concentration of carbon dioxide would reduce the yield of the cellulose, which was restored to normal when the airflow was increased.

On the other hand, the conversion of glucose to gluconic acid and gluconic ketoacid in the oxygen-rich environment by *Acetobacter* spp. would reduce the yield of cellulose. Optimizing fermentation conditions, such as pH value, temperature, and inoculum amount; choosing a suitable source of nitrogen; supplying sufficient oxygen and increasing the enhancing intensity of oxygen in culturing; and adding cellulase, ethanol, agar, and other substances to the culture medium will be advantageous in producing bacterial cellulose and increasing its yield.

7.8.3.3 Solid-State Fermentation on Inert Adsorption Carrier to Produce Bacterial Cellulose

Solid-state fermentation on an inert adsorption carrier is a new type of solid-state fermentation that overcomes problems in conventional solid-state fermentation such as formation of substrate caking, low efficiency of mass transfer and heat transfer, and substrate unevenness. Meanwhile, the high porosity of the solid carrier provides a great interface for microbial growth so that the microorganisms can obtain the oxygen needed for growth from outside under the conditions that a small amount of air or oxygen or none is passed. The solid carrier provides a more conducive environment for microbial growth and metabolism and overcomes the problem of high energy consumption in liquid agitation aerobic fermentation, as well as the damage by shear stress [142, 143].

Chen and Weng [144, 145] studied the factors that affected cellulose production in the carrier solid-state fermentation process with *Acetobacter xylinum* using polyurethane plastic foam as an adsorption carrier. The results showed that bacterial cellulose production reached 4.86 $g \cdot L^{-1}$, and the productivity of the entire volume of fermentation reached 1.62 $g \cdot L^{-1} \cdot d^{-1}$ after 72 h at the following conditions: solid-liquid ratio of 1:16, carrier particle size less than 1.25 cm, bed depth of 3 cm, and initial glucose concentration of 20 $g \cdot L^{-1}$. Compared with conventional liquid static fermentation, the yield and volumetric productivity increased 5.65 times and 3.16 times, respectively. By comparing liquid fermentation and flask fermentation, the two liquid fermentation productions of bacterial cellulose were not improved because of a DO limitation when the initial carbon source concentration increased. The introduction of adsorption carrier solid-state fermentation solves the problem of the low carbon utilization rate to a certain extent, and the product is of finer microstructure and a low degree of polymerization. But, there is no change in the composition and structure. In addition, a pressure pulsation solid-state fermentation device was used to further promote mass transfer to increase the product yield 7.8 %.

As a kind of polysaccharide polymer of enormous commercial prospects in industrial production, if bacterial cellulose can be produced with biomass resources of low cost and wide range of sources to a large-scale production, it will be applied to act as a high-value-added bio-based material. A preliminary investigation was conducted by Weng and Chen that studied the fermentation performance of the enzymatic hydrolysis of steam-exploded straw with *Acetobacter xylinum* to produce bacterial cellulose. As the enzymatic hydrolyzate of steam-exploded straw

contains a high content of organic acid, the effects of the initial pH value of the fermentation medium on the amount of product was studied, and the results showed that the optimal pH value to produce bacterial cellulose was 6.3–6.5. There was no inhibition of *Acetobacter xylinum* growth and product accumulation in the enzymatic hydrolyzate of steam-exploded straw, and its production increased by 91 % through the controlled trials. The pH value showed less reduction in the fermentation process than glucose as a carbon source. It is suggested that the enzymatic hydrolyzate of steam-exploded straw is a valuable alternative carbon source for fermentation production of bacterial cellulose.

7.9 Biotransformation of Lignin into Chemical Products

Lignin is a type of abundant natural polymer material that can be used as a basic chemical raw material and can be modified into sulfonated lignin, amine lignin, nitrocellulose lignin, phenolic lignin, and so on. In addition, phenolic resin, epoxy resin, polysulfone, and polyurethane can be synthesized by lignin and other chemicals. Meanwhile, some phenolics, aldehydes, and a variety of vapors can also be made through lignin degradation, so lignin utilization is of great importance. The recovery of a large amount of lignin from stalks can be widely used in various processing and utilization operations, which opens a wealth of raw material resources for industrial development.

7.9.1 Enzymatic Synthesis of Lignin-Based Polymer Materials

Another development of enzyme-catalyzed polymerization of phenolic resin is lignin-phenol copolymer. Lignin-phenol copolymer can be used as a polymer dispersant, soil amendment, and especially as an alternative for versatile phenolic resin. Some properties of this copolymer are better than traditional phenolic resin. What is more, producing lignin-phenol copolymer by the method of enzyme-catalyzing polymerization can avoid pollution to the environment. On the other hand, the use of lignin produced in other industrial processes instead of phenolic compounds at high prices can reduce the cost of production. Blinkovsky and Dordick [146] reported that copolymerization of lignin-phenol copolymer with horseradish peroxidase in a water-organic solvent system could obtain lignin-phenol copolymer with up to 80 % lignin content and with a lower glass transition temperature and higher curing temperature. This means that less available phenol could be used to produce more lignin-phenol polymer. However, in this kind of water-organic solvent system, the utilization of phenol is low, with only 38 % selectivity used for copolymer resin, and the others form oligomers. If these oligomers can be transformed into low molecular weight oligomers with photoelectric effects, the reaction could provide not only lignin-phenol copolymer but also organic functional materials. The key to

realize this vision is the control of the molecular weight distribution. It will bring considerable social and economic benefits from the high-value utilization of lignin that consists of phenyl propane. Partial use of the lignin in black liquor produced in the paper industry to make lignin-phenolic copolymer not only could provide an alternative to the existing phenolic resin technology that produces a large amount of pollution but also would bring economic benefits for the black liquor. The enzyme-catalyzed lignin-phenol copolymerization may also provide new utilization for the lignin produced in ethanol and uronic acid production.

It will open up a new field for the industrial synthesis of polymer materials if the lignin resources can be used as basic raw materials. The modification of lignin with a conventional chemical copolymerization method has already been reported. The new method to produce phenolic resin using enzyme catalysis is safe and reliable; it is undertaken at room temperature and atmospheric pressure, regardless of the risk of the traditional craft. Therefore, the enzymatic method provides a broad, realistic application prospect for lignin-phenol resin production technology.

7.9.2 Lignin Biodegradation Products

There are three enzymes that exist in nature that can decompose lignin. They are lignin peroxidase (LiP), manganese peroxidase (MnP), and phenol oxidase (also known laccase, Lac). LiP and MnP can break the Cβ–Cα bond in lignin molecules and form phenoxy residues.

Some progress has been made in ligninase and lignin biodegradation over the past decade. But, there are still many problems in microbial transformation and biodegradation of lignin. As most enzymatic catalysis reactions are undertaken in a water-organic medium interface, the recent development of enzyme reactions in the organic phase has an important guiding significance for lignin enzymatic organisms. When microemulsion was used as a reaction medium, the water-soluble enzymes could be solubilized in the small water pool of water-in-oil microemulsion to avoid direct contact of enzymes with organic solvent and prevent the denaturation of the enzymes. At the same time, the hydrolyzates of lignin were extracted into the organic phase, which can reduce the inhibition to enzymes and repolymerization of enzyme hydrolysis products. The water-in-oil microemulsion is just like a microreactor that provides an ideal environment to study the enzyme activity and enzymatic mechanism. Chen and Li [147] studied ligninase activity and lignin hydrolyzates in microemulsion preliminarily, which laid a good foundation for further industrial development and use of lignin resources. Lignin was extracted by ethanol in this study, and the detail of the method is as follows:

Cellulosic feedstock → steam explosion → water extraction → ethanol extraction → filtration → distillation of filtrate to recover ethanol → adjusting pH of residue to 4.0 → lignin precipitation

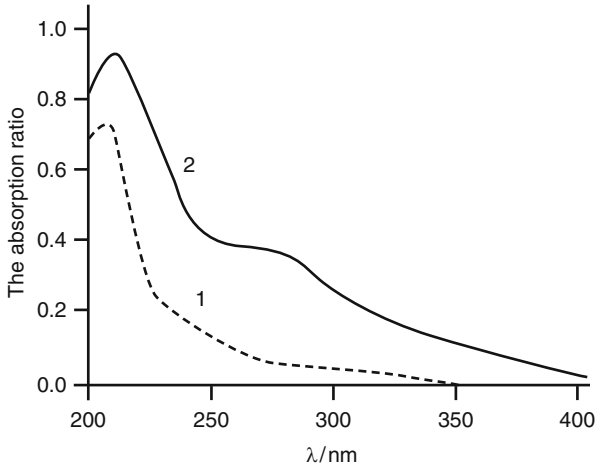

Fig. 7.6 Ultraviolet spectra of ethyl acetate extract of lignin before and after enzymatic hydrolysis, *1* before enzymatic hydrolysis, *2* after enzymatic hydrolysis

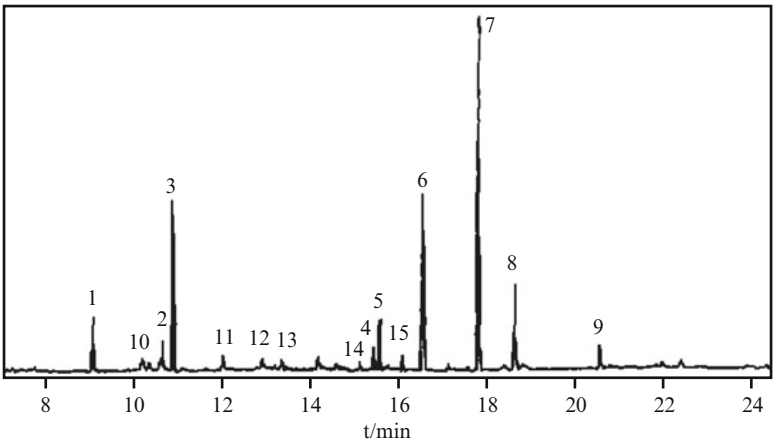

Fig. 7.7 Chromatography—mass spectrometry spectra of ethyl acetate extract of lignin enzymatic hydrolysis [147]

Ethyl acetate was used to extract lignin enzymatic hydrolyzate, and then the supernatant was characterized by ultraviolet spectra. As shown in Fig. 7.6, the product in lignin enzymatic hydrolyzate showed a significantly stronger absorption at 270–340 nm than the absorption of lignin at 290–340 nm. This means there were some small molecular organic acids, phenols, and esters generated in the lignin enzymatic hydrolysis.

The supernatant of the extraction of lignin with ethyl acetate was analyzed with gas chromatography–mass spectrometry (GC-MS) (Fig. 7.7). The detailed

analysis of the 15 major peaks that appeared in the GC-MS spectrum is as follows: The first peak belongs to the saturated straight-chain hydrocarbons, which were produced because of the complete enzymolysis process in which the aromatic ring is disconnected and methoxy is eliminated. The second peak is resveratrol (dimethoxy benzyl alcohol, $C_9H_{12}O_3$, Mw 168), produced because of the cleavage of the C_α–C_β bond. Initially, LiP is considered to be hydrogen peroxide peroxidase. When catalyzing the C_α–C_β cleavage in the β-l lignin model compounds, the labeled oxygen is added to the β-C atom. It was found that this process occurs in a pure chemical process between the molecular oxygen and the phenyl radical intermediates; later, Kirk et al. found that the reaction between oxygen and hydroxyl group–substituted benzene radical can cause the activation of oxygen. That is, oxygen is reduced to superoxide ions and then reacts with a hydrogen proton to generate H_2O_2 and O_2. This reaction also occurs in the catalytic cycle of LiP, and O_2 becomes the ultimate electron acceptor. The enzymatic hydrolysis contains high content of butylated ρ-cresol (peak 3), which is generated by rupture of the bond between the C_α–C_β. Peak 4 may be attributed to 1,2-benzoate, methyl-n-propyl ester, which is formed in the following way: Aromatic radical cations are formed through one-electron oxidation after the fracture of the C_α–C_β bond. Then, aromatic radical cations react with water or other nucleophilic reagents in a variety of types of reactions according to the substrates. The other peaks are as follows: Peak 5 is a straight olefin product; peak 6 is an ester product. The strongest peak in the spectrum (peak 7) is a straight-chain alcohol; peak 8 represents a straight-chain acid; peak 9 is oleic acid amide; peak 10 is dimethoxy benzaldehyde; and peak 11 is syringic acid with lilac group structure and was generated because of the action of the enzyme to its oxidative cleavage of C_α–C_β. Peak 12 is methyl vanillate, the result of wood prime side-chain C_α–C_β fracture through enzymatic hydrolysis. Peak 13 is acetyl ferulic acid produced because of the aromatic epoxidized cracking that occurred in the enzymatic process. Peak 14 is a type of straight-chain esters with branches, and peak 15 represents substances with a quinone structure.

The enzymatic hydrolysate of lignin consists of veratryl alcohols, low molecular weight phenolic compounds, ferulic acid, lilac acid, vanillic acid, and other small molecular acids, as well as some neutral substances, such as ketones, alkanes, and so on. LiP could be used as a catalyst for one-electron oxidation and lead to a series of free radical reactions in lignin model substance degradation, which would explain the chemical reaction mechanism of the lignin degradation process. It was found that the emergence of LiP activity in the white-rot fungus culture was similar to that in the lignin degradation process. But, the enzymatic hydrolysis effect of LiP cannot fully reflect the microbial degradation of lignin, and the microbial degradation of the lignin mechanism has to be studied continually.

As mentioned, lignin resources not only can be used as the basic raw materials for polymer making, which opens a new field of industrial polymer material synthesis, but also can be used as raw materials for some small aromatic molecule products. Because lignin degradation mechanisms, metabolic intermediates, and pathways are not clear at present, the large-scale industrial applications of lignin still needs time and effort.

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Chapter 8
Applications of Lignocellulose Biotechnology in the Pulp and Papermaking Industry

Abstract The high-energy consumption and environmental pollution caused by traditional mechanical and chemical pulp impels the papermaker to attempt new clean pulping methods. Biopulping has gained the attention of many researchers because it is friendly to the environment. The application of biotechnology in the pulp and papermaking industry not only can improve the pulping properties and slurry ability but also can fundamentally improve the quality of the ecological environment by mitigating or reducing pollutants. This chapter elaborates the biotechnology application of natural lignocellulose in the pulp and paper industry from four aspects: biopulping, biobleaching, enzymatic deinking, and others.

Keywords Pulp and papermaking industry • Biopulping • Biobleaching • Modification of papermaking materials

8.1 Introduction

The application history of biotechnology can be traced to the period of ancient civilization, at the time that fermentation technology was used in the food and drink industry. Biotechnology in the pulp and papermaking industry is not new. For example, the anaerobic and aerobic wastewater treatment and fermentation of sulfite pulp liquor have been used for many years. Recently, with the rapid development of biotechnology in other fields, application of biotechnology achieved considerable progress in the pulp and papermaking industry. In addition, the papermaking industry needs a new technology that has less environmental pollution and more economic benefits. Thus, it promotes the new development of biotechnology in the pulp and papermaking process. Most of these new biotechnologies are still in the research stage, but some have realized industrial application. The developing biotechnology, if successful, will have far-reaching effects on future pulp and papermaking technologies [1, 2].

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The high energy consumption and environmental pollution caused by traditional mechanical and chemical pulp impels the papermaker to attempt a new clean pulping method. Biopulping gains the attention of many researchers because it is friendly to the environment. However, only using the microorganism or enzyme to remove the lignin of lignocellulosic materials cannot reach the same effect as traditional chemical pulp. So, biological pretreatment is combined with the traditional mechanical method, chemical method, and organic solvent pulping. The potential advantages of biological pretreatment for the environment and energy saving indicate the direction of future clean pulping development [3–6]. Compared with mechanical pulp, this can save 30–40 % energy consumption, and under the same delignification conditions, the lower alkali can improve the physical indicators of paper compared with chemical pulping [7].

The application of biotechnology in the pulp and papermaking industry not only can improve the pulping properties and slurry performance but also can fundamentally improve the ecological environment by mitigating or reducing the generation of pollutants [8]. This chapter elaborates the biotechnology regarding natural lignocellulosic materials in application in the pulp and papermaking industry from aspects including biopulping, biobleaching, and others.

8.2 Biopulping

Biopulping takes advantage of the decomposing ability of microorganisms on lignin to remove lignin in the pulp raw material or pulp plant tissue and separate fibers. According to the ways lignocellulosic materials decompose, the microorganisms that can corrode plant tissues in nature can be divided into three categories: brown-rot fungi, soft-rot fungi, and white-rot fungi. White-rot fungi, which are commonly used in biopulping, are basidiomycetes; there are about 2–3 million in nature, such as *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Ceriporiopsis subvermispora*, and so on. The microorganisms used in biopulping must have a fast reaction rate, high degradability to lignin, and little possibility of decomposing cellulose. During biopulping, before biological treatment, the raw material usually requires high-temperature or steam sterilization to ensure that the inoculation of white-rot fungi is not inhibited by other species.

The research of biopulping includes the following aspects. (1) In the screening of specific microbial strains, strains with high selectivity for the degradation of natural lignin compounds, less damage to lignocellulosic materials, and strong antibacterial activity would be the main breeding strains. The majority of the selected strains are basidiomycetes, while a small number of strains are ascomycetes or adelomycetes; brown-rot fungi and bacteria are rarely used. (2) Lignin is completely broken down by the enzymes in the metabolic process of microorganisms, which is generally considered to be the inherent mechanism of biopulping. The enzymes involved in the reaction process at least include lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac). It is reported that the enzymes do not directly act on

the lignin; they exert catalysis through the small-molecule intermediate products, reduce the cross-linking degree, and greatly improve the solubility of lignin. In fact, the mechanism of biopulping has not been fully understood. (3) Also researched is the biopulping process and the development of related bioreactors.

8.2.1 Mechanism of Biopulping

8.2.1.1 Enzymatic Degradation of Lignocellulose by White-Rot Fungi

White-rot fungi can secrete a variety of extracellular enzymes. Most of them can secrete cellulase, hemicellulase, ligninase, pectinase, protease, and other enzymes. In 1983, Tien [9] and Glenn et al. [10] found LiP. In 1984, Kuwahara et al. [11] found MnP. In the 1990s, research on the catalytic characteristics of enzymes and molecular biology were popular. Lac was used to remove pulp lignin in 1993, and MnP was used for pulp [12] in 1996. Plant tissues are degraded by extracellular enzyme used by the white-rot fungi. Ligninases are the most important enzymes used in biopulping; they include LiP, MnP, Lac, aromatic-ring-splitting enzymes, and the lactoperoxidase system [13].

The substrates that are catalyzed by Lac are not limited to nonphenolic lignin; according to statistics, the number of substrates has reached 250 [14]. The types of substrates according to their structures include phenols and their derivatives, aromatic amines and their derivatives, carboxylic acids and their derivatives, metal organic compounds, other nonphenolic substrates, and so on. Phenols and their derivatives account for about half of the total substrates, including *o*-dihydroxybenzene and *p*-dihydroxy benzene and their derivatives. Lac cannot oxidize phenol but catalyzes α-naphthol and some phenol derivatives. Lac is a copper-containing protein, and it can catalyze polyphenols through four successive one-electron transfer reactions to form quinones and free radicals using O_2 as the recipient. In vitro, lignin monomers form polymers under the condition of the Lac/O_2. In the bacteria, it can degrade lignin with the help of other ligninolytic enzymes.

8.2.1.2 Growth Process of White-Rot Fungi in Wood Chips

White-rot fungi can grow in the cells of all conifer and broadleaf trees. During the growth process of white-rot fungi in wood chips, the mycelia enter into the cell cavity of wood chips; they first grow in the ray parenchyma cells and then penetrate into other cells through the pits or directly through the cell wall. The cell wall immediately begins to be degraded after the decrease of the metabolic nutrients (Figs. 8.1, 8.2, 8.3, and 8.4) [15].

White-rot fungi attack the cell wall components in the uneven distribution areas and further pass through the primary wall and middle lamella. When white-rot fungi

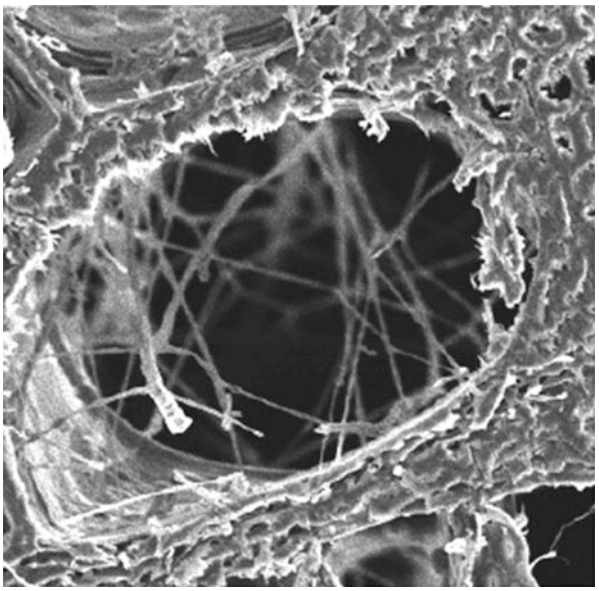

Fig. 8.1 White-rot fungi mycelial growth in the catheter

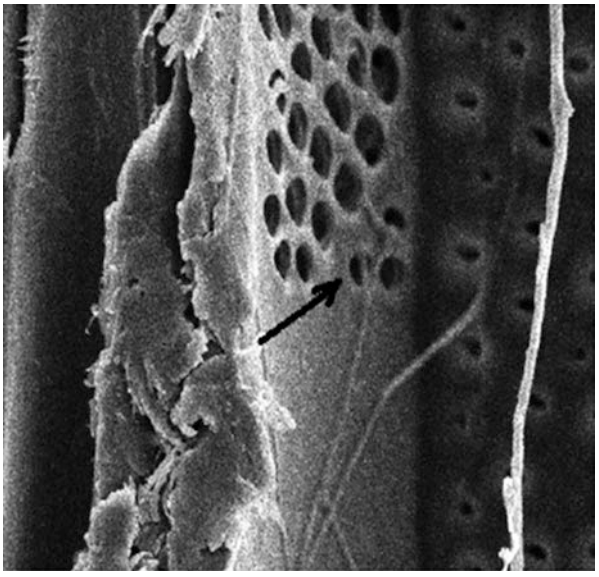

Fig. 8.2 White-rot fungi through the pit into adjacent cells

selectively degrade lignin, a wide range of cell wall erosion is observed, and many holes are generated among adjacent cell walls laterally. The holes and natural pits offer the pathway for mycelia to enter into the cell wall. Mycelia in the cell cavity further extend from the edge of the secondary wall to the middle lamella. White-rot fungi degrade lignin first in the secondary wall instead of the middle lamella, and

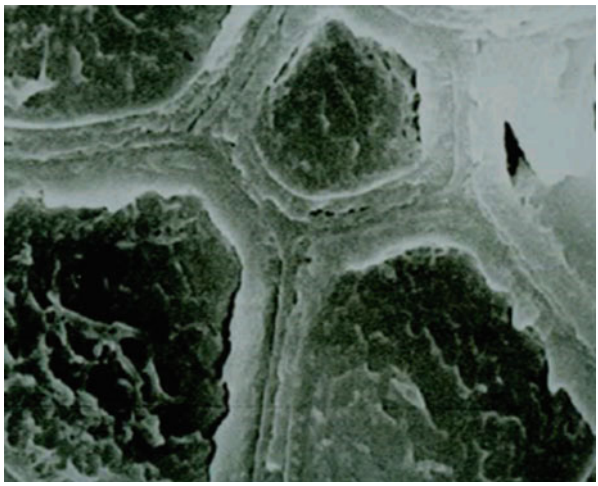

Fig. 8.3 White-rot fungi cell walls become rough

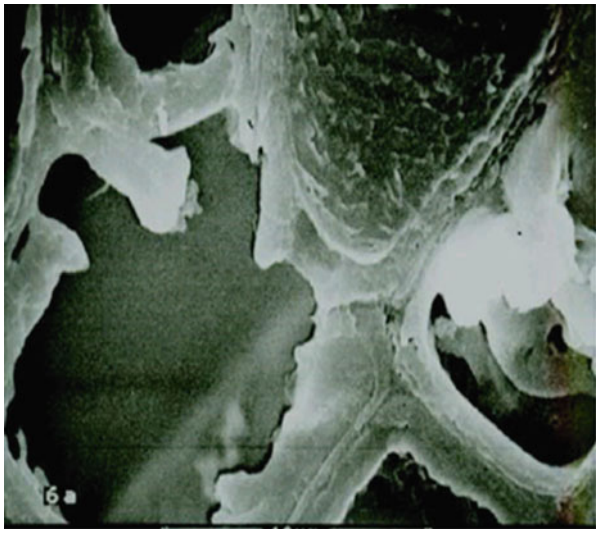

Fig. 8.4 Fiber cell walls were destroyed

then the cells will be separated from each other. White-rot fungi can make the cell wall thin from the cell cavity to the middle lamella. The extracellular mucous sheath around white-rot fungi makes the mycelia closely combine with fibrous material. After the further decomposition of fibrous material, microfilaments and mucous complex become the connection of mycelia and fibrous material accompanied by expansion of the cell wall and delignification. Through X-ray analysis, it was shown that white-rot fungi selectively degrade the lignin, first degrading the cell cavity, then the secondary wall, and finally the middle lamella; they almost do not degrade the cell corner [16].

8.2.2 Microorganisms and Enzymes of Biopulping

Chen and Liu [17] used steam-exploded wheat straw as raw material for biopulping. The raw material contains a large amount of easily used hydrolysates generated from hemicellulose, so cellulose degradation is reduced, and the selectivity of lignin degradation is improved. When this material was fermented by *Phanerochaete chrysosporium*, the lignin degradation rate reached 60 % in 5 days. Lin et al. studied the degradation of ligninsulfonate by *Phanerochaete chrysosporium* BKM/1767 and *Coriolus versicolor* Lu-II; the results showed that the degradation ability of Lu-II was stronger, reaching 74.5 % in 8 days; the relative molecular weight peak ranged from 3,000–1,500 to 500–100. The maximum degradation rate of BKM/1767 was 65.6 % in 10 days, and there were two lignin relative molecular weight peaks, ranging from 3,000–1,500 to 500–100. The degradation of lignin mainly occurred on water-insoluble lignin. There was a certain degree of condensation reaction during water-soluble lignin degradation. Meyers et al. [18, 19] indicated that, compared with nonbiological treatment, in the same freeness degree the biomechanical tensile strength and the burst index of poplar wood chips treated with white-rot fungi increased by 47–60 % and 33–46 %, respectively. Call [20] indicated that the kappa value (19.2) of wood cooking pulp treated by white-rot fungi was much lower than that of untreated pulp (kappa value was 28.7). The study also showed that the degradation rate of lignin would be enhanced by adding a delignification agent (sodium bisulfate, ascorbic acid, vitamin C, etc.) and oxidant (O_2, O_3, H_2O_2, and others).

8.2.3 Industrialization and Economic Analysis of Biopulping

Economic evaluation of biopulping is built on the thermomechanical method model. The results indicated that treatment by fungi could reduce energy consumption by 25 % and save costs by USD21 (for 40 %, USD33 were saved) per ton of dried pulp. It is estimated that saving energy for every one percentage point can reduce the investment by about USD250,000 [a typical price of electricity is USD0.035/(kW·h)]. Therefore, it is appropriate for the scale investment in biological treatment. Recently, an economic model basis on quality and energy balances was proposed. It was applied in the process of the packed bed bioreactor and chip pile system. The controlled reactor process obtains a 21 % return on investment (ROI), but the ROI of chip piles is up to 106–217 %, depending on the cost of the inoculum.

8.2.3.1 Economic Analysis of the Packed Bed Bioreactor

Taking the treatment of *Populus davidiana* with *Phanerochaete chrysosporium* at the capacity of 300 $t \cdot d^{-1}$ as an example, the economic analysis of the packed

bed bioreactor was studied. After treatment for 2 weeks, the dry weight loss of *Populus davidiana* was 5 %. Assuming that the airflow rate was 0.59 $L \cdot min^{-1}$, the optimum ROI could be 21 %, considering the amount of steam, inoculum, ventilation, electricity, and vaccination costs (Table 8.1).

8.2.3.2 Economic Analysis of the Wood Chips Pile System

The wood chips pile system includes ventilation pipe, steam pipe, and a sprayer for adding inoculum to the conveyor belt. The ventilation rate and the amount of inoculum for the wood chips reaction system are the same as for the packed bed bioreactor. The investment costs include those for ventilator, pipes, inoculation box, and humidifying equipment. The analysis showed that the ROI was 106–217 %, depending on the cost of inoculum (Table 8.2). Recently, it was found that for the treatment of *Pinus taeda* L. by *Cerporiopsis subvermispora*, sterilization, inoculum, colonizer bacteria environment, and so on would have an impact on the economics of the chip pile system.

(1) Sterilization: In laboratory biopulping, wood chips were sterilized by autoclave in early research. *Cerporiopsis subvermispora* cannot compete with local microorganisms in unsterilized wood chips. Recently, *Ceriporiopsis subvermispora* could be effectively cultivated on the wood chips after simple steam treatment; this is now under study from the view of engineering.
(2) Amount of fungal inoculum: The fungal inoculum amount is one of the major cost factors in large-scale biopulping. Therefore, the relationship of the inoculation amount with the energy consumption and the mechanical strength of paper should be considered. Compared with a control (Table 8.3) [22], 0.3 % inoculum (dry weight basis) saved 19 % of energy consumption and significantly improved the paper mechanical strength, such as the tear index. The inoculum number can be reduced to less than 0.5 ‰ (dry weight basis) by adding cheap and commercial nutrition, such as corn leaching solution. Such a low inoculum amount is good enough in the commercial range. Subsequent studies suggested that *Ceriporiopsis subvermispora* can save energy up to 38 % and improve 51 % of the tear index. Other nonchemical additives, including yeast extract and molasses, can also be used in biopulping but have less effect than corn leaching solution.
(3) Chip pile environment: Imitate the wood chips pile using the bioreactor and perform research on the control of temperature and ventilation to make the fungi treatment effective. These data can be used for the design of the chip pile system.

8.2.4 Problems of Biopulping

Lignin is one of the main components of papermaking raw materials such as wood, grass, and other higher plants. The essence of the biopulping process is

Table 8.1 Economic feasibility of a packed bed bioreactor [21]

Project	Example 1	Example 2	Example 3	Project	Example 1	Example 2	Example 3
Equipment installation fee (USD)	5,000,000	10,000,000	17,000,000	Yield loss (USD)	2.46	2.46	2.46
Circulating capital (USD)	206,750	206,750	206,750	Depreciation (USD)	4.96	9.72	16.39
Total investment (USD)	5,206,750	10,206,750	17,206,750	Total operation fee (USD)	13.64	18.40	25.07
Raw material fee (USD)	2.46	2.46	2.46	Pretreatment fee (USD)	23.49	23.49	23.49
Vaccination fee (USD)	3.00	3.00	3.00	Gross profit (USD)	9.85	5.09	−1.57
Salary (USD)	0.76	0.76	0.76	ROI (%)	21	5	−1

Table 8.2 Economic analysis of chip pile system [21]

Project	Example 1	Example 2	Example 3	Project	Example 1	Example 2	Example 3
Equipment installation fee (USD)	5,000,000	10,000,000	17,000,000	Yield loss (USD)	2.46	2.46	2.46
Circulating capital (USD)	206,750	206,750	206,750	Depreciation (USD)	0.76	0.76	0.79
Total investment (USD)	5,206,750	10,206,750	17,206,750	Total operation fee (USD)	9.35	11.35	16.35
Raw material fee (USD)	2.46	2.46	2.46	Pretreatment fee (USD)	23.49	23.49	23.49
Vaccination fee (USD)	3.00	5.00	10.00	Gross profit (USD)	14.14	12.14	6.14
Salary (USD)	0.76	0.76	0.76	ROI (%)	217	180	106

Table 8.3 Energy savings and paper strength [22] of *Pinus taeda* L. chips after treatment by *C. subvermispora* for 2 weeks

Processing (dry basis inoculum size) (%)	Energy saving (%)	Mechanical strength	
		Burst index ($kN \cdot g^{-1}$)	Tear index ($mN \cdot m^2 \cdot g^{-1}$)
Control		0.62 ± 0.05	1.67 ± 0.13
0.01	4	0.63 ± 0.04	1.89 ± 0.09
0.05	11	0.71 ± 0.04	2.16 ± 0.20
0.10	12	0.74 ± 0.03	2.13 ± 0.14
0.15	12	0.70 ± 0.06	2.04 ± 0.15
0.30	19	0.70 ± 0.05	2.14 ± 0.15

applying lignin-degraded microorganisms to decompose the most lignin and part of the hemicellulose and to separate the fibers from each other to form paper pulp. Hemicellulose and other polysaccharides are easier to decompose; lignin has complex molecular structures and biological stability, so it is difficult to be broken down by microorganisms. That makes it difficult to study and apply for biopulping, and the application development of biopulping worldwide is slow. The following discussion is about the research and technical difficulties of biopulping and the main problems that need to be settled.

8.2.4.1 Existence of Lignin

In lignocellulose, the content and structure of lignin differs according to the type of raw materials and even differs from the different regions where the cell wall exists. The mixture of lignin and hemicellulose fills the areas among the microfibers. The chemical connections of lignin and hemicellulose form the lignin-carbohydrate complex (LCC). In the alkali chemical pulping process, with the decrease of solution concentration in the late cooking period, part of the dissolved hemicellulose reabsorbs on the fiber surface, which inhibits the further dissolution of the residual lignin, and forms the LCC with residual lignin, resulting in more difficulties in the removal of residual lignin.

8.2.4.2 Major Obstacles in the Biopulping Process

(1) There are difficulties in the biopulping process. Lignin is the main component of lignocellulosic biomass and is exceeded in natural abundance among renewable polymers only by cellulose. From the point of fixed energy, lignin is a natural polymer with maximum energy on Earth. By plant photosynthesis, lignin is massively accumulated each year. At the same time, lignin is degraded by microorganisms to participate in the carbon cycle. Biopulping requires that large amounts of lignin should be biodegraded in a short time, which brings more difficulties to biopulping.

(2) Lignin is the product of plant tissue evolution. Lignin was formed in vascular plants from biological evolution. The organization of vascular plants plays evolutionary roles of transporting moisture and nutrients and increasing wood mechanical property. The original plants with no differentiated cells and tissues did not contain lignin, such as fungi, algae, and moss. In addition, in the initial formation of the cell wall, the cell wall only contains cellulose and hemicellulose. With the differentiation and maturation of cells and tissues, lignin is gradually deposited between cell walls to connect cells with each other so that the chemical and physical properties of the wood are strengthened and the ability to resist harsh environments is enhanced because hydrophobic lignin fills the cell wall through the water delivery channel organizations, which prevents channel leakage in the process of water delivery. Lignin is an evolutional product of plant tissue, so it plays a strongly resistant role in biodegradation.

(3) The activity of ligninolytic enzyme is low. Basidiomycetes (white-rot fungi) are the major microorganisms for secreting ligninolytic enzyme in nature. In comparison with microorganisms that can generate polysaccharidases, it grows slowly and is more difficult to cultivate. The activity of ligninolytic enzymes produced by wild strains is low. Usually, the pretreatment of wood chips by white-rot fungi needs more than 10 days. It is difficult to realize industrial production of ligninolytic enzymes.

From the point of view that microorganisms use substrates for growth and reproduction, the nutrient sources at the beginning are carbohydrates with low molecular weight, monosaccharides, or extracted components. After a long time, only when microorganisms become wood-rot fungi such as basidiomycetes and ascomycetes can they take wood chips as a nutrient source for growth and reproduction and secrete large amounts of ligninolytic enzymes.

From the view of metabolism, microbial metabolism uses lignin as the substrate, which belongs to "secondary metabolism." The enzymes secreted by white-rot fungi are LiP, MnP, and Lac. There is no coupled relationship between the secretion of enzymes and mycelial growth. During the period of mycelial growth and reproduction, lignin-degrading enzyme has been secreted into the culture mediator. Its secretion is limited by carbon, sulfur, and nitrogen and inhibited by glutamate and other amino acids. Moreover, it is sensitive to the equilibrium relationship between trace elements and the mycelial requirement and is influenced by the oxygen concentration in the environment. The optimum range of pH is also small (pH 4–5). These factors greatly limit the ability of the microorganism to secret ligninolytic enzymes.

From the view of the environmental effects on microorganisms, the growth of microorganisms on wood chips or the surface of pulp and enzyme secretion are influenced by the pH value and redox potential of the substrate, nutrient requirements of mycelia, and temperature and humidity. In fact, the environment is not suitable for producing ligninolytic enzyme because of the low enzyme activity. It is also part of the technical challenges that industrial applications of biopulping technology face.

Table 8.4 Mn(II) regulation of ligninolytic enzymes of *Phanerochaete chrysosporium*

	Ligninolytic enzyme activity ($nmol \cdot mL^{-1} \cdot min^{-1}$)	
Mn(II) concentration	LiP	MnP
Low	251 (7^a)	86 (8^a)
High	0	1,789 (6^a)

[a]Incubation time of the highest enzyme activity (days)

(4) Ligninolytic enzyme is a complex system controlled by many regulatory factors. Ligninolytic enzyme mainly includes LiP, MnP, and Lac. Currently, the typical white-rot fungi that produce ligninolytic enzymes are *Phanerochaete chrysosporium* and *Tramentes versicolor*. The classification of ligninolytic enzymes is based on the secretion mode of enzymes. The enzyme system secreted by *P. chrysosporium* and *Phlebia radiate* mainly includes LiP and MnP; it has the highest biodegradation efficiency of lignin (can degrade DHP, short for dehydrogenase polymer, which is a lignin model compound, into CO_2). The enzyme system secreted by *Dichmitus squalens* and *Rigidoporus lignosus* mainly includes MnP and Lac; it has the highest selectivity of lignin among ligninolytic enzymes, but its DHP degradability is relatively modest. The enzyme system secreted by *Phlebia ochraceofulva* and *Junghuhnia separabilima* mainly includes LiP and Lac; it has weak DHP degradability.

MnP is a kind of heme glycoprotein. Under the existence of H_2O_2, Mn(II), and α-hydroxy carboxylic acids (such as lactic acid) or dicarboxylic acids (such as oxalic acid), it can oxidize Mn(II) to Mn(III) through single-electron oxidation with an organic substrate. Phenolic units of lignin are oxidized to phenoxy radicals through the highly oxidizable mediator [Mn(III)], further leading to the oxidative degradation of lignin. LiP is a kind of glycoprotein containing heme, under the condition of low-concentration H_2O_2, through two-electron oxidation, which can oxidize the nonphenolic lignin substrate to cation radicals. The oxidation is followed by a series of nonenzymatic reactions of cation free radicals, resulting in the rupture of the aliphatic side chains and breaking of the aromatic ring and partial depolymerization of methylated lignin. So, the LiP p-phenols substrate of lignin is oxidized to phenoxy radicals. The mechanisms of LiP and MnP are different. LiP is one of the key enzymes for the depolymerization of natural lignin, oxidizing nonphenolic lignin to cation radicals, directly involved in the lignin biodegradation process. But, it is difficult to spread to the plant cell walls, more difficult to reach the secondary cell wall S_2 layer, and almost impossible to biodegrade the S_2 layer. In the catalytic reaction of MnP, Mn(III) attacks or oxidizes the lignin molecules, mainly depending on the chelating agent to keep itself stable. In limited nitrogen culture, Mn(II) plays an important role in regulating the enzyme activity of LiP and MnP secreted by white-rot fungi. Mn(II) has an inductive effect on MnP activity and the resistance to LiP activity. The Mn (II) regulation of ligninolytic enzymes is shown in Table 8.4 [23].

Laccase is a glycosylated polyphenol oxidase. The catalytic mechanism of Lac is to obtain electronics from the phenolic substrate, then transfer to oxygen molecules to reduce oxygen and oxidize phenolic substrates to semiquinone radicals. Consequently, the free radicals experience several uncatalytic reactions, such as oxidation of C_α, breakage of alkyl-aromatic, breakage of the C_α–C_β bond, and a polymerization reaction. Because of its low redox potential, Lac can oxidize nonphenolic substrate only with the presence of a reaction mediator [such as ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline oxazole-6-sulfonic acid)]. The reaction mediator activates enzyme through electron transport, which itself forms a stable radical cation. Benzyl alcohols such as veratryl alcohol are typical nonphenolic lignin model compounds. Studies have shown that the laccase mediator system (LMS) can catalyze the lignin model compounds and lead to the oxidative dehydrogenation at C_α to form the corresponding aromatic aldehydes. Substituents on the aromatic ring have great influence on the oxidation reaction. Veratryl alcohol has two methoxys, so Lac has a rapid oxidation rate and high conversion. In addition, Cu(II) and 2,5-xylidine can act as the inducer for Lac secretion by accelerating the transcription rate of *lccm* RNA.

When lignin is degraded by a ligninolytic enzyme of white-rot fungi, cellulose would also be degraded. Ligninolytic enzymes are inducible, and their regulatory roles occur on the messenger RNA (mRNA) levels. The secretion of *Phanerochaete chrysosporium* LiP and MnP is regulated by a nitrogen source in mRNA levels. Meanwhile, the secretion of *Coiolus versicolor* Lac is also influenced by the nitrogen source because the transcription rate of *lccm* RNA is accelerated with the increase of nitrogen concentration. Therefore, nitrogen plays a key role in the activity regulation of ligninolytic enzyme secreted by white-rot fungi. In addition, veratryl alcohol, lilac aldehydes, *p*-coumaric acid, and vanillin are effective inducers of white-rot fungi.

(5) Natural lignin is difficult to prepare, and the degradation of lignin is hard to detect. Preparation of natural lignin is difficult, so taking lignin as the culture substrate for white-rot fungi has become another obstacle in lignin biodegradation. The culture substrates for lignin biodegradation mostly use milled wood lignin, cellulase-degraded lignin, lignin model compounds, and synthetic lignin compounds. So, it is difficult to measure the capacity of lignin biodegradation. The lignin biodegradation ability can only be indirectly estimated by CO_2 produced by the ^{14}C lignin model compound decomposition.

(6) Amplification culture of microorganisms generating ligninolytic enzymes is difficult. At present, the microorganisms cannot be cultivated in actual production. The main reasons are as follows: (1) The chemical structure of lignin is extremely complex. The content and structure of lignin are not the same in different materials and different parts of the same raw material, even different regions in the same cell. It is difficult to degrade lignin and to understand the lignin biodegradation mechanism. (2) Lignin-degradable microorganisms are few in nature because lignin is a polymer compound that is extremely difficult to use by biodegradation. (3) Microorganisms producing ligninolytic

enzymes are mostly basidiomycetes. Their growth is slow, and amplification culture is difficult. They are difficult to cultivate in a large amount in a short time. (4) There is poor selectivity of strains on the mediator. For example, when degrading lignin, it consumes some carbohydrates, such as cellulose, which is extremely detrimental to the pulp and paper. (5) The requirement of culture mediator for white-rot fungi is strict, including forced ventilation with limited carbon and nitrogen and addition of hydrogen peroxide or ABTS, trace elements, and veratryl alcohol as the inducers of the lignin-degrading enzymes secreted by white-rot fungi. (6) Catabolites cannot become an effective carbon source and energy source, so glucose should be added as the carbon source. (7) Because the lignin biodegradation mechanism has not been fully understood, there is a shortage in the use of genetic engineering technologies to re-form the strains and perform some strain screening; as a result, reports on the successful separation of strains producing lignin-degrading enzymes with high activity in the laboratory have not materialized. (8) Industrial-scale fermentation is much smaller than for the pulp and papermaking industry. If the pulp yields are 700 t·day^{-1}, 1,000–1,500 t of raw wood chips are needed each day. So, the amount of $(1–15) \times 10^4$ t wood chips cultured for 10 days by the wood-rotting fungi is large. To prevent microorganism pollution, 20–30 bioreactors that have 500 m^3 should be used.

It is probably sufficient for microorganisms to be cultured in a bioreactor with a volume of 100 m^3. The cultivation can be achieved in the fermentation industry, and it is technically feasible. The culture of white-rot fungi requires a high oxygen consumption rate by deep ventilation and higher stirring speed and consumes large amounts of electricity. The higher stirring speed harms enzyme production.

At present, study is still at the laboratory level and small-scale development stage. The low enzyme activity cannot meet the needs of the actual biopulping process. The greatest difficulty of the biopulping process is the rapid and massive production of ligninolytic enzymes. At present, there are shallow stationary fermentation, immobilized fermentation, and biological rotating disc fermentation in biopulping. The shallow stationary fermentation is applied in biomechanical pulping, and biological rotating disc fermentation is used in the bleaching wastewater of biological treatment.

(7) A major obstacle is the biopulping process along with the degradation of cellulose. Cofactors $NADPH_2$ and ATP during the growth of microorganisms secrete ligninolytic enzymes; this relies on supplementation by consumption of polysaccharide material. This may result in the degradation of carbohydrates in the biopulping process. If cellulose and lignin coexist, the lignin degradation is also accompanied by the degradation of cellulose by *Sporatrichum pulverulentum*. This phenomenon is more evident in the initial stage.

In short, biopulping research has made significant progress in recent years and has been greatly improved in both technology and industrialization. But, considering the actual needs of the pulp and papermaking industry, many difficulties need to be overcome. Therefore, biopulping technology will continue

to be a hot topic in the future. In this area, the existing strains still cannot meet the requirements of large-scale industrial pulping, so the primary issue is still to screen effective strains. The effective strains can simplify the operating processes, reduce production inputs, and significantly improve the pulp-making efficiency. Along with the advance of biotechnology, artificial mutagenesis, gene cloning, and general recombination in bioengineering techniques, biotechnology will be more widely used in strain screening and improvement to improve biopulping efficiency. In addition, researchers need to resolve low efficiency from the aspect of raw materials, such as steam explosion pretreatment of raw materials to improve enzyme action sites. Now, the application of steam-exploded raw materials has been proved effective in pulp and has a broad development area [24–26]. Moreover, research on the biopulping mechanism should be taken seriously. The inherent biopulping mechanism is clear, which not only can provide a theoretical basis for the screening of an efficient strain but also can provide guidance for bioreactor design and determination of the best pulping process. Most of the existing research work focuses on developing the biological pulping technology and ignores the biopulping mechanism, which constrains the efficiency of biopulping technology research and development to a certain extent. In the whole of the current biopulping technology, good social, economic, and environmental benefits are achieved in small-scale production, but there are still some difficulties in large-scale industrial production (usual daily handling amount of raw materials is more than 100 t). It is difficult to effectively control and regulate the process conditions in the biological treatment process of papermaking materials. For example, the heat generated by microbial metabolism, as well as the humidity, ventilation, and other environmental conditions, change because the placement of raw materials hinders the improvement of the biopulping industry. Thus, the realization of optimum process conditions for large-scale production and the research and development of equipment are important parts of the biopulping industry.

8.3 Biobleaching

With the increasingly stringent requirements for environmental protection, chlorinated organic compounds contained in chlorine bleaching are harmful to the environment, so chlorine and hypochlorite bleach are increasingly restricted. Pulp bleaching is developing toward the direction of no elemental chlorine. People use oxygen, hydrogen peroxide, and ozone to replace chlorine. A new bleaching technology called biobleaching has aroused widespread concern in the past 20 years. In biobleaching, enzymes produced by microorganisms interact with some components in pulp to form delignification or be conducive to delignification and improve pulp bleachability and brightness. The main purpose of biobleaching is to save chemical bleaching agents, improve the performance of the pulp, and reduce bleaching pollution [27].

In 1986, at the International Paper Process Biotechnology Seminar, the Finnish scientist Viikari first reported that the xylanase pretreatment of pine and birch kraft pulp (KP) can reduce the consumption of bleaching chlorine and improve pulp brightness [28]. In 1989, the xylanase bleaching KP industrial test was first performed in Finland [29]. Use of the xylanase preparations as bleaching aids can reduce the bleaching waste pollution lignose, with significant benefits and easy industrialization. Currently, it has been applied by more than 30 large-scale paper mills in Europe and North America, which is the most successful example of biotechnology in biopulping [30, 31].

Biobleaching is described from the following three aspects: First, microorganisms play a direct role in pulp biobleaching. Second, hemicellulase is involved in pulp biobleaching. Third, biobleaching is conducted by ligninolytic enzymes.

8.3.1 Hemicellulose in the Raw Materials and Pulp

(1) Lignin-carbohydrate complex. The main components of plant materials are cellulose, hemicellulose, and lignin. Cellulose is the skeleton of substances that makes up plant cell walls, and the mixture of lignin and hemicellulose fills among the microfibers. It has been proven that there are chemical connections between the lignin and carbohydrates, forming the LCC.

(2) Hemicellulose in pulp and its reabsorption. In the cooking process, part of the xylan is dissolved in the cooking liquid. With the decrease of the liquid concentration, the dissolved xylan is reabsorbed on the fibers to form the xylan deposition. The mass fractions of galactoglucomannan and xylan in softwood and spruce are 22.9 % and 10.4 %, respectively. However, when the yield of the cooking pulp gained by the KP method was 46.6 %, their contents were reduced to 8.3 % and 6.9 %, respectively. The loss of hemicellulose in xylan is minor, mainly because uronic acid exists at the xylan branches chain, reducing the rate of the peeling reaction, and xylan has higher alkali stability than galactoglucomannan.

The dissolved xylan is reabsorbed to the fibers again in a later stage of cooking. The ratio of polygalactose glucose mannose reabsorption is less than xylan. In samples of pulp, the reabsorbed xylan was estimated to be from 4 to 12 %. Reabsorbed xylan in the pine pulp was estimated as 50 % of the total amount. Many hemicelluloses are reabsorbed and deposited on the fiber surface to reduce the permeability of the fiber surface and hinder the dissolution of residual lignin in pulp washing and bleaching.

Part of the reabsorbed hemicelluloses and residual lignins can form the LCC, which makes it more difficult to remove the residual lignin in the subsequent pulp washing and bleaching process. This part of the LCC has been analyzed by the enzymolysis method, chromatographic separation and mass spectrometry (MS), ultraviolet, and so on. During the continuous cooking process, black liquor was continuously removed and replaced by new cooking liquid; the

Table 8.5 Effects of enzymes and alkaline pretreatment on the lignin and kappa values of birch wood pulp [32]

Treatment of pulp	Kappa values	Residual lignin (%)	Carbohydrate content (%)
Contrast	20.7	3.1	96.9
Alkali treatment	18.4	2.8	96.2
Xylanase treatment	19.9	3.0	96.0
Xylanase treatment coupled with alkali treatment	16.1	2.4	96.6

results showed that reabsorbed xylan in the fiber surface was extremely rare, and the amount of the LCC was also slight. Therefore, continuous cooking, compared to batch cooking, had large differences in hemicellulose content, the distribution of hemicellulose in the fiber, LCC content, and pulp properties.

Based on this analysis, a model describing hemicelluloses reabsorbed to the fiber surface in the pulping process was proposed, showing that xylan is absorbed on the surface of fibers to reduce the permeability of the fiber surface; some adsorbed xylan and residual lignin form LCC to decrease the residual lignin dissolution rate.

8.3.2 Mechanism of Xylanase in the Bleaching Process

8.3.2.1 Mechanism of Auxiliary Function of Xylanase in Removing LCC

Hemicellulase is the general term for enzymes that selectively break down the hemicellulose. It mainly catalyzes the breakage of the main chain of the hemicellulose and is most commonly used in biobleaching; the major enzyme is xylanase.

In the pulp biobleaching process, the mechanism of delignification in LCC by xylanase may occur in several ways; these are discussed next.

(1) Xylan reabsorbed or deposited on the fiber surface is decomposed by xylanase
Xylanase was found to act especially on xylan on the pulp surface. After the dissolution of reabsorbed xylans, the pulp brightness is not significantly improved, which is similar to the extraction of xylan in the fiber surface by dimethyl sulfoxide (DMSO). Therefore, it was proposed that xylanase could not play a direct role in removing residual lignin. Hortling et al. [32] reported that xylan adsorbed on pulp fiber was treated by the xylanase, and then lignin was dissolved by 0.01–0.1 M NaOH. The results showed that the residual lignin content and pulp kappa value were significantly reduced (Table 8.5). Therefore, xylanase can improve the bleachability of the pulp but cannot completely replace the chemical bleaching of pulp.

Zhan and Su [33] performed a production test using xylanase as an aid to bleach the sulfate pulp of *Pinus massoniana*. The results showed that, under the same conditions, the xylanase could improve the brightness of the pulp (in the

case of high brightness, the brightness value added was about 2 % ISO (International Organization for Standardization)). When achieving the same brightness, the amount of Cl_2 was decreased by 14.2 %, and Cl_2O was decreased by 16.1–18.7 %, decreasing the pollution load.

(2) LCC is decomposed by hemicellulase
LCC is a complex of lignins and carbohydrates. Part of the LCC naturally exists in pulp materials, and the other part is formed in the pulping process. The other role of hemicellulase in the biobleaching process is to decompose the hemicellulose in LCC, reducing the degree of polymerization and volume and contributing to the dissolution of the residual lignin. Some scholars treated spruce KP with xylanase or acid and extracted the KP by alkali or DMSO, then studied the molecular weight distribution of carbohydrates and lignin. They found that the molecular weight of the treated samples was smaller than the untreated samples, indicating that hemicellulose in the LCC can be degraded by the xylanase and was easier to dissolve.

(3) Synergism of xylanase and other enzymes

① Synergism of xylanase and galactoglucomannan enzyme
The previous discussion indicated that the recovery and deposition of galactoglucomannan are less on the surface of KP fiber. The dissolution rate of galactoglucomannan pretreated by galactoglucomannan enzyme was low (about 5 %). The dissolution rate of galactoglucomannan pretreated by xylanase and then galactoglucomannan enzyme was six times higher than the dissolution rate without xylanase pretreatment. The reason is that hemicellulose is absorbed and deposited in KP fiber surface mainly contains xylan. The xylan is dissolved by xylanase, increasing the permeability of the fiber surface and then is treated by galactoglucomannan enzyme, which is conducive to dissolving galactoglucomannan in the LCC and KP fibers.

② Synergism effect of xylanase and ligninolytic enzymes
The xylan of pulp fibers is pretreated by xylanase to increase the permeability of the fiber surface and reduce the limitation and shielding effects of the diffusion of enzymes. Then, the ligninolytic enzyme promotes the decomposition of lignin and LCC, which is conducive to the dissolution of the residual lignin.
As can be seen from the results in Table 8.6, xylanase treatment may improve the brightness by 2.6 %, and then ligninolytic enzyme treatment may improve the brightness by 3.6 %. Ligninolytic enzyme treatment of lignin alone may increase the brightness only by 2.3 %. This shows that xylanase is helpful for delignification [34].

8.3.2.2 Mechanism Model of Xylanase-Assisted Delignification

From the previous analysis, in the pulping process, when the solution concentration is high, the xylan first would be dissolved in the cooking black liquor. In later

Table 8.6 Effects of enzyme treatment on brightness of bagasse mechanical pulp [33]

Item	Contrast	Treated by xylanase	Treated by ligninolytic enzymes	Treated by xylanase and ligninolytic enzymes
H_2O_2 (%)	1.5	1.5	1.5	1.5
Consumption of H_2O_2 (%)	0.98	1.06	1.06	0.94
Brightness (%ISO)	46.2	49.8	49.5	50.8
Increased brightness value		2.6	2.3	3.6

cooking, when the concentration of solution is low, the xylan would be reabsorbed or deposited on the fiber surface. This part of reabsorbed xylanase cannot be extracted easily by hot or cold alkaline solutions, which indicates that xylan and lignin form LCC to reduce the solubility of xylan.

Xylanase can decompose the reabsorbed xylan, increasing the permeability of the fiber surface, which is conducive to the dissolution of lignin. Furthermore, xylanase reduces the polymerization of LCC, which is beneficial to the dissolution of lignin.

8.3.3 Efficiency of Xylanase in the Biological Bleaching Process

8.3.3.1 Reduction of the Consumption of Chemicals in the Bleaching Process

Treatment of pulp by xylanase is applied in the multistage bleaching process (in the process, ClO_2 is substituted for Cl_2). In the test of hardwood, based on the high brightness, it can greatly reduce the consumption of chemicals (Cl_2), and in the chlorinated segment, 50 % ClO_2 is used to replace Cl_2. To obtain the same brightness, the consumption of the total available chlorine will be greatly reduced.

8.3.3.2 Reduction of the Harmful Ingredients in Bleaching Liquid Waste

The consumption of Cl_2 is reduced by pretreatment of pulp with xylanase, so the content of absorbable organic halogens (AOX) decreases in the bleaching waste liquid, reducing environmental pollution. Twenty percent of ClO_2 is substituted for Cl_2 in the chlorination section in the multistage bleaching process. When the pulp brightness is 91 % (ISO), the treatment of xylanase may significantly reduce the content of AOX in the bleaching waste liquor.

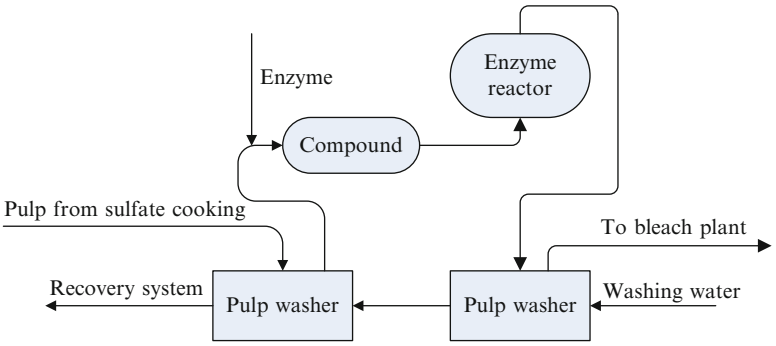

Fig. 8.5 Enzyme-assisted bleaching processes

8.3.4 Biological Bleaching Process

8.3.4.1 Operation of Bioassisted Bleaching

As shown in Fig. 8.5, the biobleaching process can be divided into the pulp-washing, enzyme-assisted bleaching, and chemical bleaching processes.

(1) Pulp washing
After cooking and washing of the pulp, the lignin, hemicellulose, and degraded cellulose are removed, so the pulp brightness is improved. This provides a favorable environmental condition for the enzymes.
(2) Bioassisted bleaching process
After washing, the pH is nearly neutral or slightly acidic, and the temperature is at 40–60 °C. The enzyme is added to the enzyme reactor, acting on the xylan that was sucked back and the decomposing part of LCC. Enzymes play an assisted role in the bleaching.

Then, the pulp is washed again to improve the pulp brightness and the permeability of the fiber surface, provide a favorable condition for the subsequent chemical bleaching processes, and reduce the content of AOX in the bleaching waste liquor.

(3) Chemical bleaching process
Enzyme pretreatment can only improve the bleachability but cannot achieve higher brightness. The pretreated pulp is treated by a chemical bleaching process, so the residual lignin is deprived and the pulp can meet brightness requirements.

8.3.4.2 Bioassisted Bleaching Process

There are two main means for xylanase treatment of the bleaching process:

(1) Enzyme treatment is the first stage in the process of biobleaching.
Biobleaching usually adopts multistage bleaching, generally without the aerobic delignification process. The common bleaching steps include L (DC) ED, L (DC)

EDED, LCEHH and LCEDED, of which L, D, C, E, and H represent Lac, chlorine dioxide, chloride, alkali treatment, and sodium hypochlorite treatment, respectively. The enzyme treatment is generally arranged before the conventional bleaching processes, thereby saving subsequent chemical bleaching agents. The development of the traditional chemical bleaching process is going toward the nonpolluting bleaching direction, so enzyme treatment can be used as a pretreatment for the chemical bleaching process.

(2) Enzyme treatment is after aerobic delignification.

In the study of the bleaching process containing oxygen bleaching (O), ozone bleaching (Z), and peroxide bleaching (P), to achieve the bleaching effect in the aerobic delignification process, the L step can be arranged after the aerobic delignification to use the xylanase, such as OLDED OLZED, OL (DC) ED OLDP and OLPDP. The L step is prior to the Z step. Yang et al. proposed a new enzyme treatment process that was totally chlorine-free (TCF) bleaching, such as OLZP, sometimes called the EnZone method.

8.3.5 Bioassisted Bleaching Technology

The parameters of the xylanase-involved biological process include the optimum pH value of enzyme, a temperature of 30–60 °C, a pulp concentration of 5–10 %, an enzyme dosage of 1–10 IU·g^{-1}, and a reaction time of 1–3 h. Usually, the pH value of xylanase produced by bacteria is about 6–9, which is higher than that of fungi. The parameters of enzyme treatment must be appropriately selected to obtain the best treatment effect.

8.3.6 Development of and Prospects for Xylanase Technology

A large amount of xylan from the pulp and papermaking process is discharged into rivers, causing environmental and ecological pollution. There is also a considerable amount of xylan in agricultural residues. The transformation of xylan into a useful product is the future development direction of xylanase, and xylanase will have broad prospects in the process of lignocellulosic biomass as well as the development of renewable resources and energy.

Xylanase has made some progress in biochemistry, synthesis regulation, and molecular biology research. To make the xylanase applied in the industrial production on a large scale, a xylanase that is effective in a variety of operating conditions must be found. So far, the use of xylanase in biobleaching has experienced three generations: acidic enzyme, neutral enzyme, and alkaline enzyme. Currently, the research and development of the third-generation xylanase (alkaline enzyme) is at its peak. With protein engineering identifying the xylanase activity site residues by chemical modification means, X-ray crystallography, and site-directed mutagenesis,

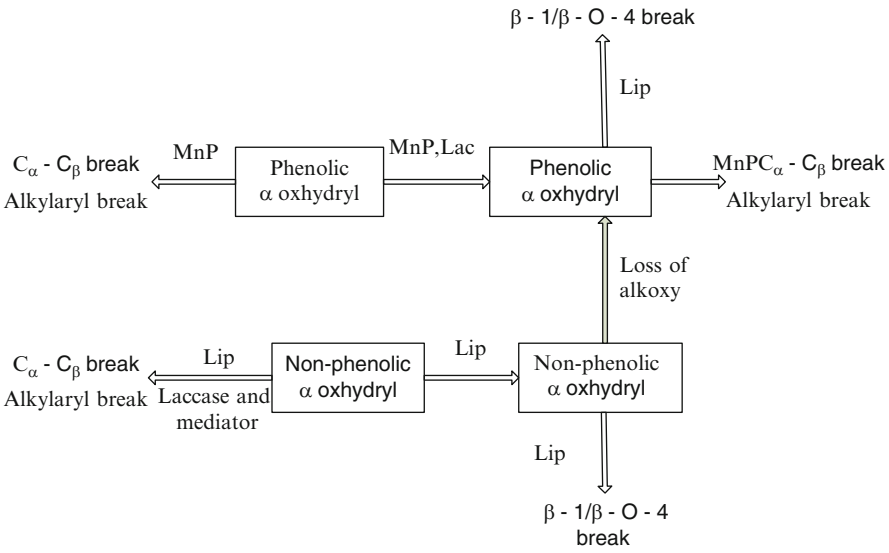

Fig. 8.6 Route of the lignin biodegradation process [35]

obtaining xylanase tolerant to heat and alkali with strong activity has become the current research focus. Under the joint efforts of microbiologists, biochemists, and related technologists, it is expected that a series of mature applications of economic and effective xylanase technology will be developed.

8.3.7 Biobleaching of Lignin-Degrading Enzymes

LiP, MnP, and Lac are the major enzymes in the lignin biodegradation process. The LiP and MnP can degrade phenolic and nonphenolic lignin, and Lac, because of its low redox potential, can only oxidize phenolic lignin. However, if used with a suitable redox mediator, Lac can oxidize the nonphenolic structural unit of lignin. The possible route is shown in Fig. 8.6 [35].

8.3.7.1 Lignin Peroxidase Biobleaching

LiP is a series of isozymes containing an Fe(III)–porphyrin ring (IX) heme, of which the most important feature is the ability to oxidize the electron-rich nonphenolic aromatic compound.

Zhou [36] used Fourier transform infrared (FTIR), gas chromatography (GC)/MS, and gel permeation chromatography (GPC) technology to study the synergy mechanism of lignin peroxidase–hydrogen peroxide bleaching. The results showed that the pretreatment of lignin peroxidase can increase the syringyl structural unit, p-hydroxyphenyl group, methoxy group, and phenolic hydroxyl group to enhance the reaction ability of lignin and degrade the dissolved low molecular weight lignin to provide favorable conditions for hydrogen peroxide bleaching. Enhancing the reaction of the bleaching agent with the phenolic-type structural unit and syringyl structural unit can reduce the amount of chromophore, improve the dissolution amount of lignin, and greatly increase the relative molecular mass and pulp brightness.

8.3.7.2 Biobleaching of Manganese Peroxidase

Some white-rot fungi with high bleaching ability produce MnP and Lac; some produce MnP and LiP. MnP is considered to play a key role in the bleaching of KP. MnP, which was first discovered in *Phanerochaete chrysosporium,* is secreted by many fungi, including *T. versicolor,* and *P. sordida*. MnP is one of the important extracellular enzymes, and its role in the lignin degradation process has drawn increasing attention.

Under the presence of hydrogen peroxide and dicarboxylic acid chelating agents (such as malonate and oxalate), Mn^{2+} can be oxidized to Mn^{3+} by MnP; then, Mn^{3+} is further oxidized to the various phenolic-type compounds, such as 2,6-dimethoxy phenolic, vanilla acetone, and phenol. In many fungi, MnP is the key enzyme in the initial degradation of lignin because MnP can form Mn^{3+} with a strong oxidation state. Mn^{3+}, as the diffusible redox mediator, further splits the aromatic ring of lignin polymer and then, with the synergy of other enzymes, eventually breaks the macromolecule [37].

8.3.7.3 Laccase Biobleaching

Laccase is a phenol oxidase containing copper and is widely produced by *Coriolus versicolor,* which is one of the main producing microorganisms. With oxygen as the oxidant, lignin units containing phenolic structures are oxidized to phenoxy radicals, and oxygen molecules are reduced to water molecules. Because of the low redox potential of 0.5–0.6 V, Lac cannot oxidize nonphenolic lignin with a high redox potential. When Lac coexists with mediator, the mediator can have high activity and stability, transferring an electron from oxygen to the lignin molecules, so the lignin is degraded. Thus, the mechanism is that Lac oxidizes the mediator to produce substances with strong oxidizing ability first, and the oxidized mediator is the real bleach.

Mo et al. [38] obtained a strain of white-rot fungi that produced Lac in the laboratory; they studied the fruiting body and basidiospore morphology as well as the molecular characteristics, and they analyzed the amplified 18srDNA sequence. They found that the strain belongs to *Panus rudis*. In the solid fermentation of straw through white-rot fungi, the producing Lac peak appeared in 21 days; the activity was 3,895 IU·L^{-1}. The Lac collaborating with ABTS were used to treat sulfate bamboo pulp, then used hydrogen peroxide to bleach. The results showed that the pulp brightness through the Laccase biobleaching, chelating treatment and peroxide bleaching (LQP) and aerobic delignification, Laccase biobleaching, chelating treatment and peroxide bleaching (OLQP) bleaching was higher than that through chelating treatment and peroxide bleaching (QP) or aerobic delignification, chelating treatment and peroxide bleaching (OQP) bleaching without enzyme treatment (by 4–7 %), with the Kappa value decreasing, and a high viscosity was maintained.

Wang et al. [39] applied Lac to treat the lignin of needlebush KP in the mediators of 1-hydroxyphenyl benzotriazole (HBT), N-hydroxy-acetyl-aniline (NHA), and violuric acid (VA). The degradation ability in the VA mediator was superior to the other two. Residual lignin was analyzed by nuclear magnetic resonance (NMR) before and after the biobleaching; the results showed that lignin was greatly oxidized, and its structure was mostly changed in the Lac-VA system after the biological bleaching treatment. In the three LMSs, the carboxyl group content was increased, and the methoxy group was correspondingly reduced. The condensed C_5 phenolic lignin was directly acted on by the biological bleaching.

Liu et al. [40] used two Lacs or an Lac/mediator system to treat kraft bamboo pulp, then carried out the TCF bleaching. The results showed that kraft bamboo pulp took the OLQP bleaching by LMS to obtain the bleached pulp; the bleached pulp had a similar brightness compared with traditional CEH bleaching including chlorination, alkali treatment and hypochlorite bleaching, and was maintained at good pulp strength. The KP was treated by Lac under the existence of hydroxybenzene and HBT. After OLQP bleaching, the pulp brightness was 80.7 % ISO, and the viscosity was more than 800 mL·g^{-1}. In the case of no additional mediator, the Lac-treated pulp remained with a better delignification effect after OL^0QP (bleaching steps with aerobic delignification, then Laccase biobleaching without adding external mediator, then chelating treatment and peroxide bleaching; L^0 means Laccase biobleaching without adding external mediator) bleaching. The pulp brightness was up to 76.7 % ISO and increased by 23.2 % compared with OQP bleaching.

At present, the enzyme of the lignin pulp biobleaching needs to be further researched, and the regulation mechanism remains unclear. Once the research progress of ligninase biobleaching has a breakthrough, it will become possible to put the industrialization of biobleaching into practice, achieving the goal of biological pollution-free bleaching.

8.4 Application of Biotechnology in Other Aspects of Pulp and Paper

8.4.1 Genetic Engineering Technology in Modifying Papermaking Materials

Wood is the main raw material for the pulp and paper industry. To ensure the continuous supply and perfect properties, people expect to directly cultivate species with good pulp and papermaking properties, fast growth, and high yield. The time of the asexual reproduction of fine tree species is shortened by new cell culture or tissue culture techniques. Genetic engineering can be used to develop new varieties. The essence of genetic engineering is hybridization at the molecular level, unlike general hybridization being limited by the phylogenetic distance of different species. It can make the recombination between near kinship or far, according to people's need and hope to breed new fine species. In the United Kingdom and Japan, recombinant DNA technology is used to control the biosynthesis of lignin in tree growth. In the British company Zencea, an inhibitor of cinnamyl alcohol dehydrogenase gene was isolated that can inhibit the growth of lignin in trees. It was implanted into the fast-growing poplar and eucalyptus, and a new generation of pulp and paper timber was bred. The lignin of that kind of wood is easy to dissolve in the chemical pulping process, which can save chemical usage and energy consumption and reduce the damage to the fiber, thereby improving pulp yield and quality, saving fiber raw materials, and reducing the wastewater pollution load and the cost of wastewater treatment.

In 2009, Ellis successfully transferred a foreign gene into the embryos of white spruce and found that many modified embryos grew into seed plants and quickly grew to 3 or 4 in. In addition, the nutrition and growth of trees can be improved and new pest-resistant varieties can be bred through mycorrhizal fungi, nitrogen-fixing bacteria, and biological control. The research into biotechnology improving species is still in a primary but rapidly developing stage and will have a profound impact on the development of the wood pulp industry.

8.4.2 Application of Microbial Fermentation in Sulfite Pulp Waste Liquid

So-called red liquid is withdrawing liquid waste after acidic sulfite cooking; it contains not only lignin sulfonic acid (salt) with different sulfonation degrees, but also many other organic matters. The maximum use of all kinds of red liquid is shown in Table 8.7.

The comprehensive utilization of red liquid has a long history in China. For example, the sulfite softwood pulp red liquid contains not only lignosulfonates

Table 8.7 By-products that may be obtained from sulfite softwood pulp red liquid [21]

Compound	Origin	Yield [kg·(t)$^{-1}$]
Methyl alcohol	OCH_3 of glucosyl xylan	7–10
Acetic acid	Xylan acetyl	30–90
Methane acid	Oxidation of formaldehyde acidic sulfite	0.5–1
Formaldehyde	$HOCH_3$ of lignin	2–6
Methyl ethyl ketone aldehydes	Hexose decomposition	5–6
Furfural	Pentose dehydration	5–6
Glucuronic sulfonic acid, uronic acid	Replacement and oxidation in the sulfites of saccharides	150–250
Saccharides	Degradation of hemicellulose and cellulose	200–400
Cymene	Oxidation of the terpene acidic sulfite	0.3–1
Lignin sulfonate	Lignin	600–800

but also monosaccharides generated by the hydrolysis of partial cellulose and hemicellulose. About 60–65 % of the monosaccharides are reducing substances (this usually refers to hexose) that can be used for ethanol production, and the other 35–40 % are reducing substances (this usually refers to pentose) that cannot be fermented to produce ethanol. Therefore, this red liquid is used to produce ethanol, and the pentose is used to cultivate yeast.

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Chapter 9
Applications of Lignocellulose Biotechnology in Other Industries

Abstract Natural lignocellulosic materials are the most abundant renewable biomass on earth. The effective utilization of cellulose can offer cheap raw materials for agriculture, feed, light industry, and the chemical industry. Besides applications in ecological agriculture, bioenergy, the chemical industry, and the pulp and paper industry, cellulose biotechnology is vitally important in the textile industry and for environmental protection. The irresistible request for ecology protection, energy saving, and resource-optimized utilization in textiles and the thirsty wishes of environmental protection methods and establishment of healthy ecosystem in environmental protection have forced people to pay increased attention on the development and exploitation of lignocellulose biotechnology. The deepening of research will involve increasingly extensive domains to speed up economic development. This chapter mainly describes the biotechnologies of natural lignocellulose in the textile industry and for environmental protection.

Keywords Straw • Regeneration of cellulose fiber • Environmental protection • Preparation of restoring materials for ecological engineering

9.1 Application in the Textile Industry

In recent years, with the development of the textile industry, the advocation on the environmental protection is increasing; green textiles have become an important foundation for the sustainable development of the textile industry. Today, new products, new technology, and new equipment have been widely developed and utilized, followed by the guidance of environmental protection. At the same time, with the development of science and people's living standards, people's concept of consumption also constantly changes. The consumption concept in the past was to keep warm and be practical, but now, increasing attention is on being fashionable, unique personality, keeping comfortable. The pursuit of health, comfort, and an outstanding personality has become the trend of the global clothing

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industry. Thus, improving machining accuracy and the depth of natural fiber products, enhancing product quality, and adding value are inevitable. Meanwhile, environmental protection, low energy consumption, and utilization of resources by recycling have become the main themes of the development of the modern economy. The innovation of the textile industry, focusing on the principal line of ecology protection, energy saving, and resource-optimized utilization, has become inevitable for its future development. In this process, the importance of cellulose biotechnology has caused widespread concern [1, 2].

9.1.1 Structure and Performance of Cotton Fiber

Cotton fiber is a natural fiber with a high fiber content. It contains 94 % cellulose, 1.3 % protein, 0.9 % pectin, 1.2 % ash, 0.6 % wax, and 0.8 % organic acids. Cellulose is a straight-chain macromolecule composed of thousands of glucose molecules linked by the β-1,4-glycosidic bonds. The molecular weight of the natural cellulose is 600–1,500 kDa. The cotton fiber is composed of long chains of fiber molecules, not arranged in parallel in a certain direction, but arranged in two or three layers with different degrees of crystallinity and polymerization. The long chain of 40 cellulose molecules constitutes the basic unit, a fiber bundle, with an average diameter of 3.5 nm. Several fiber bundles cross with each other to form the network structure.

The section of cotton fiber from outside to inside includes the cuticle, primary cell wall, secondary cell wall, and cell lumen. The cuticle is a thin layer with cotton wax and pectin that has filamentous wrinkles on the surface. When the primary cell wall is prolonged to some extent, the cell wall gradually precipitates from outside to inside to form the secondary cell wall, which is almost entirely composed of celluloses. Cellulose is precipitated to form the slender bundles (microfilaments); many microfilaments are connected to form microfibril bundles. They tend to form the spiral structure with the fiber uranium, which presents spiral wrinkles on the fiber surface. Crystallization regions and amorphous regions are in each of the microfilaments. The X-ray diffraction spectrum shows that a series of holes, submicroscopic capillary tubes, run through the primary cell wall and secondary cell wall. Capillary tubes in cotton fibers include two categories. One is the coarse capillary tube, such as the cell lumen and pits; the other is the cell wall capillary tube, such as the gap between microfilaments and the gap between molecular chains in amorphous regions (there are a thousand more superficial areas of this kind than of coarse capillary tubes).

The supramolecular structure of the cotton fibers is also called a microstructure, referring to cellulose in the cell wall. The common structure theory is a model of two-phase structure, which states that the natural cellulose is a defectively complete crystalline material. The crystallization region is composed of some high-ordered parts with complete crystalline grains; the amorphous region consists of some defective, low-ordered parts with incomplete crystalline grains. In addition,

there is also a transition part between these two regions. These different regions in chemical reactions exhibit different reactivity. For instance, in the enzymatic hydrolysis process, the amorphous region is the most vulnerable to damage; the transition part is also affected, but the crystallization region is less affected.

9.1.1.1 Application of Cellulase in Modification of Cotton Fabrics

Enzyme, as a natural degraded biological agent, plays an increasingly important role in the textile industry [3]. Bioprocessing of cellulose fiber and fabric is one of the main application areas for enzymes. Cellulase, as a biological enzyme, has experienced rapid development since introduction into the textile industry in the 1980s. The utilization of cellulase in the textile industry has the following advantages: (1) as a biological catalyst, it is nontoxic and harmless; (2) it requires mild processing conditions (temperature, pH, etc.); (3) only a low dose of cellulase is necessary; (4) the cellulase released from one reaction can catalyze another reaction successively; (5) wastewater generated during reactions can be biodegraded, which can reduce pollution and conserve energy; (6) the effect of some special features can be acquired, and the performance of fabric can be improved [4].

(1) Decrement and softening of fabrics with cellulase
Treating cotton textiles with cellulase would cause reduction and weight loss of cotton fiber and changes of fiber properties, including softness, water absorption, dyeability, drapability, smoothness, mechanical properties, and so on. The main aim of weight reduction treatment is to improve the softness, flexibility, and drapability of cotton fabric, making the fabric silky and causing changes in other performance. Because the enzyme treatment may damage the mechanical properties of cotton fibers, more attentions should be taken during the processing.

The weight loss rate of cotton fabric after cellulase treatment is related to many factors, such as types and concentration of the enzyme, pH of the reaction solution, temperature, processing equipment, velocity of flow, organizational structure of textiles, and pretreatment (mercerization, bleaching) conditions. In cellulase treatment, the cotton fabric weight loss rate and various properties should be well balanced. It is good to control the weight loss rate of cotton fabric within the range of 3–5 %.

(2) Polishing of fabrics with cellulase
Polishing, first proposed in Japan, is novel biochemical processing of fabrics. It deals mainly with cotton fabrics to make the surface smooth and to improve softness. Asferg and Videback extended the basic principle of polishing to knitting and other cellulosic fabrics and found additional effects, which were the reduction of lousiness on the surface, thus also reducing the tendency of pilling. Pederson and Sasserod also proved the durability of the polishing effect of cellulose fabrics.

Polishing of cotton fabrics by cellulase would decrease pilling and stiffness, increase compressibility, and improve fabric handling. Thus, the drapability of fabrics would be improved; also, luster and color are enhanced, so the fabrics have a silky gloss similar to that produced by traditional mercerization, and the lines of the fabric are delicate and clear [4].

(3) Washing and stone-milling treatment of fabrics by cellulase
Denim, as a popular textile, is a heavy cotton twill fabric that is dyed by yarn. Cellulase finishing was applied earliest in the washing arrangement of indigo jeans garments to obtain the same effects of decolonization and bleaching, so it was called enzymatic washing. Enzymatic washing can reduce the amount of pumice from the stone-milling treatment, protect the machine from damage, avoid pumice and dust, and reduce environmental pollution. Enzymatic washing uses cellulase to generate controlled corrosion on the surface of denim that has been dyed by indigo and sulfide vat dyes and obtains a uniform fading effect that relies on the synergistic effect of rubbing and friction of the washing machine. Enzymatic washing makes fabrics have a unique and colorful surface and soft feel because it does not cause excessive damage to fabric strength. If different cellulases are combined and different processes are used, hundreds of appearance effects can be produced [5].

9.1.1.2 Selection of Cellulase Preparations

Because cellulase has binding specificity and catalytic efficiency, allowing the enzymatic treatment to obtain a good effect, the first step is to select appropriate cellulase preparations and take strict control of processing conditions to avoid excessive loss of fabric strength. The cellulase on the market mainly can be divided into acidic, neutral, and alkaline cellulase. At present, the most applied cellulase is the neutral cellulase [6] or acid cellulase. Treatment with acid cellulase can lead to heavier staining than neutral cellulase. But, acid cellulase treatment can generate heavy denudation of the cotton, which can produce a chemical wear effect in a short period of time. Neutral cellulase treatment can generate lower denudation of the cotton than acid cellulase and provide a long duration and low staining. Usually, denim is treated by neutral cellulase because of the requirement of high contrast ratio and low strength loss. Generally, acid cellulase is more economical if the quality is acceptable. But, the reproducibility of the finishing effect of the acid cellulase is poor, with the vitality sensitive to pH. If the pH changes by 0.5, the enzyme activity would change by about 0–20 %. There are diversiform cellulase products, so the final choice should be in accordance with different requirements.

9.1.1.3 Mechanism of Cellulase on Cotton Fibers

Increasing research on cellulase has been based on the purpose of converting lignocellulosic resources into glucose. To improve the conversion rate and obtain more glucose, β-glucanase with high activity is essential. As for cellulase used in the textile industry, decomposing cotton cellulose into glucose is not necessary. However, it is necessary to destroy the hydrogen bonds between the molecules of the cotton fiber bundles, lose structure, partly degrade the long chains of cellulose molecules, cut cotton fibers, remove the floating hairs, and so on. Therefore, the

activity of β-glucanase is not high. Because of the low concentration of enzyme and short reaction time, even in the case of low β-glucanase activity, the feedback inhibition of enzyme would be avoided, which would not reduce the activity of C_1 and C_x.

The reaction of cellulase starts from the adsorption of enzyme and substrate. There are two types of cellulase molecules; one is a spherical enzyme with an average diameter of 5.9 nm, and the other is an elliptic enzyme of 3.5×20 nm and 2.6×16.5 nm. If the cellulose does not have some special treatment, it is difficult for the whole enzyme molecule to enter the fiber interior. Therefore, generally, cotton fibers would not be completely decomposed by cellulase. Appropriately controlling the enzyme concentration and reaction time could fluff the structure of cotton fibers and remove some cotton fibers, consequently reducing quantity and improving texture and aesthetic values.

Cellulase has high specificity and catalytic efficiency. Active sites are located in the surface of the cellulase. In the reaction, the active sites of the cellulase are specifically bound with substrate (i.e., cellulose fibers) to form complexes first. So, cellulase molecules should be able to be close to fibers and absorbed in certain parts of the cellulose fibers (typically, they are first adsorbed on the surface) and then have coordination complexation to form the intermediate complex. Then, specific catalysis occurs in the active site of the enzyme, prompting the controlled hydrolysis of the cellulose molecule chain, hydrolyzing cellulose molecules.

The cellulose molecule is a linear chain macromolecular compound linked by a β-1,4-glycosidic bond. The cellulase has special catalysis on it. Cellulase not only can hydrolyze cellulose polymer seriately from one end but also can cut the long cellulose polymer into small molecules. Cellulose in both the primary and secondary walls of the cotton fiber can be hydrolyzed to produce cellobiose and glucose and dissolved in hydrolysate. The cellulose fibers are made from the crystalline and amorphous regions; water molecules easily enter the inside of the amorphous region to form hydrogen bonds, leading to the full extension of cellulose and making cellulose easily go through the pores and be adsorbed on the surface to make effects. Cellulose molecules in the crystalline region are arranged regularly with a compact structure, so it is difficult for cellulase to enter. When treated by cellulase, the fine fibers exposed on the cellulose surface are hydrolyzed, making it easy to be peeled off from the fibers, so that the fabric becomes smooth and clean. Meanwhile, enzyme treatment of fiber would decrease the surface tension, weaken the flexural strength, decrease the friction coefficient between the fibers, increase the area of the amorphous region between the fibers, and loosen the structure so that the fabric also becomes bulky, soft, and antipilling and has high hygroscopicity [4]. Although the strength of fabric would be reduced because the degradation starts from the amorphous regions, the loss of strength can be adjusted within a reasonable range so it has little impact on the durability of the fabric if enzyme treatment is controlled properly.

In fact, there is also high-value cellulase (EG, endo-β-1,4-glucanase), which is enabled to effectively combine with cellulose molecules in the crystalline region and cut some cellulose molecules, finally making cellulose degraded, hydrolyzed, and

dispersed. According to reports, this enzyme also can be attached to the region that has a poor degree of crystallinity so that the molecule arrangement is more relaxed and generates pores that facilitate the action of the enzyme. When this enzyme acts on cellulose with a 60–70 % degree of crystallinity, its hydrolysis capacity is 14 times higher than that of the low-value cellulase, but for amorphous regions or low-crystalline cellulose, the hydrolysis ability is almost similar.

9.1.1.4 Problems and Prospects

With the development of technology and civilization and the increasing awareness of environmental protection and a healthy lifestyle, green ecological textiles have been favored by global consumers. Cellulase finishing is the most active area of biological enzymes in textile applications. It is the application of modern biotechnology in the textile industry. Cellulase in the textile industry mainly involves environmentally friendly postprocessing. It is necessary to deeply study the finishing process of cellulase at the same time to study applications of cellulase in the pretreatment (such as desizing and scouring of cotton) and compounding finishing of cellulase to broaden its application in cotton textile processing and develop cotton textiles that are diverse, functional, and high grade. It is also an effective way to make cotton textiles with top quality and additional value.

9.1.2 Regenerated Cellulose Fiber and Related Textiles

Regenerated cellulose fiber is made from natural polymer by chemical methods and basically has the same chemical composition as the original polymer; also, cellulose as a raw material can produce regenerated cellulose with the structure of cellulose II. Regenerated fiber is a man-made fiber that has the same chemical composition as natural cellulose and protein; it can be divided into regenerated cellulose fiber and regenerated protein fiber.

Although traditional natural fiber is still a main raw material for the textile industry, the man-made fiber has become the main force of the chemical fiber and is gradually becoming a replacement for the natural fiber. Regenerated cellulosic fiber might become the largest source of raw materials for textiles in the future and will be the focus of development.

9.1.2.1 Development of Regenerated Cellulose Fibers

Since the late 1960s, with increasingly stringent environmental regulations in developed countries and the increasing cost of pollution treatment, coupled with the increase in production costs as well as the decrease in the competitiveness of products, traditional craft production of cellulose fibers in Europe, America,

Japan, and other developed countries is gradually reduced. Some enterprises for regenerated fiber gradually are being transferred to developing countries, mainly in Asia.

Regenerated cellulose fibers mainly include viscose fibers, acetate fibers, and cuprammonium fibers.

(1) Viscose fiber
The production process for viscose fiber generally is as follows: First, make the plant cellulose to form the cellulose xanthate, and the cellulose xanthate is dissolved in dilute sodium hydroxide to make viscose, which is extruded into a coagulation bath from a spinneret hole, then is solidified and decomposed to form regenerated cellulose fiber. Viscose fiber is the most productive regenerated cellulose fiber, but because of heavy pollution and other problems, there is gradual reduction of the traditional process of viscose fiber production in developed countries. The production capacity growth worldwide for viscose fiber is mainly centralized in China; the output of viscose fiber accounts for about half of the total output of the global regenerated cellulose fiber.

Now, the varieties of viscose fiber include modal, high-strength fibers, and conventional fibers. Despite environmental pollution, the viscose fiber will continue to be produced for a long time and trend toward the high-quality modern viscose fiber, which has improved performance.

(2) Acetate fiber
First, in the production process for acetate fiber, cellulose is formed into cellulose acetate, and then the cellulose acetate is saponified into acetate fiber. The production of acetate filament is mainly centralized in Europe, the United States, Japan, and other developed countries. The United States was the first country to product acetate fiber; it also has the largest productivity. But, in recent years because of the decline of the domestic market and the aging of production equipment, acetate fiber used in textiles in the United States gradually has decreased. The same situation also applies to Western Europe. The worldwide production of acetate filament is estimated to remain at 20 million tons for the next few years.

(3) Cuprammonium rayon
In the production process for cuprammonium rayon, first the pulps are dissolved in cupric tetramminohydroxide solution to prepare a viscous solution that is extruded into a coagulation bath and then regenerated to cellulose fibers. Cuprammonium rayon is a type of cellulose fiber with low productivity; it is mainly produced in Japan, but production has declined in recent years. The productivity of cuprammonium rayon is not expected to have much fluctuation in the next few years.

9.1.2.2 New Technologies for Regenerated Cellulose Fiber

Viscose fiber occupies an important position in textile raw materials and occupies about a 90 % market share of regenerated cellulose fiber because of the mature technology and low cost, but with the strengthening of environmental awareness on

the international level, the development of nonpolluting new renewable cellulose fiber has become the consensus of researchers.

(1) N-methylmorpholine-n-oxide technology
N-Methylmorpholine-n-oxide (NMMO) technology is a new process for the production of cellulose fibers without chemical reactions. The process makes the best use of the characteristic that cellulose can be dissolved by tertiary amine oxides in NMMO. Cellulose pulp first is dissolved in the spinning solution with suitable viscosity, and then cellulose fibers are prepared by dry-jet wet spinning. In this technology, the solvent is removed through a coagulation bath; the NMMO in coagulation liquid and cleaning solution is purified and recycled. The entire production process forms an inner circulation with more than 99.5 % NMMO solvent recycled.

NMMO technology was developed in the 1970s by the German Enka Company; the German Akzo Nobel Company obtained the patent in 1978, and a pilot plant with an annual productivity of 100 t of filaments was set up in Germany in 1994. Germany TITK also developed a self-technology for lyocell production and established the pilot plant. In 2006, the Lenzing Company, which mainly engaged in the industrialization of the lyocell fiber, had grown to a company with a capacity of 150,000 t annually.

(2) Cellulose carbamate
In the 1980s, Finland Nested Oy's research group invented the production technology for cellulose carbamate with liquid ammonia used for the production processing of Cellca cellulose fibers. Cellulose carbamate is a potential intermediate product that serves as a substitute for cellulose xanthate in the viscose production process. Cellulose carbamate in the dry state is directly dissolved in the sodium hydroxide solution to form the cellulose carbamate solution required by spinning, and then the cellulose carbamate solution is moved into a permeate bag placed in low-temperature purified water. Part of the sodium hydroxide is removed to obtain the cellulose carbamate/sodium hydroxide spinning solution. In low-temperature protection, through filtering and vacuum debubbling, the solution is compressed by nitrogen, pumped by a metric pump, then ejected from the spinneret mouth into the acid coagulation to precipitate cellulose carbamate fibers. Cellulose carbamate fibers are immediately mechanically stretched in a water bath and sent to the high-temperature dilute alkali bath to regenerate, thereby obtaining the cellulose carbamate fibers.

9.1.2.3 Prospects for Regenerated Cellulose Fibers

In recent years, the development of China's renewable lignocellulosic resources achieved some progress; productivity increased steadily, and fiber production

technology and the "three wastes" treatment technology were constantly evolving. However, there are still many problems that need to be solved. First, the development of regenerated cellulose fiber resources lacks an overall planning and promotion scheme at the national and industry levels.

Second, the related core technology of regenerated cellulose fiber resources needs to be strengthened. Now, regenerated cellulose fiber has irreparable defects brought about by techniques: The structural stability is poor; the wet strength accounts for 38–42 % of the dry strength, especially with textile shrinkage more than 10 %; the strength is low, with the strength of regular viscose generally less than 24.7 cN/dtex; and the pollution is serious as the conventional viscose production routes have the large amounts of waste gas and waste water [7].

Third, the stable supply of the regenerated cellulose fiber resources is a problem. In the current production of viscose fibers in China, cotton pulp accounts for more than 60 %. Because of the slight growth of international cotton output, the growth of cotton pulp resources is also limited. Wood pulp is the best supplement for cotton pulp, but wood resources in China are small, especially wood available for pulp production. Productivity of bamboo and hemp pulp is limited because of high costs.

Finally, the development of renewable cellulose fiber and its products lacks a view of scientific development. Some enterprises have quick success in the promotion of new products. They exaggerate some properties of the product, avoiding their shortcomings and deficiencies. These are detrimental to sustained and healthy development [8].

To solve these problems, researchers need to solve the following problems in the development of regenerated cellulose fiber: (1) The contradiction between functional materials and fiber strength needs a solution. Generally, to ensure the strength of the regenerated fibers, lignin and other low molecular weight substances need to be removed for the most part. In this process, antibacterial substances are likely to be easily reduced, causing the loss of active ingredients of the natural materials and reducing the utilization level of resources. (2) There is need for increased research in implant technology of functional substances. To study nonblended grafting technology, it is necessary to appropriately increase the content of useful substances in the pulp and the technical content of regenerated cellulose fiber and to expand its applications. (3) The differentiation of structure must be solved. The differentiations of synthetic fiber have partially reached or overstepped the degree of natural fiber function; however, the differentiation of regenerated cellulose fiber far from initiation. The differentiation of structure in the regenerated cellulose fiber system has some technical difficulties; especially in the case of low strength, differentiated processing is more difficult. Cross-linking, synthesis, and other combination techniques for producing new viscose fiber can be used to explore a new fiber-processing line and promote the differential production of regenerated cellulose fibers.

9.2 Application in Environmental Protection

Natural lignocellulosic materials are the most abundant renewable biomass on earth; microbial degradation and transformation of them is the main aspect of the carbon cycle in nature. The effective utilization of this process not only can offer cheap raw materials for agriculture, feed, light industry, and the chemical industry, but also can play an important role in the protection from environmental pollution and establishment of a healthy ecosystem.

9.2.1 Application of White-Rot Fungi

The white-rot fungi are fungal groups that vary greatly. They can degrade all components of the cell wall, including lignin, no matter the location. They will cause the corrosion of the cell wall with the presence of the mycelia. With the degradation process, a larger gap would be formed, which is often filled with mycelia; the fungi that cause this type of white corrosion are often considered to be nonselective and facultative white-rot fungi. Some kinds of white-rot fungi show high degradability of lignin but a low value for cellulose.

White-rot fungi have an important role in the degradation of organic pollutants. Because many organic pollutants have a similar structure as the lignin structural unit, the degradation of various organic pollutants (mainly aromatic organic compounds) in industrial wastewater by white-rot fungi has become an important research direction. Research showed that white-rot fungi not only could degrade structural analogues of lignin but also could degrade a variety of other organic compounds, including PCBs (polychlorinated biphenyls). This work caused a great deal of attention in both academia and industry. Because of the strong financial support from industry, the research and application of white-rot fungi and its technology have achieved great development within last 10 years; its influence has expanded increasingly and gradually shifted from the research laboratory to industrial applications. In this area, research in China started in the 1990s, which was almost 20 years later than some countries. Therefore, it is necessary to have distinctive and innovative research work according to China's national conditions, learning from worldwide experience.

9.2.1.1 Advantages of White-Rot Fungi for Environmental Control

Compared with other organisms, especially bacteria, white-rot fungi have the following advantages for environmental control.

(1) Bacteria could synthesize the desired degrading enzyme only when induced by a certain concentration of substrate. So, they cannot degrade organic pollutants in low concentrations and can only reduce the pollutants to a limited level.

The induction of a degrading enzyme of white-rot fungi has nothing to do with the presence and number of substrate, so it initiates the degradation process relying on nutrient limitation. In this way, the white-rot fungi can degrade pollutants in low concentrations in the environment, even those with almost undetectable levels.

(2) *Advantage of kinetic properties.* The degradation of chemicals by bacteria is mostly enzymatic conversion, which follows Michaelis-Menten kinetics. Thus, the degradation to pollutants should be considered with the K_m (Michaelis constant) of various degrading enzymes in the bacteria. Bacteria are essentially repellent to and have low compatibility (high K_m values) with refractory xenobiotics, which determines the lack of thoroughness and adequacy of the degradation process. In contrast, white-rot fungi fulfill the chemical conversion process via free radicals and degradation of compounds appears to be first-order kinetics. The enzyme to launch the initial oxidation reaction of the substrate has no real K_m values and is conducive to the formation of oxidation products, which means white-rot fungi can degrade pollutants with low concentration to near-zero levels.

(3) *Advantage in competition.* White-rot fungi can maintain a competitive advantage by producing free radicals, oxidizing other microbial proteins and DNA, taking advantage of the oxidation reduction system of plasma membrane, regulating the environment pH to low level, and inhibiting the growth of other micro-organisms.

(4) *The extracellular degradation.* Generally, enzymes can metabolize cyanide when the cell first inhales cyanide because microbial metabolic enzymes are present in the cell, which could seriously inhibit cell metabolism. However, the degrading enzyme system of white-rot fungi is present extracellularly and produces a highly efficient oxidant (such as veratryl alcohol radical cation VA•+ and •OH); toxic pollutants need not first enter the cell metabolism, avoiding the toxicity to cells. For example, cyanide as a strong inhibitor of respiratory oxidase can inhibit the growth of microorganisms in the municipal wastewater treatment system with a concentration of only 4 $mg{\cdot}L^{-1}$. But, the extracellular system of white-rot fungi enables the white-rot fungi to endure and mineralize a high concentration of cyanide and present apparent inhibition until 260 $mg{\cdot}L^{-1}$.

(5) *Nonspecific degradation of substrate*. Because pollutants in the environment are almost mixed together, their degradation usually requires cooperation of a variety of microorganisms. White-rot fungi can degrade compounds with different chemical structures, even completely mineralized pollutant mixtures such as creosote and chloroaromatic compounds. So, the nonspecific degradation of the white-rot fungi makes them have a good prospect for application.

(6) *Adapt to both solid and liquid phases*. Most microorganism systems are only suitable for a soluble substrate, but many pollutants (such as dichlorodiphenyl-trichloroethane [DDT]) are not soluble in water and have poor biodegradability. White-rot fungi can be used in soil remediation and water pollution control because they can use a water-insoluble substrate.

(7) White-rot fungi do not ask for much of the nutrient and can be cultivated with sawdust, wood chips, agricultural waste, and other cheap sources as substrate. White-rot fungi have a high application value in the degradation of xenobiotic biomass because of the advantages that distinguish them from general microorganisms, such as high efficiency, low energy consumption, broad-spectrum coverage, and wide applicability. However, there is a certain distance from direct application to large-scale wastewater treatment and land treatment of white-rot fungi because some special physiological and biochemical characteristics are not yet understood clearly.

9.2.1.2 Degradation Mechanism of Environmental Pollutants

The degradation mechanism of white-rot fungi to pollutants mainly depends on the extracellular degradation system, consisting of enzymes secreted by the cells; this system is aerobic and activated by autogenerated H_2O_2 to initiate a series of free radical chain reactions, finally achieving the nonspecific oxidative decomposition to the substrate thoroughly.

9.2.1.3 Research Progress for White-Rot Fungi in Environmental Control

Applied researches of white rot fungi are mainly divided into two categories, the study on the degradation of different types of target compounds and the choice of engineering application parameters.

(1) Application of white-rot fungi in cleaner production
To meet the needs of sustainable development, great concerns have been applied to the strategy of cleaner production. Cleaner production can achieve the reduce pollution, make it harmless, and provide resource recovery; this has not only environmental benefits but also good economic efficiency and development prospects. Cleaner production is the best choice for modern industrial enterprises to achieve resource conservation and pilot technology and provide intensity, quality, and efficiency. It is also the only way to prevent and control industrial pollution. It is known that the papermaking industry produces large quantities of pollutants in the pulping and bleaching processes. Therefore, minimizing the generation of pollutants in the production process to achieve cleaner production is the focus of attention for environmentalists.

The white-rot fungi can secrete a variety of special degradation enzymes to degrade cellulose, lignin, and so on, which can be reused in wood pulping and bleaching in the paper industry.

Study has confirmed that white-rot fungal pretreatment could reduce the energy consumption of mechanical pulping, reduce wastewater, and achieve pulp with good physical properties. However, to realize industrialization, there are many problems that need to be solved, such as shortening the period of biological pretreatment,

controlling the problem of other microbes, and managing the ventilation problem in the pretreatment of wood chips.

(2) Application of white-rot fungi in the treatment of recalcitrant pollutants
White-rot fungi have great application prospects in clean production and in the treatment of recalcitrant pollutants and the degradation of pollutants emitted into the environment. It also has received a wide range of research.

① Application of white-rot fungi in the treatment of papermaking wastewater
Because white-rot fungi can degrade lignin, people first thought to use it to treat wastewater in paper mills. The results showed that it could discolor the wastewater discharged from the first alkali extraction procedure and degrade residual lignin in wastewater. This kind of wastewater is the major source of water pollution, which is a complex mixture containing the lignin fragments, phenol, anisole, and other low molecular weight components.

② Application of white-rot fungi in the degradation of dyes
With the development of organic chemistry, synthetic dyes have appeared. To date, thousands of varieties of synthetic dyes have been synthesized and widely used in the printing and dying, leather, printing and photographic industries. Especially with the rapid development of the printing and dyeing industry, the variety and quantity of dyes keep increasing. At present, China's dyestuff wastewater has a large impact on the environment. This wastewater contains a variety of biologically toxic organics that can also cause the decrease of water transmittance and lead to the destruction of the water ecosystem even in small concentrations. So, if the wastewater that is not properly treated is emitted into the water, it will cause serious environmental pollution and ecological destruction.

Most dyes are synthetic macromolecular aromatic compounds, have a wide variety of complex structures, and are difficult to degrade. Because the synthetic dyes do not inherently exist in the biosphere and nature, they are difficult to degrade in nature. Although some bacteria have been screened, their degradation ability is not strong, and they have a strong specificity for substrate. In addition, bacteria mostly conduct complete degradation of dyes under anaerobic conditions; the resulting intermediate products always have high toxicity. White-rot fungi have a unique mechanism of enzyme degradation, with the degradation ability unaffected by the substrate structure. The conversion of pollutants catalyzed by the key enzyme system of white-rot fungi belongs to mediators involved in the catalytic oxidation reaction, which has a broad spectrum and high conversion efficiency of pollutants [9]. Thus, using the white-rot fungi to handle the pollution caused by dyes has a unique advantage. The dyes that can be degraded by white-rot fungi involve azo dyes, triphenylmethane dyes, heterocyclic dyes, polymeric dyes, and so on.

Although white-rot fungi have a good decoloring effect on various dyes, some of the characteristics of white-rot fungi also cause many difficulties in practical application. (1) The growth period of white-rot fungi is long, generally 5–6 days from inoculation to maximum enzyme activity, which is not conducive to the industrialization of wastewater treatment. (2) The extracellular enzymes that have a bleaching role are easily lost during the actual wastewater treatment process.

(3) Because of the low removal ability for chemical oxygen demand (COD), it must be combined with other methods to make the wastewater meet the discharge standard.

(3) Application of white-rot fungi in the degradation of pesticides
Application of white-rot fungi in cleaner productionIn agricultural pollutants, recalcitrant pesticides have characteristics of wide range, strong biological toxicity, easy bioconcentration, and so on. The migration and transformation of chlorinated pesticides, such as benzene hexachloride (BHC) and DDT, has been a constant hot research topic.

Some research indicated that most of the chlorinated pesticides can be completely mineralized by the white-rot fungi under appropriate culture conditions. Zou and Zhang [10] used gas chromatographic/mass spectrometric (GC-MS) analysis of partly degraded products and discussed the possible degradation mechanism of different types of chlorinated compounds, determined the static culture conditions of white-rot fungi in the water, and explored the biological degradation conditions and mechanism of chlorinated pesticides on these conditions. The experiments showed that white-rot fungi grew well in medium composed of 0.02 % glucose, 0.03 % ammonium tartrate, and a proper amount of inorganic salt when cultivated statically at 35 °C. The mycelia of white-rot fungi gained at 5–7 days had the maximum degradation efficiency (more than 90 %) of chlorinated pesticides.

(4) Application of white-rot fungi in chemical wastewater degradation
Nitroglycerin is a highly toxic substance commonly in wastewater from chemical and pharmaceutical plants. Bhaumik et al. [11] used a sequencing batch fixed-bed bioreactor to degrade nitroglycerin by a mixture of bacteria and *Phanerochaete chrysosporium* in aerobic conditions and anaerobic digestion sludge in anaerobic conditions. The sequencing batch reaction showed that the mixture of bacteria and *P. chrysosporium* could degrade trinitrin into senitroglycerin and nitroglycerin, and the degradation efficiency had a close relationship with coenzyme concentration. Huang and Zhou [12] took white-rot fungi isolated and purified from TNT-contaminated soil to conduct aerobic biodegradation tests of wastewater polluted by TNT. After 5 days, the degradation rate of TNT in wastewater was higher than 99 %.

Several countries have performed much research in the application of white-rot fungi in environmental engineering and achieved encouraging progress. But, so far, this research only focused on a few strains, such as *P. chrysosporium*, lacking examples of large-scale applications. To accelerate the resolution of removal of recalcitrant pollutants, more work on the biological characteristics and application technology of the various types of white-rot fungi is essential.

9.2.2 *Applications of Straw in Restoring Materials of Ecological Engineering*

9.2.2.1 Application in Preparation of Greening Materials of a Rocky Slope

In recent years, with the increasing strength of infrastructure construction and high demand for the protection of the ecological environment in China, roads, railways, and construction of hydropower projects all require ecological protection on a rocky slope. Nearly 4×10^5 km^2 of rock surface exist in China, which increases with increasing yearly construction and development. According to requirements of engineering construction and ecological restoration, the ecological protection technology of rocky slope wounds has a huge market demand.

The rock surface refers to the exposed surface formed by excavation and movement of rock and soil body, which is caused by basic infrastructure (such as roads, railways, electricity, water and municipal engineering construction, etc.) and industrial and mining production processes (such as in mine areas, quarries, etc.). With the rapid development of economic construction, because of vegetation destruction and dissipation of surface soil, soil erosion and ecological damage of the wound slope gradually have become severe, becoming a hot topic of ecological restoration. At present, the main measure of ecological protection is to combine engineering measures with vegetation.

In the ecological slope protection technology applied in China, the thick-layer base material spraying (TBS) technique accounts for about 20 % of the market. In 2002, China independently developed this technology by combining the application of basic materials with engineering measures [13]. Greenery basic materials are the core of this technology; they are mainly prepared from highland peat, straw fiber, organic fertilizer, and other functional materials. However, because most existing greenery substrate products take peat and other natural organic fertilizers as the main functional materials, the cost of this technology is still relatively high [14]. At the same time, straw without any processing has limited effect on stabilization of the soil body, and straw after a specific pretreatment can replace most of the traditional greenery substrate, but to now, no reports exist about taking pretreatment products of straw to produce greenery substrates of rocky slopes.

Existing greenery substrates need to be added with many natural organic materials, and product characteristics of natural plant straw are poor. To solve the problems mentioned, Chen et al. [15] provided a method using steam-exploded straw through solid-state fermentation to prepare greenery substrates for rocky slopes. Through the pretreatment of steam explosion and solid-state fermentation, based on of stable growth of ecological recovery vegetation, new-style greenery substrates were produced to replace the peat or organic fertilizer because they can increase the stability of soil and have resistance to erosion and provide prevention of plant disease, reducing application costs and ecological resource damage.

From the point of view that straw and other renewable resources can effectively enter into the natural carbon cycle, for the specific needs of the existing greenery for

the specific needs of product attributes, through the combination of pretreatment, the straw is not only a carrier of greenery substrate but also constitutes the main functional materials with the specific activity. By steam explosion, straw fibers are puffed and humified with the increase of water absorption and humic acid content, which could reduce the amount of peat in the product. After solid-state fermentation, products are enriched with a large number of beneficial microflora in the soil and have the effect of ecological fertilizer and can control plant diseases. The content of organic matter and humic acid are further improved, avoiding or reducing the dependence of the previous products on inorganic fertilizers. These products are able to meet the conservation capacity and water-holding capacity of rocky slope wound recovery, which can effectively reduce application costs.

The technical route of the rocky slope greenery substrate preparation method via solid-state fermentation of steam-exploded plant straw solid is described as follows:

(1) Preparation of steam-exploded straw. Cut the whole plant straw to 3–5 cm, and add water with the same weight of raw materials to infiltrate the straw for 10–20 min. Next, put the material into a steam explosion tank, input the high-pressure steam, then maintain the pressure in the steam explosion tank at 1.5–1.7 MPa for 3–8 min. Quickly open the outlet valve to release the pressure, and steam-exploded straw is discharged from the discharge port.

(2) *Solid-state fermentation of mixed bacteria*. The solid-state fermentation substrate is prepared by evenly mixing steam-exploded straw with bran (weight percentage of straw is 10–30 %) and then adding aseptic water. Adjust the solid-liquid ratio to 1:4–1:6; next, inoculate with seed liquid of *Trichoderma* (weight percentage of solid-state fermentation substrate is 3–6 %) to conduct the solid-state fermentation. The culture is cultivated at 25–28 °C for 2–3 days. After fermentation, inoculate (weight percentage of solid-state fermentation substrate) 5–7 % of *Azotobacter chroococcum* seed liquid, 5–7 % *Azotobacter vinelandii* seed liquid, 3–5 % *Bacillus megatherium* seed liquid, 3–5 % *Bacillus mucilaginosus* seed liquid, and 5–7 % *Trichoderma harzianum* seed liquid to conduct the solid-state fermentation, which is cultivated at 25–28 °C for 4–5 days. After fermentation, 2–4 % of *Xanthomonas campestris* seed liquid (weight percentage of solid-state fermentation substrate) is inoculated and cultivated at 25–28 °C for 2–3 days.

(3) *Add clays* that are two to four times the weight of fermented materials, and some functional materials are also added. Then, stir them at room temperature to obtain the rocky slope greenery substrate.

The agricultural straws mentioned include agricultural wastes such as cornstalks, wheat straw, rice straw, or canola stalks. When carrying out the solid-state fermentation, *Trichoderma viride* was inoculated first; then, *Azotobacter chroococcum Beijerinck*, *B. megatherium* var. *phosphaticum,* and *Trichoderma harzianum* were added at the same time and *Xanthomonas campestris* was added last. Among them, xanthan generated by *Xanthomonas campestris* can increase the viscosity and water absorption of the substrate. In the prepared rocky slope greenery substrate, organic matter content was more than 50 %,

humic acid content was more than 10 %, and the total number of living bacteria was more than 2×10^8 g^{-1}. It not only can replace the past large-scale use of peat, but also can control plant diseases to satisfy the demand of conservation capacity and water-holding capacity.

9.2.2.2 Application in Preparation of Modified Material of Desertification Land

Land desertification has become one of the most serious ecological problems because that area of deserted land in China is 1.7397 km^2, accounting for 18.12 % of the total land area of China. Although in recent years China has increased the intensity of management and improvement in desertification land, it is still in the stalemate phase, keeping equivalence between governance and desertification. In China, using straw for desertification control began in the 1990s. The initial practices were to bury straw into a sand windbreak or cover the land with straw to prevent desertification, similar to practices in foreign countries. However, these actions cannot solve the problem and only act as limited auxiliary measures.

Because water shortage, damage of vegetation, and poor soil texture have become the main driving forces of desertification, scholars have recognized that improving the soil texture, water conservation, and vegetation construction are fundamental measures to combat desertification. Regarding these aspects, much research has been carried out, with the modification of desertified land using chemical materials becoming a hot research topic. Owing to the high cost and limited absorption rate of the plants on chemical improvers, the long-term application not only will pollute groundwater but also can cause soil compaction and the destruction of the ecological environment. Therefore, the function of straw in the control and improvement of land desertification has drawn much attention again. From the standpoint of existing research, the effect of using straw as the main raw material of desertified land modified materials is equivalent to those using chemical materials. Compared with simple chemical materials, straw is readily available and has low cost, long duration, full function, and other advantages.

Currently, in China, mechanical grinding is always used as the pretreatment method before using straw as additives in desertification land modified materials. This kind of pretreatment is simple, but has some shortcomings, such as a longer period of composting, poor quality of products, and the low efficiency of fertilizer. Although some methods aim at improving soil fertility, the water absorption capacity of the soil does not significantly improve and energy consumption in the product process is high [16]. There are also reports about preparing a bioactive water-retaining modified agent with agricultural straw, but the preparation of active water-absorbing material needs chemical treatment, which would lead to certain pollution of the environment [17]. In addition, because of the imbalance of the nutrient content of straw, it is necessary to add a certain proportion of inorganic fertilizers, organic nitrogen, humic acid, and rare metal compounds when used for land improvement, which cannot reduce the cost of the product fundamentally [18].

To resolve the problems that exist in the present pretreatment technology using straw to prepare desertification land modified materials, such as unsatisfying technique, environmental pollution, and high cost because of the extra addition of inorganic fertilizer, humic acid, nutrients, and other ingredients, Chen et al. [19] proposed a preparation method for desertification land modified materials by combining solid-state fermentation with steam explosion pretreatment of straw, which can improve the bioconversion properties of straw, increase the efficacy of straw as a biofertilizer to reduce the amount of chemical fertilizers and environmental pollution, and decrease the costs of prevention and control of desertification land.

Straw fiber properties can be used to produce functional products that are similar to chemically modified material. Straws can be treated by a steam explosion and solid-state fermentation method to improve water absorption, humification, and the nutritive element content of the material. Then, the treated straw can form a preparation with strong, biodegradable absorbent material, namely, bacterial cellulose preparations under certain conditions, which can improve the microstructure of soil, significantly increase soil water storage capacity, and reduce the amount of bioactive water-retaining agent. Also, a large number of beneficial microorganisms are enriched in soils after solid-state fermentation, which can enhance the moisture conservation and sustained release of nutrient elements, becoming suitable for large-scale application in prevention and control of deserted land.

The preparation of modified materials for desertification land by the combination of solid-state fermentation and steam explosion of straw is described as follows:

(1) *Preparation of steam-exploded straw*. Cut the whole plant straw to 3–5 cm, add water with the same weight as the raw materials to infiltrate the straw for 10–20 min. Next, put the material into a steam explosion tank, then maintain the pressure in the steam explosion tank at 1.5–1.7 MPa for 4–8 min. Quickly open the outlet valve to release the pressure, and steam-exploded straw is discharged from the discharge port.

(2) *Solid-state fermentation of complex microbial system.*

① Preparation of inoculation of single bacterial strain. The liquid inocula of *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Bacillus megatherium* var. *phosphaticum*, *Bacillus mucilaginosus*, and *Trichoderma harzianum* were prepared by inoculating one circular lawn from each strain to liquid media and shake cultivating at 25–28 °C for 2–3 days at 120 rpm.

② Solid-state fermentation of complex microbial system. First, the steam-exploded straw is ground to 20 grind and then mixed with 15–25 % bran uniformly to obtain a solid-state fermentation substrate. Add water into the solid-state fermentation substrate and stir uniformly; adjust the solid-to-liquid ratio to 1:4–1:6, then sterilize at 121 °C for 20 min. The cooled substrate is inoculated with 4–6 % of *Trichoderma* inoculum and cultivated at 25–28 °C for 2–3 days. Then, the culture is inoculated with 5–7 % of *A. chroococcum* inocula, 5–7 % of *A. vinelandii* inocula, 4–6 % *Bac. megatherium* var. *phosphaticum* inocula, 4–6 %

of *B. mucilaginosus* inocula, and 5–7 % of *T. harzianum* inocula and cultivated at 25–28 °C for 4–6 days.

(3) *Fermentation of bacterial cellulose.* Acetic acid bacteria liquid medium is inoculated with 2–4 % *Acetobacter xylinum* and statically cultivated at 28–30 °C for 7–9 days; to improve bacterial cellulose production, the fermentation period may be extended.

(4) At the end of fermentation, the fermented residues are mixed with bacterial cellulose at a ratio of 1:0.03–0.05 and are dried at room temperature to obtain the modified materials of desertification land.

The raw materials used for the preparation of modified materials for desertification land can be cornstalks, wheat straw, rice straw, or rape straw. Compared with mechanically ground straw, the steam-exploded straw has 32–40 % higher water absorption and an 80–100 % higher water retention rate. For the modified materials prepared by solid-state fermentation of steam-exploded straw, the organic matter content is higher than 60 %, the humic acid content is higher than 15 %, the total number of living bacteria is higher than 300 million g^{-1}, and the content of nitrogen, phosphorus, and potassium is higher than 5 %. The product not only can improve the microstructure of soil and reduce the amount of bioactive water-retaining agent but also can be beneficial for enhancing the function of moisture conservation and sustained release of nutrient elements. This technology is suitable for large-scale application in prevention and control of soil desertification.

9.2.2.3 Application in the Preparation of Belt-Seeding Carrier

In recent years, with increasing attention to the living environment, green space has become an important indicator of the sign of city beautification and urban ecological environment improvement. The surface area of urban green land has shown an upward tendency in recent years; especially, the annual growth rate of greening lawn in China is more than 30 %, increasing at a speed of more than 20,000 ha annually. However, shortcomings of lawns and high management and maintenance costs have become a huge burden on cities. New technology that can reduce the establishment and maintenance costs has become an urgent requirement for the construction of urban green space.

For now, artificial vegetation is the most effective means to solve this problem; the main characteristic is to use the artificial vegetation in combination with growth substrate to form rolled or strap-shaped products, facilitating artificial placement or planting, also known as belt seeding or artificial vegetation roll. Artificial vegetation has the advantages of rapid speed of construction and no limitation on soil conditions; it can be used in urban landscaping, roof greening, slope protection for highways, establishment of sports turf, water and soil conservation engineering, and other areas.

Researches on artificial vegetation in China began in the late 1990s; it started later than in foreign countries, but with similar production technology to those countries, mainly blanket-like or ribbon products produced using natural fibers such as straw, coconut shell, a variety of hemp, and so on as the raw material and then mixing with seeds, fertilizers, and net in certain proportions. However, the low-technology level results in low mechanization and efficiency of artificial vegetation, which further limits its application because of the high costs compared with traditional greening technology. The renewable fibers are used to produce nonwoven fabrics, and plant seeds are mixed with fertilizers and pesticides, although in certain roles, extensive use would pollute the environment and groundwater [20]. Some products take waste newspapers, waste cotton, and wastepaper as carriers and then add the seeds and fertilizers into them, but these untreated urban wastes are resistant to degradation resulting in the low use value, but some heavy metals in them would massively accumulate to cause pollution of the soil environment [21]. Coconut also can be a belt-seeding carrier; the seeds and water-retaining agents are adhered in meshes of coconut, but the product nutrient contents are low, and the seed germination cycle is long; the germination rate also is not high [22].

On the problems of the high cost of existing artificial vegetation technology and the product effects that are not obvious, with the products unsuitable for a large-area spreading application, Chen et al. [23] provided a preparation method of belt-seeding carriers via solid-state fermentation of steam-exploded straw that can avoid or significantly reduce the use of pesticides and chemical fertilizers, decrease the amount of original carrier materials and additives, and effectively lower the cost and environmental pollution.

This method takes straw, the waste of the agriculture industry, as the main carrier; it uses steam explosion to humify straw and lowers the crystallinity of straw, increasing water absorption and degradation. Steam-exploded straw is washed and divided into washed matter and water-washed liquid. Through the solid-state fermentation of the complex microbial system in washed matter, carriers have the effect of biofertilizer and disease resistance. Xanthan gum, the fermentation product of water-washed liquid, can be used for adhesion of carriers and seeds and shape forming of the carrier. This technology has some advantages; for example, there is no need for additional pesticides and fertilizers, reducing the amount of retaining agents and adhesive, decreasing the demand of fine soil and the cost of construction and management, and promoting the protection of the environment.

The technical route of belt-seeding carrier preparation via solid-state fermentation of steam-exploded straw can be described as follows:

(1) *Preparation of seed liquid of a single bacterial strain*. Whole straw is cut to 3–5 cm, then water with the same weight of raw materials in it is added to infiltrate the straw for 10–15 min. Next, put the material into a steam explosion tank, input the high-pressure steam, and maintain the pressure in the steam explosion tank at 1.3–1.6 MPa for 5–7 min. Quickly open the outlet valve to release the pressure, and the steam-exploded straw is discharged from the discharge port.

(2) *Solid-state fermentation of a complex microbial system.*

① Preparation of solid-state fermentation substrate: Steam-exploded straw is washed with water; after filtration, the material is divided into washed matter and water-washed liquid. Then, 15–30 % bran is added to the dried washed matter and mixed uniformly to obtain a solid-state fermentation substrate.
② Water is added to the solid-state fermentation substrate; then, the solid-to-liquid ratio is adjusted to 1:4–1:6. After sterilization, inoculate 3–6 % of *Trichoderma* inoculum and culture at 25–28 °C for 2–3 days.
③ Inoculate 3–6 % inoculum of *A. chroococcum*, 3–6 % inoculum of *A. vinelandii*, 3–5 % inoculum of *Bac. megatherium* var. *phosphaticum*, 3–5 % inoculum of *B. mucilaginosus,* and 4–7 % inoculum of *T. harzianum*; culture at 25–28 °C for 4–6 days. At the same time, inoculate 3–5 % inoculum of *Xanthomonas campestris* and culture at 25–28 °C for 2–3 days.

(3) Combine the solid-state fermented material with xanthan gum at a rate of 1:0.005–0.01, then dry at room temperature to obtain the prepared belt-seeding carrier.

Straw, such as cornstalks, wheat straw, rice straw, or rape straw, can be used for preparing the belt-seeding carrier. Through the steam explosion treatment, the humification degree of straw would be improved, fiber crystallinity would decrease, water absorption and degradation would increase, and the carrier after solid fermentation would have the effect of biological fertilizer and resistance to disease. Meanwhile, xanthan gum has strong water absorption. It also can be used for adhesion of carriers and seeds and shape forming of the carrier. For the belt-seeding carrier prepared by solid-state fermentation of steam-exploded straw, organic matter content is higher than 53 %, humic acid content is higher than 12 %, and the total number of living bacteria is higher than 200 million g^{-1}. The demand of fine soil and the cost of construction and management are effectively lowered. This technology would significantly promote the protection of the environment.

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Chapter 10
Ecological Industry Model for Biotechnology of Lignocellulose

Abstract Natural lignocellulose are the most important part of biomass and have the potential to convert into a variety of intermediate or final products. According to the requirements of ecological engineering and ecological agriculture, the significance of bioconversion and comprehensive utilization of natural lignocellulose has been studied. The major eco-industrial productions by modern biotechnology are renewable clean energy, pollution-free biological pesticides, biological nitrogen fixation of organic fertilizer, and microbial feed and feed additives, which can advance the level of eco-agriculture. Therefore, the technology system of comprehensive utilization of natural lignocellulose is proposed in this chapter, and the key technology and its features to break through the barriers of economy and technology are involved. Also, some typical examples for an ecological industry model of biotechnology of lignocellulose are introduced.

Keywords Ecological industry model • Comprehensive utilization of natural lignocelluloses • Typical demonstration projects

10.1 Introduction

Survival and development are the eternal themes of human society. The traditional development viewpoints unilaterally pursue the economic and social prosperity at the expense of the natural environment. The industrialization process resulted in predatory exploitation of natural resources and environmental pollution, especially from the twentieth century, which has greatly undermined the ecological balance of nature and made the survival of humankind fall into a deep crisis. An article on "sustainable industrial development strategies" was reported in the Scientific American of American Popular Science Monthly in September 1989. Robert Frosch and Nicolas Gallopoulos proposed a new mode of production in industry for the first time. The industrial ecosystem can completely work like a biological ecosystem; that is, mass and energy work in the following continuous cycle:

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plant → herbivore → carnivore → microorganisms → plant. Moreover, the concept of industrial ecology was proposed. Since then, the ecological view began to penetrate into the industrial field.

The proposal of a sustainable development strategy is a revolutionary transformation of the traditional concept of development. As strategic thinking, it was a deep reflection and revolution of the human view of nature and values based on an ecological crisis. The rational use of resources and environmental protection were inevitable requirements and ways for achieving sustainable development. Therefore, the industrialization process should be cycled like a biological ecosystem, complying with the following processes: (1) Plants absorb nutrients; (2) foliage for herbivores is synthetic; (3) herbivores are preyed by predators; (4) the excretion substances and the body are degraded and then used by plants. This mode of operation is the goal of the so-called eco-industrial systems.

The basic principle of the industrial ecology system is the "4R" technical principles (reduce, reuse, recycle, and replace). The reduction principle is to reduce the matter and energy flow to enter the processes of production and consumption. The reuse principle is achieved through exchange of by-products and multistage comprehensive utilization of materials. The recycle principle relies on the recycling of conversion technology's matter and energy during integration technology. The replace principle requires renewable resources as the raw materials during the process. A series of ecology methods was formed based on the 4R principles, such as industrial metabolism oriented at raw materials; cleaner production oriented at the reaction process; evaluation of the life cycle oriented at production; system integration of energy and material oriented at the whole process research; and the construction of an eco-industrial park oriented at regional systems.

The emergence and wide application of biotechnology play an important role in the process of industrial ecosystems because of the two following reasons: The essence of biotechnology is to efficiently use Earth's resources and biological resources to provide adequate food, energy, and a healthy environment for humans. Biotechnology emphasizes the efficient and reasonable use of materials, mainly biomass. The biomass is multistage used and manifested in the form of large-scale industrial production based on the organism as a catalyst [1].

Natural lignocellulose materials are the most important part of biomass and have the potential to be converted into a variety of intermediate or final products. I have made breakthrough progress in the ecological industry for the biotechnology of lignocelluloses. This chapter mainly introduces the ecological industry model for the biotechnology of lignocelluloses.

10.2 Cleaner Production Theory

Cleaner production is an active model for environmental pollution control and governance; its goals are energy conservation and pollution reduction. Through the whole process of production, cleaner production is to design the optimal production

process and to eliminate or reduce the adverse effects on human health and the ecological environment of industrial production based on technology management.

There is no uniform definition of the cleaner production concept in the international arena. The more common definition was proposed by United Nations Environment Program (UNEP) Industry and Environment Program Activity Center in 1989. Cleaner production is an integrated preventive environmental strategy applied in production processes, products, and services to improve efficiency and reduce the hazards to humans and the environment. Cleaner production also was defined in China's Cleaner Production Promotion Law. Cleaner production means to improve the design, use clean energy and raw materials, and the advanced technology and equipment, then to improve management and comprehensive utilization; improve resource utilization efficiency; reduce production, services, and products to avoid the production and emission of pollutants in the process; and to reduce or eliminate hazards to human health and the environment based on the reduction of pollution from the source [1, 2]. It can be seen from the definition of cleaner production that cleaner production can be summarized as "three cleaners and one control," that is, cleaner raw materials and energy, cleaner production processes, cleaner products, and whole process control of cleaner production.

(1) Cleaner raw materials and energy

The premise and foundation to achieve cleaner production is to have cleaner raw materials and energy. At first, the raw materials must be pure. Compared with impure raw materials, the content of raw materials that are pure is higher, so conversion in the production process could be higher with fewer waste emissions; therefore, resource utilization would also be higher. The raw materials must not contain toxic substances. If the raw materials contain toxic substances, toxicity and pollution would appear in products in the production process. Clean production should eliminate toxic raw materials and energy through technical analysis and the use of nontoxic or low-toxicity raw materials and energy and cleaner fuels in the production process. Technological transformation for energy-saving clean production should improve the efficiency of energy use and increase the use of solar, wind, biomass, geothermal, and other renewable energy.

(2) Cleaner production processes

Cleaner production processes include the following: (1) use of toxic and hazardous raw materials as little as possible or eliminate their use to change the ratio of raw materials or to reduce their usage; (2) minimize the use of scarce energy resources and improve energy efficiency; (3) have less waste process and efficient equipment; (4) have as few as possible risk factors, such as high temperature, high pressure, low temperature, low pressure, flammability, explosive factors, strong bursts, strong vibrations; (5) apply simple and reliable production operation and control methods; (6) and internally recycle materials.

(3) Cleaner products

Cleaner products are conducive to the efficient use of resources and do not produce harmful products in the entire process of production, utilization, and disposal. Clean products could be achieved through product life cycle assessment (LCA) and green

design. The conservation of raw materials and energy should be considered in the product design stage. Less expensive, scarce, and hazardous materials are used. In the stage of the production process, re-forming the production technology, updating the equipment, and improving the utilization of raw materials and energy in every production process reduce waste and toxicity. The product should not damage human health and the ecological environment during and after use. The product packaging should be reasonable, avoiding excessive packaging. The product should be easy to recycle, reuse, and regenerate after the product has been used. The service life and use function of the product should be reasonable.

(4) Control of cleaner production process
The whole process of the production organization and measures taken in all links for necessary pollution prevention should be controlled. The specific practices are to improve management, including materials management, equipment management, production process management, product quality management, and on-site environmental management [3].

10.3 Circular Economy

The circular economy is also known as and is the short title for a resource recycling economy. The circular economy is relative to the traditional linear economy. It is essentially an ecological economy; cleaner production and comprehensive utilization of waste are blended together. It requires the use of ecological rules to guide the production activities of human society. In accordance with the natural ecosystem, the material cycle and energy flow pattern restructure the economic system. The circular economy also blends the economic system harmoniously into the natural ecosystem and material cycle to establish a new form of economy. The economic activity of the traditional process based on resources, products, and waste as a single linear process should be shifted to resources, products, and renewable resources according to the feedback process. Waste means any substances which were produced and remained in process of producing target products, and possibly harmful to the environment. Therefore, the circular economy is an important innovation of the traditional linear economy. Its characters are the low consumption and high utilization of the natural resources, and the low emission and recycling of waster. Its aims is to maximize the use of materials and energy efficiency, and then to reduce the impact of production activities on the natural environment to the minimal extent, and solve the sharp contradiction between the long-standing economic development and environmental damage from the fundamental, finally, to really achieve the “win-win” of economic development and ecological protection [3].

The circular economy is an eco-economy. A circular economy is referred to as a closed-loop flow of material based on the features of the material and energy echelon and closed-loop use, which show efficient use of resources in terms of those resources, low-pollution emissions, and even zero-emission pollution in the environmental aspects. It integrates clean production, comprehensive utilization

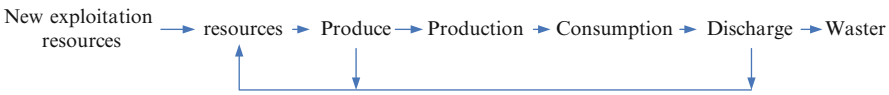


Fig. 10.1 Schematic diagram of circular economy [4]

of resources, eco-design, sustainable consumption, and use of ecological rules to guide the economic activities of human society. It is shown in Fig. 10.1. Compared with a traditional economy, the differences of the circular economy can be described as follows: The material one-way flow linear economy was based on resources, products, and pollution emissions under the traditional economy; the circular economy establishes constant material recycling based on the economic development model, which requires technical and economic activities of a natural ecosystems mode organized as the mode of the material cycle resource, product, and renewable resources. Therefore, the environmental and economic system basically does not produce waste or generates less waste. The basic trend of the circular economy is to efficiently use natural resources, protect the environment, achieve eco-efficiency, and realize eco-economic activities in the capacity of the ecological environment in accordance with the laws of ecology [4].

An eco-industrial park is established according to the theory of a circular economy and principles of industrial ecology, which indicate that a new industrial park is in harmonious coexistence with the ecological environment. In the park, companies reduce their waste emissions and use the by-products from another enterprise as raw materials to form a continuous cycle of energy, reduced emissions of the final waste, and eventually minimized destruction of the environment. At present, the terminal remediation technology can only ensure that industrial pollution discharge, while pollution still exists, and cumulative pollution might be more hazardous.

The circular economy is a systemic industry revolution and a fundamental change in the pursuit of maximizing profits from the products to follow the sustainable development of ecological laws. The 4R principles are the basic principles for the development of a circular economy. The 4R principles involve reduce, reuse, recycle, and rethink.

(1) Reduce principle

Based on the feed-in resources, the industrial chain requires a reduction in raw materials and energy, especially to control the use of environmentally harmful resource inputs and then to achieve reduced material and energy flows in the production and consumption process and conserve resources and reduce pollution from the source of economic activity. Implementation of the minimization principle requires companies to pay attention to small-size and lightweight products in the production process while minimizing the overexploitation and utilization of nonrenewable resources. If possible, companies select alternative renewable resources for product packaging to pursue the simple and plain rather than luxurious waste; moderate consumption of the product is required to achieve reduced resource requirements and waste disposal.

(2) Reuse principle
For the middle part of the industrial chain, the product, the goal of reuse is to maximize the product. According to the reuse principle, products and packaging are reused in their initial form, which avoids premature relegation to the garbage, extends the lifetime of the products and services, and improves efficient use of the products. The reuse principle requires the manufacturers during the production process not only to provide product durability and long service life but also to use standard size design for repair or replacement parts so the entire product does not have to be replaced; also, the development of a recycling and remanufacturing industry is encouraged. In daily life, the reuse principle promotes people to buy products and packaging reused as much as possible and to find other uses for the product before the items is disposed as waste, reducing pollution of disposable supplies.

(3) Recycle principle
The recycle principle is based on the output (waster), which requires the production to recycle resources after completing its function. The recycle principle can be reclosed multistage waste recycling and a resource virtuous circle and then minimizing waste. There are two levels of recycling. One is the original level; waste would be used to produce the same types of new products, such as wastepaper recycled paper, waste plastic recycled plastic scrap, and steel recycled steel, and so on. The other recycling, that is the secondary level recycling, is waste resources used to produce other types of products, such as waste plastics used to produce diesel. In contrast, the original level recycling on the efficiency of resource use is much higher than the secondary level recycling, which is the ideal pursuit for recycling in a circular economy.

(4) Rethink principle
The rethink principle belongs to the feedback method, which was first proposed by Michigan State University in the United States. The rethink principle indicates the desire not only to achieve complete material and energy closed-loop flow but also to continue to deepen, and constantly improve and think deeply about, how to avoid and minimize waste and maximize resource utilization to achieve pollution emission minimization and waste recycling maximization in the production process.

The principles of reduction, reuse, recycle and rethink are not of the same importance in the circular economy. The circular economy is not simply to recycle waste as renewable resources but stresses the priority to reduce resource and energy consumption and reduce waste generated based on the integrated use of the 4R principles.

10.4 Industrial Ecology

Industrial ecology is a new and vigorously developing discipline and is integrated and interdisciplinary. Research on industrial ecology includes the interaction and relationship between human industrial systems and the natural environment.

According to industrial ecology, the new theoretical framework of coordination development is achieved between human industrial systems and the natural environment; the operations are provided to solve problems in all disciplines and all sectors of society between industrial systems and natural ecosystems. Industrial ecology has laid a solid foundation for the theory of sustainable development [5]. The pursuit of industrial ecology is the harmonious development of human society and natural ecosystems, seeking unity of economic, ecological, and social benefits and ultimately achieving the sustainable development of human society.

Industrial ecology studies industrial production using ecological theory and methods, in which the industrial system is considered a closed system similar to the natural ecosystem. In this system, the waste generated by or by-products of an enterprise are the raw materials of another enterprise. Then, the industrial enterprises geographically proximal can form an interdependent industrial ecosystem, similar to natural ecosystems. Usually, the concepts of "industrial symbiosis" and "industrial ecological chain" are used to identify the relationship between industrial enterprises in the industrial ecosystem.

According to industrial ecology, the entire industrial system is regarded as an ecosystem. In the system, the flow and storage of material, energy, and information are not isolated relations of simple superposition. In contrast, they can run like a biological ecosystem. The interdependence of interaction and mutual influence form the complex and linked network systems. In addition, industrial ecology is the ideal industrial ecosystem and should be able to run as a full cycle, such as "zero pollution" and "zero emissions." In this state, there is no waste in absolute terms; there is waste in a sector, but it is likely the resource for another department. In an ideal industrial ecosystem, the flow of material between the four main actors can reach efficient circulation, and a variety of material flow in an input system and waste flow in an outflow system are lower than the material within the system's circular flow (Fig. 10.2).

The overall characteristics of industrial ecology include the following aspects:

(1) Through studying and optimizing overall material recycling throughout the entire process, industrial ecology is indicated from raw material extraction to production, packaging, use, and final disposal of waste. This "cycle" idea is not confined within an enterprise; it is more focused on a wide range of overall industrial system optimizations, for example, the industrial ecology park mentioned in the material that follows in this chapter. (2) Industrial ecology requires people to use a systematic mode of thinking, analysis, and design of the natural system as a whole model. (3) Industrial ecology emphasizes linking theory with practice. Because this discipline is an executable tool for sustainable development, industrial ecology also has been referred to as "sustainable science." At present, the main implementation measures taken by industrial ecology include optimizing internal recirculation in the production process, utilizing renewable resources and materials, and minimizing waste emissions and the absorption of toxic substances.

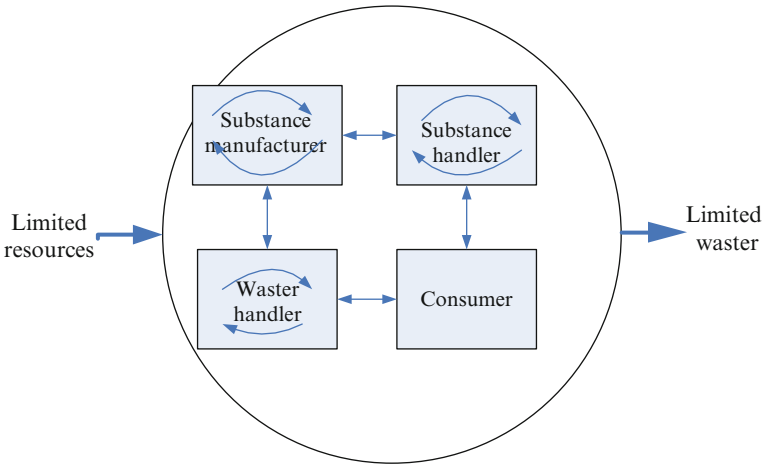


Fig. 10.2 Material circulation flow of ideal industry ecosystem [6]

10.4.1 Concept Development for Industrial Ecology

Robert A. Frosch simulated biological metabolic processes and first proposed the concept of "industrial metabolism." The modern industrial production process is a metabolic process of transformation of raw materials, energy, and labor to the product and waste. After further development, Gallopoulos put forward the concept of an "industrial ecosystem" and "industrial ecology" from an ecosystem approach. The U.S. National Academy of Sciences and Bell Labs coorganized the world's first "industrial ecology" forum in 1991. A comprehensive and systematic summary of the industrial ecology concept, content, and methods and application prospects has basically formed the framework of the concept of industrial ecology. Bell Labs thought that "Industrial ecology is an interdisciplinary study of the interrelationships between a variety of industrial activities and its products and the environment."

The world's first *Journal of Industrial Ecology* was published by Yale University and Massachusetts Institute of Technology in 1997. The journal editor, Reid Lifset, further clarified the nature and the object of study of industrial ecology in the foreword. He emphasized that "industrial ecology is a rapidly developing branch of science systems, from the three levels of local, regional, and global systems, to research products, processes, industrial sectors, and economic sectors in energy flow and logistics. The focus of industrial ecology is to research the role of industrial circles in reducing environmental pressures in the life cycle process of a product. Product life cycle includes raw material extraction and production, manufacturing, product use, and waste management."

10.4.2 Methods of Industrial Ecology

The ideas of industrial ecology include the management system concept of the whole process "from cradle to grave." That is, industrial ecology does no harm to the environment and ecology in the entire product life cycle, including raw material extraction, production of raw materials, manufacturing, product use, and product processing [7]. Industrial ecology correspondingly forms three methods: material-oriented analysis methods–industrial metabolism; product-oriented analysis methods–LCA; region-oriented research methods–regional industrial ecosystem construction [8].

10.4.2.1 Analysis of Industrial Metabolism

Industrial metabolism is a systematic analysis approach simulating the biological and natural ecosystem metabolism. Industrial metabolism focuses primarily on simulation and analysis of the material and energy flows in industrial systems according to the principle of mass conservation during the whole process, including the material as it proceeds from the initial extraction, to industrial production, to product consumption system, to the ultimate waste. Industrial metabolism would track and establish a material balance sheet that measures or estimates the quantity of material flow and its physical and chemical states, depicting the route and kinetic mechanism. An industrial metabolism application is successful or not depending on access to appropriate data and depth understanding of metabolic processes. Compared with LCA, industrial metabolism stresses describing the metabolic processes (flow and changes of mass and energy) and is not concerned about its impact on the environment [9].

10.4.2.2 Life Cycle Assessment

Life cycle assessment is an objective process to evaluate the pressure on the environment from products, production processes, and activities. The LCA identifies and quantifies the use of energy and matter and the resulting environmental waste emissions. Its purpose is to evaluate the impacts of energy and material use and waste discharge on the environment, seeking to improve the environment. This evaluation runs through the entire life cycle of products, processes, and activities, including raw material extraction and processing, product manufacturing, transportation and sales, product usage, reuse and maintenance, recycling, and final waste disposal [7].

LCA was the most important product-oriented tool of the International Organization for Standardization (ISO) 14000 environmental management system.

The ISO14000 standard was issued in June 1997. LCA is summarized as four organic associated parts: defining goals and determining the range; inventory analysis; impact assessment; and interpretation. LCA is one of the international standardization systems.

The main purpose of LCA for the product is to seek opportunities and methods to improve the environmental impact of products, to provide technical support for product eco-design and development. A series of new design concepts and methods became a hot topic of research and application in industry, such as life cycle design (LCD), life cycle engineering (LCE), design for the environment (DfE), dismantling design (DD), design for recycling (DfR), and so on [10]. LCA is the most important method in industrial ecology currently.

10.4.2.3 Eco-industrial Park Construction [11]

The material cycle works between producers, consumers, and decomposers in the ecosystem. If all materials are used, the concept of waste is meaningless. However, materials are moved in a linear fashion in industrial systems, from the manufacturer to consumer and then directly into the air, water, or garbage dump. The waste can be said a meaningless output by human activities. Usually, an eco-industrial park optimizes the overall balance of matter and energy between the different industrial enterprises in a certain geographical space, as well as industrial enterprises, residents, and the natural ecosystem, and then forms a sustainable regional body that shows efficient use of internal resources and energy and minimized emissions of external waste.

Establish an efficient eco-industrial park simulates the natural ecosystem to establish industrial logistics and a supply network, and the network is a circulation within the logistics of closed-loop recycling. Specific measures can be taken, such as the following: The new production process should maximize use of recycled materials, use raw materials and energy efficiently, and minimize waste generation and redetermine the value of the waste. In short, an efficient supply of food web relationships in the eco-industrial park should be formatted between the individuals (corporations). Therefore, there is no waste in the system, which should ensure that all waste is understood and used as a product.

Different organisms through the food chain are interdependent or mutually beneficial or beneficial for one side in accordance with the principles of ecology "symbiosis." The input and output of each production system (or business) may have a close relationship with other production systems, which can be interconnected, thereby reducing the input and output, reducing costs, and mitigating the pressure released to the environment. Of course, the actual ability of cooperation and symbiosis also depends on other factors, such as economic viability and policy support [11].

10.4.3 Research Fields and Characteristics of Industrial Ecology

In the late 1980s, academia and industry began to carry out the research and practice of industrial ecology theory from different angles and gradually formed the characteristics and main contents of industrial ecology. However, industrial ecology is a young subject. The theory and research of industrial ecology are constantly improved with the expansion of further research and practice.

10.4.3.1 Research Field and Main Contents of Industrial Ecology

The subject of industrial ecology is promotion of the modern industrial system to convert it into the three ecosystems. Conversion strategies include reusing waste as a resource, closing the material loop system and minimizing the use of consumable materials, dematerializing industrial products and economic activity, and decarburizing energy [5].

Basic research for industrial ecology mainly concentrates on the specific technical measures to reduce industrial impact on the environment, including waste discharge systems, material substitution and dematerialization, functional economic analysis, monitoring, and evaluation. Industrial ecology studies the ecological processes of the entire industry, including logistics equilibrium analysis, life cycle analysis and evaluation of the product or process, establishment of an industrial ecology index system, and so on. Industrial ecology also promotes realization of the eco-industrial system, including how to make the appropriate adjustments in market rules, the financial system, laws, and regulations to establish the idea of eco-industrialism throughout the production and living process.

The field of industrial ecology is extensive. It includes theoretical study of industrial systems and natural ecosystems, material and energy flows analysis, dematerialization and decarburization, LCA, LCD, DfE, extended producer responsibility, a product-oriented environmental policy, eco-efficiency, eco-industrial park, technological change, and the environment [12].

One of the core issues of industrial ecology research is the use of systems engineering theory and methods to achieve material integration and energy integration between processes in the industrial system. The eco-industrial integration of substances includes the integration of material transformation and exchange in the reaction process and integration of purifying substances in the separation process. The two issues need to be addressed in the integration of material transformation during the reaction process. One is optimization of the environment and economy from raw materials to the product in a single-production process; the other is the material integration of the multiple-production process. For a single-production process, its products can often have different raw materials generated through

different reaction paths and different reactor systems and operating conditions. Each path will have different wastes, so an environmentally friendly reaction path integration method and reactor network synthesis method obtained by systems engineering methods are needed to find the economic and environmental impact of a multiobjective optimal production program [13].

The industrial ecosystem with a closed loop of logistics is the emphasis of industrial ecology, which has become an important environmental policy in the United States and Europe. Industrial ecology emphasizes the closed loop of material to reduce the demand of the environment as a source or sink. Xerox Corporation used this integrated approach, and their asset management plans were involved in every aspect of manufacture, use, and handling of substances through comprehensive methods such as reusing, remanufacturing, and recycling to close the loop of substances. Kalundborg in Denmark, under the constraints of environmental regulations, formed a closed loop of material flow through economic levers.

The purpose of the dematerialization of industrial products is that the investment of resources in industrial production should be equal to the environmental capacity. An important way to achieve dematerialization is to provide the functions or services instead of providing the product itself. It is necessary to develop a "functional economy" to exert the maximum "usage" rather than the material goods "consumption." A consumer society requires shorter product life cycles. In industrial ecology, some methods can be designed to achieve these requirements, such as the recycling of products and upgrading technology; integrating different products, contracts, and services supply; transferring products to other users (such as developing countries) or returning them to the manufacturing chain; and so on, finally making products a factor in the economy and generating benefits. The concept of remanufacturing and circulation is different. The former refers to the extension of the production chain, not just the material flowing in a closed loop. To achieve remanufacturing, the economic structure should be adjusted accordingly. In general, the technological content and added value are higher at the end of the production chain. Therefore, the extension of the production chain should correspond to human resources and technical level [14].

The energy integration of the eco-industry is to achieve effective use of energy within the ecosystem, which not only includes the effective use of energy within each production process, usually composed of the steam power system and heat recovery network, but also includes energy exchange in each process. Increasing energy efficiency and reducing energy consumption not only can save energy but also can mean reduction of environmental pollution [13]. The systematic use of energy is the most important part of industrial ecology. Energy production and consumption are the core of industrial economy. Energy characteristics implied in the product are not apparent as features of the product itself; the use of energy is not in the processing and manufacturing process of products but in the process of the use of products, such as cars, refrigerators, and air conditioners. The energy consumption of a manufacturing process is negligible compared with the service life.

10.4.3.2 Characteristics of Research on Industrial Ecology

It can be seen from the brief introduction that the object of the study of industrial ecology is a complex system. To solve the problem, industrial ecology must use different methods to carry out research from a different perspective. Therefore, an obvious feature of industrial ecology is the broad research area. During the research and development, the basically theoretical framework of industrial ecology has been gradually established, forming its own characteristics, mainly in the following aspects [15]:

(1) Observing and solving problems in view of system theory
First, the industrial system is regarded as a subsystem of the natural ecosystem, and the subsystem is a low level of the evolutionary subsystem. System analysis is used to study the interaction and relationship of the industrial system and natural ecosystem to promote the industrial ecosystem to a high-level ecosystem so that the industrial ecosystem has coordinated development with the natural ecosystem. Second, in terms of the industrial ecosystem, the industrial system is still a subsystem of the natural ecosystem in the human socioeconomic system. To resolve the conflicts between the industrial system and the natural environmental system, it is necessary to understand the contradiction between the natural ecosystem and the industrial system and address the various problems between the industrial system and the socioeconomic system and other subsystems. Therefore, we must adopt comprehensive and integrated research methods of complicated systems to deeply understand the relationship and problems and propose solutions to problems. In addition, industrial ecology emphasizes the global system of research, not only to consider and solve the environmental impact of human industrial activities on local and regional systems but also to focus on considering and resolving the impact on the life support systems of Earth.

(2) Multidisciplinary cross and integration
The object of industrial ecology is the relationship between the natural ecosystem and the human social-economic system; the issues involved are extremely complex, involving not only the problems of natural sciences but also engineering and technical disciplines and the humanities and social scientific problems. Therefore, the study of industrial ecology has surpassed the boundaries of disciplines and become multidisciplinary research. Then, the complexity will gradually fuse to form a new discipline with research continuing in depth.

(3) Core research of industrial products and services
The industrial system is the subsystem that provides products and services to the social-economic system. The matter and energy of the industrial system and the natural ecosystem are exchanged and thus have an impact on the whole movement of the product (including raw material collection, raw material production, product manufacturing, product usage, product reuse, final disposal of products). Therefore, the current research in industrial ecology is carried out concerning the entire movement of products, for example, the flow analysis of raw materials and energy, design and evaluation of product, product-oriented environmental policy, and so on.

(4) Quantitative analysis method
The quantitative analysis method is a prominent feature of industrial ecology. The research emphasizes that industrial ecology must go beyond the descriptive analysis of the past, borrow quantitative analysis tools of other disciplines, or create new quantitative analysis methods and analyze the environmental impact of industrial systems and give quantifiable results. At present, industrial ecology has made considerable progress in the quantitative analysis method.

(5) Strong operability
The method of industrial ecology has strong operability, such as the assessment of the product life cycle and eco-efficiency.

(6) Challenging work
The object of industrial ecology is a complex system, with many areas and the issues that need to be explored and resolved, providing challenging work.

10.4.4 Significance of the Study of Industrial Ecology

The study of industrial ecology is of great significance, which is mainly presented in the following aspects [16]:

(1) Fundamental solution to the problem of conflict between industrial systems and natural ecosystems
The industrial system is regarded as a subsystem of the natural ecosystem, and the subsystem is a low level of the evolutionary subsystem. To maintain the harmonious development of the natural ecosystems, the senior ecosystem evolution was studied theoretically. Thus, industrial ecology involves the fundamental problem in the long development of the industrial system and not just environmental issues, so that fundamentally solving the contradictions between industrial systems and natural ecosystems provides a solid foundation to improve not only the theoretical basis but also the development of the theory of sustainable development.

(2) Significant impacts on other disciplines
Industrial ecology proposes many problem theories and methods to be studied with a new way of thinking. These problems are related to the major issues of sustainable development, involving all disciplines, requiring study and enrichment by researchers of various disciplines. The further research of industrial ecology will have a profound impact on many disciplines, such as environmental science, engineering, economics, management, and law. With the in-depth development of industrial ecology, there will be a new group of industry experts: designers of the eco-industrial park, analysts of matter and energy flow, experts on dematerialization and industrial nutrition, and so on.

(3) Powerful solutions to improve the competitiveness of enterprises
By means of complex and scientific theories and the awareness of the ecosystem, industrial ecology could find innovative ways to make the industrial system and

the natural ecological system normally run by considering the entire life cycle of products, optimizing material and energy flow, and advocating comprehensive and integrated concepts to solve the biological-ecological benefits of enterprises and so on. Therefore, industrial ecology achieves a comprehensive solution for the full use of resources and pollution control; in the meantime, it improves the competitiveness of enterprises and provides a theoretical approach and practical strategy.

10.5 Industrial Ecology Park

An eco-industrial park is a new form of industrial organization designed according to the circulation economic theory and principles of industrial ecology. The logistics and energy flow of eco-industrial parks are designed with efficient economic process and harmonious ecological functions of network-based industrial evolution through proper simulation of natural ecosystems based on the carrying capacity of the ecosystem. An eco-industrial park is the gathering place of eco-industry. It consists of a number of enterprises, natural ecosystems, and residential areas. These components cooperate with each other and develop into a regional system with local communities. The eco-industrial park is the fundamental path to solve the dilemma of limited resources and low development level. It is beneficial for increasing employment, controlling environmental pollution, and reducing pressure on the environment [17].

10.5.1 Construction Objectives and Features of an Eco-industrial Park

The construction of an eco-industrial park needs to rely on local resources to expand the industry metabolic chain and waste metabolic chain among the major industries according to the guidance of the recycling economy and industrial ecology theory. Then, a perfect network of an ecological industry chain is formed to achieve efficient use of resources and minimize pollutant emissions. This further strengthens a region's transformation and industrial restructuring to establish a quality management system and environmental management system and build an eco-industrial park with sound infrastructure, optimized structure, rational layout, and complete equipment to coordinate the development of economy, society, and environment.

The State Environmental Protection Administration (SEPA) has issued a number of important documents and clearly proposed the basic requirements to promote the construction and development of eco-industrial parks since 2003. SEPA released a comprehensive eco-industrial park standard in 2006, the industry categories of an eco-industrial park and an industrialization-based eco-industrial park; it further proposed eco-industrial park development goals. Goals and indicator systems for comprehensive eco-industrial park development are shown in Table 10.1.

Table 10.1 Comprehensive eco-industrial park development goal indicators system [17]

Project	Number	Index	Unit	Index value
Economic development	1	Industrial added value	Million Yuan/unit	≥15
	2	Industrial added rate		≥25 %
Material reduction and circulation	3	Comprehensive energy consumption per unit industrial added value	t/Million Yuan	≤0.5
	4	Freshwater consumption per unit industrial added value	M^3/Million Yuan	≤9
	5	Wastewater generation per unit industrial added value	t/Million Yuan	≤8
	6	Waster per unit industrial added value	t/Million Yuan	≤0.1
	7	Repeated use rate of industrial water		≥75 %
	8	Comprehensive use rate of industrial waster		≥85 %
	9	Water reuse rate		≥40 %
Waster control	10	COD per unit industrial added value	Kg/Million Yuan	≤1
	11	SO_2 of per unit industrial added value	Kg/Million Yuan	≤1
	12	Hazardous waste treatment rate		100 %
	13	Life centralized sewage treatment rate		≥70
	14	Domestic garbage harmless treatment rate		100 %
	15	Waste collection system		Yes
	16	Waste disposal facility		Yes
	17	Environmental management system		Perfect
Management	18	Perfect information platform		100 %
	19	Environmental report		One/year
	20	Public satisfaction with the environment		≥90 %
	21	The public on ecological industry recognition rate		≥90 %

Data sources: http://www.zhb.gov.cn/tech/hjbz/bzwb/other/qt/20060901_77200.htm

10.5.2 Industrial Ecology Park Design

The detailed understanding of industrial location and existing enterprises is the first work in the design of an industrial ecological park. The overall plan should emphasize the inner loop and strive to form a closed-loop approach. The method is to reasonably introduce industry and enterprise potentially synergistic and symbiotic with the original business. In addition, it is also important to improve

the background system of these enterprises, optimizing the material, energy, and information flow [18].

(1) *Material flow.* There is no absolute "waste" in industrial ecology park because a company's waste is likely another potential raw material [19]. Companies strive to optimize the use of materials in the industrial ecology park, minimize the use of toxic materials, and use a common processing equipment for toxic waste. In addition, designers of an industrial eco-park will be conscious to attract a number of companies to engage in resource recovery and recycling, to deal with by-products, and to provide renewable raw materials for manufacturing enterprises in the park.
(2) *Energy flow.* Companies in an industrial ecology park will achieve the purpose of improving the efficiency of resources through the design of the building, lighting, and infrastructure [19]. For example, the cooling water from a company is oriented to provide heat for another company or civilian heating system. In addition, many infrastructures in an ecological park are experimenting with renewable resources, such as wind and solar energy.
(3) *Park management and service support systems.* The management system of an industrial eco-park must be mature and powerful and able to coordinate all intercompany exchange of by-products. The management system could help the company adapt to the sudden change of the entire cycle or certain parts. An industrial ecological park could also share some support services to reduce costs, such as training centers, restaurants, transportation, logistics, as well as the purchase of common supplies [19].

10.5.3 Building of Industrial Ecology Park

An industrial ecology park needs to attract different "residents," not only large companies, but also incubator companies, local businesses, and environmental technology companies. The location of an industrial eco-park, natural environment, resources, transportation, infrastructure, material flow of information, and so on constitute a comprehensive set for an industrial ecosystem, which is significant for attracting new members and remaining existing business sense. Offering good conditions is a convincing means to enhance attraction the industrial eco-park.

With long-term effects of the development of the traditional industrial model, the problems of environmental awareness are serious. If people want to promote the plan of an industrial ecology park and achieve long-term results, they have to deepen ecological thinking, not only to strengthen environmental education and training to member companies in the industrial ecological park but also to introduce environmental and ecological literacy to every inhabitant. People should be made to realize a common mission that industrial ecology will create more employment opportunities for the whole community, protect the environment, and achieve higher social value.

10.6 Comprehensive Utilization of Lignocellulose and Process Analysis of the Ecological Industry

Research showed that comprehensive utilization of lignocelluloses has become the key factor of biomass industrial development. The key of oil utilization is to use and exploit all parts of oil, so it is the same for biomass utilization. According to the research mentioned, the following section mainly introduces the comprehensive utilization technology and ecological industry process of lignocelluloses and explores the eco-industrial model and feasibility for the comprehensive utilization of lignocelluloses from the perspective of green chemistry. The guidance of research is cleaner production and sustainable development.

Because the chemical composition and structure of the cellulose are basically clear, it is natural to consider the possibility of sugar utilization. These sugars can be converted to ethanol and feed by microorganisms; the production process is more mature. The saccharification technology for lignocelluloses was been introduced previously, so the following section mainly introduces the production process for lignocelluloses.

10.6.1 Direct Conversion of Lignocelluloses

Full utilization of lignocelluloses has been used in production processes, such as methane fermentation and mushroom cultivation, which are the early stage of lignocellulose complete utilization. Full utilization of lignocelluloses developed a more advanced three-component mode after the introduction of biomass fractionation, making separated component use reasonable. Direct conversion changes materials into products in only one step with a simple process. Direct conversion will avoid the cost of more preprocessing and not consider the production process of different components. But, direct conversion still has some disadvantages, such as low rate, single product, and low additional value. The main ways of direct conversion include direct combustion, thermochemical conversion, methane fermentation, mushroom cultivation, and feed fermentation directly from straw.

(1) Direct combustion

Direct combustion of lignocellulosic materials is the most widely used method of biomass energy conversion. In addition to the direct heat, direct combustion can achieve electric power. Compared with fossil fuels, the heat output of direct combustion is low (in particular, some sugars have lower heat output during direct combustion), and the energy efficiency is low; these would cause environmental pollution. Therefore, direct combustion would not be advocated. But, direct combustion is still a form of end environmental governance, such as combustion of solidified municipal waste and black liquor from pulping industry.

(2) Thermochemical conversion

Thermochemical conversion decomposes natural lignocelluloses into liquid and gaseous fuels at high temperature. Thermochemical conversion can improve fuel quality and energy efficiency and enhance energy density, making them easy to store and use. In addition, the natural cellulose have a relatively high content of hydrogen and carbon, almost no sulfur, and low ash, so they are easier to handle than coal. Thermal decomposition is the center of thermochemical conversion. The natural cellulose materials are converted into a variety of products by thermal decomposition in the absence of air or a small amount of air or steam. The pyrolysis process of thermal decomposition is carried out without oxidants or under low-oxidant conditions, which is generally an endothermic reaction. The polymer compound is decomposed into a low molecular product, such as hydrogen, methane, carbon monoxide, carbon dioxide, and other gases. The liquid portion of polymer compound includes methanol, acetone, acetic acid, and other organic matter, such as tar, solvents, oil, and aqueous solution. The solid part is mainly carbon black. Burning is an exothermic reaction whose main products are carbon dioxide and water.

The composition and ratio of pyrolysis product vary with thermal decomposition conditions.

① Gasification

The gas composition of thermal decomposition varies with materials, gasification gas medium (gasification agent), devices, and heating conditions. There could be 20–40 % fuel gas received from coal in the process of gasification, while from natural cellulose materials, it is 70–80 % fuel gas. Using air as the gasification agent, because of nitrogen mixed with the generated gas, the gas has low calorific value (700–1,800 kcal/m^3). With oxygen as the gasification agent, the calorific value of gas is 2,500–4,500 kcal/m^3. The difference of gasification and carbonization is that, in the gasification process, the pyrolysis material and carbonized residue continue to react with air, steam, oxygen, carbon dioxide, or hydrogen; that is, some materials are burned in controlling conditions, so that the rest is converted into a gas with low calorific value. But, the heat generated in the exothermic process can be used to maintain the reaction temperature required in the gasification system. However, gasification is different from direct combustion because of its extremely rare smoke. A gas furnace is the equipment to achieve the gasification process.

② Liquefaction

Gases from high-speed thermal decomposition are converted to diesel, oil, or gasoline, or CO_2 and H_2 are converted to a mixture of ethanol-containing methanol in the presence of catalysts and pressure conditions. A liquefaction reaction can run in the presence of hydrogen or carbon monoxide.

③ Dry distillation

Dry distillation can produce a mixture of solid, liquid, and gaseous fuels. The solid product is wood vinegar. The wood vinegar and flammable wood gas can be separated from the gas mixture through condensation in the dry distillation kettle.

The gas product is wood gas, whose main components are carbon dioxide, carbon monoxide, methane, ethylene, and hydrogen.

In short, research of the thermochemical conversion of natural cellulose materials has taken place since the 1940s. More than 100 research investigations on gasification and thermal decomposition were performed in the United States from 1984 to 1990. However, studies in this area are still in the development stage and has not yet industrialized. Generally, the requirements for thermochemical conversion technology is high, and the cost is also high.

(3) Methane fermentation

Methane fermentation is the process of producing mixture gas mainly of methane and carbon dioxide with the crop straw, livestock and human waste by microorganisms under anaerobic conditions. Methane is used as a fuel for life; its thermal efficiency is up to 60 %, six times higher than the thermal efficiency of straw direct combustion. Biogas slurry and residues can be directly used for feed and high-quality organic fertilizer. The calorific value of an ordinary methane fermentation pool is about 22–25 MJ/m^3 (5,200–5,900 kcal).

It is a fundamental change in the fuels history of China and the world to utilize straw and human and animal feces to produce biogas instead of firewood and coal. Methane fermentation not only can solve fuel problems but also can expand the source of feed and fertilizer, which is beneficial to the control of pests and diseases.

Methane fermentation technology has been applied widely. Enhancing the fermentation of efficient bacteria and shortening the fermentation cycle need further research in the future, which will help to explore the wide application of raw materials and efficient bioreactor systems.

(4) Edible mushroom cultivation

Some edible fungi, such as oyster mushrooms, dried mushrooms, and other wood-rotting and grass-rotting fungi that belong to the basidiomycetes of white-rot fungi, have the strong decomposition ability of crude cellulose. They can directly use and degrade cellulose, hemicellulose, and lignin in agricultural waste. Waste materials from the harvested edible fungi fine fibers, especially lignin in them, can be greatly reduced and thus can be directly used as feed additives. The equipment for edible mushroom cultivation is simple, and raw materials are wide and cheap. Combining the production of edible mushrooms and the improvement of straw feeding value is important in rural areas in China [20]. Zhen studied the feasibility of the cultivation of rare edible *Pleurotus geesteranus* in cornstalks; results showed that cottonseed hull could be completely replaced by corn stovers to cultivate mushrooms, and the biological efficiency was not significantly different. The biological efficiency improved 10.47 % in cornstalks instead of sawdust. So, this method not only can save wood but also is conductive to maintaining ecological balance [21]. Yao studied the cultivation feasibility of *Flammulina velutipes* by rape straw stalks; the results showed that the hyphae had the strongest growth with a substrate of 66 % rape straw, 22 % cottonseed hull, 10 % wheat bran, 1 % sucrose, and 1 % superphosphate. The bud stage, the bud-pressing stage of the fruit body, and the fruiting period were all shortened, which would increase economic efficiency [22].

(5) Biological protein feeds from straw fermentation
Straw is a low-quality concentrated feed with a crude fiber content of 30–50 %. So, the digestibility for direct use as ruminant beverages is low (40–50 %), which shows that straws are not suitable for monogastric animals and have a limitation in further use. Therefore, exploring straw microbial fermentation to improve the nutritional value of straw and expand the feeding range has become a major topic of widespread concern in all countries of the world.

There are three key points of straw microbial fermentation of protein feed: the microbial degradation rate of crude fiber, the improved rate of nutritional value (crude protein), and the cost of production. It is necessary to first breed microorganisms that could degrade cellulose, hemicellulose, and lignin, the crude fiber degradation rate of which is at least 50 %, and the crude fiber content is controlled below 25 %. The second point is to improve the protein content up to 20 % and accumulate the corresponding organic acids, and vitamins and cellulase, starch enzymes, and lignin enzyme activity, as well as substances that are beneficial to flora growth. Third, considering the production cost, the current one-step solid-state fermentation has much lower costs than the liquid fermentation process. In addition, we should correctly understand the multinutritious nature of bioprotein feed, such as the presence of dietary fiber, digestive enzymes, and bifidus growth factors.

10.6.2 Industry Overview of Lignocellulose Comprehensive Utilization

The comprehensive utilization of lignocellulose is centered on the fractionation of components to convert three components individually into the needed products. As shown in Fig. 10.3, the comprehensive utilization of lignocellulose has been widely applied in all kinds of industries, such as the energy, food, feed, pharmaceutical, paper, chemical fiber, materials, flavor, construction, oil, fertilizer, and chemical production industries and so on. The ultimate goal is that lignocellulose can be the alternative for materials in the chemical and fermentation industries. Eventually, the new ecobiological industry system without oil and food as substrates would be explored.

(1) Eco-industrial model of lignocellulosic microbiological conversion
Material production in nature is a clean process. Through the bionics principle, the rules of the nature should be learned and applied in industry. This production process is called ecological technology. Eco-industry is a combination of industry and ecology, and through ecological technology, it accelerates industrial material recycling and resource utilization in industrial ways. The ecological industrial model of lignocellulose microbial conversion is shown in Fig. 10.4.

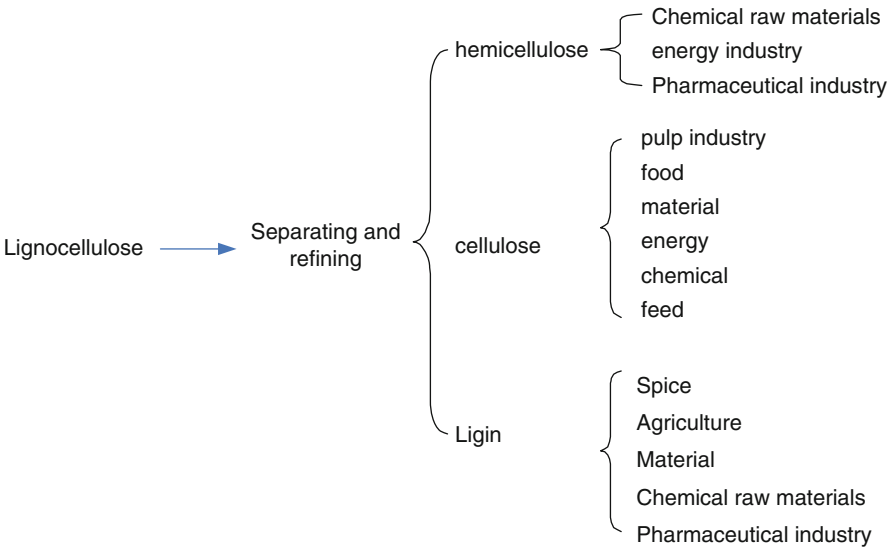


Fig. 10.3 Comprehensive utilization industrial system of lignocellulose

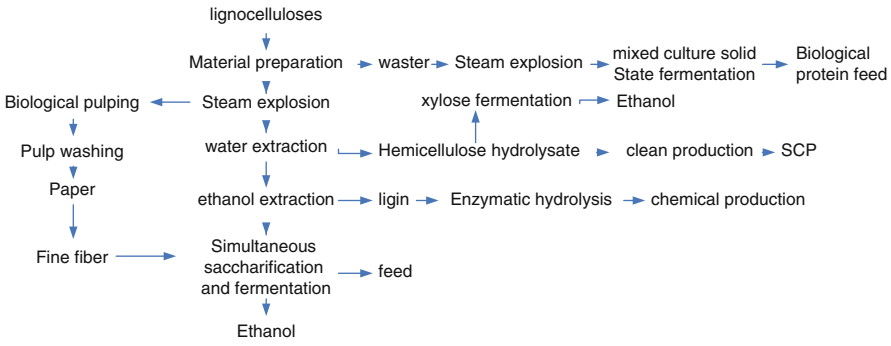


Fig. 10.4 Ecological industrial model of lignocelluloses

(2) Cellulose industrial system

Based on the separation of components, each component of lignocellulose can be synthetically and efficiently utilized. The following is a description of products of the industrial production sector in cellulose, hemicellulose, and lignin industrial systems and an analysis of the status and role of the microorganisms in several industrial sectors:

① Paper industry

In the paper industry, the purpose of comprehensive utilization of biomass refers to the comprehensive utilization of lignocellulose which would ease serious pollution problems. The process of the paper industry combined with new engineering

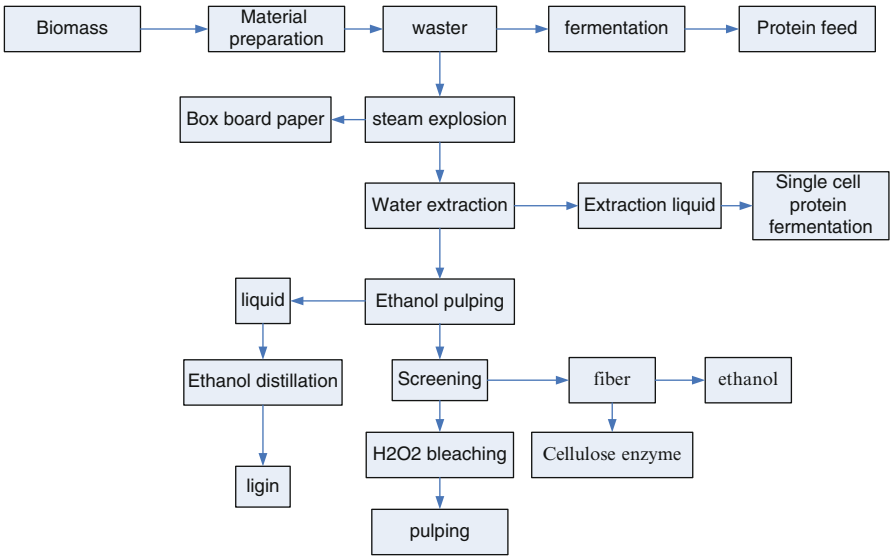


Fig. 10.5 Ecological industrial model of biomass comprehensive utilization in the papermaking industry

technologies is to achieve the commercialization of biomass comprehensive utilization and to become suppliers of chemical raw materials, organic fertilizers, and protein feed.

The main research on microorganisms applied in the paper industry involves biopulping and biobleaching. Biopulping is the process of plant fiber segregation under microorganism treatment. Microorganisms can degrade lignin and keep cellulose and hemicelluloses at a maximum degree; therefore, raw materials and energy are saved, and the process is helpful to ecological and environmental protection. Biobleaching decomposition would remove the residual lignin to achieve bleaching pulp with microbial treatment, which would prevent the destruction of cellulose and hemicellulose in chemical bleaching and increase pulp yield; in particular, it avoids the environmental pollution caused by the chemicals. Biopulping and biobleaching have been included in the scope of the leading-edge technology in biology and the paper industry. The new technology on the paper industry of lignocellulose with the principle of comprehensive utilization is shown in Fig. 10.5.

From Fig. 10.5, it can be seen that selecting the appropriate production process and route would effectively separate components and primary products. In the meantime, the raw materials are maximally used to achieve by-products that are more valuable, thereby realizing the complete utilization of materials. This actually is the manifestation of a recycling economy and ecological engineering in the lignocellulosic industry.

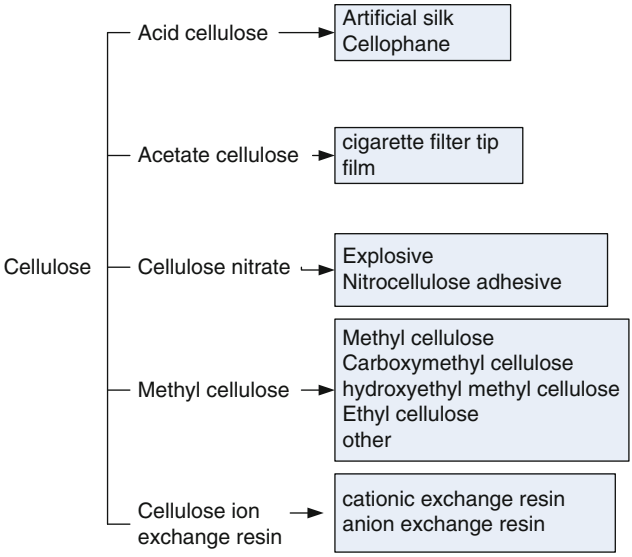


Fig. 10.6 Applications of lignocellulosic resources in the chemical fiber industry

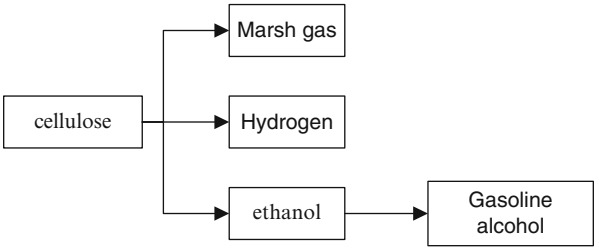


Fig. 10.7 Production from lignocellulose in the energy industry

② Chemical fiber industry
Figure 10.6 shows the application of lignocellulosic resources in the chemical fiber industry.

③ Energy industry
As shown in Fig. 10.7, the products of the energy industry from cellulose are primarily achieved through microorganisms. The energy used is clean energy that is part of the natural energy cycle, so the application of lignocellulosic resources in energy is a promising industry.

④ Feed industry
As shown in Fig. 10.8, production in the feed industry is completed by microbial fermentation, and some have already started industrial production, such as cellulose enzyme in feed, single cell protein, but some need a further breakthrough on cost, such as artifical rumen.

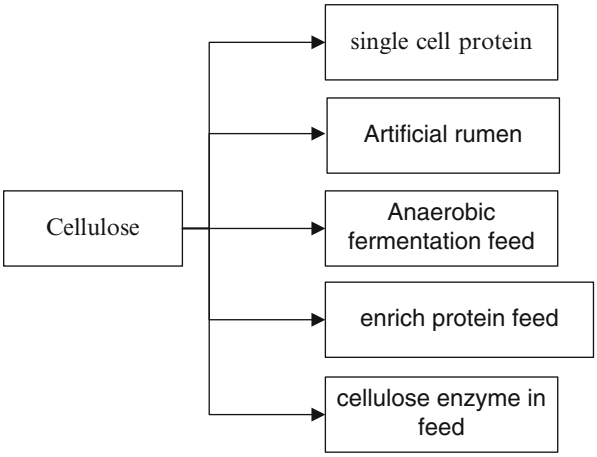


Fig. 10.8 Production of lignocellulose in the feed industry

⑤ Food industry
Cellulose is mainly processed into dietary fiber in the food industry. Modern medicine has proved that people are susceptible to digestive diseases, especially intestinal disease, when the body lacks fiber. Dietary fiber is one indispensable ingredient of future nutrients.

⑥ Materials industry
As shown in Fig. 10.9, the production of various products from lignocellulose would be a new development direction in the materials industry. Especially, the development of cellulose copolymerization technology is attractive.

⑦ Chemical raw materials
As shown in Fig. 10.10, the production process for chemical raw materials is mainly achieved through fermentation. Lignocellulose can be broken down to monosaccharides by enzymes, which not only can be used to produce chemical raw materials but also can produce a range of fermentation products as substrate for the fermentation industry.

(3) Hemicellulose industrial systems
Hemicellulose can be used for the production of chemical raw materials that can be used in the pharmaceutical, energy, and feed industries.

① Chemical raw materials
As shown in Fig. 10.11, using hemicellulose to produce xylose, xylitol, and furfural is the main form of production in the hydrolysis industry. In addition, glycerol and ethylene glycol can be produced through the degradation. These products should be gradually replaced by microbiological processes.

② Energy industry: Products are as shown in Fig. 10.12.

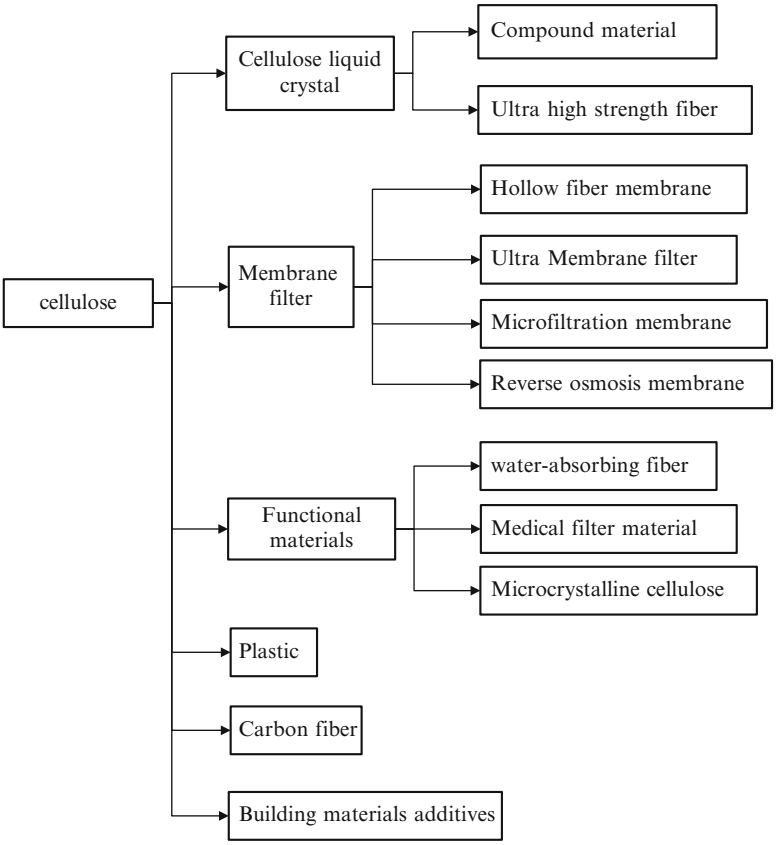
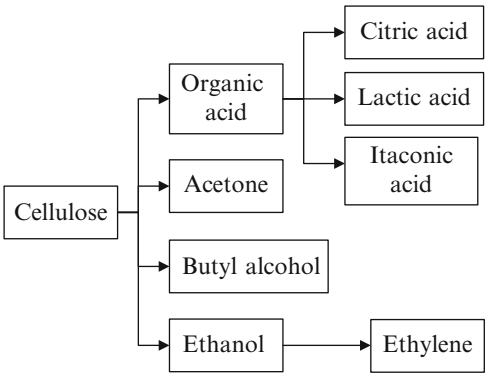


Fig. 10.9 Products from lignocelluloses in the materials industry

Fig. 10.10 Chemical materials from lignocelluloses



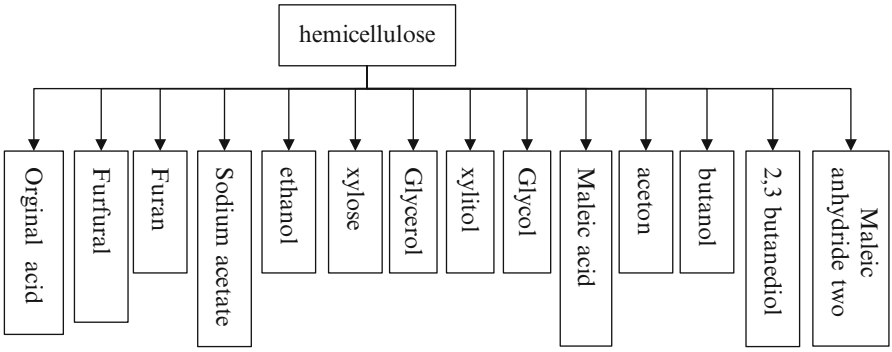


Fig. 10.11 Chemical materials from hemicelluloses

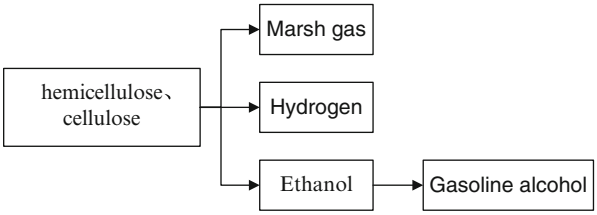


Fig. 10.12 Energy productions from hemicellulose

③ Feed industry
Production of single-cell protein (SCP) through hemicellulose fermentation is the main application of hemicellulose in the fermentation industry. This is especially suitable for the processing of paper waste.

(4) Lignin industrial system
Lignin is a natural organic polymer that widely exists in seed plants. It is also the enhancing system in the plant skeleton. The amount of lignin is more than that of hemicellulose but less than that of cellulose. About 50 billion tons of lignin can be generated on Earth through photosynthesis in plants per year. Over the past few years, with the resource crisis and the growing awareness of the human environment, how to effectively utilize the natural renewable resources has been strategically mentioned by many countries. Considering efficient management of water pollution and high-value utilization of solid waste, plus considering the saving of polymer materials and other nonrenewable resources, the utilization of lignin has a significant economic value and far-reaching social influence [23].

① *Fragrance industry.* Vanillin was synthesized from lignin more than 100 years ago. The production process is mainly via the glyoxylic acid and guaiacol route. Vanillin can be used as flavoring for chocolate, ice cream, cookies, and drinks.

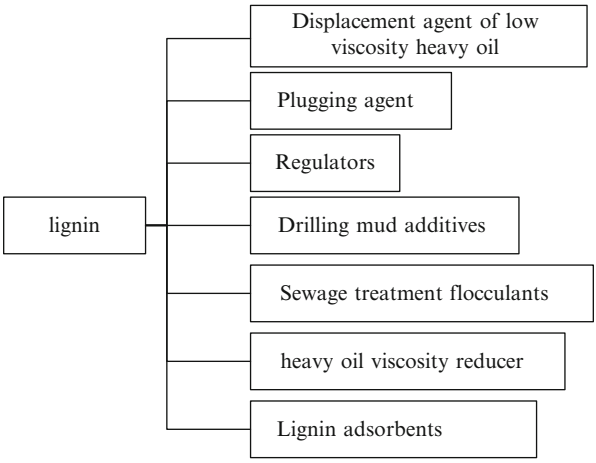


Fig. 10.13 Application of lignin in the petroleum industry

② *Petroleum industry.* Petroleum has a close relationship with lignin; they have many similar structural units and linked bonds. Therefore, the white-rot fungi are applied in oil biotransformation and biological processing, with advantages of cleanliness and efficiency, which is a promising research direction. Many of the existing oil industry products can take advantage of lignin for production, as shown in Fig. 10.13.

③ Pharmaceutical industry
Lignin as a raw material can be produced by a variety of pharmaceutical preparations, which has great significance for the rapid development of the pharmaceutical industry today. Application of lignin in the pharmaceutical industry is shown in Fig. 10.14.

④ Application in agriculture
Figure 10.15 shows that the lignin molecular structure contains a variety of active groups, and the lignin can be slowly degraded by microorganisms in the soil, converting it to humus, leading to the inhibition to soil urease activity and the promotion of plant growth and soil improvement [24].

⑤ Chemical raw materials. Production of chemical raw materials is indicated in Fig. 10.16.

10.7 Problems in the Comprehensive Utilization of Lignocellulose

The comprehensive utilization industry for natural lignocellulose is a promising industry, but there are still many issues [26].

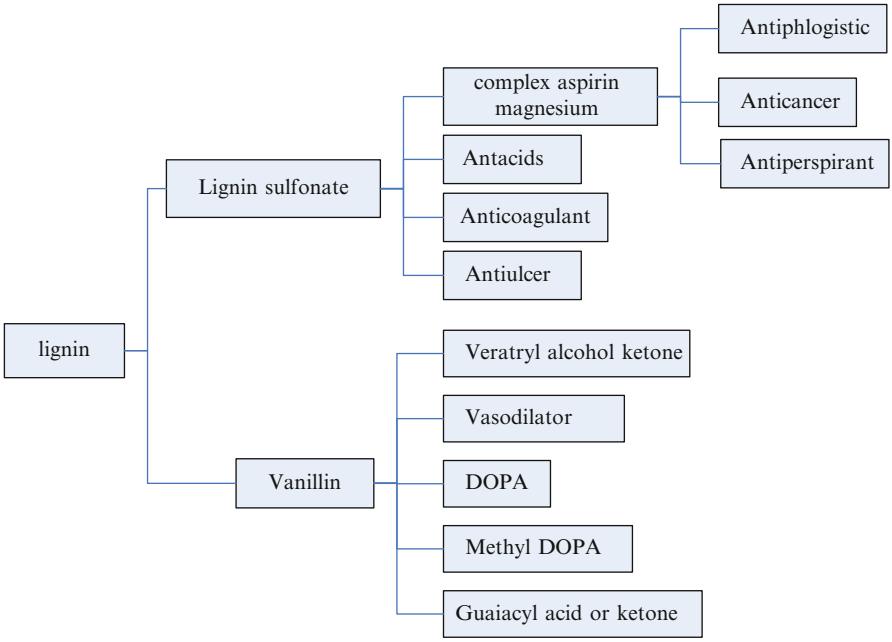
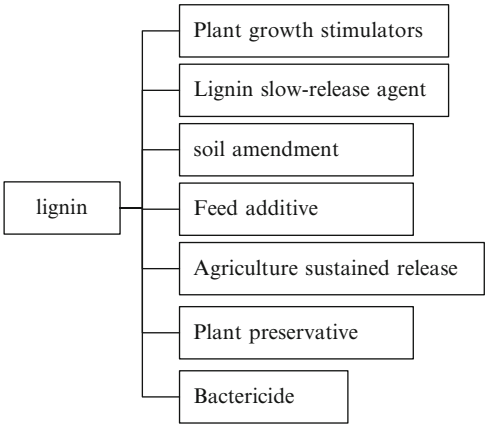


Fig. 10.14 Application of lignin in the pharmaceutical industry

Fig. 10.15 Application of lignin in the agriculture industry [24, 25]



(1) Utilization of a single component

Existing factories only use single components of natural lignocellulose, such as furfural plants, paper mills, xylitol plants, and so on. Therefore, the factories should fully understand that the other components are not waste but resources and even have potential productions to be explored. But, these utilization model provide a start for the use of natural lignocellulose feedstock.

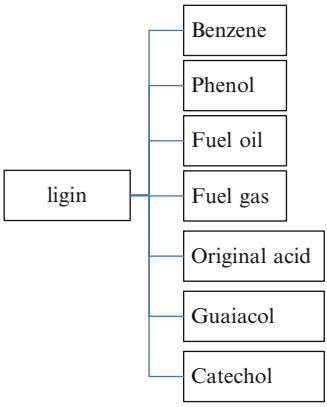


Fig. 10.16 Chemical productions from lignin

(2) Imbalanced development of technology
The concept of comprehensive utilization of lignocellulose enables people to gradually attach importance to the study of hemicellulose and lignin. Although great progress has been achieved, there is a certain distance from the research and development of cellulose technology. The complex structure of the lignin molecule varies greatly with different sources of lignin, but it has broad application prospects as an aromatic compound; it broadens the usable range of natural lignocellulosic feedstock. Therefore, lignin deserves more attention.

(3) High cost of enzymolysis to conversion
There are many reasons for the high cost of enzymolysis to conversion, mainly shown in the high cost of cellulose pretreatment, the low cellulase activity, and high operating costs. With the development of science and technology, the problem of high cost would be gradually resolved.

All analyses mentioned show the comprehensive utilization of lignocellulose will be the application field of pulp manufacturers. Their aim is to improve the utilization rate of raw materials to alleviate the serious air and water pollution problems. Only pulp manufacturers may achieve full commercialization of biomass. At the same time, the pollution problem is a typical characteristic of modern pulping technology. Pulp suppliers will need new engineering technology for their processes, so the pulp industry has the opportunity to become the supplier of chemical raw materials, organic fertilizers, and protein feed.

The comprehensive utilization of lignocellulose would not be achieved in one night in pulp production based on processing and utilization. But, when the factories need to create or transform an old factory, the comprehensive utilization of lignocellulose would play its proper role, so that the pulp manufacturer would become the supplier of precursor chemicals [27].

10.8 Application of Ecological Industry Concept in the Comprehensive Utilization of Lignocellulose

According to the requirements of ecological engineering and ecological agriculture, the significance of bioconversion and comprehensive utilization of natural lignocellulose have been studied. The major eco-industrial productions by modern biotechnology are renewable clean energy; pollution-free biological pesticides; and biological nitrogen fixation of organic fertilizer, microbial feed, and feed additives, which would advance the level of eco-agriculture. Therefore, the technology system for comprehensive utilization of natural lignocellulose is proposed in the following section, and the key technology and its features to break through the barriers of economy and technology are involved.

10.8.1 Eco-industry and Its Role in the Industrial Structure of Ecological Agriculture

According to socioeconomy, human activities are divided into three industries: agriculture, industry, and tertiary industries. From the original point of view, agriculture is an integral part of the natural ecological cycle. Industry is not an inherent process in a natural ecological cycle; it is a product of human activities and wisdom. Industrial ecology is proposed according to ecological principles, which organize the industrial production process for sustainable development, such as resource regeneration and recycling, the harmless production process to the ecological environment. In an agricultural society, activities are completely limited within the natural ecological cycle. In an industrial society, human activities have jumped out of the scope of the cycle. It seems that "everything becomes possible," but the result is that survival risk appeared. To deny the survival basis would result in denying existence itself. But, human development cannot be passively limited to the range of nature. The only way out is to create an artificial ecosystem within the Earth's bearing capability, which is the connotation of industrial ecology. Similarly, the development of ecological agriculture cannot be confined within the scope of the agricultural natural ecosystem, involved with the agricultural artificial ecosystem, which can coordinate with the agricultural natural ecosystem. The eco-industry is a branch of the agricultural ecosystem in the modern biotechnology industry. The biotechnology industry includes the following: the biomass renewable energy industry, the organic fertilizer industry of biological nitrogen fixation, biopesticide industry, and feed industry of the new microbial feed additive industries. In short, the biotechnology industry will provide services to the farming, aquaculture, and agricultural products process and promote and enhance the ecological agriculture circulating level. All the main raw material of eco-industry mentioned is lignocellulose.

10.8.1.1 Biomass Energy Industry

All fossil fuels (coal, oil, and gas) are the remains of biomass in ancient times, is nonrenewable, and increasingly dried up. People can only use renewable energy and the nucleus (artificial sun) in the future, which is inevitable. Renewable energy is transformed by solar energy in nature, and hydroenergy and biomass are relatively concentrated, which facilitates large-scale development and utilization. Hydroelectric power belongs to electrical and mechanical technology, which are mature. The utilization of biomass energy belongs to biotechnology, which is not yet ripe, so it becomes the first choice of significant basic research in renewable energy projects, such as the National Basic Research Program of China (973 program).

The same as for fossil fuels, the conversion forms of lignocellulose include gas, liquids, and solids. As a solid fuel, the generation of heat by burning is the most primitive method and has been used since ancient times; some new progresses has been made in thermal power generation and biofuel cells. There are two routes of lignocellulose conversion into gas fuel; the chemical transformation is an incomplete combustion system for gas; the biotransformation method is methane fermentation. Lignocellulose can be transformed into liquid fuels to replace oil, which is the easiest form of fuel for existing agricultural machinery and equipment. There are also two technical routes; the chemical conversion method is fast pyrolysis to make bio-oil, and biotransformation is fermentation to produce ethanol, acetone, and butyl alcohol.

The comprehensive utilization not only refers to comprehensive recycling and comprehensive utilization of the three components of lignocellulose but also refers to a design-stratified multistage recycling mode as bioenergy according to ecological engineering principles. Therefore, the biotransformation technology route is the main direction of research, with the liquid fuel form is a key point.

10.8.1.2 Microbial Feed Industry

Expansion of feed protein sources is an important condition for the development of the cultivation industry. China accounts for one fifth of the world's population. The development of a cultivation industry is the most effective way to ease a serious food situation. Straw-fermented feed as a replacement for food is an important task of the modern biotechnology industry. The Chinese government recommendation of returning straw to the field is based on the microbial transformation in herbivorous animals and is in line with the multilevel utilization of ecological engineering principles. Straw-fermented feed has been a major issue in drawing people's great concern but has not achieved satisfactory progress for a long time. The main problems are the low protein content, the instability of product quality, and the high cost. The technical route can be divided into liquid fermentation and solid-state fermentation or aerobic fermentation and anaerobic fermentation. SCP produced by liquid fermentation has received a large amount of attention worldwide . The former

Soviet Union built a 100,000-t large-scale production plant, but the price could not compete with soybean and fishmeal. So, liquid fermentation to produce SCP has failed to spread to date. Solid-state fermentation is an important way to reduce costs; it is currently used for the processing of starch-processing waste. Anaerobic fermentation products of silage and microbial silage of straws are still used, but have low protein content, which is always used for improving palatability and safe storage. The cost breakthrough for e separation of lignin and cellulase production is the key for straw-fermentation industry. The straw-fermented feed could make decisive progress and become the development direction of the microbial feed industry.

10.8.1.3 Biopesticide Industry

Pesticides are a powerful agricultural weapon to prevent natural disasters, but chemical pesticides also would cause environmental pollution, resistance to drugs, and side effects to humans and animals, which has become a major social problem. Biopesticides and biological control worldwide have received increased attention since the 1970s and are important parts of ecological agriculture.

According to their function, biopesticides include insecticides, fungicides, herbicides, and growth-promoting agents. According to microbial taxonomy, biopesticides can be divided into bacterial insecticides, mold pesticides, virus pesticides, and so on. Products have been produced in industry, such as *Bacillus thuringiensis* (BT), validamycin, kasugamycin, and gibberellin. The biopesticide industry is an emerging industry. Based on several years of research, Chen et al. proposed new solid-state fermentation instead of liquid fermentation and made significant progress in controlling production costs and toxic effects.

10.8.1.4 Industry of Organic Fertilizer by Biological Nitrogen Fixation

Fertilizer is obtained by chemical nitrogen fixation; it is a huge branch in the chemical industry and is one of the main pillars of modern agriculture. Biological nitrogen fixation is undoubtedly the future direction of development. Traditional organic fertilizer is mainly composed of fibers; it actually uses microorganisms to fix nitrogen. Because of the low efficiency of nitrogen fixation, it is difficult to compete with chemical and efficient nitrogen fixation. But, chemical fertilizers would damage the soil. Sulfur ammonia and chloramines have been banned and replaced by carbon ammonia and urea. But, the lack of organic matter in the soil will affect soil fertility (microbial flora), which increases the amount of fertilizer and the cost of production. Lignin in the lignocellulose is the best carrier for nitrogen fixation. Meanwhile, it is also the most scientific way of lignin utilization, which has just begun to attract the attention of scientists. Lignin by slow microbial degradation can produce plant growth hormone and has an unexpected effect on seedlings.

10.8.1.5 Microbial Feed Additive Industry

Feed additives have played an important role in the full-price feed formulation. In addition to the carbohydrates and amino acids, feed still needs trace elements, vitamins, and other bioactive substances. Appropriate enzymes and microbial ecological preparations also will improve the effects. Therefore, feed additives are widely used. The intensification of the feeding industry make efficiency and disease prevention important indicators of production. But, extensive use of hormones and antibiotics has become a major social nuisance. Feed additives with the target of health care and the microecological preparation could replace the hormones and antibiotics. Research and development of feed additives for an intensive feeding industry will play an indispensable role in the improvement of the main structure of ecological agriculture.

10.8.1.6 Eco-industry and Environmental Projects

As mentioned, the specific content and function of ecological engineering has been discussed based on the internal structure of ecological agriculture. In addition to agriculture, the industrial organic wastewater, waste materials, and waste generated from the service industry and municipal wastewater can go into the virtuous cycle of ecological agriculture through eco-industrial conversion. For example, the fermentation of industrial wastewater can be processed with lignocellulose to improve efficiency and reduce handling costs.

10.8.2 Technical System and Research for Comprehensive Utilization of Natural Lignocellulose

The waste of lignocellulose has received a good deal of attention but has not yet been utilized by comprehensive, effective, and economical approaches, which becomes a technical problem recognized worldwide. It has been proved that traditional chemical methods are not economical and not conform to ecological engineering principles. Therefore, it is inevitable to rely on the progress of modern biotechnology. Biotransformation is an inherent part of the natural ecological cycle. Using or emulating natural ecological processes is not efficient. Obviously, this is not only a biological issue but also an engineering problem. Because the technical and economic issues are not considered by theory scientists, they belong to the biochemical engineering disciplines. Based on several years of research, Chen and Li achieved in-depth understanding of the comprehensive utilization of lignocellulose. The comprehensive utilization of lignocellulose does not involve a single technology or aim at a single product; also, it would not achieve its economic benefits with only one isolated factory. Only in accordance with the principles of ecological engineering will the comprehensive utilization be incorporated into the

ecological agriculture system to find its right place [28]. Eco-industry has different connotations with the agricultural and subsidiary product processing industry. It is a high-level new biotechnology industry. The cycle of organic nitrogen would be achieved with livestock manure and slaughter plant waste returning to the fermentation of straw. This is high-level ecological agriculture. It does not need too large a scale but should be high tech and high quality and emphasize the completive utilization of biomass and economic efficiency to make the new products and new technology system centered on the biotransformation and complete utilization of straw. Characteristics of this technology system are high efficiency, comprehensiveness, and applicability. The total technical ideas are presented as follows:

① Define the main line based on the solid-state fermentation for biotransformation. The main research involves the solid-state fermentation reactor and its new theory, such as momentum, quality, heat, information transfer, and biological reaction kinetics.
② Solid-state fermentation to produce cellulase and fermentation-separation coupled technology of other products are key breakthroughs in technology and cost. Researches involves the method of fermentation and separation coupling and its mathematical model.
③ The utilization of lignin and hemicellulose is the key to achieve completive utilization of lignocellulose. Research on the transformation of lignin and hemicellulose should be strengthened to produce high-value-added products, which would make more progress in utilization of lignocellulose.
④ Strain selection is the base of completive utilization. Therefore, the gene pool on microorganisms capable of degrading cellulose and genetic engineering of ethanol fermentation of hemicellulose should be built and applied.
⑤ A certain scale industrial simulation plant should be established based on the system mentioned and previous work. The eco-industrial park for lignocellulose would achieve the pattern of multichannel, multiproduct, and comprehensive utilization, which would be helpful for economic feasibility analysis.

Thus, this chapter only does some preliminary exploration of these technical ideas in a few specific issues, and much work is still not complete. Basic research should begin, and multidisciplinary efforts and technology should be integrated to achieve a new breakthrough.

10.9 Typical Examples for an Ecological Industry Model of Lignocellulose Biotechnology

10.9.1 Ecological Plate and Fertilizer of Straw

Based on the differences of chemical composition and structure of straw and wood, I and my researchers proposed the new concept of denatured straw [1]. The method of ecological plate and fertilizer is established from straw degeneration methods

and the thermal curing process. The structure and properties of natural straw are changed by physical, chemical, or biological methods to form denatured straw. The cellulose, hemicellulose, and lignin are degraded effectively, then form thermal-curing bioresin plastic, which would make straw have self-adhesion and meet the needs building board molding. The steam explosion treatment would increase the active lignin, whose properties are similar to polyphenol properties, so the lignin in straw treated by steam explosion can be used as a natural adhesive. The hemicellulose would be degraded into soluble sugars, dehydration sugars, and furfural. It can replace formaldehyde to form a cross-linking reaction with lignin. The cellulose is not degraded, but its crystalline degree is improved during the process of steam explosion. This technology includes two main types of molding technology. First, the heat curing technology is to control the thermal curing process by the control of water content, in which the hydrogen bond would be rearranged; a heat curing bioresin is are generated and uniformly distributed on the surface of the straw fiber. The second is fiber restructuring molding technology. The straw is accompanied with thermal curing bioresin and bonded under certain temperatures and pressures in the straw fiber prototype devices to form plates with a certain mechanical strength that meet architectural requirements. The ecological plate without glue has attraction in economy and environment. The production process without glue is simple. It can remove the mixing process, save equipment, and reduce production costs. The ecological plate can be completely biodegraded in nature without any pollution. The strength of ecological materials is greatly increased because of the denatured treatment [29–31].

Straws can be used to produce ecological plates through degeneration technology, which will enrich straw application. Experts predict that new building would reach up to 1.5 billion m^3, and each area needs 10 m^3 of building materials. Therefore, the annual need for building board can be 15 billion m^3. The market capacity is extremely significant. Ecological plate may become the economic growth point of the new century. The steam-exploded straw can also produce corrugated paper, green wall materials, green fiberboard, green packaging material, disposable tableware boxes, and so on.

10.9.2 Ecological Industrial Chain of Enzymatic Hydrolysis and Fermentation Fuel

Controversies on bioethanol produced from lignocellulose mainly revolve around the unfitted economical feasibility and environmental concerns of the process, which attribute mainly to unilateral researches from own specialties of each scholar without regard to the characteristics of the straws themselves. To achieve an economical and environmentally-friendly system of bioethanol production from straw, a number of breakthroughs are needed, not only in individual process steps, but also in the balance and combination of these processes. Ecological industrial chain of enzymatic hydrolysis and fermentation fuel is established in this section,

by which the maximum efficacy and benefit of process can be achieved due to the production of many high-value co-products simultaneously with ethanol. The major difficulties for the production of ethanol from lignocellulose focus on the technical and economic issues [32]:

- It is difficult to realize the completive utilization of cellulose, hemicellulose, and lignin with a single technology. Emphasis on the use of cellulose and the ignorance of other components will undoubtedly cause environmental pollution and a waste of resources.
- The research on the key breakthroughs of technology is insufficient in the lignocellulose conversion process. For example, application of traditional acid pretreatment results in high costs and serious environmental pollution. In addition, indiscriminate application of technology and equipment of ethanol fermentation from starch results in the high cost of ethanol because of the high dosage of cellulase and low ethanol conversion efficiency.
- There are difficulties in technology integration and research. The economical and highly efficient conversion of straw is a systematic engineering problem because of the highly complicated structure of raw material and the shortage of appropriate techniques.

To achieve an economical ethanol production system, Chen and Wang proposed the concept of composition classification and oriented conversion. Each component of raw materials can be converted efficiently to realize the simultaneous conversion to ethanol and other high-value coproducts from agricultural residues [33, 34].

Based on these ideas, a demonstration project with annual straw ethanol output of 3,000 t has been successfully established at Shandong Province in China. This demonstration project achieves the high-efficiency and environmentally friendly production of ethanol by integrating the following technologies: steam explosion technology, gas double dynamic solid-state fermentation (GDD-SSF) of cellulase, solid-state enzymatic hydrolysis coupled with liquid ethanol fermentation and CO_2 gas stripping, and organic fertilizer production technology using fermentation residue [32].

As Fig. 10.17 shows, ethanol, organic fertilizer, and xylooligosaccharides would be produced simultaneously. In this process, hemicellulose in straw is extracted after corncobs are pretreated by steam explosion and can be used to produce xylooligosaccharide. A small portion of straw residues can be utilized for the production of cellulase, and a majority of residues are hydrolyzed by the crude enzyme preparation. Then, the hydrolysate is converted into ethanol, and the unhydrolyzed residues enriched in lignin are used for the preparation of organic fertilizers. In this process, hemicellulose is separated from straw and converted into high-value products instead of low-value or low-yield fuel ethanol. Meanwhile, fermented residues enriched in lignin are further used for bioconversion for organic fertilizers. Lignin mainly acts as an inert carrier with a characteristic of slow release to further enhance the practical application of bioorganic fertilizer.

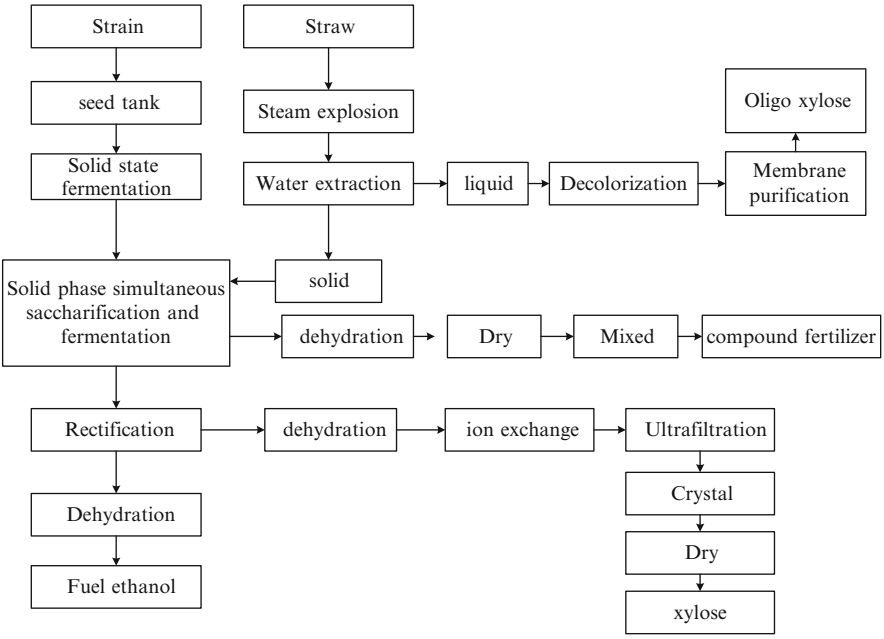


Fig. 10.17 Straw directional multilevel conversion to ethanol

The major equipment for this demonstration project include a 5-m^3 steam explosion tank, two 100-m^3 GDD-SSF reactors, a 110-m^3 solid-state simultaneous enzymatic hydrolysis and ethanol fermentation device, and four ethanol distillation towers. The overall production cost of the demonstration project was around 5,900 RMB for each ton of ethanol, having an annual production of 3,000 t of bioethanol and simultaneous production of 200 t of xylooligosaccharides and 3,000 t of organic fertilizers.

10.9.3 Butanol Fermentation from Hemicellulose of Straw and Its Comprehensive Utilization

Butanol as an excellent organic solvent and chemical raw material that is widely used in chemicals, plastics, organic synthesis, paint, and other industries. With the high-calorific value, low vapor pressure, facility to pipeline transportation, and blending with gasoline in any proportion, butanol has more application prospects than ethanol and is considered to be the next generation of biofuel. Low-cost biobutanol production is a hot topic in both academia and industry. Lignocellulose, of which cellulose and hemicellulose are converted into substrate for butanol production, is considered to be one of the raw materials with the most potential to reduce the substrate cost.

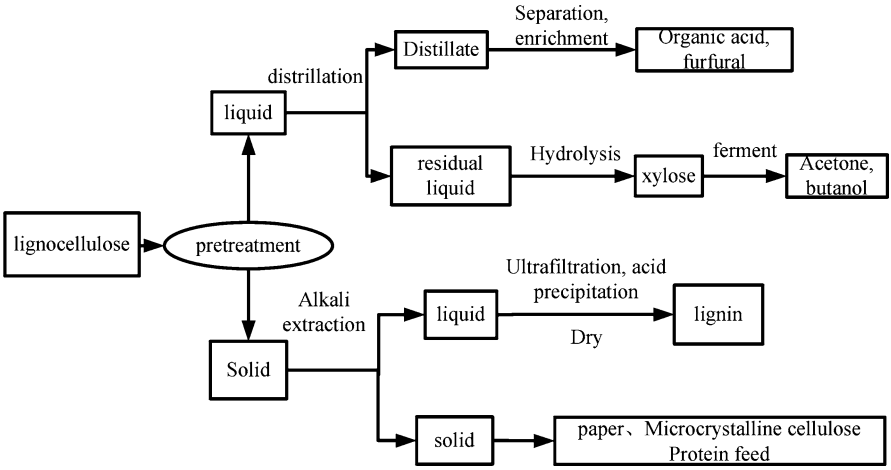


Fig. 10.18 Acetone-butanol fermentation from straw

According to research, I developed a new technical route for the corn stover refining industry. Specifically, the hemicellulose and pith cells are used as substrates for butanol production; the fiber cells are applied in the material industry, and the lignin is converted to high-value lignin-derived products. The industrial equipment in this process have been designed and assembled in a pilot plant in China's Jilin Province; production capacity is 600 t of butanol per year. It has been successfully enlarged to 50,000 t of butanol per year with coproduction of ethanol, acetone, polyether polyols, and pulp. This process will provide a new industrial way for low-cost bio-butanol production from corn stover (Figs. 10.17, 10.18, 10.19, 10.20, 10.21, and 10.22).

Key technologies for butanol production from steam-exploded cornstalk with independent intellectual property rights were achieved in this project. The main achievements are as follows [34–44]:

① The industrial technology system of butanol production from steam-exploded cornstalk was established for the first time at home and abroad; it had achieved large-scale production and established the independent processing device.
② The method of nutritional balance of medium instead of the synthetic medium was invented in which the main carbon source was hydrolyzed into sugars from straw and supplemented by corn leaching solution and starch milk. The medium could avoid the high cost and nutritional imbalance caused by the basic mixture of xylose and glucose.
③ The new technology system of butanol production from steam-exploded corn stover was established in which not only the hydrolysate of hemicellulose was fermented to acetone-butanol but also furfural and organic acids were produced, and the recycling of inorganic acids was achieved.

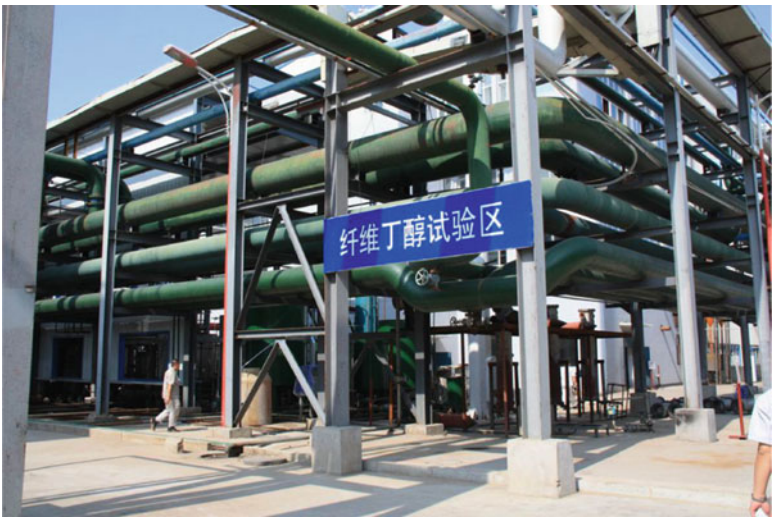


Fig. 10.19 Workshop of hemicellulose fermentation and lignin extraction

Fig. 10.20 Pilot solvent fermentation tank

④ A new method of lignin production was proposed. The lignin was extracted from the straw hemicellulose residues through alkaline extraction, ultrafiltration, and spray drying. The purity of lignin was up to 80 %. This method solved the water pollution and other problems compared with the traditional method. The cellulose after extracting lignin could produce microcrystalline cellulose. Therefore, the high value of lignin and cellulose was achieved.

Fig. 10.21 Lignin extraction tank

Fig. 10.22 Distillation tower of butanol and acetone

⑤ A new method of mixed solid-state fermentation to product protein feed was established. The wastewater of acetone-butanol fermentation was dried to 20–30 % and then added to the steam-exploded straw or straw residue after hemicellulose hydrolysis. The method solved the problems of wastewater and difficulties of evaporation and concentration, achieving the clean production.

10.9.4 Ethanol Fermentation and Coproduction of Organic Fertilizers from Sweet Sorghum

As an energy crop, sweet sorghum has remarkable advantages. First, sweet sorghum has a high photosynthetic efficiency, and its stems can reach 5 m high, which is the world's highest biomass yield of crops. Sugar in sweet sorghum stalks is the best substitute for grain alcohol production. Sweet sorghum per hectare can produce about 7,000 L of ethanol, whose biomass production is about twice that of corn, sugar beet, and wheat and a quarter more than sugarcane. Second, the utilization value of sweet sorghum is high. The grain not only can be eaten but also can be used for feed and industrial raw materials. Stalk residues after the production of ethanol are flavorful, are easily digestible, and have good nutrition, which can be used to develop animal husbandry. Because the fibers of sweet sorghum stalks are long, the quality of ethanol residue is higher than that of reed, so that residue can be used to produce paper. The residue also can be used for gasification power generation and production of fiber sheets without glue. Third, the water requirement is only one third that of sugarcane, and sweet sorghum has wide adaptability to soil conditions, especially its remarkable resistance to drought and salt tolerance, so sweet sorghum is known as "the camel of crops." Last, the growing season of sweet sorghum is short compared with other plants, and it can be harvested in two or three quarters. To produce fuel ethanol from sweet sorghum, it would neither affect the grain market nor account for grain farmland, but also it would significantly increase farmers' income. Thus, sweet sorghum is a broad prospect for the development of energy crops, which are major crops for Chinese agricultural areas with drought and saline soils.

The traditional technology of producing ethanol from sweet sorghum mostly is liquid fermentation using the juice. Because water loss is serious in the postharvest storage, the sweet sorghum must be immediately squeezed and needs to be brought together to generate the juice. These instant and concentrated processes would result in the additional transportation costs and juice costs. What is more, wastewater treatment in liquid fermentation is a tough problem that causes serious pollution in the environment. The water shortage of Chinese natural conditions does not allow the use of a liquid fermentation process.

Based on a technology platform of component separation and the new solid-state fermentation technology, I produced ethanol from sweet sorghum stalk. The mature sweet sorghum is transported to the fermentation plant and fermented to produce fuel ethanol under solid-state fermentation with yeast after crushing. The fermentation residues would be separated by mechanical treatment; then, the small fiber is used to produce feed. The long fiber is used to produce paper, and the other parts are used to produce feed additives. The process is clean and achieves the completive utilization of sweet sorghum (Fig. 10.23). This technology has the following advantages [45]: (1) The production costs are lower than for liquid fermentation. The sweet sorghum directly produces ethanol by solid-state

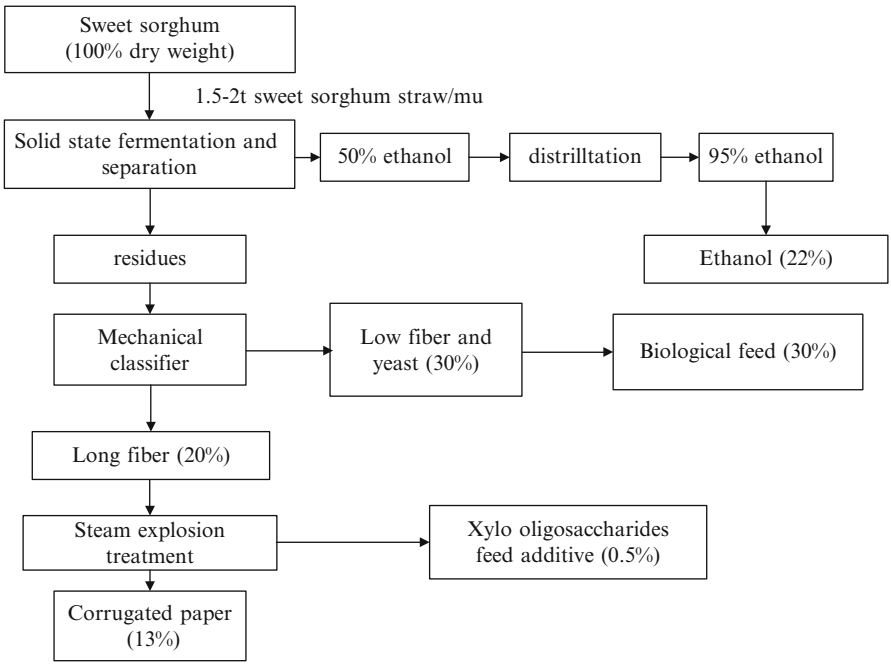


Fig. 10.23 Production process of sweet sorghum

fermentation, saving the cost of a juicer and centralized storage. (2) The decreased processing of solid-state fermentation is much more convenient, so the problem of molasses alcohol waste would be solved, and cleaner production processes would be achieved. (3) The water content of fermentation residue is low, which is helpful for comprehensive utilization [46, 47].

10.9.5 Production of Ethanol and Isoflavones from Steam-Exploded Radix puerariae *by Solid-State Fermentation*

The current fermentation ethanol technology from a starch resource includes the following processes: milling and mixing, gelatinization, liquefaction, saccharification, liquid fermentation, and distillation. The gelatinizing process is an operation with high-energy consumption, accounting for 30–40 % of the total energy consumption of the production process. *Radix puerariae* is rich in starch, fiber, and isoflavones; it contains about 50–60 % starch on a dry weight basis. Based on the process of producing ethanol from *Radix puerariae*, the fiber (9–15 %) will greatly enhance

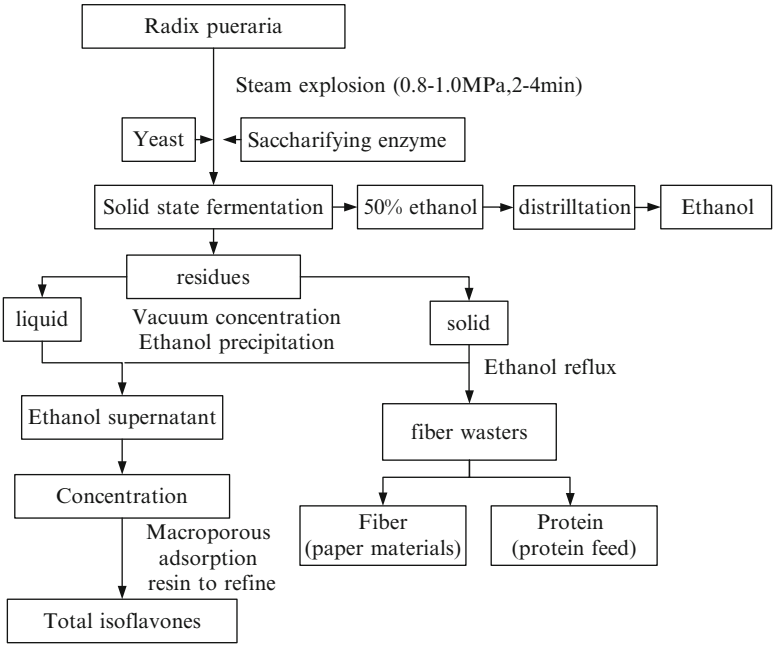


Fig. 10.24 Ethanol and isoflavone production from steam-pretreated *Radix puerariae* by solid-state fermentation

the smash energy consumption and hinder the highly effective dissolution of the starch from *Radix puerariae*. Furthermore, liquid fermentation easily produces a great deal of wastewater, which would cause serious environmental pollution and make it difficult to extract *Radix puerariae* isoflavones.

In the last 20 years, I and my colleagues started a promising journey in biomass utilization through the development of solid-state fermentation and steam explosion technology. Aiming at the foregoing problems of the fermentation of *Radix puerariae*, low-pressure steam explosion was introduced to pretreat *Radix puerariae*, then the production of ethanol by simultaneous saccharification and fermentation of steam-pretreated *Radix puerariae* was presented. The isoflavones were extracted from the fermentation residues. The gelatinization process of the starch is replaced by an unpolluted steam explosion pretreatment, reducing energy consumption. The fiber structure of the materials would be destroyed to improve the dissolution efficiency of the *Radix puerariae* starch and isoflavones. Solid-state fermentation is a clean technology by which it is easy to extract isoflavones from the fermentation residues [48–52]. The process was shown in Fig. 10.24.

Optimal technological conditions were obtained: *Radix puerariae* was steam pretreated at a saturated vapor pressure of 0.8 MPa for 3.5 min; glucoamylase (65 $U \cdot g^{-1}$), cellulase (1.5$U \cdot g^{-1}$), 0.1 % $(NH_4)_2SO_4$, 0.1 % KH_2PO_4, and activated yeasts were added, and fermentation took place at 35–37 °C for 60 h. Under

these conditions, the yields of ethanol and isoflavones were 9.2 % and 1.48 %, respectively; the starch utilization rate was 95 %. In comparison with the traditional fermentation technology, the simultaneous saccharification and fermentation of steam-pretreated *Radix puerariae* is clean and energy saving, which can provide a new way for ethanol production from nonfood starch material and will be worthwhile to explore and implement in industry.

This technology has the following advantages. (1) Water saving: Compared with the traditional starch ethanol fermentation process, the process three to five times less water will be needed in the liquefaction unit. The solid-to-liquid ratio is 1:5; it is 1:10 for liquid fermentation. It will save about 10 t of water to produce 1 t of ethanol. (2) Energy saving: The gelatinization process of the starch is replaced by an unpolluted steam explosion pretreatment, whose energy cost is only about 30–40 % of the total energy consumption.

The technology of a continuous separation coupled solid-state fermentation heat pump can isolate 30–50 % of ethanol, and the ethanol can be directly distilled to 95 %, which can reduce the energy consumption of primary distillation by 30–40 % [51, 52].

10.9.6 Clean Retting of Marijuana Fiber by Steam Explosion Pretreatment

Marijuana fiber can be divided into cellulose and noncellulose parts. The latter also includes hemicellulose, pectin, lignin, soluble material, waxes, and ash, which collectively are referred to as gelatin. To make marijuana have spinability, it must first be unglued. Because the fiber cells are woven into the mesh with hemicellulose and other chemical bonding, that increases the difficulty of degumming. A marijuana single fiber is short (12–25 mm) and has different uniformity; if the entire intercellular layer materials were removed (i.e., "unglued"), it would cause flocking and lost spinability. Therefore, an "appropriate degumming process" should make the single fiber bonded to form the technical fiber. At present, there are three kinds of degumming methods: chemical, microbial, and enzymatic. Chemical degumming is the common method in industry, and the other methods are still in the research stage. These methods have some shortcomings: high energy consumption, heavy pollution, and poor degumming effect.

Because of these shortcomings, a new and clean degumming method was proposed by Chen et al. [53]. First, the hemicellulose and lignin of marijuana are partly degraded by steam explosion, then the steam-exploded marijuana is further unglued with a microbial enzyme. This combined pretreatment method can realize the selective separation of each component, with both degumming and maintaining material morphology and chemical composition [31, 54–56]. The process of marijuana meets the needs of actual production, and some related industries have been established, such as papermaking, fuel ethanol fermentation, and extraction of active substance.

10.9.7 Converting Pennisetum hydridum *into Fuel Ethanol with Coproduction of Electricity and Paper Pulp*

Pennisetum hydridum is a high-quality fodder grass with high output and high protein content; it was introduced from Colombia, South America [59]. It belongs to the Angiosperm phylum, Monocotyledoneae class, Gramineae family, and *Pennisetum* genus. The yield of *Pennisetum hydridum* is high. The high-quality gramineae fodder grass is cultivated by hybridizing the elephant grass and American *Pennisetum. Pennisetum hydridum* grows straight in a cluster and has a tall body, and advanced root system; and it is a perennial plant in areas with adequate temperature. The plant has a height of up to 4–5 m and a length of 9–15 cm between joints; there are 15–30 effective sprouts, and one axillary bud is grown on each joint and is encapsulated with leaves with a length of 60–132 cm and a width of 3–6 cm. *Pennisetum hydridum* is cultivated in the areas of Guangdong Province and Guangxi Province, which have advantages of fast growth, long harvest period, and stable and high output. *Pennisetum hydridum* can be reaped in 2–3 months after planting in spring and will keep growing after reaping. It can be reaped four to six times and for 6–7 years once planted. The output per year for each hectare is up to 375 t.

The main chemical ingredients of *Pennisetum hydridum* include cellulose, hemicellulose, and lignin, and these three ingredients constitute the supporting skeleton of the plant body. The cellulose forms microfibers that constitute the netlike skeleton of the cell walls of the plant; the hemicellulose and lignin are the "adhesive" and "filler" filled between the fibers. In *Pennisetum hydridum*, the total content of cellulose is 75 % or more, the content of Klason lignin is more than 20 %, and the content of ash is about 3 %. In addition, *Pennisetum hydridum* further comprises pectin substances, lipids and waxes, lipins, and low-molecular carbohydrates.

At present, the main application of *Pennisetum hydridum* in industry is use as a fine-quality fodder grass and a new raw material for feedstuff, papermaking, and construction material. (1) As the raw material for fodder grass, *Pennisetum hydridum* has the best succulence for feeding phytophagous livestock, fowls, and fishes. It has a large harvest amount per year and can be reaped for a long period. However, when *Pennisetum hydridum* is used as feedstuff directly, it has the disadvantages of difficult digesting absorption, poor palatability, and low added value. Chinese Invention Patents 200410040941.4 and 200610138365.6 have introduced methods for producing protein feedstuffs utilizing *Pennisetum hydridum*. (2) As the raw material for papermaking, *Pennisetum hydridum* is a fast-growing papermaking raw material with great potential and good papermaking performance. *Pennisetum hydridum* has the advantages of long fiber length, high aspect ratio, good beatability, soft fibers, relatively high tearing strength, and so on. It is suitable for preparing top-grade paper pulp and dissolved pulp and can be used as a raw material for papermaking instead of wood to save wood for the country. A method for preparing bleached pulp utilizing *Pennisetum hydridum* as a raw material was introduced by Zhang from the Nanjing Forestry University of China. (3) As the raw material for building material, Chinese Invention Patents 200410016746.8

and 200610011006.4 introduced that building material boards of fiber boards, medium-density boards, molded board, and the like with excellent quality and low price, as well as various artworks, can be manufactured utilizing *Pennisetum hydridum*.

However, the applications of *Pennisetum hydridum* mentioned are only aimed at the utilization of a single component in *Pennisetum hydridum* or the utilization with low added value. For example, as a papermaking raw material, only the cellulose in *Pennisetum hydridum* is utilized, and the other components of hemicellulose, lignin, and others are discharged as papermaking waste liquid; therefore, not only the resource is wasted, but also the environment is seriously polluted. Thus, in view of the integrated aspects to increase the added value of *Pennisetum hydridum*, save the resource, or the like, the situation makes it imperative to develop a processing route for the comprehensive utilization of *Pennisetum hydridum*. Planting and managing *Pennisetum hydridum* as an energy source crop will provide a new route for solving the energy problem in China [60].

In addition, in recent years, the technology for biomass electricity generation has developed rapidly. In the countries of Austria, Denmark, Finland, France, Norway, Sweden, America, and so on, biomass has drawn increased attention for use as a fuel for electricity generation. In China, since 1987, research work on biomass energy miniaturized gasification electricity generation technology has been carried out and was regarded as a key project of the Ministry of Science and Technology. In 2000, a demonstration project for straw gasification electricity generation was performed in China. However, because the low thermal value and low density, electricity generation utilizing biomass cannot make a breakthrough in economical cost. Therefore, to satisfy the national energy source demand and increase the comprehensive utilization value of *Pennisetum hydridum*, further in-depth research on producing fuel ethanol with coproduction of a biomass fuel for electricity generation by fermenting *Pennisetum hydridum* is needed [60].

In summary, the invention has advantageous effects as follows:

① According to the characteristic of compact structure for *Pennisetum hydridum*, it is subjected to steam explosion pretreatment that increases the fiber dispersion degree greatly and favors the growth of microorganisms and the separation of the long and short fibers.

② Aiming at the previous disadvantage of a single product for *Pennisetum hydridum*, a full utilization technology for *Pennisetum hydridum* is emphasized that increases the utilization efficiency and saves the resource greatly.

③ By producing fuel ethanol from *Pennisetum hydridum* fermentation with coproduction of xylooligosaccharide, paper pulp, alcohol-soluble lignin, recovered CO_2, and electricity generation, the comprehensive utilization route for *Pennisetum hydridum* is optimized.

④ By overcoming the disadvantages of the pollution of the traditional methods for component separation and single-component utilization, a clean full utilization of *Pennisetum hydridum* is realized (Fig. 10.25).

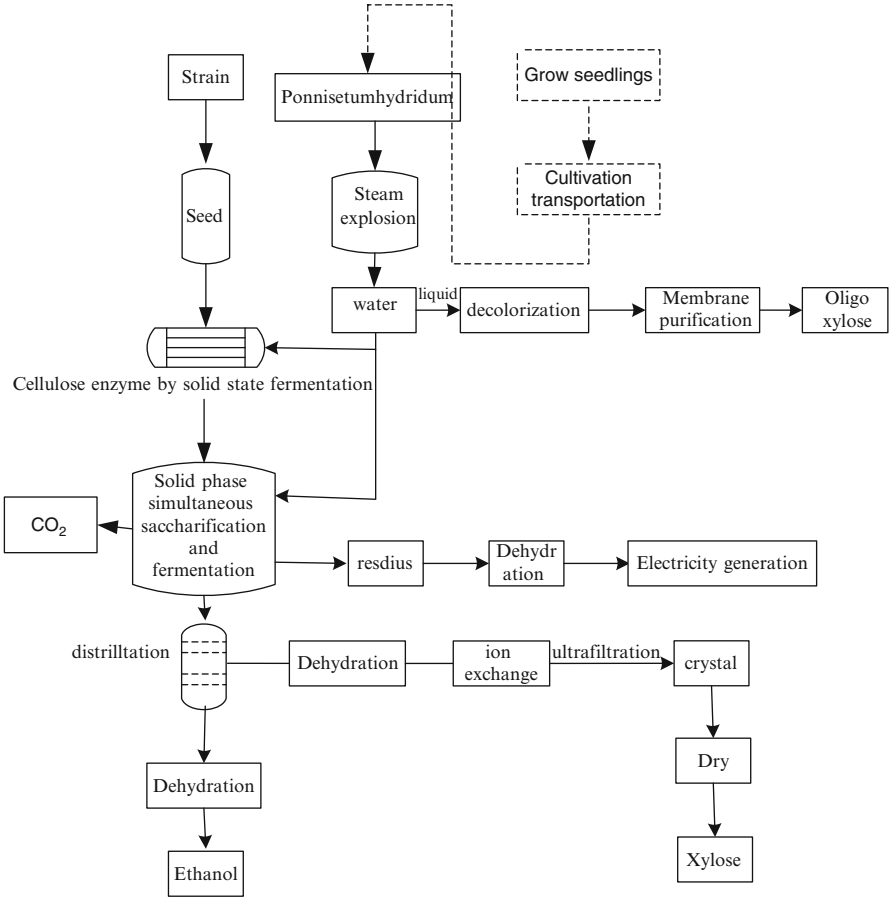


Fig. 10.25 Converting *Pennisetum hydridum* into fuel ethanol by producing electricity power and paper pulp simultaneously

10.9.8 Selective Liquefaction of Lignocellulosic Materials and Its Ecological Industry Chain

Liquefying lignocelluloses with phenol is important. Lignocellulose, which is regarded as a homogeneous material in complete liquefaction, is actually inhomogeneous. Currently, phenol plays an important role in the liquefaction of lignocellulose. Liquefaction of lignocellulose with phenol is characterized by the easy liquefaction of lignin and hemicellulose. In contrast, cellulose is liquefied with difficulty, retained in the residues, and then discarded. Based on these features, phenol-selective liquefaction of lignocellulose for multilevel conversion is proposed. Hemicellulose and lignin can be liquefied as much as possible, whereas cellulose is retained selectively. Lignin and hemicellulose react with phenol to

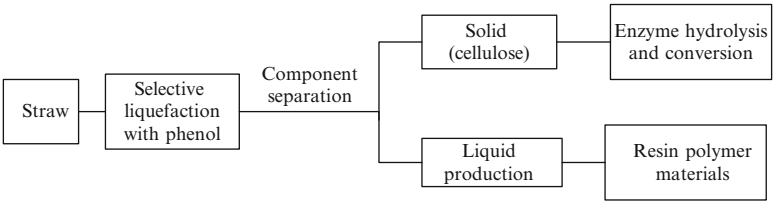


Fig. 10.26 Selective liquefaction of straw with phenol

generate phenolic compounds (those combined with phenol). Liquefied products can also be further synthesized into resin substances. Retained high-value cellulose is used in enzymatic hydrolysis to prepare sugar platform intermediates.

Based on the heterogeneity of lignocellulose, the technology of phenol-selective liquefaction of lignocellulose for multilevel conversion is presented for the first time in this section (Fig. 10.26) [61, 62]. The effects of all four variables (liquefaction temperature, time, catalyst concentration, and phenol dosage) on the change in the straw components and enzymatic hydrolysis yield of liquefaction residues are demonstrated. The performance of synthetic resin adhesive from the liquefied products is also assessed.

First, the liquefaction products and solid insolubles are preliminarily separated through selective liquefaction. Second, according to the components, the insoluble solid product (mostly cellulose) is used to produce cellulose, and the liquid products (mainly hemicellulose and lignin) are used to synthesize resin polymer materials. Using phenol-selective liquefaction, lignin ($\geq$70 %) and hemicellulose ($\geq$85 %) are liquefied, with large amounts of cellulose ($\geq$80 %) retained in the unliquefied residue. In phenol-selective liquefaction, not only can the liquefied products be used for the synthesis of resins, but also the unliquefied residues with high cellulose content are useful for enzymolysis. Phenol-selective liquefaction effectively achieves fractionation and biomass full utilization of lignocellulose, thus opening up a new avenue for bio-based product development.

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Chapter 11
Research Methods for the Biotechnology of Lignocellulose

Abstract Biotechnology for lignocellulose conversion is developed with its research and application. During this process, the research method plays an important role in discovering, exploring, and establishing theories. The research method for lignocellulose conversion is also necessary for industrialization testing and control. In this chapter, research methods for the biotechnology of lignocellulose are presented. According to the biological conversion process of lignocelluloses, research methods are classified into three types: research methods for primary refining, research methods for hydrolysis with microorganisms, and research methods for fermentation engineering. Finally, research methods for the whole biotransformation process are also summarized. In each part, the related research advances of my group are also given.

Keywords Research methods • Biochemical methods • Fermentation engineering methods • Primary refining methods • Life cycle assessment

11.1 Research Methods for the Cellulose Primary Refining Process

11.1.1 Introduction

Cellulose materials should be converted into middle products first and then converted into final bioenergy and biomaterials. However, for cellulose materials, the compact structure formed by cellulose, hemicellulose, and lignin results in high energy cost while converting cellulose materials into sugar. Moreover, it is hard to fulfill the requirements of further conversion into bioenergy and biomaterials. Therefore, it would be important to research the changes of lignocellulosic materials in the process of primary conversion and the effect of different methods on these

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changes. This way, the recalcitrance of hydrolysis would be solved, and refining technology would be set up for the conversion of different materials into related products [1].

The recalcitrance of lignocellulosic materials includes two aspects: structure and components. Currently, characterization methods for lignocellulosic material components are usually from the papermaking area. In addition, near-infrared, X-ray diffraction, and electrical scanning technologies are applied to analyze the crystal structure and surface characteristics of cellulose materials. However, advanced characterization technology should be introduced to form a complete characterization technology system for deep and realistic recognition from different levels and aspects.

Cross-discipline examination and re-creation are necessary to overcome difficult problems in the sciences. Now, discoveries are usually from a visual description or modeling. So, the scientific rules should be followed to find the recalcitrance of cellulose materials in the primary refining process: from visual to abstract, from macro to micro, from outside to inside. In this section, the research methods are introduced gradually according to this order.

At first, the morphological and structural changes should be characterized because it could provide direct recognition. Especially, different changes could be observed in the process of primary refining, including special structure, crystal structure, and fiber cell with three-dimensional (3-D) scanning and optical microscopy. Then, the component changes should be analyzed, including main and trace components. Moreover, the changes of functional group should be explored. Finally, the research methods for energy consumption and processes are introduced.

The quality of products would be affected by cellulose materials changing in the primary refining process. Cellulose materials are usually applied in papermaking. Pulping mainly uses cellulose, so analysis methods for cellulose are mature regarding cellulosic material analysis in primary refining. Research methods for lignin and hemicelluloses should also be set up to realize fractionation and high-value conversion of lignocellulosic materials.

11.1.2 Research Methods for Morphology and Structure of Lignocellulose in Primary Refining

11.1.2.1 Research Methods for Tridimensional Morphology and Structure

Different components are connected to each other tightly, so it is hard to separate different components or deconstruct a special structure in the process of converting cellulose materials into high-value products. If the morphology changes at different conversion stages could be demonstrated in three dimensions, the recalcitrance for pretreatment and enzyme hydrolysis might be discovered. This would be helpful for setting up the relationship between pretreatment and enzyme hydrolysis and to find

an effective refining process. Therefore, 3-D scanning technology and computed tomographic (CT) technology could be introduced.

In 3-D scanning technology (3-D digital technology), stereoscopic material is taken as a description object and a 3-D special axis is exported, including every sampling point of the object's surface and a color model along with color information. Hundreds and millions of measuring data could be obtained by noncontact stereo scanning equipment according to the property of the stereo scanning equipment, scanning parameters, and size of object measured. The large amount of stereo data is called the point cloud. Each set of stereo data includes not only stereo axis information but also color information. With stereo scanning, the method of reverse engineering could be used. While designing and manufacturing industrial products, reverse engineering could describe specially as measuring process for accessory original shape or sample model without designing drawing or sample model to reconstruct the designing drawing or CAD (computer-aided drawing) model.

There is contact and noncontact 3-D scanning technology according to the manner of collection. For contact 3-D scanning technology, a 3-D axis is obtained by contacting an object directly with a measuring machine probe. The precision of contact 3-D scanning technology is high, but efficiency is low. It is not usually used in reverse engineering. Noncontact scanning technology making use of optics emerged in the 1990s in euramerican countries. At present, structural light 3-D scanning technology is based on a white light lamp house. This technology was introduced into the industrial scanning measurement area because of its high precision, high speed, large scanning range, and so on.

At present, 3-D scanning technology is mainly applied in the mold-making area, including the molding of teeth, body, accessories, costume, spaceflight, and so on. The distance used between points could reach 10–30 μm. It would be helpful to import this technology for stereomorphology research of cellulose materials in the primary refining process.

Computed tomographic technology scans a thickness layer of the human body with X-rays. The X-rays penetrate a thickness layer and could be accepted by a probe. Then, it would be converted to visible light, which is then converted to an electrical signal by photoelectric conversion. Finally, the electrical signal is transformed into a figure by an analog digital converter and sent to a computer for disposal. To form an image, the chosen layer is divided into a cuboid of the same volume, which is called a voxel. The scanning information is calculated to obtain the X-ray attenuation constant or absorption constant of each voxel. Then, the constant is arranged as a digital matrix that can be stored in a disk or compact disk. Digitals in the matrix are converted into small squares with different colors of gray from black to white, which is a pixel. Pixels are arranged as a matrix to form the CT image. Therefore, the CT image is reconstructing an image. The absorption constant of each voxel could be calculated with different mathematical methods.

The CT image is constructed by gray voxels as they range from black to white and are arranged as a matrix. A pixel reflects a related X-ray absorption constant. Images are obtained with different CT equipment are different in pixel size and number. The size might be 1.0×1.0 mm, 0.5×0.5 mm, and so on. The number might be

256×256 (65,536), 512×512 (262,144), and so on. Obviously, the smaller the pixel is and the more there are, the higher the spatial resolution is. However, dimensional resolution of a CT image is lower than that of an X-ray image.

A CT image is demonstrated by different grays, so it reflects the X-ray absorption extent of organs and tissues. A black shadow indicates a low-absorption area, which is a low-density area such as the lung with high air content in a human body. A white shadow indicates a high-absorption area, which is a high-density area, such as the bones of the human body. However, the density resolution of CT is higher than that of X-ray imaging.

For CT technology, the density could be demonstrated not only by the extent of gray but also by the absorption constant of the X-ray, which can provide a quantity value. In actual work, the absorption constant is converted into a CT value to demonstrate the density using the unit Hu (Hounsfield unit). For water, the absorption constant is 10, and the CT value is defined as 0 Hu.

The CT image is a layered image, and a transect is usually applied. To present the whole organ, consecutive layers are required. The image of the coronal and sagittal planes could also be reconstructed by a reconstruction sequence in the CT equipment. In the area of medicine, the relationship between an organ and its illness could be observed from more aspects.

(1) Plain CT scan is a common scan without contrast enhancement or contrast. Usually, a plain CT scan is used first.
(2) A contrast enhancement CT scan injects water-soluble organic iodine such as 60 mL of 60–76 % diatrizoate intravenously before scanning. When the content of iodine in the blood is enhanced, its content in an ill organ and a natural organ would be different. Therefore, the image of an ill organ might be clearer. This method includes mass injection, the sessile drop method, and intravenous injection.
(3) A contrast CT scan first contrasts the organ or structure and then scans. For example, 8–10 mL iotrolan or 4–6 mL air are injected into the cistern first, and then scanning is carried out, which is also called cistern contrast CT scanning. A cistern and small tumor in the brain could be presented clearly.

    It would be helpful to research the compact structure of lignocellulose by importing CT technology in the primary refining process. If different components are dyed and analyzed with CT, information that is more valuable could be obtained. Together with 3-D scanning technology, changes of lignocellulose in the primary refining process could be illuminated directly and visually. Moreover, the recalcitrance of hydrolysis could be explored.

11.1.2.2 Research Methods for Morphology and Structure of Tissue

Tissue is an ensemble of similar cells from the same origin; together, they carry out a specific function. If the cells are the same kind, it is called simple tissue. If the cells are different kinds, it is called compound tissue.

In the primary refining process of lignocellulose, milling technology is often used first to destroy the tissue structure. The mill type usually includes a mechanical mill, jet mill, grinder, and cryogenic comminution mill [2]. The mechanical mill includes the gear-type mill, hammer mill, cutter mill, turbine mill, pressure-grinding mill, and milling mill. A grinder includes the ball mill, mortar mill, and colloid mill. Different mills and grinders affect striking, cutting, grinding, hammering, and so on differently. It would be helpful to finding out an efficient destruction manner to research the deconstruction effect of different milling methods.

It is important to learn about plant tissue for plant anatomy. For example, information on a stem is learned by optic microscopy with a low-power lens by observing a crosscut section. Rind, ground tissue, and vascular material could be observed clearly. Moreover, bundle sheath, phloem, and xylem in vascular tissue could be observed with a high-power lens.

There are two kinds of slice-making methods according to time saving [3]:

(1) *Temporary slide method*: A slice is used for temporary observation without saving it for a long time, such as by temporary slide mounting, freehand slicing, wood slicing, ultrathin slicing, and so on.
(2) *Permanent slice*: A slice could be saved for a long time, such as by paraffin slicing, semithin slicing, freehand slicing, wood slicing, and ultrathin slicing.

Slice-making methods for plants mainly include different slicing methods (freehand slicing, paraffin slicing, semithin slicing, frozen slicing, vibration slicing, sliding slicing, and ultrathin slicing) and nonslicing methods (maceration method, smear method, squash method, whole slicing, and scanning electron microscopy sample preparation method). Steps to prepare samples include

$$\text{get sample} \rightarrow \text{fix} \rightarrow \text{wash} \rightarrow \text{slice} \rightarrow \text{dye} \rightarrow \text{dehydrate} \rightarrow \text{penetrate} \rightarrow \text{seal}.$$

11.1.2.3 Research Methods for Morphology and Structure of Cells

In the primary refining process of lignocellulosic materials, it is important to deconstruct the fiber cell wall. Therefore, an optical microscope (resolution limit is 0.2 μm) and an electron microscope (resolution limit is 0.2 nm) could be used to observe cell and subcellular changes. At the same time, a special structural change of different components could also be analyzed, especially the position of cellulose, hemicellulose, and lignin.

The resolution ratio of an electron microscope is 0.2 nm, and the thickness of the cell wall is 10 μm. The ultrastructure of the cell wall is introduced in the following material using wood as an example. The connecting layer among the fiber cells is called the middle lamella (ML), which is composed of amorphous pectin and lignin. The ML is 0.1–0.2 μm thick. The fiber cell wall includes a primary wall and a secondary wall. The primary wall (P) is a liquid saccule formed at the beginning of cell wall synthesis [4]. The primary wall is 0.1–0.2 μm thick and connects tightly to the lamella. The wall proliferation on the primary wall is called the secondary

wall (S). According to the microfiber arrangement, the secondary wall is divided into the outer layer, middle layer, and inner layer, which are expressed by S1 (0.5–1 μm), S2 (3–10 μm), and S3 (thinner than S2), respectively. S1 is mainly composed of cellulose and hemicellulose. The microfibers of cellulose are nearly perpendicular to the fiber axis. The microfiber twists around the fiber wall regularly with high crystallinity. So, S1 is more stable to chemical and mechanical effects than S2 and is hard to dissolve. S1 is similar to a sleeve binding S2. The thicker S1 is, the stronger the binding strength is for S2. The connection between S1 and the primary wall is tight, but the connection between S1 and S2 is slack.

11.1.2.4 Research Method for Cell Wall Thickness and Lumen Diameter

Cell wall thickness and lumen diameter are main characteristics of lignocellulose. In the primary refining process, these characteristics would change, leading to different product performance. So, analysis with a microscope is necessary.

The main steps are as follows [3]:

$$\text{exclude air} \rightarrow \text{soften} \rightarrow \text{slice} \rightarrow \text{dye} \rightarrow \text{seal} \rightarrow \text{measure}$$

11.1.2.5 Research Methods for Fiber Length and Width

Fiber length and width would change with the effects of physical, chemical, and biological changes in the primary refining process of lignocellulose. Different products, such as paper, timber, and fuel, require different fiber lengths. So, it is necessary to characterize the change. GB/T 10336-1989 is used; the sample is observed by an optical microscope and projector, respectively.

As fiber length distribution of raw materials is uneven, it obviously cannot fully reflect length if fiber length is expressed with only a simple mathematical mean. Therefore, in addition to a mean value, the supplementary explanation of the other indicators is necessary, including the average fiber length, maximum fiber length, minimum fiber length, generic fiber length, and distribution frequency.

At present, the kajaani FS300 can measure fiber length and width automatically; it is made of Metso Automation Company Limited.

11.1.2.6 Research Methods for Fiber Coarseness, Fiber Number per Milligram, and Weight Factor

If the final aim of cellulose material primary refining is to produce materials; fiber coarseness and fiber number per milligram are main factors related to the properties of the produce. Fiber coarseness and fiber number per milligram are related to not only raw materials but also primary refining manner.

Milligram weight of absolute dry fiber with a 100-m length is called fiber coarseness [4]. It is expressed with decigrex (dg). Fiber coarseness is related to and reflects raw material specific gravity, fiber cell wall thickness, lumen size, cooking extent, and so on.

The basic points of different methods to measure fiber coarseness are to take a certain amount of fiber, add water in a certain proportion, obtain a small amount of the total length of the fiber in suspension, and then calculate the milligram weight of 100 m absolute dry weight.

Milligram fiber number [5] refers to the number of fibers contained in a milligram of fiber. It is related to the fiber length, width, wall thickness, the content in cells or fiber fragments, and the primary refining method. A kajaani fiber analyzer could be used to measure the number of fibers; then, the milligram fiber number is obtained by calculation.

Fiber coarseness of various raw materials is different. The weight per unit length is defined as the weight factor, also known as the quality factor. In the paper industry, the weight factor of cotton pulp is regarded as 1. The fiber coarseness of other pulp is compared to that of cotton pulp fibers to obtain their weight factors.

11.1.2.7 Research Methods for Crystal Structure

For lignocellulosic materials, lignin and hemicellulose mostly exist in an amorphous form. However, cellulose mostly exists in crystalline form. Different products have different requirements for the form of cellulose after primary refining.

Crystal structure is studied by an X-ray diffraction method. The crystallization zone content of cellulose can be calculated according to intensity distribution curves of X-ray diffraction [6]. In addition, there are the adsorption method and specific gravity method. The adsorption method is based on the fact that the adsorption rate of the amorphous region is significantly larger than that of the crystalline region. The adsorption method is more suitable for measuring the accessibility.

11.1.2.8 Research Methods for Molecular Weight

The measurement of cellulose molecular weight is divided into four categories [7] according to measuring principle: chemical methods (end-group analysis); thermodynamic methods (vapor pressure, osmotic pressure, boiling point elevation, freezing point depression); dynamics methods (ultracentrifugation settling velocity, ultracentrifugation sedimentation equilibrium method, viscosity method); and optical methods (light-scattering method). Different methods have their advantages, disadvantages, and the limitations of application scope. The average molecular weight of different types is obtained by different methods.

Lignin molecular weight [7] could be measured by the osmotic pressure method, light-scattering method, supercentrifugation method, gel permeation chromatography, and high-performance liquid chromatography (HPLC), which are combined with appropriate standard samples for measurement.

11.1.2.9 Research Methods for Polymerization Degree

The formula of cellulose is $(C_6H_{10}O_5)n$, of which n is the number of glucose groups and is called the degree of polymerization (DP). The average DP of cellulose reflects the average length of the cellulose molecule chain and directly affects pulp viscosity. Pulp viscosity can be measured by the national standard method of China, namely GB/T 1548-2004, and then the cellulose DP is calculated according to the formula.

11.1.3 Research Methods for Components in the Primary Refining Process [8]

11.1.3.1 Research Methods for Cellulose Content

Holocellulose [5] refers to all of the cellulose and hemicelluloses in plant cellulose materials, that is, the total amount of carbohydrate. The principle of the holocellulose content determination method is to try to remove lignin and keep intact cellulose and hemicellulose. The holocellulose content determination method includes sodium chlorite, chlorine-ethanolamine, chlorine dioxide, peracetic acid, and peracetic acid–boron hydride sodium methods. The sodium chlorite method is mostly used to determine the holocellulose content with the advantage of simple separation operation and rapid delignification. It is suitable for plant cellulose materials such as timber and nontimber. For specifics, refer to the national standard method of China, GB/T 2677.10-1995.

The cellulose content can be determined by the nitrate and ethanol method [5]. This method uses a concentrated nitric acid and ethanol solution to treat a sample. Lignin in the sample is nitrated, and some is oxidized. The nitrated lignin and oxidized lignin are dissolved in the ethanol solution. Large amounts of hemicellulose are also hydrolyzed and separated. The resulting residue is nitric acid–ethanol cellulose. Ethanol medium can reduce the hydrolysis and oxidation effect of nitric acid on cellulose. A sample of 2–3 g (depending on cellulose content in the sample) is accurately taken and is preground and dried. It is put it in a 250-mL grinding mouth Erlenmeyer flask. Then 25 mL of a nitric acid–ethanol solution is added, and a reflux condense apparatus is fitted. Finally, the Erlenmeyer flask is heated in a boiling water bath for 1 h. The sample is treated in this way three times. The sample is filtered and washed until neutral and dried to a constant weight. Ash is measured. Then, the cellulose content is calculated by a formula.

The Van Soest method can simultaneously measure the content of cellulose, hemicellulose, and lignin. Plant feed, such as general feed, forage, and roughage, could be decomposed by a neutral detergent (3 % sodium dodecyl sulfate). Then, most of the cell contents are dissolved in detergents, including fat, sugar, starch, and protein, collectively as neutral detergent solubles (NDS). The residue is called neutral detergent fiber (NDF). This part is mainly the cell wall, such as hemicellulose, cellulose, lignin, silicate, and a small amount of protein.

Acid detergent could further decompose each component in NDF. For plant feed, the part dissolved in acid detergent is called the acidic detergent solubles (ADS), including NDS and hemicelluloses. The remaining residue is called the acid detergent fiber (ADF) and contains cellulose, lignin, and silicate. In addition, the difference between the value of NDF and ADF is the hemicellulose content in the raw materials. ADF is digested by 72 % sulfuric acid to dissolve cellulose. The residue is lignin and silicates. So, 72 % sulfuric acid digestion residue is subtracted from the value of ADF; then, the content of cellulose is obtained. Seventy-two percent of the sulfuric acid digestion residue is then ashed. Ash is the content of silicate in raw material, and the escaping portion in the ashing process is the acid detergent lignin (ADL) content. Wang Yuwan [5] improved these methods by changing neutral detergent to 2 M HCl. Other steps are as the same presented.

Cellulose can be divided into α-cellulose, β-cellulose, and γ-cellulose according to their solubility [7]. α-Cellulose is the cellulosic portion dissolved in 17.5 or 18 % NaOH solution at 20 °C. The extract alkali solution is precipitated by acid, and the precipitation is β-cellulose. The residue in a neutral solution without precipitating is called γ-cellulose. If a viscosity method is used to measure the DP of three celluloses, the DP of α-cellulose is greater than 200, β-cellulose is 10–200, and γ-cellulose is less than 10.

If cellulose materials are processed as a feed, traditional leaching, ANKOM (ANKOM Technology Corporation, USA) bag and CAU (made by Chinese Agricultural University) filter bag technology could be used to determine the NDF, ADF, and permanganate lignin (PL) contents [9]. By comparison, it was found that three methods have no differences in determining NDF, ADF, and PL content when measuring the major components of feed. The CAU filter bag technique has advantages, including a low cost, convenient ordering, and suitability for laboratory applications.

11.1.3.2 Research Methods for Hemicellulose Content

Methods introduced in Sect. 11.1.3.1 can be applied.

11.1.3.3 Research Methods for Lignin Content

Methods introduced in Sect. 11.1.3.1 can be applied. According to the properties of lignin, lignin can be determined [10] directly by the concentrated sulfuric acid hydrolysis separation method [10, 11], spectrophotometry method [12, 13], infrared spectroscopy method [14], redox reactions titration method [10], and so on.

11.1.3.4 Research Methods of Reducing Sugar Content

There is variety of methods to determine the reducing sugar content [15], such as the optical rotation method, gas chromatographic (GC) method, liquid chromatographic

method, etc. The accuracy of the optical rotation method is poor, while the measurement process for GC and liquid chromatography is complex. The 3,5-dinitro salicylate (DNS) method is widely used because of its high accuracy, its reproducibility, and especially its suitability for determination of large quantities. DNS is an oxidizing agent, which could react with a reducing sugar. So, that nitro group is reduced into an amino group, and the solution becomes orange. The orange depth is proportional to the concentration of reducing sugar in a certain range. Therefore, the reducing sugar content could be obtained by the colorimetric method.

11.1.3.5 Research Method for Content of Non-fiber Cells

The nonfiber cells are all parenchyma cells except the fiber cells [5]. The parenchyma cell content measurement method used here is an approximation by measuring area. Slice preparation procedures are the same as that for the length and width measurements. The prepared slice is placed under a 70–100 magnification microscope, and the approximate area of each cell is measured by eyepiece-measured micrometer (calculated from the average length and width of a single cell or the average diameter). It is recorded according to different cell types. On a slice with the cell uniformly distributed, 100 various miscellaneous fiber cells and associated parenchyma cells are measured. The results are expressed by the area percentage of each type cell to all cells (the fiber cell is included).

11.1.3.6 Research Methods for Protein Content

Determination of the protein content includes four common methods: nitrogen determination, double contraction urine method (Biuret method), Folin-phenol reagent method (Lowry method), and ultraviolet (UV) absorption method. The Coomassie blue method (Bradford method) is a new rapid method recently used widely. The sensitivity of the Bradford and Lowry methods is high, and the nitrogen determination method is complex but more accurate. Nitrogen determination is taken as a reference by other methods. The Kjeldahl nitrogen determination method can be found in the national standard method of China, GB/T 5009. 5-2010.

11.1.3.7 Research Method for Wax Content

The substances contained in wax can be analyzed by HPLC as reported, and the content of waxy substances can be analyzed by GC-MS (mass spectrometry).

11.1.3.8 Research Method for Oil Content

In the primary refining process, determination of the oil content in lignocellulosic materials can refer to oil content measurement method of oil seed using, for example, the national standard method of China, GB/T 14488.1-1993.

11.1.3.9 Research Method for Ash Content

The remaining minerals of samples treated by high-temperature combustion and ashing are called ash. Ash content [5] and its composition differ from types and parts of raw materials. The ash content in lignocellulosic materials can be determined by referring to the raw materials of papermaking using, for example, the national standard method of China, GB/T 2677.3-1993.

11.1.3.10 Research Methods for Moisture

Moisture [5] refers to a mass ratio of the lost weight caused by drying to a constant weight at a specified temperature $(105 \pm 2)\,^{\circ}\mathrm{C}$ to the original sample weight, which is expressed as a percentage. Moisture determination methods include drying and distillation methods. The drying method is a national standard. It could refer to that of raw materials of papermaking, for example, using the national standard method of China, GB/T 2677.2-1993. At present, a straw moisture analyzer is sold by Dezhou Ketou Electronics Company, Limited, and Shenzhen Guanya Technology Company, Limited.

11.1.3.11 Research Method for Flavone Content

Flavonoids and aluminum ions can form a stable red complex under alkaline and nitrite conditions, and the red complex has maximal absorption at a 510-nm wavelength. So, the flavone content could be determined with a UV spectrophotometer.

11.1.3.12 Research Methods for Pectin Content

Referring to GB/T 10742-2008 of the China national standard method, which is the pectin content determination method in papermaking, there are two methods for pectin content assay: gravimetric method and carbazole colorimetry method. In addition, there are the volumetric method, titration method, and atomic absorption spectrometry indirect measuring method [16]. The ramie pectin is determined by the method in the GB5889-86 China national standard method. The near-infrared spectroscopic method [17] and microwave-assisted extraction method [18] reduce long measurement cycle.

11.1.3.13 Research Methods for Tannin Content

Tannin measurement methods include a spectrophotometric method and a volumetric method [5]. The spectrophotometric method is based on the property that tannin is not dissolved in benzene but is dissolved in ethanol. A sample is first extracted with benzene to remove organic solvent and then is treated with ethanol to dissolve tannin. A spectrophotometer is used to determine the optical density (OD) at a wavelength of 500 nm. The content of tannin can be obtained from a standard curve.

11.1.4 Research Methods for the Function Group Content in Primary Refining [5]

11.1.4.1 Methoxy Group Content

The methoxy group mainly exists in lignin and less in cellulose and pectin. The methoxy group can be studied by chemical methods (gravimetric and bulk density methods) and instrument analysis methods (chromatography, nuclear magnetic resonance [NMR] spectroscopy, and electrochemical method). For the chemical method, the basic principle is to make the sample react with concentrated hydrogen iodide acid, leading to a methoxy group in lignin pyrolysis. Alkyl iodide is generated and removed by distillation. Then, different absorbent is used to absorb alkyl iodide for determination of the content of the generated compound. In this way, the content of the methoxy group is determined. According to different absorbents, there are the silver nitrate method, bromide method, nitrogen benzene method, and so on. The silver nitrate method is a gravimetric method. The pyridine and bromide methods are volumetric methods. The bromide method measures methoxy group content by taking bromine as an absorbent. The bromide method is also known as the WeiBoke method, which has the advantages of higher accuracy, simple operation, rapid rate, and so on. Therefore, the bromide method is widely applied.

11.1.4.2 Hydroxyl Group Content

There are many groups in lignin, cellulose, and hemicellulose. There are two kinds of hydroxyl groups in lignin. One is the phenolic hydroxyl groups that exist in the structural unit of the phenyl ring. Another is aliphatic hydroxyl groups that exist on the side chain (alcoholic hydroxyl groups). The hydroxyl content can be determined by chemical and instrumental analysis methods. Instrumental analysis methods include NMR spectroscopy, GC, UV spectroscopy, or the electrochemical method. For the H-NMR spectral data, 2.50–2.17 mg/kg represents a phenolic hydroxy, and 2.17–1.70 mg/kg represents alcoholic hydroxyl groups. If GC is used

to measure the phenolic hydroxyl content, a sample is treated by dimethyl sulfate for ethylation first, then the ethane content is measured, which could be converted into phenolic hydroxyl content. In addition, the lignin phenolic hydroxyl content can also be measured using UV spectroscopy at a wavelength of 300 or 250 nm. Phenolic hydroxyl groups and alcoholic hydroxyl groups in the lignin are called the total hydroxyl content. Usually, the total hydroxyl content and phenolic hydroxyl content in the lignin are measured by a chemical method, and then the alcoholic hydroxyl content is recalculated. For determination of the phenolic hydroxyl group, the periodate oxidation method and nonhydroderivative titration method are widely used. The nonaqueous conductometric titration method can measure the carboxyl content at the same time.

11.1.4.3 Carboxyl Group Content

The carboxyl group mainly exists in the hemicellulose. The carboxyl group can be measured by a variety of methods, such as the direct alkali titration method, exchange reaction with phosphorus-nitrophenol silver salt, reaction with methylene blue, sodium bicarbonate–sodium chloride method, conductometric titration, and dynamic ion exchange method. The dynamic calcium acetate ion exchange method (GB/T 10338-1989 of China national standard method) and sodium bicarbonate–sodium chloride method are widely applied.

11.1.4.4 Simultaneous Measurement Methods for Carboxyl Group and Phenolic Hydroxyl Group

The nonaqueous titration method could determine the carboxyl group and phenolic hydroxyl group contents in lignocellulosic material simultaneously and includes the nonaqueous conductometric titration method and nonhydropower titration method. The nonaqueous titration method uses the principle of the nonaqueous component to titrate weak organic acids. Water is a strongly polar solvent, and weak organic acids express low intensity in water. Because of homogenization of water, the intensity difference between water and weak organic acid is small. Therefore, it is difficult to analyze organic weak acid in water. However, in organic solvent, the acidic property of some weak acid is greatly strengthened. So, the difference of acid property between week acid and organic solvent is significantly increased. Therefore, it could be titrated by strongly organic alkali. The lignin carboxyl group and phenolic hydroxyl are acidic groups with weak acidity. So, the electrical conduction titration or potentiometer titration method could be used in solvent of organic medium with weak catalyzing ability. According to system conductivity or potential change before and after titration, the titration end point can be obtained, thereby calculating the content of these acidic groups.

11.1.5 Research Methods for the Techniques of the Primary Refining Process

11.1.5.1 Process for Primary Refining

The research methods for the techniques of the primary refining process of lignocellulosic materials [19] include physical, chemical, and biological methods. Physical methods are mechanical grinding, steam explosion, microwave and ultrasonic, radiation, and freezing methods. Chemical methods are alkali, cellulose solvent, organic solvent (ionic liquid), dilute acid, oxidation (wet oxidation), supercritical, and supercritical water treatment methods. The biological method is the primary refining with related microorganisms and enzymes.

The research on the primary refining process is based on the composition and structural characteristics of different lignocellulosic materials and then looks for an appropriate primary refining process for the corresponding product. The primary refining process can be a single method or a combination of several methods. Based on the long-term research on the primary refining process, Chen et al. proposed the processes discussed next (Table 11.1).

11.1.5.2 Research Methods for Consumption in the Primary Refining Process

The primary refining of lignocellulosic materials includes crush, component separation, and selectively deconstructive processes. Different processes require different methods for consumption analysis. The grinding process mainly spends energy. The crushed theory researches the relationship between energy consumption and crushing level. Because the crushing operation is an extremely complex process involving a variety of factors, there is no uniform conclusion, but three hypotheses.

The German von Literlinger proposed the area hypothesis in 1867; regarding that for grinding of solid materials, energy consumption is proportional to the new surface area. In Germany, Kick proposed the volume hypothesis in1885; in this theory, when geometry similar to the material is crushed into products with similar geometry, energy consumption is proportional to the volume or weight of the broken block. In 1952, Bond from the United States proposed the cracks hypothesis. The calculation processes for these three hypotheses are as follows:

① The area hypothesis, proposed in 1867 by Germany's von Literlinger, considered that energy consumption was proportional to the new surface area in a solid material grinding process. Their relationship could be expressed by the following equation:

$$A = Q\left(\frac{1}{D_2} - \frac{1}{D_1}\right)$$

Table 11.1 Process for primary refining

Raw materials	Primary refining process	Primary products	Final products
Cornstalk	Steam explosion → filtering → mechanical fractionation of filter residues	Hydrolysate of hemicelluloses, vascular tissue (long fiber), ground tissue (short fiber)	Furfural, cellulose materials, low molecular weight chemicals (or levulinic acid), silicon dioxide
Straw of lignocellulose	Steam explosion → filtering → filter residues → enzyme hydrolysis → filtering	Hydrolysate of hemicelluloses, fermentation residues	Furfural, xanthan gum, low molecular weight chemicals, organic fertilizer, protein feed
Straw	Steam explosion → alkali dissolving → filter	Filtrate	Oxalic acid
Straw	Steam explosion	Product of steam explosion	Cellulose acetate
Straw	Steam explosion → filter → filter residues → mechanical fractionation	Hydrolysate of hemicelluloses, vascular tissue (long fiber), ground tissue (short fiber)	Furfural, carboxy methyl cellulose, carboxy methyl lignin, silicon dioxide
Straw	Steam explosion → filter	Hydrolysate of hemicelluloses, filter residues	Furfural, desertification land modification materials, lignocellulose board
Straw	Steam explosion → filter		Lignocellulose board
Wheat straw	Steam explosion → filter → filter residue fractionation → treatment with glycerin	Hydrolysate of hemicelluloses, fermentation materials, parenchyma cells	Furfural, 2,3-butanediol, low molecular weight chemicals
Leaf, sheath, root, and fragment of straw	Steam explosion → filter → filter residue mechanical fractionation	Hydrolysate of hemicelluloses, cellulose, and lignin	Xylooligosaccharide, ecological fertilizer, fuel
Stalk of straw	Steam explosion → filter → filter residue mechanical fractionation	Thermal solid, fiber cell, parenchyma cell	Cellulose materials, bioenergy, chemical products, silicon dioxide

where A is the energy consumption. D_1 and D_2 are particle size of the feed and discharge material, respectively. Q is a constant.

② The volume hypothesis, proposed in 1885 by Germany's Kick, supposed that when geometry similar to the material was crushed into products with a similar geometry, the energy consumption was proportional to the volume or weight of the broken block. The relationship was expressed using the following formula:

$$A = C \log\left(\frac{D_1}{D_n}\right)$$

where A is the energy consumption. D_1 and D_n are the particle size of the initial feed and final discharge, respectively. C is a constant.

③ The cracks hypothesis, proposed in 1952 by Bond of the United States, suggested that when particles with a size of D_1 were crushed into a particle with size of D_2, the energy consumption can be expressed as follows:

$$A = K\left(D_2^{-1/2} - D_1^{-1/2}\right).$$

where K is a constant; Bond replaced it with 10 Wi. Wi is commonly known as the Bond index. It is a parameter reflecting property resisting crushing and abrasion. The crack hypothesis also interprets D as a measure of crack length of the ruptured block. Therefore, crush energy consumption is also proportional to the crack length.

These three hypotheses have limitations in application. The area hypothesis is more suitable for grinding operations with a particle size 0.01–1 mm. The volume hypothesis is more suitable for coarse crushing and medium crushing with a particle size greater than 10 mm. The cracks hypothesis is between them and is suitable for a more extensive range, from medium crushing to rough grinding operations.

There are still some other physical processing methods, such as steam explosion, microwave and ultrasonic, radiation, and freezing methods; and chemical processing methods, such as alkali treatment, solvent treatment, organic solvent treatment, dilute acid treatment, oxidizing treatment, and supercritical extraction. However, there is no mature model currently to analyze the consumption of energy and chemical materials, but it could be calculated according to the consumption per unit of product. Greenhouse gas produced by these treatment methods could be calculated by referring to the Revised 1996 IPCC (Intergovernmental Panel on Climate Change) Guidelines for National Greenhouse Gas Inventories, in which greenhouse gas emissions from solvent used in a production process are included.

11.1.5.3 Research Methods for Products in the Primary Refining Process [20]

According to the purpose of primary refining, different research methods are required to analyze the final product. The purpose of primary refining can be

simply divided into two types: physical crushing to obtain small particles and make further conversion easy; and chemical decomposition to obtain a component or mixed component for preparing final products. For physical crushing purposes, a particle size analyzer could be used to appraise the crushing results. For assaying the separation or selective fractionation of components, Fourier transform near-infrared diffuse reflectance spectroscopy could be used [21].

11.2 Research Methods for the Microbial and Enzymatic Hydrolysis Process of Lignocellulosic Materials

11.2.1 Introduction

Many microorganisms in nature can use cellulose or hemicellulose as a carbon and energy source, maintaining the circulation of the carbon element. Cellulose-degrading microbes are diverse, which provides a realistic basis for screening cellulose-degrading strains from nature. Since Pringsheim isolated cellulose-decomposing bacteria from the soil in 1912 for the first time, research on various cellulose-degradating microorganisms and their product cellulase has made great progress. A large amount of cellulose-degrading microorganisms and their product cellulase is separated and purified. Cellulase is distributed widely because many fungi, bacteria, and actinomycetes can produce cellulase under certain conditions. Recently, cellulase was found in succession from archaea. A degradation strategy presents diversity because of different cellulose-degrading microorganisms [22].

11.2.2 Research Methods of Resource Science for Cellulose Degradation Microorganisms

11.2.2.1 Screening and Identification Methods for Culturable Cellulose-Degrading Microorganisms

1. Screening methods for cellulose-degrading microorganisms
According to the special nutritional requirement and metabolite products of microorganisms, the objective colony can be easily found from mixed flora through the design of appropriate selective media and differential media.

(1) Screening of cellulase-producing bacteria: Congo red staining method [23, 24]
Congo red could form a red complex with cellulose in medium. When cellulose is decomposed by cellulase, Congo red–cellulose complexes would not be formed. A transparent circle around cellulose decomposition microorganisms will appear in the medium. So, cellulose-degrading microorganisms could be screened by presentation of a transparent circle. The rich medium used is composed of 2.0 g K_2HPO_4,

1.4 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.3 g $CaCl_2$, 5.0 mg $FeSO_4 \cdot 7H_2O$, 1.6 mg $MnSO_4 \cdot H_2O$, 1.7 mg $ZnSO_4$, 2.0 mg $CoCl_2$, 2 % straw powder, and 1,000 mL distilled water. Congo red–cellulose differential medium is composed of 2.0 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 1.0 g KH_2PO_4, 0.5 g NaCl, 20.0 g CMC-Na, 0.2 g Congo red, 20.0 g agar, and 1,000 mL distilled water. The liquid enzyme-producing medium is composed of the following materials: straw flour and wheat bran mixed by quality ratio 4:1, 2 g mixture in 250-mL flask, 100 mL Mandels nutrient solution, natural pH value. Mandels nutrient solution is composed of 1.4 g $(NH_4)_2SO_4$, 2.0 g KH_2PO_4, 0.3 g urea, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.3 g $CaCl_2$, 7.5 mg $FeSO_4 \cdot 7H_2O$, 2.5 mg $MnSO_4 \cdot H_2O$, 2.0 mg $ZnSO_4$, 3.0 mg $CoCl_2$, and 1,000 mL water.

For strain enrichment, 5 g strain source samples are added to 100 mL rich medium with a straw as the sole carbon source; then, the strain is shake cultured for 7 days at 28 °C under constant temperature, drawing 5 mL of culture to transfer to a new enrichment medium, enriching three generations.

For strain primary screening, enriched culture medium is diluted appropriately, separation and purification of strains is performed on Congo red medium, and preservation is in a slant.

Strain secondary screening is also a liquid enzyme production test. The primary screening strain is inoculated to liquid medium for enzyme production and then shake cultured at 30 °C for 4 days. Finally, enzyme solution is filtered and enzyme activity determined.

(2) Hemicellulase-producing microorganism screening: flat transparent circle method [25]

Hemicellulose is insoluble in water, so if it is added to a medium, the plate would become opaque. Hemicellulases are generally extracellular enzymes and can be degraded into water-soluble reducing sugars, leading to a transparent circle; based on this, hemicellulase-producing microorganisms can be screened.

The following are used for the enrichment medium ($g \cdot L^{-1}$): 0.5 NH_4NO_3, 1.0 KH_2PO_4, 0.2 NaCl, 0.3 $MgSO_4 \cdot H_2O$, 0.2 $CaCl_2 \cdot H_2O$, 0.1 K_2SO_4, 15 hemicelluloses, pH 6.0. The primary screening medium contains the following ($g \cdot L^{-1}$): 2.0 NH_4NO_3, 2.0 KH_2PO_4, 5.0 NaCl, 0.2 $MgSO_4 \cdot H_2O$, 5.0 east extract, and 20.0 hemicellulose at pH 6.0. The secondary screening medium has the following ($g \cdot L^{-1}$): 20.0 hemicellulose, 3.0 NH_4NO_3, 2.0 KH_2PO_4, 5.0 NaCl, 0.2 $MgSO_4 \cdot H_2O$, and 5.0 yeast extract at pH 6.0.

For the enrichment culture, 5 g soil samples are added to 45 mL sterile water and uniformly shaken. After standing, 2 mL of supernatant are injected to a 50-mL Erlenmeyer flask containing 20 mL of enrichment medium. It is cultured at a constant 30 °C, 150 $r \cdot min^{-1}$, shaking for 48 h.

For primary screening, 1 mL of enrichment culture is coated to a plate covered with screening medium. It is inverted and cultured at 28 °C for 72 h. Then, a generated hydrolysis circle is observed during culture. Finally, colonies with a hydrolysis circle are picked and transferred to slant medium to culture. The slant is preserved.

For secondary screening, the primary screening strain is activated and a ring is picked to inoculate into 80 mL (250-mL flask) secondary screening medium.

Then, it is shake cultured at 150 r·min^{-1} in a constant temperature of 30 °C for 48 h. Then, the fermentation broth is centrifuged at 5,000 r·min^{-1} for 20 min to obtain a crude enzyme solution. Hemicellulase activity is measured using the DNS method to screen the strains producing the highest enzyme activity.

(3) Ligninase-producing strain: aniline blue decolorization circle method
Aniline blue plate decolorization method [26–28]. Aniline blue is a poly dye. Production of lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase will decolorize it. So, it is direct and convenient method to detect lignin-degrading enzymes qualitatively. The fungi determined are inoculated in aniline blue decolorization medium. Then, the culture is observed and recorded daily. LiP or MnP production is detected qualitatively by the presence or absence, generation speed, and the size of the decolorization circle in blue medium colonies.

Tannic acid (potato dextrose agar, PDA-bavendamm) plate method [27, 28]. The principle of this method is that laccase secreted by strains can make tannin and other phenolic compounds in PDA-bavendamm plates polymerize, leading to a brown round ring around the colonies. The laccase production level can be determined according to the diameter of the color circle and the generation time. When fungi are cultured in medium containing tannic acid, if laccase is produced, a brown diffusion region will appear in the medium around colonies. Otherwise, there is no color change in the medium. If there is brown discoloration around colonies, a Bavendamm reaction has occurred, indicating that the strain has lignin-degrading ability.

PDA-RB (rose bengal) blue plate method. The principle of this method is that peroxidase produced by the strains can make the RB in a PDA-RB blue plate change appears as an orange-yellow ring color circle around colonies. Productivity of LiP and MnP can be determined according to the colored circle diameter and generating time.

2. Microorganism identification methods [29]

(1) *Classic indicators for strain identification*. These include individual microorganisms and colony morphology observations, physiological and biochemical reactions, ecological characteristics, life history, serological reactions, and so on.
(2) *Miniature, simple, rapid, or automated strain identification technology*. This technology includes API (Analytic Products Inc.) bacterial numerical identification system, which can determine 20 biochemical indicators simultaneously; the "Enterotube" system; "Biolog" automatic; and a manual bacterial identification system.
(3) *Modern methods for microorganism classification and identification*

① Determination of DNA base proportion ratio of guanine to cytosine which is an important indicator now to publish any new microbial species and a reliable indicator to establish new a taxon.
② Nucleic acid molecular hybridization method, which is an effective means to determine the nucleic acid homology degree and kinship between different

species. The homology degree between species could also be determined by isotopically labeled radiation autoradiography techniques through calculating the relative radiation intensity value of heterozygous DNA between the measured strain and a reference strain.

③ Ribosomal RNA (rRNA) oligonucleotide catalog, which is a method to determine the genetic relationship and evolutionary lineage between different biologies through the analysis of the most stable rRNA oligonucleotide sequence homology degree in prokaryotic or eukaryotic cells. For prokaryotes, the 16S rRNA gene sequence is analyzed, and for eukaryotes, the 18S rRNA gene sequence is analyzed.

④ Identification indexes of cell chemical composition reaction, including the chemical composition of the cell wall, sugar type of whole-cell hydrolyzing liquid, analysis of phosphorylation lipid component, quinones analysis, and so on.

⑤ Numerical taxonomy method, which is a method based on the principle of numerical analysis to classify microorganisms according to the phenotypic traits similar extent to each other with the help of modern computer technology.

11.2.2.2 Identification Methods for Nonculturable Cellulose-Degrading Microorganisms [22]

The number of microbial species in nature is about 10^5–10^6. However, because of the limitation of the awareness level and experimental conditions, only 1 % of total microbial species in the environment are separated and identified with a traditional method based on specific selective media and culture conditions. There are many nonculturable microbial species in the environment. The biodegradation of natural lignocellulose is carried out under joint action by the known cellulose-degrading bacteria and a large number of unknown cellulose-degrading bacteria. By traditional microbiological screening methods, it has become increasingly difficult to screen new cellulose bacteria and the enzyme component with efficient degradation ability. Meanwhile, there are also many problems, such as low efficiency for obtaining a more efficient new enzyme with a molecular modification method. Apart from cellulose-degrading strains with pure culture, there are many efficient cellulose degradation flora in natural habitats, which are rich in cellulose enzyme. To find new genes or new enzymes related to cellulose degradation, the rise and development of environmental genomics and environmental proteomics provide a new idea.

For nonculturable species in a particular habitat, it can greatly extend microbial genetic resources application space to study how their genetic material system occurs or active substance diversity and to find new genes or new protein. These are some research ideas for environment metagenomics and environment metaproteomics.

(1) Environmental genomics technology and its application in looking for new cellulase genes

The emergence of environmental genomics technologies makes finding a specific sequence or functional gene directly from natural habitats or specific environments

possible and does not depend on microbial culture. The basic strategy is as follows: All the genetic materials of all microorganisms are extracted directly without isolating and culturing the related microorganisms from the environment to build an environment genomic library. Furthermore, a genomic library is set up, and the gene of the active product is discovered. There are two ways to analyze a genomic library. One is based on function analysis. Another is based on sequence analysis. The former is mainly by biochemical methods (such as enzymatic activity measurement, etc.) to screen active clones. Although rapid and intuitive, it requires a related gene or gene cluster to be actively expressed in the host cell. Therefore, the probability is low of obtaining active clones by this pathway. The latter technology is to obtain an interesting gene by designing hybridization probes or polymerase chain reaction (PCR) primers according to conserved DNA sequences. To some extent, this avoids the defects of the former technology. However, at the same time, it also limits the probability of a new gene encoding functional protein. This technology is built on complete trust of existing genome annotation information, resulting in its potential drawbacks. Environmental genomics technology has its own drawbacks, but the study of a single microbe has been extended to the study of microbial populations. Its superiority is especially demonstrated in the study of nonculture microorganisms.

(2) Environmental proteomics and its application in finding new cellulase and nonenzymatic factors

Genomics research tools based on DNA level only show the potential genetic ability of microorganisms; its true expression of genes in natural ecosystems cannot be shown. Environmental proteomics make up this deficiency. Environmental proteomics mainly study protein composition and its activity, which is expressed by the genome in different environments. It is a direct reflection of microorganism activity in natural habitats. Environmental proteomics focuses on the function of the microbial community and active substance analysis. The environmental proteomic analysis method can identify and confirm the target of hydrolytic enzymes in complex communities and expression patterns of series protein. A more complete protein library should be obtained first from the research environment to research functional protein in the natural environment. Ideally, proteins in the library should have a sufficient concentration and purity to meet the needs of existing analysis and be representative. In addition, there is still no unified approach to obtain ideal protein libraries from different samples because there are various environmental samples with complex components. Environmental proteomics research is not yet perfect, but scholars have performed a large amount of useful exploration. Benndorf established the method to extract a protein group from soil with alkali and phenol, and the protein component was analyzed by electrophoresis and mass spectrometry. With this method, microbial species from chlorobenzene-contaminated groundwater samples were analyzed; these species may be involved in the degradation of pollutants. It is still in its infancy to look for a new cellulase with environmental proteomics technology. Yu et al. extracted protein samples from rotting silage material. Through a series of purification and concentration steps, five endo-1,4-β-glucanase components were obtained using two-dimensional electrophoresis combined with Congo red activity.

11.2.2.3 Research Methods for the Relationships of the Cellulose-Degrading Microorganism Community [30–36]

Microbial ecology studies often take macromolecules, such as fatty acids, nucleic acids, proteins, and metabolites, related to microbes as life markers. These markers contain much information to reflect the characteristics of microbial populations from different aspects.

(1) Phospholipid Fatty Acids
Phospholipid fatty acids (PLFAs) are the cell membrane constant component of living microorganisms. So, they are sensitive to environmental factors and significantly different among species. The PLFA number and type and the ratio between different fatty acids may reflect the in situ microbial community structure and vitality in soil. PLFAs with different chemical structures characterize different microorganisms. Mcmahon analyzed PLFA labeled with stable ^{13}C and found that the content of 18:2 ω6 fatty acids was high in the process of ryegrass degradation in soil, which revealed that fungi played a major role in the ryegrass degradation process. PLFAs can only provide a rough generalization for microbial populations. Other methods should be applied for the analysis of microbial systems.

(2) Ribosomal RNA
Ribosomal RNA is conserved in evolution. Not only a conserved region but also some variation districts are contained in rRNA, which provides the foundation for positioning and analysis of the genetic relationship for a system. It is fairly common to analyze system characteristics of microorganisms such as bacteria, archaea, and fungi by 16S rRNA and 18S rRNA as molecular markers. The rRNA database continues to be supplemented and improved, and many ribosomal sequence analyses from microorganisms are related to cellulose degradation. Conserved primers could be designed by finding microorganisms with similar genetic relationship characteristics in the system. Then, a nucleic acid sequence could be amplified by PCR to study corresponding microorganisms. The composition of the cellulose-degrading bacteria in soil was analyzed by amplifying with specific primers from *Clostridium* genus degrading cellulose.

(3) Functional gene research methods
Cellulase is a critical functional protein for cellulose-degrading microbes to degrade cellulosic biomass. According to the cellulase gene as a reference sequence, primers can be designed to investigate the distribution of cellulose degradation microorganisms and their phylogenetic relationship. This way, actual functional characteristics could be better reflected because a functional protein enzyme is involved. Starting from a functional gene is more targeted and clear. Jacobsen used cellulase functional gene primers to amplify and analyze cellulase gene diversity and designed gene-specific primers for the cellulose exoglucosidase 7 family, as well as for the endoglucosidase of family 45. The results showed that cornstalk

habitats contained many new celluloses. These cellulase genes might be from uncultured microorganisms, and they showed the diversity of cellulose-degrading microorganisms.

(4) Proteomics methods
Compared with nucleic terms, protein, the product of genes, more directly reflects microbial function because it is the direct carrier of microorganisms to exercise environmental functions. Schulze et al. studied the proteome in forest lakes and forest soil environment. Results showed that 78 % of protein isolated from forests and lakes were from bacteria, while only 50 % of the total protein was isolated from forest soil. The study mentioned cellulase composition in protein from the forest soil environment and tried to explain microbial features in the process of soil organic carbon degradation.

(5) Genomics methods
Compared with the maturing proteomics, genomics is becoming mature gradually. Its application is fairly common in the study of molecular microbial ecology. Some useful information is presented for related research with new technology.

① *Denaturing gradient gel electrophoresis (DGGE)*. Shiratori analyzed *Clostridium* microbial dynamic change characteristics in the process of fibrous waste anaerobic degradation by DGGE.

② *Fluorescence in situ hybridization (FISH).* With FISH technology, Shinkai showed distinctively the spatial distribution characteristics of *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* in cellulose residues in the process of cellulose anaerobic degradation. Both are cellulose-degrading bacteria.

③ *Real-time PCR (RT-PCR) technology.* With RT-PCR, Denman designed specific primers and analyzed the number of two anaerobic cellulose degradation bacteria in cattle stomachs in the process of cellulose degradation to understand the ecological role of microorganisms in the different stages of cellulose degradation.

④ *Stable isotope probing (SIP) technology*. In a habitat, metabolically active microbial populations can use ^{13}C-labeled substrate to exert their function. In the meantime, ^{13}C will be assimilated and becomes composition of their structure macromolecules, such as nucleic acids, fatty acids, proteins, and so on. However, for microorganisms with weak physiological metabolic activity, nucleic acid or PLFA molecules containing heavy carbon labeling could not be accumulated in a short period of time. So, heavily carbon-labeled biological macromolecules could be recycled, in which ^{13}C stable isotope labeling of nucleic acid could be analyzed through molecular biology. Furthermore, the genetic structure and population information of active microbes related to substrate utilization could be analyzed. Classification features and ecophysiological functions of a microbial community active in degrading cellulose in a habitat could be analyzed with ^{13}C-labeled cellulose by RNA-SIP and PLFA-SIP analysis.

11.2.3 Breeding Methods for Cellulose-Degrading Microorganisms

Although cellulase-producing strains can be screened from nature, their enzyme production capacity and production performance still are far from meeting the needs of industrialization. Therefore, breeding of cellulase-producing strains is particularly important in that cellulose has enormous application potential in the feed, food, oil, pharmaceutical, chemical, and textile industries and so on.

11.2.3.1 Mutation Breeding

Mutation breeding treats microorganisms uniformly dispersed in a cell population with physical and chemical mutagen to increase mutation rates significantly and then screen the minority mutants that could fulfill the requirements by rapid and efficient screening method. There are many types of mutagenic agents: for physical factors, including nonionizing radiation of UV, laser, ion beam, and X-ray, that can cause ionizing radiation, γ-ray, and fast neutron; for chemical mutagens, including alkylating agents, nucleotide analogues, and acridine compounds. Alkylating agents applied usually include nitrosoguanidine (NTG), ethyl methanesulfonate, methyl nitrosourea (NMU), diethyl sulfate (DES), mechlorethamine, ethylene imine, and epoxy acetic acid [29].

Jenna et al. [37] screened thermostable fungal MY with high cellulase activity by sodium carboxymethyl cellulose (CMC) primary screening and 50 °C culture. The MY strain was then taken as the original strain and mutated with UV light and DES. Ninety-seven mutants were screened with an index of the transparent circle diameter to the colony diameter ratio (HC value) increasing by more than 30 % or a colony morphology significant variation. Twenty-nine strains were screened by measuring CMCase activity of the crude enzyme solution; the CMCase activity increased by more than 30 %. Finally, with mutant strain passage stability experiments, a MY004 mutant was obtained whose CMCase activity increased by 80.6 % compared with the original strain MY.

11.2.3.2 Gene Recombination and Crossbreeding

Gene recombination is defined as follows: It is a process that inherits genes from two independent genome transfers through certain channels to form a new stable genome. Recombination is a concept on the level of the nucleic acid molecule and the hybridization of genetic material at the molecular level. Therefore, it is obviously different from hybridization at the cellular level. Gene recombination is the theoretical basis of crossbreeding. Compared with mutation breeding, gene recombination is greatly controllable because it selects a donor strain and a recipient strain with known traits as parents. Furthermore, crossbreeding is an important means of breeding because it can eliminate the phenomenon of slow

speed in increasing production after the mutagenic treatment. Prokaryotic gene recombination includes transformation, transduction, conjugation, and protoplast fusion. Eukaryotic microorganism gene recombination includes sexual hybridization, quasi-hybridization, protoplast fusion, and genetic transformation.

Improving cellulase activity by protoplast fusion technology began with the work of Tayama et al., who researched *Trichoderma reesi* genetics using protoplast fusion technology. The stable CMCase activity was one time higher than that of the parent. In China, cellulose microbial protoplasm preparation, regeneration, and fusion were all explored. Qiu et al. [38] researched the progeny of protoplast fusion from *Trichoderma viride* N6 and *Aspergillus niger* 856. By passage, fermentation, and screening, three strains—AT23, AT16, and AT34—with high cellulase activity and stability were obtained from 11 screening fusants. Their CMCase activities were 2.2 times, 1.4 times, and 1.2 times higher than the parental *Trichoderma viride* N6, respectively. It was proved that AT23, AT16, and AT34 were gene recombination fusions and had the advantages of crossbreeding by nystatin resistance testing and soluble protein gel electrophoresis analysis. Zhang et al. carried out protoplast fusion with *Aspergillus kawachii* genetically engineered brain TR12 and *Aspergillus niger* 34309 as the original strain. Then, with UV radiation mutation breeding, a new strain with high enzyme activity was obtained. The acid resistance α-amylase and glucoamylase activity reach 91.2 $U \cdot mL^{-1}$ and 3216.2 $U \cdot mL^{-1}$, respectively. *Trichoderma reesei* is able to synthesize a large amount of cellobiohydrolase (CBH) and endoglucanase (ED). However, the vitality of cellobiase (CB) from *T. reesei* was low, and that from *Aspergillus niger* was high. To fully exploit the interspecific complementary strength traits between two distant sources, *Trichoderma reesei* and *A. niger*, protoplast fusion could be researched for *T. reesei* and *A. niger* to obtain fusants with two genera advantages.

But, in general, it is seldom reported that cellulase activity could be significantly improved through integration technology. Crossbreeding methods are complex. In the breeding of industrial microorganisms, it is rare to cultivate high-yield strains used in production practice by transformation, transduction, or bonding recombinant technology.

11.2.3.3 Protein-Directed Evolution Technology

Under the condition that it is not necessary to knowledge spatial structure and catalytic mechanisms of a biocatalyst, mutations could be introduced into genes encoding biocatalyst through various mutagenesis methods. A mutated gene could be reassembled to improve gene evolution efficiency by a recombinant method such as DNA shuffling or the like. These transformed genes can produce a biocatalyst with better performance. Then, a mutant strain fulfilling the requirements can be obtained in a larger library using a filter or a selection method.

Wu et al. [39] carried out directed mutation for ED *Cel1*B from extremely heat-resistant *Thermotoga maritime* by an error-prone PCR method. For the recombinant plasmid pET-20b-Cel12B carrying the *Cel1*2B gene, error-prone PCR conditions

were optimized using different concentrations of Mg^{2+} and a fixed Mn^{2+} concentration. The product could build recombinant pET-20b-Mu-Cel12B and establish mutant libraries. Finally, recombinants were screened by the Congo red plate method. Three mutants were obtained using the size of the transparent circle, whose enzyme activity was substantially increased. When a mutant gene was induced, the enzymatic activities were 2.1 times, 3.2 times, and 3.7 times higher than that of parent enzyme obtained by induction under the same conditions, respectively.

11.2.3.4 RNA Interference Technology [40]

RNA interference (RNAi) is a process that makes a specific gene effectively closed by degradating messenger RNA (mRNA) differentially in a cell with double-stranded RNA (dsRNA). dsRNA is endogenous or exogenous and has a homologous sequence complementary to a certain sequence of a coding region from an endogenous target gene transcription product mRNA. RNAi was post-sequence-specific transcriptional gene silencing.

To produce and use cellulase simply, quickly, and efficiently, RNAi technology and the maturity and perfection of the *Bombyx mori* baculovirus expression system will undoubtedly be a powerful tool. With RNAi, the protein factor gene could be silenced that inhibits cellulase gene transcription and expression when the glucose concentration reaches a certain concentration in the process of hydrolysis. Thereby, the inhibition effect of glucose on the cellulase was released, and the cellulase gene could maintain transcription and expression continuously.

11.2.3.5 Genetic Engineering Technology

With the development of molecular biology, genetic engineering techniques are increasingly applied to the screening of cellulose-degrading strains. Genes encoding cellulose could be isolated, and then gene expression could be improved by molecular biology techniques, including connecting the gene to a strong promoter, excluding the repression-sensitive manipulation gene, and improving the translation efficiency. Genetic engineering technology tries to improve the enzyme production of cellulose-degrading strains and cellulase stability. At present, the cellulase gene was cloned into bacteria, yeast, fungi, and plants by genetic engineering techniques developed both at home and abroad [41].

Wood et al. [42] expressed an ED gene *Erwinia chrysanthemi* P86021 in *Escherichia coli* Koll and finally obtained recombinant cellulase that can generate up to 3,200 $IU \cdot L^{-1}$ of medium. To improve the cellulase productivity of the strain, Zhou et al. [43] integrated *CelY, CelZ* genes from *E. chrysanthemi* into a chromosome of *Klebsiella oxytoca* MSA 1 brain P2, which can naturally metabolize cellobiose to ethanol. The ethanol yield of the improved strains increased by 22 % compared to that of the control.

11.2.4 Determination Methods for Structure and Activity of Enzymes Related to Cellulose Degradation

11.2.4.1 Determination Methods for Structure of Cellulose-Degrading Enzymes

Protein structure determination methods include X-ray crystallography (X-ray), NMR technology, and 3-D electron microscopy reconstitution. The three methods are completely independent for measuring the 3-D structure of protein at the level of atomic resolution.

(1) X-ray crystallography [44]
X-ray crystallography is the earliest and most important determination method used for protein structure examination. About 85 % of protein structure included in protein data bank (PDB) is determined by X-ray crystallography. Molecules in the crystal tend to be locked in a certain state. Molecular is mostly in the ground state or the average of different configurations while determining crystal structure. However, molecules usually exercise function at an excited state or transition state. But, X-ray crystallography technology has a difficulty in capturing molecular dynamic information. No matter what, X-ray crystallography technology has been and will be the main method for protein structure research.

(2) NMR spectroscopy [44]
NMR spectroscopy is a strong complement to X-ray crystallography. Today, NMR has become the primary means to determine the 3-D structure of biological macromolecule solutions. NMR technology has an advantage in analysis of protein-folding stability, movement, and the interactions among subunits of protein complexes. There are also some shortcomings of the NMR technique. It can only measure small proteins and proteins with molecules of medium size (relative molecular mass generally less than 30,000). Pattern analysis work is extremely time consuming, taking from several months to a year, leading to a long experimental period and slow speed. The reaction of NMR diffraction technology is performed in solution, so the object of study must be a soluble protein; determining an insoluble protein is more difficult. Samples need to be isotopically labeled. These shortcomings restrict the application of the NMR technique to a certain extent. With the discovery of some new technologies, such as the G-matrix Fourier transform NMR (GFT-NMR) technology, NMR technology developed quickly. At present, about 15 % of protein structure in the PDB are analyzed by NMR.

(3) Circular dichroism (CD) [45]
CD is another common means to study 3-D structural change of proteins in solution. In the CD signal, 260–320 nm of near-UV region is mainly from amino acid residues of aromatic clusters in protein. Each amino acid residue has a specific wavelength peak shape. Most important, the near-UV CD spectra of protein can provide 3-D structural information, which is strong evidence for a denaturation midpoint state "melt sphere."

(4) Endogenous fluorescence spectroscopy [45]
Endogenous fluorescence spectroscopy is commonly used to study the 3-D structure of the protein. CD and endogenous fluorescence spectra just use certain parameters related to the 3-D structure to assess the presence of the 3-D structure of protein.

(5) 3-D electron microscopy reconstruction [44]
With the continuous development of computer image-processing technology and microscopy equipment in the last 5 years, the 3-D electron microscopy reconstruction technique became another important method for examining protein structure following the X-ray and NMR technology.

The advantages of 3-D reconstruction techniques are as follows: (1) Molecular morphology can be obtained directly even at a lower resolution; electron microscopy can also give meaningful structural information. (2) It is suitable for samples that cannot be analyzed by X-ray crystallography and NMR techniques, such as difficultly crystallized membrane proteins and macromolecular complexes. (3) It is suitable for capturing dynamic structural changes and is easy to combine with other technologies to obtain high-resolution molecular complex structural information; it contains the phase information, therefore, while determining a phase, it is direct and more convenient than X-ray crystallography.

(6) Scanning probe microscopy (SPM)
Scanning probe microscopy is a surface analysis technique invented in the 1980s; it is available for 3-D surface structure and dynamic processes at the nano level for real-time, real-space observation. So, it has wide prospects in the study of structural biology. Exoglucan CBH enzymes isolated from *Trichoderma* S38 cultures are analyzed with SPM technology for holoenzyme molecular morphology observation. SPM images showed that CBH I molecules are tadpole shaped, composed of a large head and long tail, which are presumed to be core protein and substrate adsorption domain of CBH, respectively. The length of the whole enzyme molecule is 29 nm, and the maximum width is 9 nm [46].

In 1990, Rouvinen applied the X-ray diffraction method to analyze the catalytic domain of the *Trichoderma reesei* CBH II. In 1993, Spezio crystallized and analyzed the catalytic domain of E2 of *T. fusca*. Study of 3-D structural analysis provided a reasonable explanation for endoglucase and exoglucase substrate specificity. In 1989, Kraulis used the NMR method to study 3-D structure of the cellulose-binding domain (CBD) from *T. reesei* CBH I. And in 1995, Xu studied the 3-D structure of CBD from *Cellulomonas fimi* Cex. Tormo applied the X-ray diffraction method to study the structure of CBD from *Clostridium thermocellum* Cip in 1996 [47].

Spectral science reduction showed that there are five kinds of oxidation state for LiP. Natural-state LiP contains high-spin Fe^{3+}. Two electrons are oxidated by H_2O_2, and LiP becomes LiP I (oxygen with iron porphyrin ring radicals containing $^{\bullet}{}_{+}Fe^{4+}$). Single-electron reduction forms LiP II (oxygen with the iron porphyrin ring containing Fe^{4+}). Then, by single-electron reduction again, it returns to the natural state. His^{82} is in the cracks of the active center openings in the channel surface, and Trp^{170} is in the surface of the enzyme protein. Its electron transfer may have two different pathways: substrate-His^{82}-Ala^{83}-Asn^{84}-His^{47}-heme or

substrate-Trp^{170}-Leu^{171}-heme; MnP crystal structure includes 17 % neutral sugars and a large number of acidic amino acids. There is only one Mn^{2+} binding site in heme [48–50].

The copper number of different types of laccase is different. Generally, lacasse contains four copper ions. Copper ions can be divided into three types according to spectroscopic and magnetic characteristics. Type I has the characteristics of one Cu^{2+}, single-electron acceptor, paramagnetism, blue color, and special absorption spectra at λ614 nm. Type II has the characteristics of one Cu^{2+}, single-electron acceptor, paramagnetism, nonblue color, and no characteristic absorption spectra. Type III has the characteristics of two Cu^{4+}, dual-electron receptor, diamagnetism, coupling ions (Cu^{2+}–Cu^{2+}), and broad absorption spectra at λ330 nm. The 3-D structure of laccase is not yet clear, but it has been confirmed that copper ion is located in an active site and plays a decisive role in the catalytic oxidation process [48, 51, 52].

11.2.4.2 Determination Method for Activity of Cellulose-Degrading Enzymes

Enzyme activity is usually determined when the solubility of substrate is better and the concentration of substrate is high. So, the reaction is zero stage for substrate. Then, the specific activity of the enzyme can be obtained exactly by measuring initial velocity. However, it is difficult to compare cellulase activity determined by various methods and calculated with different methods because there is no common standard. A major problem in current cellulose basic research is how to determine cellulase activity accurately and rapidly. Problems in the determination of cellulase activity and the current method used are discussed next.

1. Problems in the cellulase activity measuring process

(1) Substrate cellulose structure is heterogeneous, including an easily degradable amorphous region and a difficultly degraded crystalline region. Most substrate is water insoluble or sparingly soluble polymers. In terms of cellulase, substrate is always unsaturated. The contact of cellulase enzyme with a solid substrate and reaction product (such as CMC) is only partially soluble. The more the substituent is, the greater the solubility is. In turn, the more the substituent is, the lower the decomposability is as a cellulose enzyme substrate. Therefore, researchers around the world determine cellulase activity in the state of unsaturated substrates. The measured enzymatic activity is always low because of unsaturated substrate. Moreover, the more the cellulose is used, the greater the unsaturation is. While determining cellulase activity, different results are obtained for different enzyme preparations or different dilutions.

(2) Because the substrate used also contains cellulose, hemicellulose, and lignin, the saccharification effect of enzyme on substrate also includes hemicellulase activity in general cellulase in addition to that of cellulose (approximately two times higher than cellulase) because the sugar produced is reducing property sugar. Therefore, impure substrate has a great impact on cellulase activity.

(3) Cellulase is an enzyme system with multiple components. A synergistic effect exists between each component. The component and proportion of cellulase are greatly different because of different strains or the same strain under different culture conditions, leading to a different degradation process for cellulose. The scientific complexity of cellulase and the degradation process affect the determination of cellulase activity; it is more difficult to determine the activity of a single component in a crude enzyme preparation.

(4) Determination of cellulase activity generally refers to the cellulase that are secreted. Although some bacteria and basidiomycetes have a strong ability to degrade cellulose, there is less research to determine their activity because its degradation mechanism is less understood. It does not reflect the actual state to study enzyme activity with a traditional activity measurement method. Generally, it is difficult to detect their activities.

(5) If cellulase activity unit is demonstrated according to the international provisions (the amount of enzyme needed to catalyze 1 μmol in 1 min), the value of cellulase activity is generally low. Some scholars apply values of $mg{\cdot}h^{-1}$, $mg{\cdot}(24\ h)^{-1}$, or $mg{\cdot}(30\ min)^{-1}$ to represent units, making it difficult to compare units.

In short, the determination method for cellulase activity relates to the structural complexity of the substrate and is difficult to standardize. In addition, problems include multicomponent cellulase preparation and different cellulase enzymatic mechanisms.

2. Method for determination of cellulase activity

Compared with the general enzymes, determination of cellulase activity is complex and difficult. It is more difficult to measure vitality of each single component of cellulase preparation. The measurement methods that can comprehensively reflect the activity of each group are different because of different structural properties of substrate used. Cellulase solubility activity, cellulase saccharification activity, and enzyme activity of each component are described next.

(1) Cellulose solubility activity

Such measuring methods generally qualitatively describe cellulase activity, and some can describe it quantitatively. The choice of measurement method is mainly based on the nature of the work. For example, such determination methods are suitable for cellulose microbial screening

① Turbidimetric method

The turbidimetric method was invented in the 1960s. Li and Nummi took 0.03 % Avicel and milled cellulose powder as a substrate, respectively, and measured turbidity or OD change to indicate enzyme activity. For the concentration per time unit determination, 20 mL appropriately diluted enzyme solution were mixed with 3 mL substrate with a turbidity of 80 and concentration of 0.04 %; 10–20 % was used at 50 °C within 10 min. The slope was the initial reaction rate; the decline of turbidity per minute was the unit of enzyme activity. For OD per

time unit determination, 0.2 mL appropriate diluted enzyme solution was added to substrate, $A_{620} = 0.400$, at 50 °C for 10 min. The change of OD value is the enzyme activity unit.

The turbidity method is based on the starting reaction rate of cellulose degradation by cellulase and is the result of a combined effect of cellulase multicomponents. So, it preferably reflects on the dissolution ability of cellulose. But, it has rarely been used previously. But, the study on the process of cellulose degradation found that the enzymatic hydrolysis of cellulose demonstrates the dissolution and saccharification processes, in which different enzyme components are involved. Therefore, the turbidimetric method is now gradually being applied, especially in a cellulase degradation mechanism study.

② Transparent ring method
Usually, cellulose is expanded by phosphoric acid and is made into a uniform agar plate. A clear transparent circle would form because of enzymatic hydrolysis of the substrate. In a certain range of enzyme concentrations, the logarithm of the enzyme concentration is linearly related to the diameter of the transparent circle. This method is simple; therefore, it is widely used for *i* strains solution.

Lignocellulosic material is mixed with 2 % acetic acid buffer to form a suspended emulsion. Glass balls with a diameter of 2 mm are added. Then, the suspended emulsion is stirred at 180 $r \cdot min^{-1}$ for 12 h to make it into particles with a diameter less than 0.1 mm. Nutrient agar is poured into the flat bottom of a petri dish first to form a 3-mm thick layer. After condensation, agar containing 2 % milling cellulose is poured in to form a 1-mm thick upper layer. Thickness should be as uniform as possible. Finally, cellulose is coated uniformly, degrading at 40 °C for 12 h.

③ Weight loss method
A certain substrate is weighed, including filter paper, cotton, microcrystalline cellulose, and so on. Then, appropriately diluted cellulase solution is added. The mixture is reacted at 50 °C for 24 h, filtered, dried, and weighed. The weight loss rate indicates cellulase activity.

④ Filter paper crash method
Filter paper in 1 cm × 1 cm strips is added to an L-shaped tube. Then, the filter paper is completely collapsed into a powder at 40 °C. The reciprocal of time (min) required is used to demonstrate cellulase activity.

(2) Cellulase saccharification activity
The ability to generate reduced sugar from cellulose is called the activity of saccharifying cellulase. Filter paper and microcrystalline cellulose, cotton, and so on are used as substrates while detecting cellulase activity which is obtained by measuring the reducing sugar after enzymatic hydrolysis. The most typical and the most commonly used method is filter paper activity (FPA).

① General determination of FPA
For general determination of FPA, 50 ± 5 mg Whatman filter paper and 0.5 mL appropriately diluted cellulase solution are added to 2 mL of 0.2 $mol \cdot L^{-1}$ acetate

buffer at pH 4.8. The mixture is reacted at 50 °C for 60 min. Then, 1 mL DNS reagent is added to terminate the reaction. The mixture is put into a boiling water bath for 5 min and immediately cooled. The amount of reducing sugar is measured at 550 nm. The concentration of the enzyme solution is adjusted repeatedly to obtain 2 mg reducing sugar generated in the reaction. It is really complicated to adjust enzyme solutions from different sources to a similar reaction. In recent years, many improved methods were provided, such as the improved method proposed by Gao et al. But, conventional methods in the laboratory adjust the OD of the generated sugar at about 0.4.

② Cotton enzyme activity
For the cotton enzyme activity method, 50 ± 5 mg cotton, 2 mL acetic acid buffer at pH 4.8 and with a concentration of 0.2 mol·L^{-1}, and 0.5 mL of appropriately diluted enzyme solution are added together. The mixture is reacted at 50 °C for 24 h. The reducing sugar is determined by the DNS method.

(3) Determination of cellulose component activity
CBH is a necessary component for the enzymatic hydrolysis of crystalline cellulose. However, if it acts alone on cellulose, it is difficult to determine physical and chemical changes. Reducing sugar would be generated only when it cooperates with EG (endo-β-1,4-glucanase). It is obvious that the enzymatic activity is the activity of different enzyme components while determining enzymatic hydrolysis with cotton as substrate. Now, there are several methods to measure CBH activity.

① P-Nitrophenol-β-D-cellobiose (PNPC) method
For the PNPC method, 2 mL reaction solution contains 0.5 % PNPC, 0.1 % glucose lactone, and 0.05 mol·L^{-1} acetic acid buffer at pH 4.8, in which glucose lactone keeps β-glucosidase (BG) from hydrolyzing cellobiose side chain. Then, 0.5 mL appropriately diluted enzyme solution is added. The reaction solution is reacted at 50 °C for 15 min. Then, 0.5 mL 10 % Na_2CO_3 is added to terminate the reaction. The amount of generated p-nitrobenzoic acid is determined at 420 nm.

② An improved method proposed by Gao et al.
Gao et al.'s improved method determines CBH according to the differences of adsorption property of CBH-CBH, EG, and BG components in cotton fiber. However, it is too complicated.

There are mainly two methods for EG: CMC method and Brix method.

① CMC method
For the CMC method, 2 mL reaction solution containing 0.5 % CMC-Na, pH 4.8, and 0.2 mol·L^{-1} acetate buffer. Then, 0.5 mL suitably diluted enzyme solution is added. The mixture reacts at 50 °C for 30 min. Finally, the amount of reducing sugar is measured by DNS.

② Brix method
For the Brix method, 5 mL 0.5 % CMC-Na are added to an austenitic sugar meter, then 20 mL of suitably diluted enzyme solution are added. The reaction is carried out at 30 ± 0.1 °C for 10 min. Finally, the Brix value change is measured.

The activity of BG is measured according to the following procedures: 2 mL 0.5 % salicin is mixed with 0.5 mL appropriately diluted enzyme solution. The mixture reacts at 50 °C for 30 min. The amount of reducing sugar is measured by DNS.

3. Determination method for lignin-degrading enzyme system activity [53]

(1) Lignin peroxidase

The mixture contains 0.1 M tartaric acid buffer (pH 3.0), 0.2 mM resveratrol, 0.4 mM H_2O_2, and sufficient quantities of enzyme solution to provide absorption at about 0.4 min^{-1}. H_2O_2 should be added finally, and then the reaction can begin. The determination of veratridine alcohol oxide absorption changes is at 310 nm and 30 °C. The amount of 1 μmol substrate generated in 1 min is defined as an enzyme activity unit. Veratryl alcohol requires vacuum distillation or extraction to remove contaminants to avoid reaction delays.

The following method is also used: 1 mL reaction solution contains a certain amount of enzyme solution, substrate solution, 4 mM veratryl alcohol, 0.4 mM H_2O_2, and 40 mM succinic acid buffer (pH 3.0). The mixture is reacted at 25 °C for 3 min. The result is measured at a wavelength of 310 nm. Then, 1 μmol of substrate generated within 1 min is defined as one enzyme activity unit.

(2) Extracellular peroxidase activity determination

To determine extracellular peroxidase activity, 0.1 mL of enzyme solution is added to 4 mL of reaction mixture (mixture is formulated as follows: 50 mL phosphate buffer solution at pH 6.0 and 0.05 M concentration, adding 0.028 mL hydrogen peroxide and 0.019 mL guaiacol lignans). The mixture is incubated at 28 °C for 30 min. The OD value is determined at 470 nm using boiled and inactivated enzyme solution as a color control. The increase of 0.0 l OD per minute is defined as one unit of enzyme activity.

(3) Laccase

Measuring methods for laccase activity include spectrophotography using 2,2′-azino-bis(3-ethylbenzothiazoline oxazole-6-sulfonic acid) (ABTS), syringaldehydazine, guaiacol, and other substances as substrate, microcalorimetry, and pulsed laser-induced photoacoustic determination. Among them, spectrophotography using ABTS and syringaldehydazine as substrate is the most popular method. Its principles are as follows: A specific substrate of laccase is used with a concentration proportional to absorption at a wavelength. So, changes of substrate concentration can be obtained by determining the changes of absorption values at the wavelength, and thereby the value of laccase activity is calculated.

4. Hemicellulase activity measurement (DNS method) [25]

For measurement of hemicellulase activity using the DNS method, 0.5 mL crude enzyme solution is pipetted in a 20-mL colorimetric tube, and 0.5 mL hemicellulose suspension of 1 % mass concentration is added. The mixture is reacted at 50 °C for 30 min. Then, 3 mL DNS solution are added. The mixture is placed in a boiling water bath for 5 min, and 10 mL deionized (DI) water is added. Another 0.5 mL crude enzyme solution is inactivated in boiling water for 5 min as a blank control.

Absorbance is measured on a 550-nm wavelength spectrophotometer. The xylose micrograms of xylan enzymatic hydrolysis are calculated according to the standard curve.

For the unit of enzyme activity, xylose is taken as the standard. The amount of enzyme required to generate 1 μg xylose per minute is defined as one enzyme activity unit (U). The calculation formula is $U = N \times G/(0.5 \times 30)$, where N is the dilution time of the enzyme solution; G is the xylose content in the enzymatic solution (μg); 0.5 is the enzyme added (mL); and 30 is the hydrolysis time (min).

11.2.5 Research Methods for Lignocellulose Hydrolysis Mechanism

11.2.5.1 Deconstruction of Cellulase Molecule Structure Domain [47]

In 1986, Tilbeurgh obtained two structural domains that have independent activity by limited digesting *T. reesei* CBH I molecules with papain. One of these domains has a catalytic function and is called the catalytic domain; the other has a cellulose binding function and is called the cellulose-binding domain (CBD). Since then, a similar structure has been found in a variety of bacterial and fungal cellulase with a similar method. In the cellulase, the CBD is located in the amino terminal or carboxy terminal and connects to the catalytic domain through a section of highly glycosylated linker. Because the whole cellulase molecule is tadpole shaped with a highly glycosylated linker and has strong flexibility, it is difficult to obtain a CBD crystal. But, the deconstruction of the CBD makes it possible to obtain a spherical catalytic domain, which opens the way for structural and functional studies of cellulase.

11.2.5.2 Research on Structure and Function of the Catalytic Domain [47]

Catalytic domain research about CB structure is as following: In 1990, Rouvinen used an X-ray diffraction method to researched the catalytic domain of *T. reesei* CBH II; in 1992, Juy researched the catalytic domain of *Clostridium thermocellum* CelD; in 1993, Spezio researched the catalytic domain of *Thermobifida fusca* E2; and in 1994, Divne analyzed the catalytic domain of CBH I of *T. reesei* by making crystal. The 3-D structure research provides a reasonable explanation for the substrate specificity of endoglucanase and exoglucanase. The endoglucanase active site is located in an open cleft, and it can combine with any part of cellulose chains to cut the cellulose chain; the exoglucanase active site is located in the tunnel formed by a long loop, and it can only cut cellobiose from the nonreducing end of the cellulose chain. In 1995, Meinke deleted a loop of exoglucanase CbhA molecule from *Cellulomonas fimi* with protein engineering methods and found that endoglucanase activity improved, which further confirmed the analysis mentioned previously.

In 1990, Sinnott discussed the mechanism of enzyme catalysis of glycosyltransferase from chemistry. He regarded that aglycon loss relates to a double substitution reaction of the acid-base catalysis of two amino acid residues, which is actually similar to the mechanism of lysozyme action. From 1988 to 1994, much research was performed using site-directed mutagenesis techniques and enzyme-specific inhibitors. Finally, it was proved that glutamic acid is located in the active site of endoglucanase, exoglucanase glucosidase from bacteria and fungi. The catalyzing reaction was completed by the conversion or reservation of the configuration of anomeric carbon atoms bits. Two conserved carboxy amino acids were proton donor and nucleophile, respectively, so the acid/alkali catalytic double-replacement mechanism was proved.

11.2.5.3 Research on the Structure and Function of the Cellulose-Binding Domain

The CBD removal experiment revealed that removal the CBD of cellulase affected vitality for soluble substrate less but decreased significantly the adsorption and hydrolysis activity on crystalline cellulose. Chemical mutagenesis and site-directed mutagenesis demonstrate that aromatic amino acids play an important role in cellulose adsorption on crystal cellulose, and their mutation makes the adsorption capacity of the CBD on crystalline cellulose decrease greatly. Sequence comparisons showed that several CBDs from same family of CBH I had Phe or Trp instead of Tyr. It was speculated that the CBD adsorbs on cellulose by the accumulation force of the aromatic ring and the glucose ring. Residues formed by the rest of the hydrogen bonds in the CBD and adjacent glucose chains form hydrogen bonds to ease a single glucose from the cellulose surface to facilitate hydrolysis of the catalytic zone.

The CBD has functions of destroying the fibrous structure nonhydrolyzably to form short fibers. A scanning tunneling microscopy image demonstrated that after adsorption of the exoglucanase binding domain, the arrangement of microfibers exhibited disorder, and the nanofiberils separated from each other. With an automatic fiber length and roughness measuring instrument, the fiber length and coarseness changes further confirmed that the hydrogen bond between microfiber chains weaken and water absorption ability increases after the CBD effect, leading to fiber swelling and increased diameter.

11.2.5.4 Research on Hydrogen Bond Enzyme [54]

As long as a half century ago, Reese and other researchers proposed that there should be a hydrogen bond enzyme to deconstruct crystalline cellulose; unfortunately, it has not been confirmed in microorganisms to date. In 1994, McQueen-Mason and others separated a protein "expansin" in cucumber hypocotyls that could elongate the separated plant cell wall and weaken filter paper strength without

generating reducing sugar. Gao separated protein in *Trichoderma pseudokoningii* filtrate with a molecular weight of about 24×10^4, pI 7.0. It could make cotton fiber and chitin expand without producing reducing sugar. The infrared spectrum showed that it indeed could weaken the absorption intensity of the cotton fiber hydrogen bonding zone. It is now generally believed that natural cellulose opens the hydrogen bond among the cellulose chain and intrachain at first with a nonhydrolyzable factor and hydrogen bond enzyme. Then, disordered noncrystalline cellulose is formed and consequently hydrolyzed into fiber dextrin and glucose under the synergy effect of three kinds of enzymes.

11.2.5.5 Research on Nonenzyme Catalysis and Oxidation of Cellulose [54]

In *T. pseudokoningii* and *Gloeophyllum trabeum* culture filtrate, pure capillary electrophoresis peptide components are separated, including single-fiber generation factor (SFCF) and *Gloeophyllum trabeum* (Gt). With the development of electron spin resonance (ESR), the capture of hydroxyl radicals ($HO^{\bullet}$) and superoxide anion radicals ($O_2^{-\bullet}$) becomes possible. They lead to changes in physical and chemical properties of cellulose, and the following mechanism was proposed: The short-peptide compound complex reduces iron (Fe^{3+}), activates molecular oxygen, triggers a Fenton reaction and oxidizes cellulose hydroxyl, promotes glycosidic strand breaks, and forms short fiber. It was proved that oxidative degradation is an integral part of cellulose biodegradability.

11.2.5.6 Research on Characterization of the Topological Structure of the Cellulase Molecule [54]

After limited digestion by papain and a series of separation and purification, the catalytic domain and CBD are obtained from *Trichoderma* endo- and exocellulase. Whole-enzyme molecules of CBH I and EG I are measured. The adsorption and desorption capacity of the two domains on the fiber material and the specific activity of hydrolyzing several fiber materials are also detected. Results showed that although a single catalytic domain or CBD still has hydrolysis and adsorption capacity, when any single structural domain or two domains, is mixed with the same amount of substance, the hydrolysis or the adsorption capacity is much less than its corresponding enzyme molecules. For example, the CBD and catalytic domain of CBH I can adsorb on cotton fiber, but it is easily eluted. While the whole enzyme molecule adsorbed, it could not be eluted down even with 1 M NaCl. The synergetic effect of CBH I and EG I on the degradation of crystalline cellulose can only be reflected when the two enzyme molecules react together. The synergistic capacity can be significantly reduced while using four domains mixed with an equal amount.

11.2.6 Cellulase Recycling and Immobilization Methods

11.2.6.1 Cellulase Recycling Methods

The disadvantages of cellulase include poor stability, short life, and low catalytic efficiency, which result in the high cost of cellulase in the process of saccharification and limit the industrialization of cellulase. Immobilization can effectively improve the stability and service life of cellulase and consequently reduce the cost of cellulose application.

(1) Tannins method [55, 56]
Tannins are added directly into the saccharification solution or permeated liquid. Enzymes and tannins combine to form enzyme-tannin complexes in precipitation. After stirring for 15 min at 5,000 r·min^{-1}, supernatant (sugar) and precipitant (containing substrate residue and compounds formed by tannins and free enzyme or adsorption enzyme) are obtained. Then, polyethylene glycol (PEG) is added to precipitate with the amount of 0.8–1.0 times the equivalent tannins. PEG combines with tannins in enzyme-tannin complexes. Then, the enzyme is freed from the enzyme-tannin complexes. After sufficient stirring and centrifuging, the supernatant is enzymes liquid recovered. The immobilized enzyme on the membrane tube can be directly eluted without separation.

(2) Superfiltration method [54, 56, 57]
After centrifuging, the saccharification supernatant is filtered by 10 picometer (PM) superfiltration membranes (φ43 mm) at 3.5 kg/cm^2. Recycled enzyme is above the membrane, namely, the free enzyme in saccharification supernatant.

(3) Enzyme immobilization and reuse [58]

(4) Readsorption method to recycle cellulase [58]
Ultrafiltration membrane recycling can realize recycling of both cellulase and CB. It has the disadvantages like a high equipment requirement and complicated operation. There are many transfer barriers between immobilized cellulase and solid particles, so only CB is immobilized and recycled among the lignocellulose enzyme degradation system. Cellulase has high stability and a strong adsorption to cellulose, so recycling adsorption of cellulase has become a potential mode for reducing the application cost of cellulase. There are advantages to recycling cellulase with adsorption; for example, it is a simple process, has low equipment requirements, and can provide suitable operations at a large scale. Thus, it has broad prospects for industrialization. Xu and Chen designed a cellulase-recycling method by repeated adsorption and desorption of cellulase. A certain amount of cellulase solution is mixed with steam-exploded straw at a certain temperature. The system is separated after adsorption and desorption equilibrium. The solid residue (steam-exploded straw which has adsorbed enzyme) is mixed with liquid to recover the ratio of solid to liquid and reacted at a certain temperature. The supernatant continues to be mixed with fresh steam-exploded straw to reach the adsorption-desorption equilibrium.

Then, the second system separation is carried out. Such cellulase can be adsorbed by fresh substrate by repeating the steps. High enzymatic hydrolysis efficiency of cellulose is obtained this way. The minimum average amount of cellulase reaches 6 IU/g substrate, which is lower than the minimum amount of industrial standard of cellulase used for ethanol production, 10 IU·g^{-1} substrate [59].

(5) Recovery method for the enzyme component [55]
① Residues and free cellulase are used together. Residue obtained from saccharification is separated from the liquid by centrifugation and then is used together with free cellulose recycled from the supernatant.

② Residue immersion liquid and free cellulose are used together. Acetic acid buffer solution with ten times the amount of residues leaches down cellulase adsorbed on the residue. Then, the leaching cellulase solution is used together with free cellulose recycled from supernatant.

③ Saccharification-terminated liquid is directly recycled. Tannins are added directly into the saccharification-terminated liquid. Supernatant (sugar) and precipitant (containing substrate residue and a compound formed by tannins and free enzyme or adsorption enzyme) are obtained by centrifugation. Then, PEG is added to the precipitant. After sufficient stirring and centrifuging, the supernatant is the recovered cellulose liquid.

11.2.6.2 Cellulase Immobilization Methods

The immobilization of cellulase enzyme includes physical and chemical methods. The physical methods include the physical adsorption method, ionic bonding method, and entrapment method. The advantage of immobilizing enzyme with a physical method is that the enzyme does not participate in the chemical reaction. So, the overall structure of the enzyme is maintained, and the catalytic activity of the enzyme is well retained. However, because the embedding or a semipermeable membrane have a certain spatial or steric hindrance effect, the physical method is not suitable for some reactions. The chemical methods include the cross-linking method and the covalent bonding method. Enzyme is chemically bonded to a natural or synthetic polymer carrier using a coupling agent to cross-link the group on the surface of the enzyme. Then, insoluble immobilized enzyme is formed that has a relatively greater molecular mass. Different enzymes have different fixed strategies. For certain enzymes, orientation may be important. For others, the environment caused by the carrier may be important. Therefore, the optimum conditions of immobilization for each enzyme are still specific. There is no uniform theory; the reasons may be that there are too many mutual effect factors, and the enzyme is composed of a protein with complex composition and spatial structure, but there is no obvious causal link [60].

(1) Cross-linking immobilization method for cellulase [61]
Wu took chitosan as a carrier to immobilize cellulase through a cross-linking method. The effect of glutaraldehyde concentration and the amount of enzyme on immobilized cellulase activity was investigated. The optimum temperature, pH value, and K_m were also analyzed. Results showed that, compared with the free enzyme, there were many advantages for immobilized cellulase obtained by the cross-linking method. Dong prepared PEG–modified chitosan carrier (PEG-CS, chitosan) by the cross-linked method to immobilize cellulase. Compared with the free enzyme, the thermal stability of immobilized cellulase was significantly improved, and the stability of the operation and storage was good.

(2) Immobilization of cellulase by the magnetic adsorption method [62]
For immobilization of cellulase by the magnetic adsorption method, 1.0 g magnetic chitosan is weighted and added to 50 mL acetic acid–sodium acetate buffer (0.2 M) to swell. Then, it is filtered. A certain amount of cellulase solution of 1.0 mg mL^{-1} is added. The mixture is shaken at 30 °C and 150 $r{\cdot}min^{-1}$ for a certain time on the constant-temperature oscillator. Microspheres are precipitated under a magnetic field. Then the supernatant is spilled out and washed with acetic acid–sodium acetate buffer until no protein can be detected. Freeze-dried immobilized enzyme is conserved in a 4 °C refrigerator for application.

(3) Superparamagnetic nanoparticle immobilization method for cellulase [63]
For the superparamagnetic nanoparticle immobilization method for cellulase, 50 mg magnetic nanoparticles are added to 2 mL phosphate buffer (pH 6.0, containing 0.1 M NaCl), and 1 mL 1-ethyl-(3-dimethyl-aminopropyl) carbodiimide hydrochloride solution is added. Then, the mixture is treated with ultrasound for 10 min. Enzyme solution (2 mL) is added. The new mixture is treated with ultrasound again for 30 min. Then, it reacts at 25 °C for 24 h in a shaker at 150 $r{\cdot}min^{-1}$. Finally, separation is carried out with 3,800 Gs of surface magnetic strength. The magnetic particles obtained are washed with deionized water. The hydroxyl groups on the magnetic particles can cross-link the amino group on cellulase molecules effectively. Magnetic particles of cellulase before and after immobilization both have a superparamagnetic property.

Cellulase immobilized with superparamagnetic nanospheres can uniformly suspend in a reactor and can achieve full contact with insoluble cellulose substrate. Because the immobilized enzyme has a magnetic property, after hydrolysis, cellulase can be separated and recycled rapidly in the reaction system by applying a magnetic field. In addition, the movement manner and orientation of immobilized magnetic cellulase can be controlled using an external magnetic field instead of the traditional mechanical stirring. In this way, the catalytic efficiency of the immobilized enzyme is enhanced. By the method of polymerization of monomer or direct adsorption, cellulase can covalently couple with functional groups on the surface of magnetic particle polymer.

11.3 Research Methods for Fermentation Engineering of Lignocellulose

11.3.1 Introduction

For the process industry, lignocellulosic biomass is a raw material with the most potential and is low cost. It is not suitable for direct use as a carbon source because of its complex structure. Cellulose degradation is the central step of the natural carbon cycle. Utilization and conversion of cellulose have significance for solving the world's energy crisis, food shortages, environmental pollution, and other issues. But, the main application bottleneck for cellulose is that it cannot be used in a large scale commercially as a fermentable sugar source. At present, the most promising approach to use this principle is that the cellulosic materials are pretreated first to make them easier to be hydrolyzed by cellulase, transforming the fermentable sugar source.

Cellulase is a general term for a group of enzymes that degrade lignocellulose. By degradation, lignocellulose can produce an economic, abundant raw material. This is expected to solve the problem of solid waste generated in nature. At the same time, it is important in the natural carbon cycle how the organic carbon in the form of cellulose can be converted into inorganic carbon.

11.3.2 Generation and Removal of Fermentation Inhibitors During Primary Refining of Lignocellulose

In the primary refining process of cellulosic raw materials, a variety of fermentation inhibitors are generated that are harmful for microbial fermentation. These inhibitors are mainly three categories including week acid, furan aldehydes, and phenolic compounds. They include formic acid, acetic acid, furfural, hydroxymethyl furfural, vanillin, benzaldehyde, p-hydroxybenzoate, guaiacol, and so on. These compounds influence the growth of microorganisms and their fermentation performance in subsequent fermentation. Fermentation yield and production would be reduced. So, they are a major obstacle for cellulosic material application on a large scale as fermentation industrial raw materials.

11.3.2.1 Generation of Inhibitors

Lignocellulose usually contains three main substances: cellulose, hemicellulose, and lignin. In addition, there is a small amount of ash and extractives. Primary refining not only can reduce cellulose crystallinity and increase its porosity, but also can produce a variety of microbial growth-inhibiting compounds. At present,

a variety of pretreatment methods have been investigated, such as steam explosion, acid hydrolysis, ammonia explosion, and a wet oxidation method. Many compounds are generated in each pretreatment process. Their types and contents change with the property of the lignocellulosic materials and pretreatment conditions. In the process of acid hydrolysis pretreatment, cellulose is mainly converted into glucose. At the same time, hemicelluloses and lignin will degrade to a certain degree. Different monosaccharides generated are not stable under acidic conditions and continue to degrade, generating fermentation inhibitors, including 5-hydroxymethylfurfural (HMF), formic acid, and acetyl propionic acid. Some fermentation inhibitors will be generated when lignocellulose is hydrolyzed in acid.

(1) *Inhibitors generated from cellulose*. Glucose is produced in the cellulose hydrolysis process. Glucose continues to degrade under acidic conditions and generates HMF, formic acid, levulinic acid, and so on.

(2) *Inhibitors generated from hemicellulose.* Hydrolysis of hemicellulose can generate a variety of monosaccharides, mainly including xylose, arabinose, mannose, galactose, glucose, and a small amount of acetic acid. A variety of monosaccharides generated by the hydrolysis of hemicellulose in acidic conditions are not stable. They continue to degrade and generate HMF, formic acid, levulinic acid, and so on.

(3) *Inhibitors generated from lignin*. Lignin can also degrade by a small amount. The main products of degradation are a variety of single-ring aromatic compounds. In these substances, some of the low molecular weight phenols are generally regarded to inhibit fermentation.

(4) *Inhibitors generated from ash and extracts.* These mainly generate phenols.

The inhibitors of microbial growth can be mainly divided into three categories.

(1) *Weak acids*. These include acetic acid, formic acid, levulinic acid, and so on. Acetic acid is generated by hemicellulose deacetylation. Formic acid and levulinic acid are degradation products of HMF. Formic acid may also be generated by furfural in an acidic environment. Weak acid acidifies the intracellular environment, which is the main reason for inhibiting cell growth.

(2) *Furan aldehydes*. These are mainly furfural and HMF. Furfural and HMF are generated by the pentose and hexose, respectively, in an acidic environment. A furan aldehyde compound mainly inhibits microorganism growth, which extends the lag phase of microorganism growth.

(3) *Phenolic compounds*. These are mainly generated by lignin degradation. Phenolic compounds have the highest inhibition effect on fermentation among inhibitors generated by lignocellulose degradation. Phenolic compounds with low molecular weight (LMW) are more toxic. The position of the replacement group (para, ortho, meta) also affects the toxicity of phenols. It is generally regarded that phenolic compounds can penetrate into the cell membrane and destroy the integrity of the cell membrane structure, thereby the phenolic compounds affect the normal growth of microorganisms and reduce the fermentation efficiency [64] (Fig. 11.1).

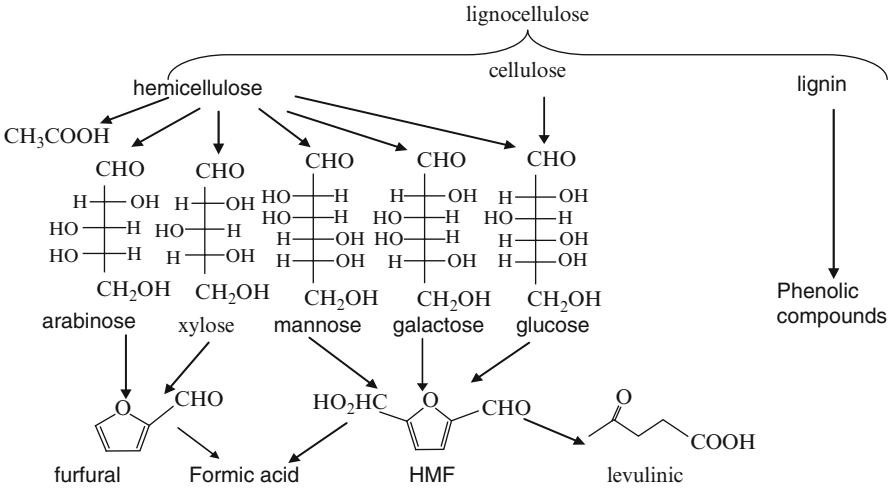


Fig. 11.1 Reaction in different levels in the lignocellulose pretreatment process and the formation of inhibitor [65, 66]

For different fermentation products, inhibitors are different. For xylitol production with fermentation, there are usually monosaccharides, including xylitol, xylose, glucose, and arabinose in hemicellulose hydrolysis. These sugars have different impacts on fermentation to produce xylitol. In the process of hemicellulose acidic hydrolysis, a certain content of acetic acid, furfural, phenols, and other toxic substances is generated and can obviously inhibit the fermentation performance of the hydrolyzate. A variety of metal ions and anions in the hydrolyzate can also influence xylitol metabolism [67]. Acetic acid, formic acid, and furfural, which are the main inhibitors in the hemicellulose acid hydrolyzate, can influence *Rhizopus arrhizus* growth with xylose as a carbon source and subsequent fermentation with glucose as a carbon source for fumaric acid production. Growth of *Rhizopus arrhizus* is sensitive to the concentration of formic acid and furfural, while acetic acid significantly inhibits subsequent acid-producing fermentation of *R. arrhizus*. In seed medium, the concentration of acetic acid, formic acid, and furfural should be controlled within 1.5, 0.5, and 0.5 $g \cdot L^{-1}$, respectively [68].

11.3.2.2 Measuring Methods for Inhibitor

Measuring of inhibitor type and concentration is crucial for selecting strains, determining inhibition degree, and removing inhibitor in the cellulose bioconversion process.

(1) Weak acids

Usually, gas chromatography is applied to analyze organic acids such as acetic acid and formic acid quantitatively. Liu et al. [69] used GC for quantitative analysis

of acetic acid, furfural, methanol, and other substances. The chromatographic conditions are described as follows: Innovax column; 30 kPa column pressure; split ratio 1:100; gas nitrogen carrier; inlet temperature of 250 °C; detector temperature of 280 °C; temperature program: initial temperature 60 °C (2 min), 7.5 °C·min^{-1} to 200 °C, holding for 2 min, 0.1 μL injection volume, direct injection.

(2) Furan aldehydes

At present, it has been reported that the furan aldehydes furfural and HMF are determined by chromatography and spectrophotometry.

The chromatography methods include GC [69] and liquid-phase chromatography. The liquid chromatographic method has been widely used in recent years. Levin et al. [70] measured the HMF content in milk by liquid chromatographic techniques. For this examination, 5 mL 0.15 M oxalic acid solution was added to a 15-mL sample. This was mixed and put into a boiling water bath for 25 min. The mixture was cooled to room temperature; 3 mL 40 % TCA (trichloroacetic acid) was added and sufficiently shaken. Then, it was centrifuged (2,000 *g*, 15 min). The supernatant was collected. Next, 10 mL of 4 % TCA was added to the precipitate. The mixture was centrifuged, and the supernatant was collected. Two centrifugal supernatants were merged, and the volume was measured. Then, the total supernatants were filtered by a microfiltration membrane (0.45 μm) and detected by HPLC. Chromatographic conditions were as follows: column resolve C18 column (150 × 4.0 mm); mobile phase was 0.1 M pH 3.6 sodium acetate buffer: methanol = 92:10, 0.1 M pH 3.8 sodium acetate buffer: methanol = 92:10; UV detector 280-nm detection wavelength; 20-μL injection volume.

Compared with the chromatographic method, spectrophotometry produces a colorimetric measurement error because of the presence of both furfural and HMF, so it cannot detect the true content. The result also is relatively high. Chang et al. [71] proposed using 2-thiobarbituric acid (TBA) as a derivative of the reagents to determine the contents of HMF and furfural in cellulose hydrolyzates by first-derivative spectrophotometry. An appropriate amount of standard solution or sample solution was added to a 25-mL volumetric flask, together with 8.5 mL concentrated hydrochloric acid and 7 mL 0.03 M TBA solution. The mixture was placed in a 40 °C water bath for 30 min. At the end of the reaction, it was cooled to room temperature. Then, it was adjusted to a constant volume and shaken to uniformity. The blank was the same amount of hydrochloric acid without TBA standard solution or a sample solution. The sample was scanned in the wavelength range of 370–510 nm. The first-derivative spectra were recorded.

Then, 20 mg·mL^{-1} furfural and HMF standard solution were prepared as a series of mixed standard solution. The first-order derivative spectrum diagram of each mixed standard solution was scanned and recorded according to the analysis steps. Peak values at 412- and 428-nm wavelengths were measured, respectively, with a zero-crossing measurement method. Then, take peak value as the y axis and take concentration as x axis. A standard curve equation was obtained by linear regression and used to calculate the content of these two components in mixture. Zhang et al. [72] proposed a rapid determination method for furfural and HMF content based

on the UV spectrum. They found that 276 nm was the isosbestic point wavelength for both furfural and HMF in concentrated acetic acid medium. In extract, acid-soluble lignin is a major confounding factor. At the same time, acid-soluble lignin is absorbed in a spectral range of 250–500 nm. However, furfural substances have no absorption beyond 325 nm. The influence of acid-soluble lignin can be removed by correcting the absorbance of acid-soluble lignin in the 325-nm wavelength. Ultimately, the contents of furfural and HMF could be quantitatively detected by a three-wavelength method based on the equal absorption wavelength of 276 nm, furfural maximum absorption at 272 nm, and acid-soluble lignin in the wavelength of 325 nm.

(3) Phenolic compounds
4-Hydroxy-benzoic acid, vanillin, and catechol are typical substances of phenolic compounds that inhibit the fermentation process. In practice, the total phenolic content is generally measured in solution.

Determination of the concentration of phenolic compounds [73] can use a spectrophotometer for detection. Vanillin and tannin are taken as standards for monophenol compound and polyphenol compound, respectively, to draw standard curves. The determination method is to take 25 mL of the sample at 30 ± 2 °C; 0.5 mL tannin-lignin reagent and 5 mL sodium carbonate–sodium tartrate reagent are added into the sample quickly for 30 min. The absorption value is measured at 700 nm. The concentrations of monophenol compound and polyphenol compound in the sample are obtained according to the absorbance values and the standard curve of vanillin and tannin. Tannin-lignin reagent is prepared as follows: A 2,000-mL flat-bottom flask is filled with 100 g of sodium tungstate ($Na_2WO_3 \cdot 2H_2O$), 25 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$), 700 mL of distilled water, 50 mL 85 % H_3PO_4, and 100 mL of concentrated hydrochloric acid. The mixture is connected to a reflux condenser and boiled for 10 h. Then, 150 g $LiSO_4$, 50 mL of distilled water, and a few drops of liquid bromine are added. The mixture is boiled for 15 min to remove excess bromine. After cooling to 25 °C, the volume of the mixture is set to 1,000 mL and filtered. The sample is kept under seal. Sodium carbonate–sodium tartrate reagent is prepared as follows: 200 g of Na_2CO_3 and 12 g sodium tartrate ($Na_2C_4H_4O_6 \cdot 2H_2O$) are put into 750 mL distilled water. The mixture is heated to dissolve. Then, it is cooled to room temperature, and the volume is set to 1,000 mL.

11.3.2.3 Inhibitor Removal Methods for Cellulose Bioconversion

As early as the 1940s, scientists discovered that the fermentation of a simple product from cellulose dilute acid hydrolysis was not as simple as that of fermentable sugars. There are certain fermentation-inhibiting substances in cellulose hydrolysates. Therefore, the removal method of these substances began to be investigated. In the past nearly six decades, a variety of biological, physical, and chemical methods has been used to remove the inhibitors in cellulose hydrolysates to improve their

fermentation property. These methods mainly include active carbon adsorption, organic solvent extraction, ion exchange, molecular sieves, steam stripping, and other methods [74].

(1) Biological methods

① Enzyme treatment

Because an enzyme has specificity, enzyme treatment can only remove specific inhibitory substances. After pretreatment by peroxidase and laccase, the hemicellulose hydrolysate of willow wood is fermented by rot fungi (*Trametes versicolor*), and the yield of ethanol was increased by two to three times compared to an untreated group [75].

② Microbial treatment

The fermentation microorganism which can absorb benzene compounds should be taken as the culture strain. *Trichoderma reesei* could degrade an inhibiting substance produced by the hydrolysis of willow hemicelluloses. In this way, the maximum yield of ethanol was increased by three times, and maximum production increased by four times [76]. Compared with the liquid treated with laccase, for liquid treated with *T. reesei*, the absorption at 280 nm was reduced by 30 %, indicating the difference of the inhibitory substances removed. By *T. reesei* treatment, acetic acid, furfural, and the benzoic acid derivative were removed from the hydrolysate.

(2) Physical methods

Physical methods include vacuum drying and concentration, cooking, activated carbon adsorption, ion exchange, adsorption, solvent extraction, and so on. Vacuum concentration and cooking can significantly reduce a volatile inhibitor. Ion exchange and solvent extraction can effectively reduce the content of acetic acid, furfural, and phenolic compound.

① Rotary evaporator

The volatile portion (10 %, V/V) in willow hemicellulose hydrolysate can be removed by rotary evaporation. The yield of the obtained mixture is just a little lower than that of control samples contained glucose and nutrients [77]. By comparison research, it was found that the nonvolatile part has high inhibiting efficiency. When acid hydrolysis of poplar was fermented with *Pichia stipitis*, the ethanol yield increased from 0 to 13 % if the hydrolysate was almost evaporated to dryness with rotary evaporation and then the residue was subjected to fermentation [78]. The reasons for the decrease of inhibition was considered to be that the concentrations of acetic acid, furfural, and vanillin were reduced by 54 % (to 2.8 $g{\cdot}L^{-1}$), 100 %, and 29 % compared with the original hydrolysate.

② Extraction

David extracted the acetic acid in dilute acid hydrolysate liquid of lignocelluloses with membrane extraction. More than 60 % of the acetic acid was removed with octanol and alanine 336 (1:1, v/v) as organic phase. Lignin and the low molecular weight phenolic compound were also separated, and the pH of the hydrolyzate increased to 4.0 from 1 to 2, which is more suitable for the next fermentation process [79].

It is reported that if spruce hydrolyzate with a strong inhibitory effect is extracted with ether continuously in a pH 2 environment for 24 h, the ethanol yield is equivalent with samples containing glucose and nutrients (0.40 $g \cdot g^{-1}$) [80]. Ether can extract acetic acid, formic acid, Jerusalem artichoke sugar acid, furfural, HMF, and benzene compounds. If the extract is redispersed in a fermentation medium, after the fermentation, the ethanol yield and the production drop to 33 % and 16 %, respectively, compared with reference fermentation samples. If inhibitor is extracted with acetate salt, the fermentation result is consistent with the reports mentioned. That is, in the hydrolysate fermented with *P. stipitis*, the removal rate of acetic acid was 56 %, and the furfural, vanillin, and 4-hydroxy benzoic acid were removed in their entirety. So, ethanol production increased from 0 to 93 %. When inhibitor was extracted with ether, it was found that the inhibitory effect of low molecular weight benzene compounds was maximum. Then, water was used to wash the dilute acid hydrolysis product of spruce treated with ether three times; inhibitor was also found in the aqueous phase, indicating that there was a water-soluble inhibitory substance.

③ Resin adsorption

According to the literature [81], with polymeric resin XAD-4 adsorption, HPLC detection revealed that the concentration of furfural decreased from the original 1–5 $g \cdot L^{-1}$ to 0.01 $g \cdot L^{-1}$. Then, the inhibition of furfural could be ignored with recombinant *E. coli* K011 in the fermentation process. The speed of the reaction was the same as the fermentation with purity sugar in the same concentration. Ethanol production reached 90 % of the theoretical yield. The polymeric resin XAD-4 can be reused after desorption.

④ Activated carbon adsorption

Jiang et al. [82] investigated a decolorization and detoxification method with active carbon. Optimal decolorization conditions are summarized as follows: activated carbon dosage to hydrolysate volume ratio of 2:1, temperature of 35 °C, and reaction for 60 min at pH 4.0. The decolorization rate was 88.11 %, and the loss yield of the reducing sugar was 48.54 %. Furfural removal reached 74.91 %, and phenolic compounds could be completely removed.

⑤ Ion exchange

Villarreal et al. [83] studied detoxification pretreatment of eucalyptus hemicellulosic hydrolyzate for xylitol fermentation. Activated carbon adsorption and ion exchange methods were used to pretreat the hydrolyzate. It was found that ion exchange methods more significantly increased the fermentation performance of hydrolysate than the activated carbon adsorption method. Under optimum operating conditions, the final xylose yield was 0.57 $g \cdot g^{-1}$ with *Candida guilliermondii* for 48 h of fermentation.

(3) Chemical methods

① Excessive alkali method

Inhibitor in cellulose hydrolysate can be removed by alkali treatment. With excess alkali (pH 10), much precipitate is formed, and the yield of ethanol is further improved. Excess alkali can effectively remove inhibitor because it makes some inhibitors precipitated and some inhibitory substances are unstable at high pH value.

In 1945, Leonard and Hajny adjusted the pH from 9 to 10 with $Ca(OH)_2$ and then adjusted it back to 5.5 with H_2SO_4. The hydrolysate with pH adjusted with $Ca(OH)_2$ had a better fermentation performance than that of NaOH. The reason may be that the former has a precipitation effect [84] for inhibiting substances.

② Associated method with sulfite and excess alkali
Larsson et al. [85] used sodium sulfite to treat the dilute acid hydrolysis product of handle fir. The concentrations of furfural and HMF were effectively reduced. It was confirmed by experiment that an associated method with sulfite and excess alkali was effective for treating hemicellulose hydrolysate of willow for fermentation with *E. coli* [86]. After 40 h fermentation, only 24 % of the xylose was consumed. However, if the hydrolysate was treated only with excess alkali, xylose would be completely consumed. Compared to treating only with excessive alkali, if 0.1 % sulfite was added to the hydrolysate and heated at 90 °C for 30 min, the fermentation time would shorten by 30 %.

As weak acids, furan derivatives and benzene-based compounds inhibit the fermentation of cellulose hydrolysis products; the interactive negative effect would enhance the inhibition effect [87]. If benzene compounds are removed by laccase, inhibition could reduced significantly [75], suggesting that benzene compounds are major inhibitory substances in the process of cellulose hydrolysate fermentation.

The inhibitor removal methods mentioned are detoxing methods before fermentation. Another favorable method is to breed highly resistant strains and improve internal tolerance. The inhibitory influence is reduced or eliminated by controlling external factors through the fermentation process. High-resistance strains mainly are bred by evolutionary engineering and genetic engineering [88].

11.3.3 Cellulase Production with Fermentation and Its Isolation Methods

Since Seillieve discovered cellulase that hydrolyzes cellulose in the digestive juices of the snail in 1906, cellulase has received much research and discussion. In the 1970s, the United States, Japan, West Germany, and other developed countries produced cellulase industrially. In the 1970s, China began to research cellulase and to apply it in alcohol, white wine, soy sauce, and other industries. However, the expensive price of cellulase limits its application in industrial production. Therefore, breeding of strains with high cellulase activity has become a critical factor.

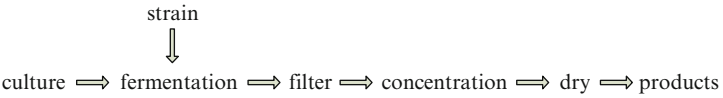
Cellulase is a collective name for a class of enzymes that degrade the β-1,4-glucoside bond. According to the different functions, cellulase can be divided into three categories: endoglucanase, exoglucanase, and β-glucosidase. Many microorganisms produce cellulase, and the enzyme that is most suitable for cellulose hydrolysis is from wood-rot fungus, *Trichoderma*. The cellulase production methods mainly include solid-state fermentation and deep liquid fermentation, and the isolation methods mainly include salting-out method, organic solvent method, and tannin extraction method.

11.3.3.1 Fermentation Process for Cellulase Production

The cellulase production technologies are mainly two types: solid-state fermentation and deep liquid fermentation. These two processes are introduced next.

1. Liquid fermentation

(1) Liquid fermentation manner
Cellulase liquid fermentation can be basically divided into two forms: batch fermentation and continuous fermentation. The general process is as follows:



The medium composition, fermentation conditions, and enzyme extraction methods are slightly different.

① *Batch fermentation*. Batch fermentation is a quasi-closed system. After the seed is inoculated into a fermentation medium, only the gas flows, and the fermentation broth always remains in the fermentation tank without other material exchange with the outside world. Medium is added once, and products are harvested once. It is widely used. Its advantages are as follows: (a) Temperature requirements are low, and the process operation is simple; (b) bacterial contamination and bacterial degradation problems are easier to solve; (c) nutrient use efficiency and product concentration are higher than for continuous fermentation.

② *Continuous fermentation*. For continuous fermentation, on one hand, fresh feed solution is complemented; on the other hand, the medium is discharged at the same flow rate to maintain a constant volume of fermentation broth. Compared with batch fermentation, continuous fermentation has advantages in yield, production stability, and automation ease. However, the contamination opportunities and the possibility of bacterial degradation are increased. Continuous culture can improve the productivity of cellulase, but cellulase concentration in the medium is low.

③ *Fed-batch fermentation*. Fed-batch fermentation is also known as semicontinuous fermentation. Some materials are added as a complement to the fermentation system in some way, but the fermentation broth is not continuously released outward. It is a fermentation technology between batch and continuous fermentation. Fed-batch fermentation is applied widely almost throughout the fermentation industry. The method has the following advantages: (a) Inhibition of substrate, feedback inhibition of product, and catabolite inhibition effect are relieved. When the yield or production rate of metabolites is visibly affected by the concentrations of some substrate component (such as acetic acid, methanol, phenol, etc., as a fermentation-based component has an inhibitory effect on substrate concentration), the fed-batch technique is more beneficial than batch fermentation. (b) The amount of mycelial growth can be reduced, and the conversion yield of useful products is improved. (c) Strain variation and bacterial contamination problems are easy to control. (d) It is convenient for automation control.

Cellulase with high activity is usually obtained by batch and fed-batch fermentation. Cellulase with the highest activity is obtained by fed-batch fermentation. Fed-batch fermentation has been increasingly widely used in industrial production and scientific research. Fed-batch fermentation can avoid the inhibition of high substrate concentration or catabolic repression. The highest strain concentration and maximum yield per unit volume could be achieved, leading to low cost of the downstream extraction [89].

Yong et al. [90] prepared cellulase with *Trichoderma reesei* by fed-batch fermentation using pulp as a carbon source. In a 10-L fermentor, cellulase was produced by batch fermentation. The concentration of the carbon source in the medium was 15 $g \cdot L^{-1}$. Filter paper activity, cellobiose enzyme activity, enzyme production rate, and enzyme yield were 2.15 $FPIU \cdot mL^{-1}$, 0.20 $IU \cdot mL^{-1}$, 16.3 $FPIU \cdot (L \cdot h)^{-1}$, and 143.3 $FPIU \cdot g^{-1}$, respectively. If carbon source concentration was increased to 27.5 $g \cdot L^{-1}$ by adding the carbon source with a batch method, filter paper enzyme activity, cellobiose enzyme activity, enzyme production rate, and enzyme yield were 3.90 $FPIU \cdot mL^{-1}$, 0.35 $IU \cdot mL^{-1}$, 23.2 $FPIU \cdot (L \cdot h)^{-1}$, and 141.8 $FPIU \cdot g^{-1}$, respectively. Studies revealed that by fed-batch fermentation technology, enzyme activity and enzyme production rate were enhanced with the increase of carbon source in the culture medium. Enzyme yield remained the same. So, the cost of cellulase production was reduced.

(2) Research on liquid fermentation process factors

In the process of liquid fermentation for cellulase, the main factors affecting fermentation yield and efficiency include medium ratio, fermentation temperature, fermentation pH, stirring speed and foam, and other factors in addition to the bacteria. Different strains have different fermentation requirements. Several major factors affecting the production of cellulase are discussed next.

① Strains

During fermentation, the speed of strain growth and the amount of product synthesis depend largely on the quality of the strain. Generally, an enzyme-producing strain must comply with the following conditions: First, it is not a pathogen. Second, it is able to use cheap raw materials with a short fermentation period and high enzyme yield. Third, it hardly mutates and degrades and is not easily infected with phage. Fourth, it is better to produce extracellular enzymes, which are helpful for separating enzymes with a high recovery rate. In addition, if enzyme is used for medicine and food, safety issues should be considered.

② Inoculum size, inoculum age, and inoculation manner

An appropriate inoculum size has a great impact on the production of enzymes. Usually, the fermentation inoculation amounts are as follows: 1–5 % for bacteria, 5–10 % for yeast, and 7–15 % and sometimes 20–25 % for mold. When the inoculation amount is too low, the activity of the enzyme is not high, and the production cycle is extended. When the inoculum amount is high, microorganisms immediately enter the logarithmic growth phase, so the cellulase synthesis is advanced, and the opportunities for the growth of various bacteria can be reduced. However, excessive

inoculation is not necessary because seed culture is time consuming, and too much metabolic waste affects normal fermentation.

Inoculum age is the physiological age when culture in seed pots can begin to be planted to the next tank or fermentor. Generally, it is better to plant at a late logarithmic growth phase. Too young an inoculum age will make offspring culture growth slow, extending the fermentation period, but too old an inoculum age will cause the strain to decline prematurely, leading to a decline in production capacity.

Inoculation manner has a certain influence on the formation of fermentation products. Generally, inoculation manner includes seed liquid direct inoculation, spore inoculation, dug lump inoculation, mycelial inoculation, or cultured cell inoculation after centrifugal separation and washing. For aerobic bacteria, seed is apt to be cultured by baffled reactor for inoculation. Different inoculation methods lead to differences in nutrient consumption rate, the pH value change, the mycelium morphology characteristic as well as product formation way [91]. Some studies have shown [92] that, under the same fermentation conditions, the final cellulase activity is similar regardless of the spore inoculum or mycelial inoculation. Because the germination and growth stages are necessary for spore inoculum, the fermentation period is long. To improve the production efficiency of enzyme, mycelia should be inoculated appropriately.

③ Medium

The composition of the medium has a great impact on cellulase formation, which mainly includes the following aspects:

(a) *Carbon source.* Generally, cellulose-containing material is used as a carbon source for the fermentation of cellulase. In China, crude fiber raw materials are usually rice straw, stover, corncob, pulp, wheat bran, furfural residue, and so on. In the fermentation process for cellulase, a mixture of bran and other coarse fiber is usually used as a carbon source. The effect of bran on cellulase production includes two aspects. On the one hand, it provides the necessary nutrient factors for enzyme production. On the other hand, the degree of fluffiness will increase if the amount of bran is increased, reducing ventilation in the medium, which affects cellulase production. The amount of bran added is different for different strains.

(b) *Nitrogen source*. Nitrogen sources include inorganic nitrogen or organic nitrogen. There are few differences between them, and ammonium nitrate is usually used. During the fermentation process, it is necessary to pay attention to a reasonable ratio of carbon and nitrogen. A low carbon and nitrogen ratio in culture will lead to excessive bacterial growth at an early stage and excessive consumption of carbon sources. A high carbon-to-nitrogen ratio (C/N) causes slow growth and reproduction of the strain because the nitrogen source is less than needed for the emergence of strain.

Mao et al. investigated the effect of different carbon and nitrogen ratios on the synthesis of xylanase enzyme with *Trichoderma reesei* Rut C-30. The results showed that a low C/N is helpful for promoting the synthesis of endo-beta-xylanase and inhibiting the synthesis of exo-β-xylosidase, selectively producing

endo-β-xylanase. A high C/N causes xylanase synthesis to lag, inhibits cellulase synthesis, improves the ratio of xylanase activity and cellulase activity and selective synthesis of xylan sugar enzyme with low cellulase activity [93].

(c) *Inorganic salts*. Metal ions provide a class of nutrients essential for growth of microorganism. Most seed fermentation needs added inorganic salts, such as phosphates, potassium, magnesium, manganese, iron salts, chlorides, and so on. Usually, tap water or a complex medium contain the desired trace elements—zinc, copper, molybdenum, cobalt, and so on—and the calcium salt. Most of these metal ions, especially inorganic phosphates, repress biosynthesis of several secondary metabolites. In the growth process and cellulase production of *Trichoderma* mycelia, potassium, calcium, magnesium, and other elements of the ion composition are involved in cell structure and energy transfer, cell permeability adjustment, and other functions. For iron, manganese, cobalt ions, and so on, the demand of microorganism is little, so they are called microelements. But, they are closely related to enzyme activity. They can be used as the base components of enzyme activity or an enzyme activator.

(d) (d) *Inducer*. In the process of fermentation for the preparation of amino acids, antibiotics, and enzymes, inducer could be added in the fermentation medium to induce the fermentation. For microorganism that can produce an inductive enzyme (such as hydrolase), the yield of enzyme can be greatly improved from the original low level by adding inducers. Usually, inducers are the substrates or substrate analogs of a corresponding enzyme; these substances can initiate the enzyme production mechanism of microorganisms. This mechanism is usually not active without the inducer, and enzyme production is suppressed. Adding a certain amount of inducer in the medium can greatly increase the production of certain microbial enzymes. General inducers include a variety of surface-active agents (detergent, Tween 80, phytic acid, etc.), diethylaminetetraacetic acid, extract of soybean spirits, ferrocyanide, methanol, and so on.

Cellulase is an inducible enzyme. In the fermentation process, large-scale synthesis of cellulase must have the effect of an inducer. Therefore, adding an appropriate inducer in the culture and fermentation process can increase the yield of the enzyme. Here, the inducing mechanism is introduced only for cellulose synthesis of filamentous fungi. In hyphae and the surface of conidia, there is a small amount of composition cellulase that converts cellulose into cellobiose and other oligosaccharides at first. Then, β-glycosydase, which is combined in the plasma membrane, begins the transfer glucosylation action, generating sophorose and other inducers. These inducers permeate into the cell through the permeability enzyme system in the membrane. Finally, cellulase synthesis begins. It has been proved that induced synthesis of cellulase is adjusted at the transcription level.

Typically, cellulose, fiber, oligosaccharides, and other structural analogs can be used as the inducer of cellulase. A commonly used raw material rich in these substances (including the plant fiber materials, paper, various distillers' grains, lactose, starch hydrolysis sugar, etc.) is taken as the carbon source for the cellulase medium. These substances cannot produce cellulose and play the role of inducer at the same time. Nisizawa discovered that cellulose, cellobiose, sophorose, cellulose

Table 11.2 Raw materials used in the cellulase fermentation process

Carbon	Nitrogen	Inducer	Inorganic salts
Rice straw	Urea	Sophorose	Calcium chloride
Corn stover	Ammonium sulfate	Fructose	Zinc chloride
Corncob	Sodium glutamate	Sorbose	Cobalt chloride
Pulp	Sodium nitrate	Gentiobiose	Potassium dihydrogen phosphate
Bran	Peptone	Methyl β-anhydroglucose	Magnesium sulfate
Furfural residues	Ammonium dihydrogen phosphate	Cellubiose	Ferrous sulfate
Monosaccharide (glucose, rhamnose, xylose, sorbitol, mannitol, mannose, arabinose, galactose)	Ammonium carbonate	Cellobiose lactone	Manganese sulfate
Disaccharide (cellobiose, sucrose, lactose)	Ammonium oxalate	Lactose	Sodium acetate
Polysaccharide (cellulose powder, carboxymethyl cellulose, microcrystalline cellulose)	Triammonium citrate	Rice straw	
Sodium carboxymethyl cellulose	Soybean meal	Corn stover	
	Yeast extract	Corncob	
	Beef extract	Pulp	
		Bran	
		Furfural residues	
		Cellulose powder	

derivatives, and so on could induce *T. reesei* and *T. viride* to produce cellulase, with cellobiose having a special role. At lower concentrations, cellobiose induces generation of cellulase. However, at higher concentrations, it inhibits the formation of cellulase. The effect of carbohydrate with low molecular weight (LMW) varies with the strain.

The amount of inducer used should be appropriate. Within a certain concentration range, the amount of enzyme production is proportional to the amount of inducer used. When there is excess inducer, it may be used by microbes, leading to an inhibition effect on cellulase synthesis. Microbes may also be cultured at first. After the massive growth of bacterial cells, inducer is added to save inducing agent. Raw materials used in cellulase fermentation process are shown in Table 11.2.

④ Metabolites

Cellulase synthesis is not difficult to inhibit by easily metabolic substrates such as glucose, glycerol, and so on. As long as the medium contains a certain amount of inhibitor, enzyme can no longer be synthesized in the strain metabolism. Such inhibition is thought to occur in the synthesis of the translational or posttranslational level.

In the process of fermentation, if limited glucose can be added or substrate is difficult to hydrolyze, the inhibiting effect could be reduced to obtain higher enzyme production. But, the effect is limited because only the synthesis rate is changed instead of the genetic characteristics of the producing strain. Han et al. obtained an antimutant UV III with a property that can resist a high concentration of glucose by UV mutagenesis for *T. pseudokoningii* TH, which can significantly increase cellulase production [94].

⑤ Cell permeability

An increase of cell permeability has a certain effect on improving the activity of cellulose. A general method is to add surfactant to the culture medium, such as Tween 80. But, there is no fixed law to follow because of the differences of strains, surfactants, and enzyme properties. Generally, Tween 80 can increase the production of *Trichoderma reesei* cellulase. Wang and Yan investigated the effect of Tween 80 and commercial detergent as surfactants on enzyme fermentation of *Trichoderma*. The results showed that when the amount of Tween 80 in the culture medium was 0.2–0.5 %, the highest FPA and xylanase improvement was reached, 29.6 % and 31.1 %, respectively. When the amount of detergent in the medium was 0.5 %, the xylanase yield of *Trichoderma* reached the highest, 34.4 % [95].

⑥ Temperature

In the process of cellulase fermentation, strain growth and product synthesis are closely related to temperature, but the optimum temperature is often different; under normal circumstances, the optimum temperature to produce cellulase is lower than the growth temperature.

The impact of temperature on fermentation is not limited to the level of microbial growth and product synthesis. Temperature will also affect the microbial metabolic pathway and direction. Mao et al. investigated the influence of temperature on the synthesis of xylanase and cellulase with *T. reesei* Rut C-30. A lower culture temperature (25–26 °C) is helpful for the synthesis of xylanase and cellulose, but the enzyme production time is longer. If the culture temperature is relatively higher (e.g., 35–36 °C), enzyme production time is shortened, but xylanase synthesis is affected to some degree, and cellulase synthesis is severely inhibited. Variable temperature culture as follows: the pre (24 h) culture temperature is 35 to 36 °C, and the late cultivation temperature is 25 to 26 °C. It is effectively contribute to the synthesis of xylanase, and inhibits the synthesis of cellulase, resulting in high ratio of xylanase and cellulase activity. This is conducive to the selective synthesis of xylanase. The activity of xylanase and cellulose reaches the highest values, 161.69 and 0.359 IU·mL^{-1}, respectively, at 72 h [96].

In the actual fermentation process, the fermentation temperature changes mainly with microbial metabolism, fermentation ventilation, and stirring speed change. At the beginning of fermentation, the absorbing heat of the synthesis reaction is greater than the emitting heat of the decomposition reaction, so the fermentation liquor needs warming. When cell growth is vigorous, the temperature of the fermentation liquid is rises by itself. Ventilation stirring brings heat. So, fermentation broth must be cooled to keep a desired temperature for microorganism growth and

Table 11.3 Comparison of constant-temperature fermentation and segmental heating fermentation

Fermentation method	Constant-temperature fermentation	Segmental heating fermentation
Glucose (%)	4.0	10.56
Crude protein (%)	19.13	19.86
Cellulose (%)	20.07	18.65

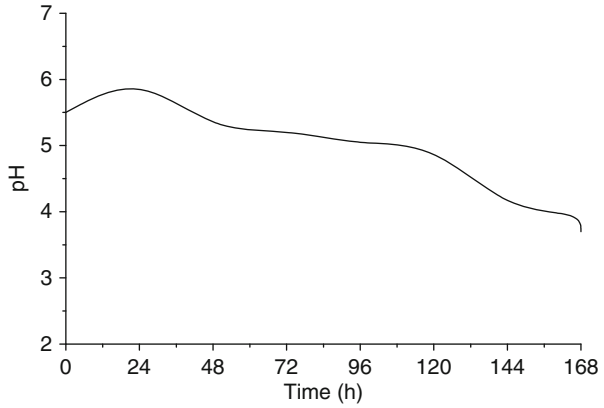


Fig. 11.2 pH changes in the liquid fermentation process

enzyme production. Generally, the optimum temperatures of microorganism growth and enzyme production are inconsistent, so segmented warming can be used to improve the yield of cellulase. Zhang et al. used segmented warming to improve the conversion ratio of cellulose [97]. Table 11.3 is a comparison of constant-temperature fermentation and segmental heating fermentation.

⑦ pH value

Any microorganism has a suitable pH range for its growth. Thus, in the early stage and the process of culture, pH directly affects the growth of microorganisms and the synthesis of enzyme. Further, with lignocellulosic feedstock, microorganisms can often simultaneously generate several enzymes. Each enzyme has its own optimum growth pH and enzyme production pH. Therefore, by regulating the culture pH at the early stage and process of culturing, a certain kind of enzyme can be selectively obtained. Zhang et al. investigated pH changes (Fig. 11.2) in the process of cellulase production with submerged fermentation. It was found that the general trend of pH value in the fermentation process was declining. Some people think that this decline is the result of strain self-regulation because the optimum pH of growth and enzyme production for general microorganisms is inconsistent. For example, the optimum growth pH value of *Trichoderma viride* is about 5.5, and the optimum pH value of enzyme production is about 3.5. So, it is not necessary to interfere with the pH changes. Acidic pH has two sides. On one hand, it is suitable for enzyme production.

On the other hand, it produces enzyme inactivation. When the pH drops below 3.0, the inactivation of enzyme becomes prominent. So, the pH value of the fermentation solution should be strictly controlled [98].

In the actual fermentation process, microorganisms continue to decompose, assimilate nutrients, and discharge metabolites simultaneously. Because these products have a direct relationship with pH value, the pH value of fermentation solution keeps changing. So, pH should be controlled according to its change.

⑧ Ventilation amount
The ventilation amount should be based on the dissolved oxygen in the medium. Generally, in the early stage of fermentation, the amounts of strains are relatively less, so the ventilation amount can be less. In strain growth stage, oxygen consumption is more, so there should be more ventilation. Strong ventilation is needed in the stage of significant enzyme production.

⑨ Stirring
For cellulase submerged fermentation, in addition to a ventilator, stirring is necessary to facilitate heat exchange, to obtain uniform contact of nutrients and cells, and to reduce the metabolites around cells, which improves enzyme generation. Air bubbles can also be broken to make the fermentation solution have a turbulent flow, thereby increasing dissolved oxygen and air utilization.

⑩ Foam
There is usually a large amount of foam in the fermentation solution. Foam not only hinders the release of CO_2 but also affects the amount of dissolved oxygen. An antifoaming agent is generally used in industrial production, mainly including some natural mineral oils, alcohols, fatty acids, acid amides, ethers, and so on. Polyethylene polyoxypropylene glyceryl ether and polyethylene oxide-propylene ethylene oxide glycerine ether are the commonly used antifoaming agents.

The liquid fermentation method can be used in large-scale production of cellulase. It has the advantages of high utilization rate of fermentation raw materials, easy control of production conditions, a low level of bacterial infection, high yield, low labor intensity, and stable quality. But, disadvantages include high requirements for medium, incomplete enzyme system, low level of enzyme production, high production costs, high equipment requirements, creation of environmental pollution, and so on, which limit cellulase industrialization.

(2) Solid-state fermentation
Solid-state fermentation is one of the oldest techniques for which humans use microorganisms. Broadly speaking, it refers to all the processes of culturing microorganisms using an insoluble solid matrix. It includes submerged fermentation with solids suspended in a liquid and the process of culturing microorganisms in no (or almost no) free water on the wet solid material. In most cases, solid-state fermentation refers to the biological reaction process with one or more microorganisms in a water-insoluble solid matrix with a certain humidity and containing no or almost no free water. The substrate can provide microorganisms not only a carbon source, a nitrogen source, inorganic salts, water, and other nutrients but also the

required places for microorganism growth. The most prominent advantages of solid-state fermentation include that it is a simple process, it requires less investment, and it has high output. Its specific characteristics are summarized as follows:

(1) The fermented cost is low because cheap raw materials can be directly used, such as industrial and agricultural residue (cellulosic feedstock, renewable resources) as substrates.
(2) It simulates natural growth environment to keep microorganism growing similarly to their natural status.
(3) The three waste discharges of wastewater, waste residue, and waste gas do not exist.
(4) Postprocessing for the product is simple. Products are easy to store and transport. (Table 11.4).

Currently, solid-state fermentation is adopted by the vast majority of Chinese manufacturers. Figure 11.3 is a production process for cellulase with rice straw as the raw material and solid-state fermentation.

(1) Solid-state fermentation type
Solid-state fermentation can be divided into shallow tray fermentation, drum solid-state fermentation, gas dual-dynamic solid-state fermentation, and so on.

① Shallow tray fermentation
Shallow tray fermentation uses straw powder, wastepaper, and corn straw powder as the main raw materials. When seed leaven is mixed into feedstock, the mixture is loaded into a dish or curtain and spread in a thin layer (a few centimeters to tens of centimeters thick). It is fermented in the training room under certain temperature and humidity (90–100 %) conditions. The heat conduction of dish fermentation is easily restricted. The presence of a medium temperature gradient will result in the generation of natural convection, which will affect the transfer of heat and oxygen and carbon dioxide and the transfer of moisture. In shallow tray fermentation, heat transfer is the biggest bottleneck. The best solution is to reduce the thickness of the substance. Reactor enlargement can only be achieved by increasing the amounts of trays, so it is not suitable for large-scale production.

Zhang et al. [115] took spent grains as the main raw material to produce cellulase by *T. reesei* Rut C230 with shallow tray fermentation (platter loading 300–400 g dry matter). The fermentation process was optimized by controlling temperature and pH. The preliminary temperature was controlled at 27–30 °C, and the maximum temperature of the late stage of fermentation does not exceed 35 °C. When the pH of feed materials rose from 4.3 to 4.5, dilute hydrochloric acid was sprayed for control. With optimized pH and temperature, FPA activity reached 508 $U \cdot g^{-1}$ substance, equivalent to 2,120 $U \cdot g^{-1}$ cellulose.

② Drum solid-state fermentation
The basic form of a drum reactor is a cylindrical container held in a rotating system. The rotational system mainly plays the role of support and provides dynamic action.

Table 11.4 Differences in the level of fermentation between solid-state fermentation and liquid fermentation

Strain	Protein	Differences of fermentation	Reference
Aspergillus niger	Pectinase	Under the same conditions, exopectinase production yield of solid-state fermentation is higher than that of liquid fermentation	[99]
A. niger	Pectinase	Under optimum conditions, the yields of endopectinase and exopectinase of the solid-state fermentation production are higher than that of liquid fermentation	[100]
A. niger	Pectinesterase	Under the best conditions, the yield of pectinesterase of solid-state fermentation is 2.3 times higher than that of liquid fermentation	[101]
Streptomyces sp.	Thermophilic protease	Under the same conditions, thermophilic protease yield of solid-state fermentation is higher than that of liquid fermentation	[102]
Aspergillus oryzae	Neutral protease	Under the best conditions, the yield of neutral protease of solid-state fermentation is 3.5 times higher than that of liquid fermentation	[103]
Panus tigrinus	Laccase Peroxidase	Enzyme productivity and stability of solid-state fermentation are higher than that of liquid fermentation	[104]
A. niger	Phytase	The yield of phytase of solid-state fermentation is higher	[105]
A. niger	β-Fructofuranosidase	Under the same conditions, the yield of β-fructofuranosidase of solid-state fermentation (149.1 $U{\cdot}L^{-1}$) is higher than that of liquid fermentation (58.3 $U{\cdot}L^{-1}$)	[106]
A. niger	Tannase	The yields of strain and tannase of solid fermentation are both two times higher than that of liquid fermentation	[107]
A. niger	Converting enzyme	The rate and yield of converting enzyme of solid-state fermentation are both higher than that of liquid fermentation, and cell growth is more vigorous	[108]
A. niger	Pectin lyase	Solid-state fermentation can highly express pectin lyase. Of extracellular protein, 65 % is the pectin lyase, and fermentation time is shorter	[109]

(continued)

Table 11.4 (continued)

Strain	Protein	Differences of fermentation	Reference
Aspergillus tamarii	Xylanase	Under the same conditions, xylanase yield of solid-state fermentation is higher than that of liquid fermentation	[110]
A. niger	Pectinesterase Polygalacturonic acid enzymes	The yields of pectinesterase and poly-galacturonic acid enzyme of solid-state fermentation are four and six times higher than that of liquid fermentation, respectively; the fatty acids C_{18} and C_{16} content of mycelia of solid-state fermentation increased compared with that of liquid fermentation	[111]
Bacillus amyloliquefaciens	Protease	The protease yield of liquid fermentation was 80,000 $U{\cdot}mL^{-1}$, and that of solid-state fermentation production reached 250,000 $U{\cdot}g^{-1}$	[112]
Aspergillus albicans	Acidic α-amylase	Acid alpha-amylase of solid-state fermentation has high yield	[113]

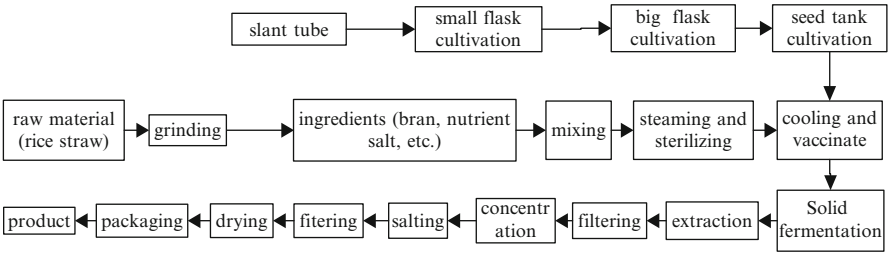


Fig. 11.3 Scheme for producing cellulase with solid-state fermentation [114]

The filling coefficient is generally 0.3. There are two main ways for heat transfer of the fermentation: direct convection by evaporation transfer and reactor conduction. Su et al. [116] investigated solid-state fermentation for ethanol production from straw with two forms of solid-state reactor: drum bioreactor and shaft agitator-type solid-state fermentor. Under the optimal process conditions of the drum bioreactor, a preliminary comparison was undertaken between two kinds of reactor performance. The strain biomass and cellulase activity of the drum reactor were both better than that of the stirred tank reactor shaft. The reason is that, in a drum reactor, substance is stirred uniformly, and there is a large contact surface between the fermentation material layer and the upper stream, leading to uniform heat and mass transfer. Shear destroying in a drum is much less than that in a shaft agitator, so the fermentation result is better.

③ Solid-state fermentation of double-dynamic gas phase

In the traditional solid-state fermentation, transfer of mass and heat is enhanced by mechanical flipping. That means that gas does not move, but the solid phase sequentially flips to mix particles and to increase the frequency of contact between the reaction particles and gas molecules. So, the gas in the material layer is involved in convection-diffusion instead of molecular diffusion. However, too much flipping is bad for the growth of microorganisms in the fermentation process because mycelia are fractured by the shear forces of flipping. There are two fatal flaws for flipping solid material in addition to difficulty with the equipment's mechanical seal, high energy consumption, and complex structure, causing infection and other issues. First, sticky, wet material contacts the mechanical rake and the walls, and the contact pressure is high. So, a dead corner is inevitable that cannot be cleaned thoroughly. It is almost impossible to achieve a strictly pure culture. The second, only positive role of flipping is to change gas phase transfer in fermented material layer from molecular diffusion to convection-diffusion. However, the medium is not like dry material which is not easy to agglomerate. Thus, a large number of particles within the clumps are hardly influenced by mechanical agitation and hence the change of the molecular diffusion into the validity of the convection-diffusion is very limited.

Chen et al. [114, 117, 118] proposed solid-state fermentation with a double dynamic gas phase for cellulase production. Vapor pressure pulsation in the tank is achieved by stamping and discharging sterile air pressure. The cycle time includes four stages: stamping time; stabilization time for peak pressure; discharging pressure time (pressure drops faster, so that the gas among the wet particles in solid medium expanded suddenly, leaving the material layer loosened); and stabilization time of valley pressure. The tank is always in positive pressure. The gas circulation speed is set according to the different microbial metabolic stages. Chen's research group [119] studied the cyclical changes of parameters in a solid-state fermentation reactor with pressure pulsation and the effect of the periodic environmental stimulation on the fermentation of *Penicillium* spp. The results showed that, in such a reactor, with air pulsation, the temperature and humidity of the air within the reactor can also change periodically. The change cycle was the same as that of the air pulsation cycle. The change amplitude increased with the amplitude of the air pulsation increases. Compared with the control experiments without adding air pulsation, the external cycle stimulation made the biomass of *Penicillium decumbens*, the total amount of CO_2 generated, and the cellulase yields increase by 104 %, 229 %, and 320 %, respectively. Data showed that the external cycle stimulation increased not only the strain biomass but also its metabolic activity.

(2) Impact factors

Solid-state fermentation approaches the natural status, so there are many differences from submerged fermentation. The most significant features are low water activity and uneven fermentation. Cell growth, in the absorption of nutrients and secretion of metabolite products, is uneven everywhere. As a result, detection and control of fermentation parameters are more difficult. Many biosensors used in liquid fermentation cannot be applied to solid-state fermentation. So far, research of

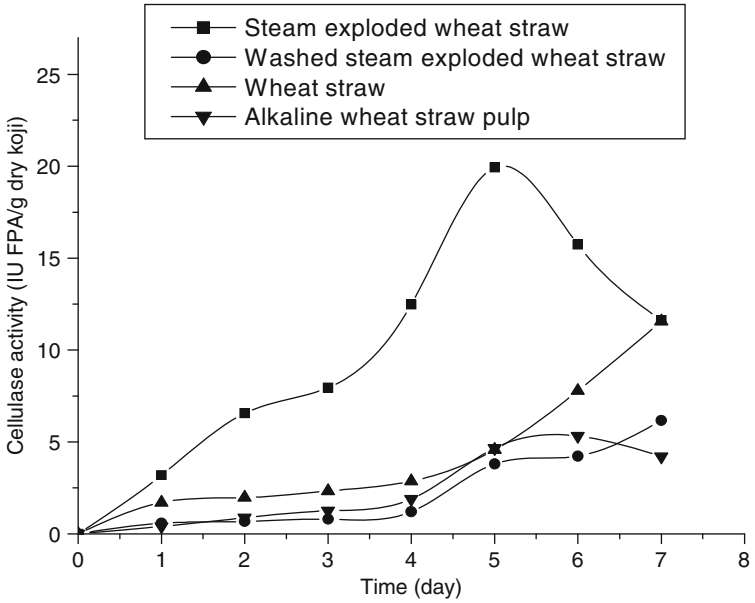


Fig. 11.4 FPA changes of cellulase from fermentation of different substrates

solid-state fermentation remains at the experience level. Parameters that can be measured or are adjustable in solid-state fermentation include medium moisture content, air humidity, CO_2 and O_2 content, pH value, temperature, and strain growth amount. The factors that have an impact on solid-state fermentation mainly include those discussed next.

① Material structure, composition, and particle size
In the solid-state fermentation process for cellulase production, materials can be divided into two parts. First, nutrition material supplies nutrients; these are materials such as cellulose-based substances, wheat bran, soybean meal, salts, and so on. Second, filler promotes ventilation; these are materials such as rice husk, corn bran, peanut skin, and so on. In the investigation of the enzymatic properties of cellulase, the enzymatic hydrolysis rate of different cellulose materials varied greatly because of different composition and structure. In general, the easier cellulose digestion is, the more easily fermented cellulose microorganisms obtain the required nutrients. This is beneficial for the production of cellulase. It is a positive-feedback effect. In addition, the structure of material may affect oxygen transfer in solid-state fermentation medium. Figure 11.4 indicates the FPA changes of cellulase in the solid-state fermentation process of different substrates. It shows that cellulase from wheat straw pretreated with steam explosion demonstrated the highest FPA activity among three substrates, reaching the highest FPA value of 19.94 IU·g^{-1} dry medium on the fifth day. For wheat straw without steam explosion, the enzyme activity was on the rise in the 7 days of the fermentation process. On the seventh day, enzyme

activity reached 11.56 IU·g^{-1} dry medium. Compared with cellulase produced by taking wheat straw pretreated with steam explosion as a substrate, the enzyme production peak was delayed, and cellulase activity was lower. The cellulase activity of water-washed steam-exploded wheat straw was up to 6.17 IU·g^{-1} g dry medium. The cellulase production peak extended; the production rose on the 7th day. The activity of cellulase from pulp fermentation reached a peak of 5.32 IU·g^{-1} dry medium on the sixth day. Because steam explosion increases the accessibility of cellulose, the cellulase activity of steam-exploded wheat straw was higher than that of wheat straw without steam explosion. When the accessibility of wheat straw pretreated with only steam explosion was the same as that of wheat straw pretreated with both steam explosion and water washing, the former had a higher cellulase fermentation level than the latter. The level of cellulase fermentation from wheat straw pretreated with both steam explosion and washing was even lower than that of wheat straw without pretreatment. Obviously, the difference of water-washed steam-exploded wheat straw and steam-exploded straw was only that washing excluded soluble sugar in the steam-exploded wheat straw. Soluble sugars decide the difference of cellulase fermentation. Steam explosion not only improves the accessibility of lignocelluloses but also produces soluble sugar that is easily used by microorganism. In water-washed, steam-exploded wheat straw, the hemicellulose content was significantly decreased, and soluble sugar was washed away, leading to the lower cellulase fermentation of washed steam-exploded wheat straw. The activity of pulp cellulase was low. The reason is that removal of lignin makes it easy to swell up by water, so ventilation of medium is reduced. The results indicate that the steam-exploded wheat straw was a good substrate for solid-state fermentation to produce cellulase [120].

In the process of enzymatic hydrolysis, the ratio of nutrients is important. The C/N of medium has a significant impact on the microbial growth and product formation. An improper C/N will affect the balance of the strain to absorb nutrients. Excessive nitrogen sources cause too strong cell growth, which is not helpful for some metabolites to accumulate. Less nitrogen source results in slow cell growth. Absence of a carbon source makes a strain age and autolyse easily.

Microorganisms grow and produce metabolites on the substrate, so these actions would be influenced by physical factors (substrate particle size, shape, porosity, fiber content, viscosity, diffusion rates among particles, etc.) and chemical factors (DP, hydrophobicity, degree of crystallinity, electrochemical properties, etc.) of the substrate. The most important factors are substrate particle size and humidity or water activity, which influence microbial growth and activity in solid-state fermentation. The particle size of the substrate directly affects the reaction specific surface area of the unit volume, the growth of bacteria in the interparticle, the oxygen supply rate, and the rate of carbon dioxide removal. Pandey investigated the effect of substrate bran particle size on product glucosidase in solid fermentation with *A. niger*. The optimum size was 425–600 μm, but if particles were smaller than 180 μm and greater than 1.4 mm, the result was the same as that of particles 425–600 μm [121].

Generally, a smaller substrate particle is considered to be the ideal choice because it can provide a larger surface area for microbial contact, significantly improving the reaction rate of solid-state fermentation. In many cases, particles that are too small are likely to cause substrate cluster and low interparticle porosity, resulting in increased resistance. So, the heat and mass transfer are adversely affected, preventing microbial respiration or ventilation and subsequently causing poor growth of the microorganism. At same time, large particles are conducive to improving mass and heat transfer efficiency because the gap increases. They can also provide better breathing and aeration conditions but a smaller surface area for microbial reaction. With the growth of mycelia, the size of the gap will be reduced during the reaction, and the effective diffusion coefficients of oxygen and carbon dioxide decrease. For some biological processes, it is essential to select the appropriate particle.

② Water activity

The greatest feature of solid-state fermentation is the lack of free water, so substrate water content changes will inevitably have a major impact on microbial growth and metabolic capacity. Whether a microorganism grows on the substrate depends on its water activity a_w instead of water content. The water activity factor a_w is defined as

$$a_w = \frac{P \ \text{(saturated vapor pressure of wet material)}}{Pa \ \text{(pure water saturated vapor pressure under the same temperature)}}$$

The water activity is related not only to the substrate itself but also to the type and quantity of the solute:

$$a_w = -VM\varphi/55.5$$

where V is number of ions, M is the molar concentration of solute, φ is the molecular osmotic coefficient, and 55.5 is the molar concentration of pure water.

The moisture level of substrate is decided by the property of the substrate, the type of final product, and the needs of the microorganism. Different microorganisms have different water activity requirements. In general, bacteria require an a_w between 0.90 and 0.99; most yeast require an a_w of 0.80–0.90; fungi and a few yeasts require an a_w from 0.60 to 0.70. Therefore, fungi are applied in solid-state fermentation because of their low water activity requirements and exclusion of other bacterial contamination. A high a_w influences the growth of fungi by reducing porosity, hindering the diffusion of O_2 and CO_2, and increasing infection probability. In the process of fermentation, the a_w decreases because of evaporation and temperature increases. To improve the a_w for the normal growth of a strain, the following methods could be applied: addition of sterile water or humid air, jet wetting by installating sprayer, and so on. In solid-state fermentation, the integration of ventilation, cooling, and water content control can effectively adjust the a_w.

③ Ventilation and mass transfer

Microorganisms used in solid-state fermentation are almost all aerobic, so the air ventilation rate is particularly important. The increase of the air rate not only can provide microorganisms desired oxygen but also can remove reaction heat and carbon dioxide, improving the efficiency of mass and heat transfer. For laboratory and industrial production, forcing sterile air through the process is the general method to achieve ventilation. But, the rate of ventilation is determined by the following factors: the characteristics of the microorganism, the demand for oxygen of the synthetic product, the heat released by the fermentation reaction, the thickness of the low-layer substrate, the amount of carbon dioxide and other volatile metabolites produced by the microorganisms, gap size in the substrate, and so on. The enhancement of the ventilation rate leads to a lower substrate a_w, resulting in dry substrates and equivalently long air-humidifying time. The concentration of oxygen can be analyzed in a timely manner using GC or an oxygen analyzer to adjust the rate of forced ventilation according to the situation.

Solid-state fermentation has no free water, so microbes draw oxygen directly from the air. But, many operating factors and the characteristics of the medium influence the oxygen transfer rate, such as air pressure, ventilation rate, porosity of the matrix, thickness of the material layer (s), substrate humidity, reaction geometric characteristics, and rotational speed of the mechanical agitation device. Liquid film is formed by the surface moisture of the substrate, which is the control factor for mass transfer. The mass transfer resistance of solid-state fermentation is smaller than that of liquid fermentation. The mass transfer rate constant is still demonstrated by $K_L a$. Ghildyal et al. [122] investigated the effect of gas concentration gradient on the product and yield in a shallow dish solid-state fermentation reactor. Results showed that the concentration gradients of O_2 and CO_2 changed seriously with material layer in the shallow dish, which greatly affected the product and yield. Along with the increase of gradient, the yield was greatly decreased. Ghildyal et al. [122] investigated the effect of gas concentration gradient on the product in a solid packed-bed reactor. It was found that forced ventilation of the solid packed-bed reactor could eliminate the gas concentration gradient. So, mass transfer can greatly enhance the activity of enzyme.

Usually, some measures can be used to improve the mass transfer conditions, for example, using particulate, porous, or fibrous material as a substrate; reducing the thickness of the substrate; increasing the gap in the substrate; applying a porous platter for fermentation; stirring the substrate; or using drum reactor. At present, the integrated forced ventilation and stirring technologies were used. But, timing ventilation or a change in gas flow direction is required to prevent the generation of channeling. Overcoming the material concentration gradient is relatively easier than overcoming the temperature gradient. Ventilation is often linked with heat dispersal, and it depends on the temperature control.

④ Temperature

The heat transfer is indivisible from mass transfer. Temperature is an important factor. Much heat is released during microbial growth and metabolic processes,

especially before fermentation when a strain grows exuberantly because of the poor heat transfer efficiency in solid-state fermentation. The temperature of the substrate is rising rapidly. Sometimes, it is as high as about 2 °C cm^{-1}. The growth and metabolism of the strain will be seriously affected if the heat generated cannot be dissipated in time. Sometimes, burning medium even happens, leading to a large amount of dead strains and complete failure of the fermentation. This is the critical and difficult point for solid-state fermentation enlargement. To reduce the product temperature, proper turning of the medium is necessary in addition to increased ventilation and spraying of sterile water. In the summer, cooling is difficult (especially in southern China, with high summer temperatures and humidity). It is desirable to use liquid ammonia refrigeration or air conditioning for refrigeration cooling for a short time.

⑤ pH

Similar to the effects of temperature on microorganisms, there is an optimum pH for microorganisms. Most fungi are less sensitive to the pH of the environment. Fungi can grow cells typically from pH 3 to pH 9. Compared with bacteria and actinomycetes, it is easier for fungi to live in an acidic environment; the optimum pH is 5–8. For some strains, however, maintaining a certain value is necessary, such as the pH for *Trichoderma reesei* needs to be 4.5 to produce cellulase. Although pH is an important parameter, in the fermentation process, pH is difficult to control effectively because of the lack of online measurement of pH in moist material. So far, pH changes in the solid-state fermentation process have been studied extensively; however, pH regulation is reported less. Commonly, a substance with buffer capacity is used as a substrate to eliminate the adverse effects brought by the change of pH. Another novel method is to use a nitrogen-containing inorganic salt (such as urea) as a nitrogen source to offset the negative impact of the acid generated during the fermentation. Lonsane [123] proposed that, as long as there is a good initial pH in the fermentation process, it is not necessary to measure and control pH in the process of fermentation. In a great deal of solid fermentation, the pH of the fermentation process, however, has a characteristic change. But the conventional method (pH electrode method and colorimetric method) is difficult to detect effectively, limiting the feasibility of pH as an important control parameter.

⑥ Humidity

Humidity refers to the humidity of the ambient air in the fermentation tank. If air humidity is too low, the material can easily become dry because of evaporation of water, which has an impact on strain growth. Air humidity affects the oxygen content in the air. The material surface usually becomes wet by condensation, affecting strain growth or infection and final product quality. The air humidity should be maintained at an appropriate value. It is generally maintained in the range of 85–97 %.

⑦ Fermentation time

The end of fermentation is important for enhancing the amount of product. In the fermentation process, the concentration of product changes all the time. Generally,

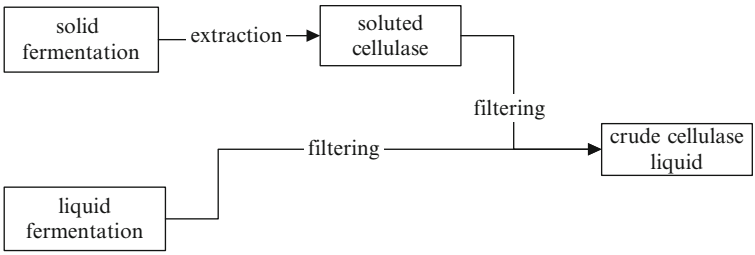


Fig. 11.5 Simple scheme of cellulase extraction

the longer the peak generation of product is, the higher the productivity is. But, the product yield decelerates and even ceases at a certain time. Therefore, whether taking strains or metabolites as the target products, microbial fermentation has a preferred time phase. If the time is too short, a desired yield cannot be obtained. If the time is too long, autolysis happens because the environment is not conducive to strain growth, and the yield is decreased while the production cost is increased. Therefore, fermentation time must be determined through experiments based on different strains, different process conditions, and different products.

11.3.3.2 Separation and Extraction Methods for Cellulase

The bottleneck for industrial application of cellulase is that cellulase is difficult to purify and crystallize. Extraction of cellulase is necessary whether it is produced by solid-state fermentation or liquid fermentation. Different extraction steps are applied according to differences in the production of cellulase between solid-state fermentation and liquid submerging fermentation (Fig. 11.5). The main difference is that a suitable solvent is necessary for cellulase-containing residue of solid-state fermentation to fully dissolve cellulase into the solvent, which is leaching. Cellulase is capable of dissolving in water, and in a certain concentration of salt solution, its solubility is increased. It Generally, cellulase is extracted in a dilute salt solution. Salt solutions used commonly include 0.15 M chlorine sodium solution and 0.02–0.05 M phosphate buffers.

The material that follows describes the extraction of liquid crude cellulase.

There are many kinds of extraction methods for liquid crude cellulase. The method most commonly used is precipitation. The precipitation method is the oldest method of isolating and purifying cellulase, but it is still widely used in industry and the laboratory. Because its enrichment effect is often greater than the purification effect, the precipitation method is usually a method used for preliminary separation, then other methods are used to further enhance purity.

(1) Salting-out method

The salting-out method uses a high concentration salt to cause the precipitation or polymerization of protein. The basic principle of this method is that the salt ions

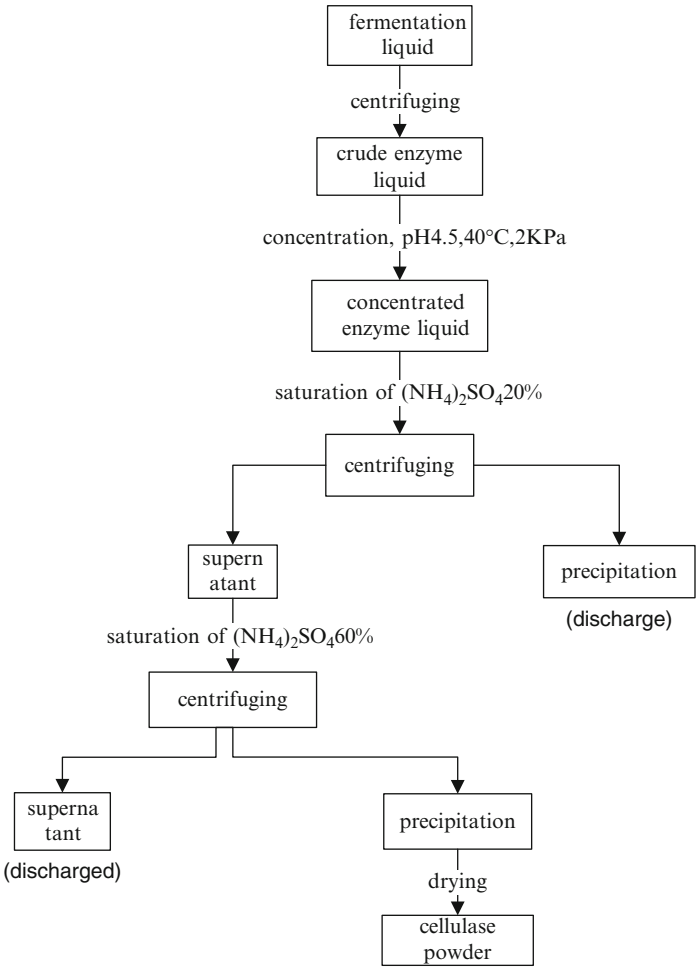


Fig. 11.6 Extraction of crude cellulase preparation

compete for the water molecules with protein molecules, reducing the degree of protein hydration by destroying the hydration shell and the protein solubility. The electrostatic charge of salt ions can partially neutralize that of protein molecules, reducing the electrostatic charge of protein to precipitate protein. In the extraction of cellulase, the most commonly used salts are ammonium sulfate. There is also a mixed ammonium sulfate and sodium sulfate salting-out method for extraction of alkaline cellulase.

Figure 11.6 is a flowchart for a specific crude cellulase extraction. It shows that the first step of salting is to concentrate a crude enzyme solution. This is because there is a large amount of liquid fermentation broth from both liquid and solid-state fermentation. This will increase not only the workload but also the amount

of ammonium sulfate. So, wastes are produced, leading to environmental pollution. Usually, the enzyme solution is concentrated to 1/10–1/15 of the original volume. Generally, a method to concentrate an enzyme solution is to adjust to pH 4.5; then, concentration is carried out under the conditions of 15 mmHg at 40 °C and reduced pressure.

(2) Organic solvent
Commonly, organic solvents used to isolate cellulase by precipitation include ethanol, acetone, isopropanol, and so on. Cellulase sedimentation obtained with organic solvent precipitation is easier to centrifugate or filtrate than that precipitated by the salting-out method. Organic solvent precipitation can exclude inorganic salts. It is commonly used in the food industry. Moreover, resolution of the organic solvent method is better than that of the salting-out method. Organic solvent is generally easy to remove and recover. The optimum concentrations of the different solvents are as follows: ethanol 70 %, acetone 60–62 %, propanol 50 %, and isopropanol 40–60 %. Application of organic solvent is easy to make its degeneration of enzyme activity, so the cellulase separation operation must be carried out at about 4 °C, and cellulase is precipitated immediately after separation. The amount of organic solvent used is generally about two times the volume of enzyme solution. The pH of enzyme solution is adjusted to near the isoelectric point. Cellulase precipitation separated out can be immediately dissolved in 0.02–0.05 M phosphate buffer for further purification.

(3) Tannin extraction
Tannin is a polyphenolic substance that can form water-insoluble complexes with proteins. When the method to resolve bacteria or protein from this complex was not found previously, tannins were a deactivator for cellulase. So, as a cellulase extraction method, tannin precipitation has not been industrialized. Later, people found several substances to resolve enzyme from the complex of tannins and enzyme. These substances are polyethylene glycol (PEG), polyethylene nitrogen pentanone (PVP), glycerides or stearic acid of PEO, and sorbitol, which open the way for the industrialization of tannin precipitation. Cellulase prepared by precipitation with tannins has high purity without containing salt. It not only meets food requirements but also can be used in oral medicine, textile processing, and the fermentation industry. Wu et al. proposed that, after precipitating cellulase with tannic, PEG can be used to resolve and activate cellulase, which can be efficiently extracted cellulase in crude broth. First, cellulase wet medium was prepared with *Trichoderma* spp. by solid-state fermentation. Then, 40 °C deionized water was added by a medium-to-water volume ratio of 1:5. The mixture was incubated for 60 min and filtered to obtain crude cellulase solution. 5.0 $g \cdot L^{-1}$ tannins were added to the diluted enzyme solution with cellulase activity FPA 22.6 $U \cdot mL^{-1}$. Cellulase in the crude enzyme solution was almost completely precipitated. The precipitation was dispersed in 0.1 M citrate buffer at pH 4.6. PEG with a molecule 6,000-U molecule was added until its content was 1.6–4.0 times higher than for tannin. The cellulase activity recovery rate reached 210–245 %. Cellulase was concentrated seven to eight times. It was regarded that PEG had an activation effect on cellulase.

PEG can resolve cellulase from a cellulase-tannin complex. The amount of tannins and PEG used is related to the protein content in the enzyme solution. Excessive PEG to some extent would activate enzyme, which makes cellulase activity recovery more than 100 %. Too much PEG causes phase change, thereby inactivating the enzyme and drastically reducing the cellulase activity [124].

(4) Other extraction methods

① *Spray drying method*. Fermentation liquid is filtered to remove solids (mainly bacterial cells and impurities). The obtained supernatant is spray dried at 50 °C. Then, it will be milled and sieved. With spray-dried extraction, the cellulase yield (ratio of enzyme dry powder weight to volume of enzyme solution) reaches 5.6 %, which is higher than that obtained by the salting-out method. But, the enzyme activity is low because the CMCase activity is 3,909 U, and the FPA activity is 306.67 U. The reason for low enzyme activity may be low purity and more slag. The advantage of extracting the cellulase with a spray-drying method is that the process is simple, and there is less salt [125].

② *Expanded bed adsorption method*. Cellulase is directly extracted from the fermented liquid with expanded bed adsorption. The principle is that cellulase is absorbed by an affinity effect of a porous structural analog of cellulase on cellulase. For absorption with an expanded bed, the effect of microcrystalline cellulose is best. Cellulase produced by *Bacillus* sp. B21 is purified by the expanded bed; as a result, cellulase is purified 18-fold with a recovery rate of 97 %. If the expanded bed is eluted by 1 M NaOH, it could be used more than ten times. The adsorption capacity and recovery rate are essentially the same [126].

③ *Aqueous two-phase extraction*. An aqueous two-phase system of PEG/$(NH_4)_2SO_4$ is used. Cellulase allocation in the PEG ammonium sulfate aqueous two-phase system is mainly because of electrostatic interactions, hydrophobic interactions, and a biological affinity effect. In an aqueous two-phase system, cellulase is an amphoteric compound. There are many acid and basic groups on the surface of cellulase, such as carboxyl acid. The concentrations of cellulase in the upper and lower phase are different because of charge action and the various forces (e.g., hydrophobic bond, hydrogen bond, ionic bond, etc.) and the presence and influence of environmental factors. When aqueous two-phase extraction system is used to extract cellulase with PEG mass fraction 18 %, molecular weight 2000, $(NH_4)_2SO_4$ 16 %, 25 °C, and pH 5.5, the distribution coefficient K and the extraction rate y are 8.23 and 0.947, respectively [127].

11.3.4 Cellulose Sugar Platform

11.3.4.1 Methods to Construct a Cellulose Sugar Platform

The sugar platform refers to the use of biomass containing an average of more than 75 % carbohydrates. These carbohydrates are the intermediates of the sugar

platform, and they also can be used as substrate for further transformation. Sugar platform technology is based on the biochemical conversion process. It focuses on fermenting sugar extracted from biomass feedstock. The sugar platform technology can produce energy, fuel, and bio-based products, as well as many chemicals that can form lineage products.

Generally, sucrose and starch can be directly used as fermentable sugar for industrial fermentation because they are easily obtained and fermented. However, the supply of sucrose and starch raw material cannot meet the needs of raw material biorefining. Cellulose can be converted into fermentable sugars and has a renewable property, so a cellulose sugar platform has increasingly attracted widespread interest. At the same time, cellulose cannot be directly used as a source of fermentable sugar. At present, the most promising method to use this material is as follows: Lignocellulose is pretreated to make cellulose more susceptible to cellulase, and then the cellulose component is hydrolyzed by cellulase [128].

Converting cellulose into sugar is the first step of biological conversion. The method used commonly includes mild acid hydrolysis and enzymatic hydrolysis.

(1) Acid hydrolysis
The acid hydrolysis method can be divided into concentrated acid hydrolysis and dilute acid hydrolysis.

① *Concentrated acid hydrolysis*. Concentrated acid hydrolysis was reported as early as 1883. Its principle is that crystalline cellulose can be completely dissolved at a lower temperature at 72 % sulfuric acid, 42 % hydrochloric acid, and 77–83 % phosphoric acid, resulting in homogeneous hydrolysis of cellulose. Cellulose is converted into oligosaccharides containing several glucose units, mainly fiber tetrasaccharide (four glucose polymers). The mixture is diluted continuously with water and heated at a certain time. Then, tetrasaccharide is hydrolyzed into glucose [129]. Concentrated sulfuric acid hydrolysis technology is relatively mature, recycling about 90 % sugar from cellulose and hemicellulose. Iranmahboob et al. [130] mixed 50 % hardwood and 50 % softwood raw material for hydrolysis. Crushed and dried raw materials (200 g) were mixed with 500 g sulfuric acid, and the mixture was diluted to 20–33 %, hydrolyzed at 100 °C for 30 ~ 20 min, to obtain 78–82 % sugar. Concentrated acid hydrolysis has the advantage of high sugar yield, up to 90 % or more. There are also disadvantages, for example, too long reaction time, serious equipment corrosion, high cost of device materials resistant to acid corrosion, environment pollution, difficulty in recycling and reuse, and so on. All of these hamper the further development of concentrated acid hydrolysis.

② *Dilute acid hydrolysis*. The dilute acid hydrolysis method usually refers to the use of inorganic acid such as sulfuric acid or hydrochloric acid with a concentration less than 10 % as a catalyst to hydrolyze cellulose into glucose and monosaccharides. The hydrolysis reaction temperature is generally 100–240 °C, and the pressure is greater than the saturated vapor pressure of the liquid, generally more than 10 atm [131]. The dilute acid hydrolysis process is simple, and was proposed by Mel Vincennes in France in 1856. As the acid concentration is low, the impact on the environment is relatively weak, and waste liquid processing is relatively

simple. Raw material cost is relatively reduced. But, because a low concentration of acid is used, the hydrolysis process requires a high-temperature and high-pressure environment, and its initial development is slow. With the development of chemical industry equipment and materials and the emergence of various temperature- and pressure-tolerant equipment as well as acid-resistant material, the advantages of dilute acid hydrolysis became more apparent. However, dilute acid hydrolysis will produce formic acid, acetic acid, HMF, phenolic compounds, and other by-products. So, various methods are studied, including a variety of physical, chemical, and biological methods to remove the fermentation-inhibiting substances in cellulose hydrolysis products to improve fermentation yield.

Factors of dilute acid hydrolysis include the pulverized degree of lignocellulosic material, liquid-to-solid ratio, reaction temperature, reaction time, the concentration of acid, and type and concentration of cocatalyst. The greater the cellulosic raw material grinding degree is, the greater the contact area of the raw material with acid catalyst will be, leading to better hydrolysis. Especially when the reaction rate is fast, the generated monosaccharide can be removed from the solid surface in a timely manner, which is helpful for carrying out the reaction. The liquid-to-solid ratio refers to the ratio of hydrolysate volume and cellulosic raw material quantity. Generally, the sugar yield of a unit of cellulose solid raw materials increases with the increase in the ratio of liquid to solid. But, hydrolysis costs rise and the concentration of the obtained sugar liquid is reduced, increasing the cost of the follow-up fermentation and distillation processes. Generally, the liquid-solid ratio is 5–20 $mL \cdot g^{-1}$. Temperature is an important impact factor for the hydrolysis rate. The hydrolysis rate will increase by 0.5–1 times when the temperature increases 10 °C. A high temperature causes the decomposition of monosaccharide. So, if a higher hydrolysis temperature is used, the reaction time should be reduced. In contrast, reaction time should increase appropriately. Theoretically, when the other conditions remain unchanged, when the acid concentration doubles, hydrolysis time can be shortened to a third or half. The acid concentration increase leads to high acid costs. At the same time, corrosion resistance requirements of the equipment will increase, resulting in high equipment costs, which is not helpful for industrialization. Generally, the acid concentration will not exceed 10 %.

Previous research for the dilute acid hydrolysis method were mainly concentrated in the field of inorganic acids, including hydrochloric acid, sulfuric acid, and so on. Compared to sulfuric acid, hydrochloric acid hydrolysis has higher efficiency, but also high difficulty of wastewater treatment. Hydrochloric acid can be taken into consideration if there is cheap source because it has problems in that it is expensive and more corrosive and requires high equipment cost. Researchers investigated phosphoric acid, nitric acid, and maleic acid hydrolysis of cellulose, and preliminary results were obtained [132].

(2) Enzymatic hydrolysis

Enzymatic hydrolysis is a new kind of cellulose hydrolysis technology; it began in the 1950s. With the development of biology, it attracts increased attentions. It obtains sugar by enzymatic hydrolysis with cellulase at atmospheric pressure at

45–50 °C and about pH 4.8. Cellulase is not a single substance; it consists of three main components: endoglucanase, exoglucanase, and β-glucanase. Endoglucanase cuts the β-1,4-glycosidic bonds randomly, making cellulose have long strand breaks. Broken molecular chains still have a reducing end and a nonreducing end. Exoglucanase has two components: hydrolyze and cellobiose from the reducing end of the cellulose long chain. β-Glucanase decomposes cellobiose and short-chain oligosaccharides into glucose. The enzymatic hydrolysis can be carried out at room temperature and under atmospheric pressure. The culture and maintenance of the microorganisms and maintenance only require fewer raw materials, reducing the energy consumption of process. Cellulase has a high selectivity and generates a single product, so a high sugar yield can be obtained (>95 %).

Cellulase requires arriving at a molecule to degrade cellulose; therefore, lignocelluloses should be pretreated to remove hemicelluloses or lignin and to destroy the crystalline structure of cellulose. Acid hydrolysis and enzymatic hydrolysis are usually combined. Acid hydrolysis is used to pretreat cellulose to facilitate enzymatic hydrolysis.

11.3.4.2 Sugar Platform Components and Content

When solid residue insufficiently reacted was removed, liquid product rich in monosaccharides and soluble oligosaccharides could be obtained. It is necessary to qualitatively and quantitatively analyze the liquid products and solid residues and to investigate the application of product and the process and mechanism of reaction. Sugar products could be analyzed by determining the reducing sugar or hydrolyzing subsequent residues with acid quantitatively into monosaccharides. The monosaccharides include glucose, xylose, arabinose, mannose, galactose, rhamnose, and other soluble sugars. In the liquid product of hydrolysis, soluble sugar is only part of the whole. Xylooligosaccharides from hemicellulose hydrolysis and oligomeric gluconate from cellulose hydrolysis are also main components of the liquid product. The health protective effects of these two types of sugar have gradually attracted attention.

(1) Determination of reducing sugar content
The DNS standard method is used for determination of the reducing sugar content; the method was introduced in Sect. 11.1.

(2) Qualitative and quantitative analysis of monosaccharides
Generally, chromatographic methods for qualitative and quantitative measurement of monosaccharides include paper chromatography, thin-layer chromatography (TLC), GC, and HPLC.

Chang et al. [133] proposed determination of the content of monosaccharides (glucose, fructose, and xylose) in the liquid mixed sugar obtained by hydrolyzing cellulosic material with a TLC determination method. Silica gel G was taken as the solid adsorbent, and glass was used as supports to make TLC plates. For eluent, the ratio of *n*-butanol, 37 % acetic acid, and water (volume ratio) was 3:2:2. The pH of

the mixture was adjusted to 4–5.0 with 200 $g \cdot L^{-1}$ NaOH solution. For aniline–oxalic acid solution, 0.93 g of aniline and 66 g of oxalic acid were dissolved in 100 mL water-saturated n-butanol. The separation effect was good, and the qualitative and quantitative analysis task could be completed.

HPLC can also be used to qualitatively and quantitatively investigate the sugar in hydrolysate. The following is an HPLC analysis method [134]: France Gilson HPLC was used with a Gilson 132-type differential refractive index detector and Spherisorb NH_2 (250 mm × 4.6 mm) column, a stainless steel column. The mobile phase was acetonitrile and water (82:18). The mobile phase was pretreated ultrasonically for 15 min to degas. The flow rate was of 2 $mL \cdot min^{-1}$. Injection volume was 20 μL. Measurement temperature was room temperature (26 ± 2) °C. The sample was quantitatively determined by the method of peak area of the external standard. As a result, this method could separate rhamnose, xylose, fructose, glucose, sucrose, and maltose.

(3) Oligosaccharides

Products of cellulose pretreated with dilute acid hydrolysis include monosaccharides and oligosaccharides. The HPLC standard method used in National Renewable Energy Laboratory (NREL) of the United States is mostly applied to quantitatively determine oligosaccharides, which is more difficult to detect than monosaccharides. Its basic principle follows Saeman's [135] methods. Lignocellulose is completely converted into monosaccharides by two-step hydrolysis of sulfuric acid with a concentration of 72 and 4 % separately. Then, the hydrolysis liquid of the biomass is quantitatively hydrolyzed into monosaccharide with 4 % sulfuric acid (that is, only the second step of the two-step acid hydrolysis). The content of monosaccharides is quantitatively determined to calculate the conversion rates of various monosaccharides. The specific operation is as follows: The first step is to hydrolyze dry feedstock at 30 °C with 72 % sulfuric acid for 2 h. Second, a hydrolysis liquid sample of the first step is quantitatively removed. The sample is diluted, or sulfuric acid is added to make the sulfuric acid concentration 4 %. It is hydrolyzed in a pressure cooker at 121 °C for 1 h. $CaCO_3$ is used to neutralize to a pH value of 5–6. Then, it is diluted and filtered with a 0.2-μm filter membrane. Common columns used are a Bio-Rad Aminex HPX-87C column and a column coupled with a differential refractive index detector. The Bio-Rad Aminex HPX-87C column can quantitatively detect glucose, xylose, and arabinose. Bio-Rad Aminex HPX-87P column can also test cellobiose, mannose, and galactose except for the monosaccharides mentioned.

Zhuang et al. [136] proposed a simple detection method for oligosaccharides that could qualitatively and quantitatively detect monosaccharides, cellobiose, trisaccharides, tetrasaccharides, and other sugars. The equipment used was as follows: Agilent 1100 HPLC chromatography detection system, French SEDERE evaporative light-scattering detector (ELSD) SEDEX75, Japan Shodex Sugar KS2802 column (8.0 mm × 300 mm), HPLC-grade water as the mobile phase at a flow rate of 1.2 $mL \cdot min^{-1}$, column temperature of 80 °C, and injection volume of 20 μL.

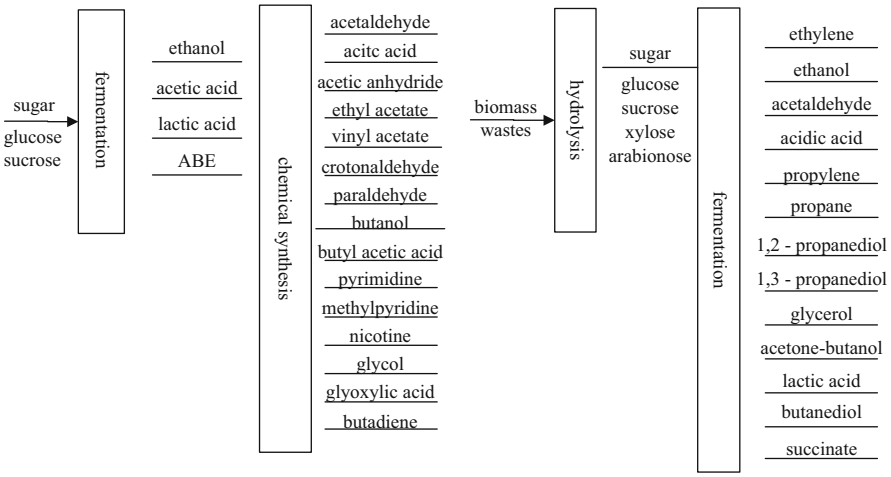


Fig. 11.7 Chemicals from lignocellulosic biomass

11.3.4.3 Conversion Products Using a Cellulose Sugar Platform

Compound prepared from a sugar platform refers to conversion of biomass into sugar and then conversion of sugar to other chemicals. Fermentable sugars from cellulose degradation as a platform can produce a variety of commonly used industrial raw materials, such as acetone, butanol, citric acid, xanthan gum, xylitol, and other important substances (Fig. 11.7). They mainly can be divided into the following product systems: The C1 system primarily consists of methane, methanol, and the like. The C2 system mainly includes ethanol, acetic acid, ethylene, ethylene glycol, and the like. A C3 system mainly includes lactic acid, acrylic acid, propylene glycol, and the like. The C4 system mainly includes succinic acid, fumaric acid, butylene glycol, and so on. The C5 system includes itaconic acid, xylitol, and the like. The C6 system mainly includes citric acid, sorbitol, and so on [134].

Alcohol from cellulose can be prepared by step-by-step hydrolysis saccharification and fermentation, which is defined as separate hydrolysis and fermentation (SHF), including two steps. First, cellulase is hydrolyzed into glucose; then, it is fermented to produce ethanol. It has the advantage that enzymatic hydrolysis of lignocellulose and fermentation of ethanol can be carried out separately under the optimal conditions of each, for example, 45–50 °C for enzymatic hydrolysis, but 30 °C for ethanol fermentation. However, the drawback of this method is that the glucose produced in the hydrolysis will inhibit the activity of cellulase and β-glucosidase, so a relatively low substrate concentration is required in the hydrolysis. The amount of cellulase used is increased, which greatly reduces enzymatic efficiency. A lower substance concentration will inevitably result in a lower concentration of ethanol fermentation, increasing the cost of fermentation and ethanol recovery.

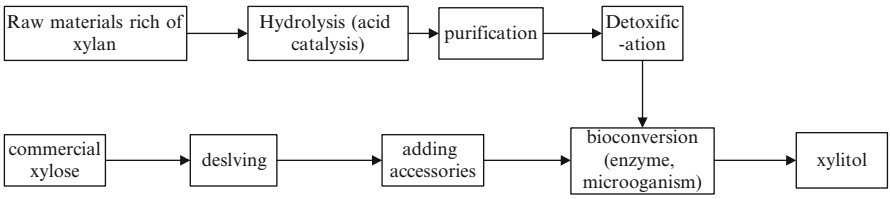


Fig. 11.8 Bioconversion schematic for xylitol

Xylitol is prepared with forestry waste as raw materials by hydrolysis, purification, concentration, bleaching, hydrogenation, concentration, crystallization, and separation processes in the system. The bioconversion process of xylitol is shown in Fig. 11.8.

Fang et al. prepared xylitol by fermentation with *Candida tropicalis,* which has tolerance domesticated by the inhibitors of hemicellulose hydrolyzate. The parameters were optimized as follows: 10 % (v/v) vaccination amount, fermentation time 24 h, 30 °C temperature, initial pH 5.5, and addition of nitrogen source in the fermentation process, according to which the xylitol yield reaches 61 % [137].

Wang et al. [138] found that *Candida tropicalis* fixed in the porous polyurethane could effectively use hemicellulose hydrolysate to produce xylitol. Optimum fermentation parameters were established with batch fermentation in shake flask conditions: 7 % inoculum size; 1.0 g·100 mL^{-1} polyurethane; temperature 30 °C; initial pH value 6.0; and shaking speed sequentially changed to regulate dissolved oxygen, 0–24 h 200 $r{\cdot}min^{-1}$ and 24–46 h 140 $r{\cdot}min^{-1}$. Polyurethane immobilization improved the inhibitor tolerance of strain fermentation. The stability of fermentation performance increased with the density of the immobilized strain by improving both the fermentation yield and volume production rate. The hydrolysate can be converted into xylitol with ion exchange but without decolorization. Immobilized cells were continuously repeated 21 days for 12 batch fermentations. The xylitol yield reached an average of 67.6 %, and the average volumetric production rate was 1.92 $g{\cdot}(L{\cdot}h)^{-1}$ [138].

11.3.5 Integration of Fermentation and Separation for Cellulase

11.3.5.1 Simultaneous Saccharification and Fermentation

To overcome product feedback inhibition, Gauss et al. [139] in 1976 proposed the simultaneous saccharification and fermentation of cellulose saccharification and ethanol fermentation within one fermentor. Inoculation of the alcohol yeast to ferment while adding cellulase can make glucose convert into alcohol. Removal of the feedback inhibition of cellobiose and glucose on the cellulase for saccharification

without impact can significantly improve the ethanol yield. The application of the simultaneous saccharification and fermentation method is not bound to separate glucose and lignin, which can avoid the loss of sugar, reduce the number of reactors, and decrease investment costs (about 20 %). In addition, the application of simultaneous saccharification and fermentation can carry out synergistic fermentation of hexose and pentose sugars. There are obvious advantages in the detoxification treatment. This is the so-called simultaneous saccharification and fermentation technology.

Chen et al. studied the factors that had an impact on simultaneous saccharification and fermentation, which indicated that the enzymatic hydrolysis process was still the major limiting factor for simultaneous saccharification and fermentation of lignocellulose because of the inconsistent optimum temperature for enzymatic hydrolysis and fermentation [140]. The optimum temperature of cellulase hydrolysis is typically about 50 °C, while the optimum fermentation temperature of ordinary yeast is usually about 30 °C. Selecting yeast with a high temperature tolerance is helpful for simultaneous saccharification and fermentation technology. The key of simultaneous saccharification and fermentation technology is to select an optimal yeast.

However, there are also some inhibiting factors for simultaneous saccharification and fermentation, such as the xylose inhibition effect, lack of coordination of the saccharification and fermentation temperatures, and so on. In simultaneous saccharification and fermentation, xylose from hemicellulose hydrolysis will remain in the reaction solution. When the concentration reaches 5 %, the xylose inhibition effect on cellulase can reach 10 %. The elimination of the xylose suppression method uses strains that can convert xylose to ethanol, such as *Candida* spp., tube capsule yeast, and so on. Current research is more about the strain that is able to make use of glucose and xylose for mixed fermentation. Compared with simply using a strain for glucose and xylose singly, ethanol production increased from 30 to 38 % and from 10 to 30 %, respectively [141].

11.3.5.2 Nonisothermal Simultaneous Saccharification and Fermentation

Chen [142] proposed nonisothermal simultaneous saccharification and fermentation (NSSF), using dispersion, coupling, and parallel systems to provide cellulose saccharification and ethanol fermentation in two bioreactors. Conveyor system of the two reactors is established by pipeline and pump at the same time. Saccharification and fermentation can be carried out under their optimum conditions by hydrolysis and fermentation in the different reaction zones. The yeast is fixed in the fermentation zone to avoid heat inactivation, which is called enzymolysis membrane–coupling fermentation isolation. The enzymatic membrane–coupling technology refers to the use of an enzymatic membrane reactor to integrate fermentation, catalysis, and separation. The enzyme membrane reactor refers to a membrane module that is introduced into the reaction appropriately. Permeable solution is separated from the reaction system using the impetus on both sides of the membrane. Thereby, biocatalysis, product separation, concentration, and enzyme recovery operations are combined into a single operating unit.

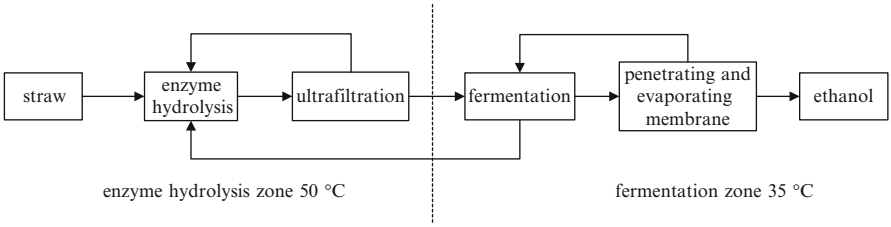


Fig. 11.9 Schematic of enzymatic hydrolysis-fermentation membrane separation coupling system for ethanol production

This method is used in the production system for straw ethanol. The whole coupling system includes an enzymatic region and a fermentation region (Fig. 11.9). The enzymatic zone is composed of the enzymatic tank and the hollow-fiber ultrafiltration membrane modules. The fermentation zone has a fermentation tank and the pervaporation apparatus. Temperatures of the two zones are controlled at 50 and 35 °C separately, so that the enzymatic hydrolysis and ethanol fermentation are carried out at the optimal temperature conditions. Straw is hydrolyzed in the enzymatic hydrolysis tank, and the enzymatic liquid circulates in the ultrafiltration membrane, so cellulase and sugar in enzymatic hydrolyzate are separated, and cellulase is sent back to the enzymatic zone continuously by hydrolysis liquid circulation. Through the ultrafiltration membrane, sugar in the fermentor is fermented by immobilized yeast, so feedback inhibition of sugar on enzymatic hydrolysis is reduce.

A permeation evaporation membrane device is started at certain times online to isolate ethanol, eliminating the inhibition effect of ethanol in fermentation broth on cellulase in the enzymatic zone and strain in the fermentation zone. It was found that only opening the enzymatic hydrolysis and fermentation cycle device without ethanol pervaporation had a faster rate of fermentation with a higher content of ethanol compared with isothermal simultaneous saccharification and fermentation results. After 72 h of fermentation, the ethanol concentration increased by 8.6 %. If the pervaporation device was opened on time, the ethanol concentration increased by 2.4 times [143, 144]. The coupling method of enzymolysis and membrane integration can separate the small-molecule products obtained by enzymatic hydrolysis online, eliminate the inhibition effect of products, and improve the enzymatic hydrolysis efficiency and yield.

11.3.5.3 Consolidated Bioprocessing

Consolidated bioprocessing (CBP), formerly known as direct microbial conversion, can complete cellulase and hemicellulase production, cellulose hydrolysis, and ethanol fermentation by one microorganism. In nature, certain microorganisms have the ability to directly convert biomass into ethanol. CBP metabolic engineering can also be carried out in two ways. One is to use microorganisms that can degrade

cellulose or engineered bacteria that can produce ethanol. Another is to use a strain whose tolerance for product has been trained, such as yeast that can also hydrolyze cellulose by exogenous cellulase. The process of CBP is helpful for reducing the cost of biological conversion, which increasingly becomes a general concern of researchers [145].

11.3.6 Research Methods for Hemicellulose Fermentation

Hemicellulose is one of the major components of plant cell walls. It has structural units that include xylose, arabinose, glucose, and so on and their methyl, ethyl unit, and uronic acid derivatives. The distribution of xylose is relatively stable. Xylose connects each other by β-glucosidase. Hemicellulose-degrading enzymes are mainly endoxylanase and exoxylanase. Hemicellulose will produce mixed sugar during the process of pretreatment and enzymatic hydrolysis. According to different sources, mixed sugars include xylose, arabinose, glucose, mannose, trehalose, and rhamnose.

11.3.6.1 Treatment Before Fermentation

(1) Pretreatment of raw materials
The pretreatment of plant cellulose materials mainly includes grinding treatment, water treatment, alkali treatment, dilute acid treatment, steam explosion treatment, and ammonia explosion treatment. The grinding treatment can only make the particles of the raw material smaller, and the component is unchanged. Water also can only remove the ash from raw materials. These two methods have only slight effects on fermentation. Ten percent of aqueous ammonia explosion pretreatment can improve the efficiency of hydrolysis of raw material under mild conditions with a slight loss of hemicellulose. Therefore, it is a preferred processing method. Ammonia explosion treatment can remove 92 % of lignin and all acetic acid, greatly improving hydrolysis fermentation performance [146]. Chen et al. [147] investigated the extraction of hemicelluloses from wheat straw pretreated with steam explosion, which showed that the concentration of sugar in hemicelluloses hydrolysate was 87.6 %, and xylose accounted for 90 %.

(2) Choice of hydrolysis conditions [148]
According to the catalytic activity of inorganic acids in the hydrolysis process, among these acids, hydrochloric acid is the best, and sulfuric acid is worst. Hydrochloric acid is used less because of its corrosive and volatile properties. At present, sulfuric acid is widely used as a catalyst. When the acid concentration is increased, the speed of the hydrolysis reaction is also accelerated, but other nonsugar organic impurities will also increase. Temperature can always promote the reaction rate. When the temperature increases 10 °C, the reaction rate generally increases one to three times. Too high a temperature will lead to xylose decomposition, producing

furfural and other harmful by-products. If the temperature is too low, hydrolysis efficiency will be affected because of the slow rate of hydrolysis. The longer the hydrolysis time is, the more complete the hydrolysis of hemicellulose is, but the amount of by-products also is greater. The higher the ratio of liquid to solid is, the more complete the hydrolysis is, but if the liquid-to-solid ratio is too large, it would lead to high acid consumption and a low xylose concentration, which will increase the cost of subsequent concentration. To make the hemicellulose xylan hydrolyze to xylose, a certain amount of acid and a high-temperature environment are required, but this will make other organics contained in some of the raw materials be dissolved or destroyed. Therefore, it is necessary to control the hydrolysis conditions, making the xylan hydrolyze as much as possible, reducing other nonsugar organic impurities into the hydrolysate, minimizing the occurrence of side reactions.

(3) Detoxification of hemicellulose hydrolysate [149]
In hemicellulose hydrolysate, substances that are toxic to yeast include furfural (degradation by-products), acetic acid (from acetylated xylan released), some of the lignin derivatives (such as phenolic compounds), heavy metal ions, and so on. So, detoxification treatment is necessary for hemicellulose hydrolysate before fermentation.

① *Lime neutralization.* Acid hydrolysate of corncob hemicellulose is neutralized to pH 5.5 at 70 °C with $Ca(OH)_2$, incubated for 0.5 h, and filtered to remove $CaSO_4$ precipitate, obtaining hydrolysate detoxified with lime.

② *Lime overneutralization.* Acid hydrolysate of corncob hemicellulose is neutralized to pH 5.5 at 70 °C with $Ca(OH)_2$ and incubated 0.5 h. $Ca(OH)_2$ is added to the mixture to adjust the pH to 9.0. $CaSO_4$ precipitant is removed immediately with filtering. The filtrate is adjusted to pH 5.5 at 70 °C with H_3PO_4. The $Ca_3(PO_4)_2$ precipitant is centrifuged to obtain the detoxification hydrolysate.

③ *Active carbon absorption.* Active carbon is added to hemicellulose hydrolysates of corncob neutralized with lime. The ratio of solid to liquid is 1:10. A decolorization reaction is carried out for 1.0 h in a shaker. Detoxified hydrolysate is obtained by a filter to remove active carbon.

④ *Ion exchange resin absorption.* Regenerated weakly basic anion exchange resin is added to an ion exchange column (Φ 2 cm × 40 cm). Hemicellulose hydrolysis of corncob flows through the ion exchange column to obtain a detoxification hydrolysate by collecting the liquid from the column.

11.3.6.2 Fermentation Process

(1) Fermentation to produce ethanol
At present, xylose in hemicelluloses hydrolysate is mainly used for fermentation to prepare xylitol, ethanol, 2,3-butanediol, single-cell proteins, and so on. In addition, hemicellulose hydrolysates can be used to produce industrial products with a higher

added value, such as xylooligosaccharides, arabinose, mannose, galactose, and so on. The traditional *Saccharomyces cerevisiae* and *Zymomonas mobilis* can easily convert glucose to ethanol, but they cannot convert xylose and arabinose to ethanol by fermentation. *Pachysolen tannophilus*, *Pichia stipitis,* and *Candida shehate* can ferment xylose to ethanol. There are many disadvantages if these yeasts are applied commercially for fuel ethanol production, such as low ethanol tolerance, slower fermentation rate, and difficulty of oxygen control in the fermentation process. Moreover, they are sensitive to the inhibitor produced in the lignocellulose hydrolysis and pretreatment process. Although xylose can be converted to xylulose by sugar isomerase, which makes it fermentable to traditional yeast, the fermenting cost is too high. Almost no naturally occurring yeast can ferment some other pentose, such as arabinose. Some bacteria can make use of these mixed sugars (e.g., *Escherichia coli*, *Klebsiella*, *Erwinia*, *Lactobacillus*, *Bacillus* spp., *Clostridia* spp.). They can produce a small amount of ethanol, which is mixed acid and organic solvent. Bacteria and yeast recombinant bacteria can be used to ferment cellulosic material through metabolic engineering to produce fuel ethanol [150].

(2) Fermentation to produce xylitol
Based on current the research, yeast is the main natural microorganism with the good performance to producte xylitol. Most others belong to *Candida* spp., *Debaryomyces* spp. and tube capsule *Saccharomyces*. Hemicellulose hydrolysate should be detoxified first, then it can be inoculated and fermented. In the xylitol fermentation process, the important impact factors include medium composition, inoculum size and seed age, pH, temperature, and ventilation in addition to the nitrogen source. In general, the growth of cell biomass positively relates to ventilation, and the xylitol conversion rate is negatively correlated with ventilation. Under aerobic conditions, the consumption of sugar is mainly used for cell growth. With increased ventilation, the cell amount also increases. Xylose consumption is mainly used to generate biomass if the growth of bacterial cells were too many. Simultaneously, increased ventilation is conducive to consumption of inhibitory substances such as glucose and acetic acid. Under microaerophilic conditions, most xylose converts into xylitol, and ethanol production is small. Nakano et al. [151] prepared high-concentration xylitol under microaerobic conditions by simulating and controlling with *Candida magnolia*. The final xylitol concentration was 356 $g \cdot L^{-1}$ with batch fermentation and strictly controlling the oxygen concentration. The theoretical yield was 82 %.

11.3.7 Research Methods for Lignocellulose Degradation

11.3.7.1 Investigation of the Lignin-Degrading Process with Model Compound [152, 153]

The structure of natural lignin is complex, which makes its degradation process difficult to study directly. Many researchers have attempted to study the conversion of substances with a structure similar to lignin to analyze the lignin degradation

mode and its metabolites. These substances are known as model compounds. Veratryl alcohol (3,4-dimethylphenylcarbinol) is a typical model compound for the study of the lignin ring-opening reaction. It is the secondary product of white-rot fungus metabolism. It plays an important role in lignin degradation. Through research, the mechanism of veratryl alcohol ring-opening was proposed, which is significant for the investigation of the aromatic ring opening of lignin monomer. Elegir used β-β and β-5 types of model compounds to investigate the degradation of laccase to lignin substances and found that the laccase mediator system could degrade phenolic lignin but not nonphenolic lignin. Choemaker proposed the fraction mechanism of the β-1 bond with the β-1 dimer model compound. Model compounds play an important role in the study of lignin degradation. It is worth noting that model compounds are only a simulation of lignin. Because of the complexity of the lignin itself, the conclusions drawn from the study of model compounds cannot be directly used for lignin. However, on the other hand, what cannot degrade the model compound also cannot degrade lignin.

11.3.7.2 Investigation of the Lignin-Degrading Process by Analysis of the Degradation Products of Natural Lignin

(1) Separation and characteristics of hydrolysates from low molecular weight lignin [154]

Low molecular weight lignin degradation products are extracted from straw treated with the white-rot fungus *Panus conchatus* with ethyl acetate as the extraction agent. Alkali with different strengths can further separate low molecular weight phenols, low molecular weight acids, and the neutral substance component. The low molecular weight phenolic compounds and low molecular weight acids are the main components of low molecular weight lignin degradation products. Their yields are 51.1 % and 34.4 %, respectively. The high yield of low molecular weight phenol is an important characteristic of straw lignin degradation with white-rot fungi *Panus conchatus*. Infrared spectroscopy and nuclear magnetic resonance spectra initially demonstrate that the oxidation reaction is dominant in lignin biodegradation. Spectroscopy of low molecular phenols showed that the oxidation reaction of the lignin side chain generates aldehyde and α-carbonyl group. The spectrum of the low molecular weight acids revealed that the C_α–C_β bond is oxidatively cleaved, forming aromatic carboxyl in addition to oxidation of lignin side chain to acids. The difference between structures of the two main components preliminarily showed that they are products of different degradation phases for rice straw lignin.

(2) Structural characteristics of aromatic acids from the low molecular weight part of the lignin degradation product [155]

The main compounds and structure of acidic components were analyzed by a color-MS integration technique. Lignin in rice straw was degraded by the white-rot fungi *Panus conchatus* to obtain the low molecular weight part of lignin degradation products. The results showed that most of this component is an aromatic compound.

The structures of the main compounds were analyzed by MS. It was found that their main ingredients were lilac acid, vanillic acid, and p-hydroxybenzoic acid and their derivatives. The result confirmed that the oxidative cleavage of the C_α–C_β bond of the side chain of the lignin occurs in the wood biological degradation process. Furthermore, it was found that there are a considerable number of the hydroxybenzoic acid derivatives with a polycondensation-type structure. They are derived from the "core" structure portion of the lignin structure. According to the relative content of the respective compounds, it was calculated that the ratio of guaiacyl to syringyl group to hydroxyphenyl was 6.1:1:2.7. It was confirmed that guaiacyl and base hydroxyphenyl structural units are preferentially degraded in the process of lignin biological hydrolysis. These results were consistent with the study results of previous electron microscopy and the polymer portion of lignin degradation.

(3) Structural characteristics of the polymer portion of lignin degradation products [156]
Rice straw was pretreated with the white-rot fungi *Panus conchatus* to obtain the polymer portion of the lignin degradation product. Structural characteristic changes were detected by quantitative ^{13}C-NMR techniques. Compared with lignin without biological effect, it was found that the ^{13}C-NMR spectra of fermented rice straw lignin had a new absorption peak, and some of the absorption peak intensity changed significantly. These changes confirmed the formation of quinone and α-hydroxy acid structure in lignin biological degradation. These types of structure are particularly conducive to the further degradation of lignin. The bond breakage of β–O–4, C_α–C_β, and C_β–C_γ and oxidation of C_γ in the process of biological hydrolysis were also confirmed. Quantitative analysis showed that the macromolecular methoxy and carboxyl content of lignin increased after biological degradation. The guaiacyl and hydroxyphenyl structural unit was preferentially degraded. It indicated that white-rot fungi attack the intermediate layer of cellulose in rice straw first. The results were consistent with results obtained by electron microscopy. The results also showed that biodegradation occurred preferentially at the noncondensed structural units of lignin.

11.4 Research Methods for the Biotransformation Process and Engineering of Lignocellulose

11.4.1 Introduction

The application of cellulose is based on efficient conversion. Cellulose biotransformation researches just stay on the conversion reaction level and lacks the law of transformation process. Therefore, it is necessary to deeply study the biotransformation process and engineering theories of lignocellulose.

The biotransformation process and engineering of lignocellulose are involved in the need for industrialization and commercialization; they are the theory and technology for cellulose conversion. The scope of study of the biotransformation process and the engineering of lignocellulose include scale-up and scale-down, integration, development of an ecological industry chain, as well as the process of conversion technological economic analysis and the life cycle.

11.4.2 Research Methods for the Scale-Up and Scale-Down of the Biotransformation Process and Engineering of Lignocellulose

11.4.2.1 Research Methods for the Scale-Up

The production process for cellulose-based products is a complex network composed of many devices, not the sum of the separate devices enlarged; there are unpredictable problems that may be exposed because perfectly amplifying each aspect does not mean that the whole process is scaled up.

The scale-up method for the biotransformation process is mainly the amplification of the bioreactor. There are usually three stages in the research and development of biological products: the laboratory, pilot, and industrialized-scale stages. The biological reaction is same in the various stages, but the mixing of reaction solution, the mass transfer, and the heat transfer often are not the same. It must be understood how to estimate the state of biological responses in different scale bioreactors, especially in the reactor amplification process, to maintain cell growth similar to the biological response rate, to summarize the inherent law and influencing factors through the application of theory and experiment, and to study and solve the transfer of material and energy. The reactor amplification process as much as possible maintains the original state of the reaction for the scaled-up bioreactor [157, 158].

Theoretically, this scaled-up bioreactor is formed by the following three steps in the development and design processes of the biological reaction and bioreactor:

(1) The cells are used in a wider culture condition to grasp the cell growth kinetics and product formation kinetics.
(2) According to the series of tests mentioned, the optimum culture medium formulations and culture conditions of the biological response are determined.
(3) Microbalance equations are solved for the quality, heat transfer, and momentum transfer and the relational model between the environmental conditions and the main operating variables (stirring rotational speed n, aeration amount Q, stirring power P_g, matrix flow rate V, etc.) is derived. Then, this mathematical model is applied to calculate the value of the main variables under the optimized conditions.

However, because of the complexity of the biological processes, the adequate description for the kinetic equations of the bioreactor process is extraordinarily complex, and the solution of some differential balance equations is still difficult. As a result, it is difficult to completely follow the ideal discussed to complete the bioreactor design and amplification. In addition to the theoretical approach mentioned, the common methods are the semitheoretical, dimensional analysis, and experience magnification methods.

(1) Theoretical scale-up

Theoretical scale-up establishes and solves the equations for momentum, mass, and energy balance. As mentioned, this scale-up method is so complex that it is difficult to apply in practice. It is the most systematic method which is based on scientific theory. Theoretically, the rate of a biological reaction does not relate to the size and shape of the bioreactor. In fact, the reaction rate is influenced by the mass transfer, momentum transfer, and heat transfer and is inevitably affected by the type of reactor and the three-dimensional structure. The theoretical scale-up is based on similarity theory, for which the characteristics of two similar reactions can be described by the same equations, their system in sync momentum, heat and mass transfer, and biochemical reactions.

For mechanical agitation ventilation, the application of the theoretical amplification method must solve the 3-D transfer equation, and the boundary conditions are complicated. Second, the transfer processes are coupling; that is, solution of the liquidity component of the momentum balance equation must be used for the mass and heat balance equations. In addition, the momentum balance often assumes that the reaction system is a homogeneous liquid, but there are a large number of bubbles in aerobic fermentation and the culture medium. In short, the main problem for the fermentation reactor theoretical scale-up is still that the momentum balance equation cannot be solved in the bioreactor system. Therefore, the theoretical scale-up method can only be used for the most simple system. For example, the scale-up principle applies to the system in which the fermentation broth is stationary or the flow state is laminar flow such as the enlargement of immobilized reactor.

(2) Semitheoretical scale-up

It is hard to solve the momentum balance equation for the theoretical scale-up method to resolve this contradiction and simplify the momentum equation. The flow of a liquid body only is considered in many flow models of a stirred tank reactor, ignoring the complexity flow in the stirring impeller or near the reactor wall. There are three types of flow model: flow type, plug flow with liquid microunit dispersed, and completely mixed flow.

A semitheoretical scale-up approach is the most common experimental research method for bioreactor design and amplification. For liquid flow, however, the main model is usually obtained only in a small experimental-scale bioreactor (5–10 L), without the advantage of the real results of a large-scale production system. So, this method has certain risk; Enlargement according to semitheoretical scale-up approach has certain risk, which should be tested through practical biological process.

(3) Dimensional analysis scale-up
The so-called dimensional analysis scale-up method maintains the dimensionless group (also known as dimensionless number) of bioreactor system parameters constant. There are strict restrictions for the application law, but this method is useful. If the momentum of the reaction system, quality, heat balance, and relevant boundary conditions, initial conditions in dimensionless form for the amplification process are listed, it is a dimensional analysis scale-up method.

For the dimensional analysis scale-up method, reasonable construction of the quasi-number is the key, and determination of the relevant parameters is the first step. During bioreactor enlargement under specific circumstances, it is necessary to analyze the system mode and identify the key control mechanism of the reaction system and then to amplify them and do not use this method mechanically.

(4) Experience scale-up
Currently, the experience scale-up is the most widely used method, such as the scale-up principle according to constant K_{La}, P/V, Re, N, τ or v_{tip}. Generally, to ensure that the individual criterion are equal at scale-up, whether the change of other criteria lead to changes of the flow pattern or damage to the microorganisms must be taken into account and changes made accordingly.

11.4.2.2 Research Methods for Scale-Down

To more accurately simulate the unevenness that exists in a large-reactor environment, the narrowed bioreactor has played a more important role than bioreactor magnification. The construction of scaled-down equipment is a beneficial supplement for the mathematical model, scale-up norms, and traditional pilot plant operation. Based on the scale-down of biological reactors, researchers find that small-scale biological reaction system could measure more parameters faster and also assess the effect of changes according to the present operation process [159].

For the narrowed bioreactor research methods, compared to bioreactor magnification research methods, the narrowing of the reactor can also based on the constant input volume power and K_{La}.

11.4.3 Integration Research Methods for the Biotransformation Process and Engineering of Lignocellulose

The integration of the biotransformation process and engineering of lignocellulose focuses on the important factors in the system, including material integration, energy integration, information integration, and water system integration.

11.4.3.1 Integration Research Methods for Substances

One of the core issues of eco-industrial research is how to apply the theories and methods of systems engineering to integrate the material between eco-industrial systems. Integration of eco-industrial material includes material transformation, integration, the material exchange, and integration of the separation process.

Material transformation of reaction process integration mainly includes two aspects: Within a single production process, from raw materials to finished products of the reaction process, first is the optimization of overall goal based on environmental and economic. The second is integration of substances between multiple production processes. Separation and purification are important for the greatest degree of recycling of waste in the process. The decontaminating separation is a mass transfer process, in which wastes are used after absorption and extraction.

Material integration is the core part of the eco-industrial system, through product system planning, raw materials for building, products, by-products, and the waste industrial ecological chain to achieve optimal material circulation and utilization. This can also be applied to the multifaceted life cycle assessment (LCA) method for the optimization of product structure [160].

(1) System Planning
For a new eco-industrial system, the main problem is the product of the planning system. According to the local resource conditions, designing a reasonable system is based on the amount of funds and market demand combined with a wide range of development planning. For transformation of an eco-industrial system, the process involves analysis of the system's existing products and processes, a proposal for a process improvement program, and then carrying out the planning of the product system.

(2) Element integration
Regarding issues for the improvement of the existing industrial system, especially with regard to the system of chemistry and the chemical industry, some key elements of the system for material recycling and waste discharge have important implications. Such elements must be deeply analyzed using mathematical methods and then integrated solutions for these elements must be proposed.

(3) Construction of an eco-industrial material chain
Construction of an eco-industrial material chain is an important step in achieving an eco-industrial system to build the optimal ecological chain of industrial raw materials, products, by-products, and waste. It is similar to the element integrated method, but the construction of an eco-industrial material chain considers the object of all process and material. Based on the original process, it will build a superstructure model by introducing process improvements, new alternative processes, alternative raw materials, complementary techniques, and so on, consequently achieving the optimum eco-industrial material chain.

(4) Multilevel life cycle assessment and structural optimization of product
At present, LCA has been used for the environmental management of product or processes and has achieved good results. First, the product life cycle multifaceted product evaluation model is divided into five stages: access to raw materials, production process, distribution process, utilization process, and recycling. Within each stage, there are multiple evaluation indicators for each factor; finally, using the method of a multi-index to synthesize these indicators, you can obtain the economic indicators, total environmental indicators, total social indicators, and composite indicator.

11.4.3.2 Energy Integration Analysis Methods

The energy integration analysis methods mainly include the integrated pinch analysis of all the energy, top-level analysis of the whole-process transformation, and a mathematical programming method for whole-energy integration and the three-link model method [172].

(1) Integrated pinch analysis of whole energy
The whole composite curves based on the whole-temperature enthalpy curve provide whole-fuel and power consumption optimization objectives. Whole composite curves visually reflect the heat transfer conditions between craft process and public works. The whole composite curves can visually analyze the amount of energy recovery, energy conversion, and utility consumption.

(2) Integrated mathematical programming methods for whole energy
A whole-energy integration method using pinch point analysis to find the amount of heat recovery of the transformation process, the consumption of public works, possible solutions for the power turbine network, and a whole-process energy-saving program and then establishing an energy integration subsystem superstructure and mixed integer nonlinear programming (MINLP) model and finally using mixed sets of continuous evolutionary algorithms for solving the optimal or near-optimal energy integration program.

The advantage of mathematical programming method when searching for the optimal process and operation conditions of energy system is that the strict optimal solution could be obtained under given conditions.

(3) Whole-energy integration of the three-link model method

Assumptions
① The main forms of energy in the process are heat, flow work and steam, which are converted via conversion equipments such as stove and pump.

② Except for the part that is converted into products, energy in the forms of heat, work and steam enters the energy recycle system after it passes into the core link of technology and pushes the completion of technology process along with the recycling energy.

③ Energy in the core part process would be lower quality after the completion of its mission. However, it still has a higher pressure and temperature and can be recovered through a heat exchanger device and switching power devices (hydraulic turbine). But, by engineering and economic constraints, the recovery energy cannot be absolute and abandoned to the environment through the final cooling and thermolysis.

11.4.3.3 Information Integration Analysis Method

Information integration analysis of the object is the exchange of information, resources, methods, techniques, and mode and is an application-oriented analysis. The specific process is from the framework integrated analysis to the self-integration analysis of elements and then the integration analysis of induction [161].

(1) Framework for integrated analysis
The framework for integrated analysis is the foundation and guarantee of information integration analysis; the aim is to establish a framework for an integrated mode, to choose the framework of integrated elements, and to determine the location and function of the elements in the framework.

① *Establishment of the framework model.* A good framework model should have pertinency, aiming at a specific framework for topic tasks; have operability that can be implemented with existing methods and tools; have flexibility that can be changed with the environment and task; and have independence to complete the various stages of the various aspects of the integration analysis. It is a complete system analysis and a dynamic cycle. The establishment of a specific framework needs the task requirements and comprehensive consideration according to the characteristics of each element and the existing conditions. In this process, a process factor is established according to the business processes; a method factor is constructed by the horizontal structural features of the methodology; protection factors are constructed according to the protection situation; an application factor is constructed according to the application efficiency; and then the integrated analytical framework for information is established in a certain way by these four factors.

② *Selection of integrated elements for the framework.* Resources, technology, equipment, policies, and many other elements are related to information integration analysis; these elements are responsible for different roles. These roles are based on the need to selectively choose the related strong elements of the integrated framework. The elements are chosen based on the task, considering the roles of the relevance of different levels, availability, and timeliness; a comprehensive evaluation is determined.

③ *Determining the location of elements.* The location of functional elements in the framework is determined by their characteristics, elements reflecting principal contradiction live in the main position. After the determination of positions,

the elements also have their own characteristic features and structural features. Framework elements have links and deconstruction and adaptive functions and complete a specific task with other elements in the same framework. Meanwhile, the location and function of the entire integration process must be relatively stable and dynamic. For a specific integration, it is stable, but after this particular integration comes to an end, its new location and new features should be determined again based on the new integration.

(2) Element integrated analysis
Any element within the framework has different aspects of the structure because an independent body is relatively balanced. Within the system, each element has strong interactions, thereby generating many other effects and coherent state with mutual restrict. The element integrates toward ordering and optimization without outside instruction and self-organizing information. Its power comes from the synergy and competition of internal pats, which is the integration of the same level. Its interference to the outside is unspecific and the information received from the external world is not mandatory. Haken said that a key of a system transformation from disorderly to orderly is not the balance of thermodynamics or the distance from the equilibrium; it is the synergy of a large number of subsystems within the open system, and the emphasis is on the internal association and interworking and coupling among the various elements when changes happen.

The Element self-integration process is carried out in two stages. (1) In horizontal integration, the factors of the element increase. It maintains the same organizational level, but the complexity of the relative growth of self-integration and the structure and function of elements go from simple to complex. (2) Jumped-style integration is an orderly integration process from unorganized to organized; its nature organizes from relatively low organization level to relatively high organization level and from disorder to order.

(3) Response integrated analysis
Response integrated analysis is based on the integration between elements and is high-level integration. The focus of attention should be comprehensive, systematic, and purposeful. Integration between the elements is to be implemented by mutual induction. The so-called induction is feelings or actions caused by an outside influence and emphasizes the outside influence between elements.

11.4.3.4 Research for Water System Integration

Water is an indispensable resource for the process industry. In recent years, with the increasing water shortage and environmental pollution, environmental protection is increasing emphasis on the process industries, which are prompted constantly to minimize freshwater consumption and wastewater emission. China has scarce water resources, and the per capita water share is only about a quarter of the world average. At the same time, there are still significant gaps when single unit consumption of fresh water and single unit emission of waste water of domestic enterprises

are compared with industrialized countries. Therefore, in China, the water crisis is severe, and water saving and pollution remediation are urgent.

The tasks of water system integration include analysis, synthesis, and transformation. The targets of analysis are the minimum amount of freshwater and the minimum wastewater. The target of water system integration is to design a water network through water reuse, regeneration, and circulation. The water system is transformed by changing the existing water network, reaching maximum water reuse and minimum wastewater.

The water system integration method generally includes the water pinch optimization techniques and mathematical programming optimization techniques [162].

(1) Water pinch point optimization technique
Pinch technology originated in the 1970s and had new breakthroughs in the 1990s. It is no longer limited to the thermodynamic problem and more widely extends to the design of a water system to solve a water crisis. The water pinch point technique is an important method for industrial water reuse, minimum amount of wastewater, and wastewater treatment system design. In foreign countries, it has been successfully applied to some of the refining and chemical enterprises, and the saving rate is up to 20–30 %.

Generally, if the wastewater from a water unit could meet the import requirements of another unit in impurity concentration and corrosivity, it can be used to achieve the purpose of saving freshwater. This wastewater reuse is the main focus of water conservation.

(2) Mathematical programming optimization technique
The mathematical programming method according to the superstructure uses computer-aided design to solve water system integration quickly and reliably, and it is another water network design.

11.4.4 Research Methods for Cellulose Biotransformation Ecological Industrial Chain

Throughout the cellulose resource development status, there are some issues in the existing cellulose industry, such as the use of a single component, single conversion technologies, and low availability of raw materials, which result in great waste of resources and transformation process pollution. Because of the relative independence of each operating unit, the process characteristics and the influence on each other are rarely taken into account, resulting in the isolation of various technical aspects and lack of coordination and complementarity. It also leads to additional energy consumption, which is not conducive for improving work and economic efficiency and potentially increases the cost of production [141].

Taking into account characteristics of cellulose, to achieve product diversification and comprehensive utilization of lignocellulosic resources, it is necessary

to establish the cellulose ecological industry chain. It requires the integration of multiple technologies, and its core is the pretreatment of raw materials as well as the key technologies of the transformation process and their optimal combination. Therefore, cellulose ecological industry chain research should focus on the following five areas [163]:

(1) Coupling of a variety of key technologies
After fully understand the nonuniformity of straw component, Chen et al. proposed straw steam explosion technology as the core of component separation and grading directional conversion. By the combination of steam explosion and solvent extraction (ethanol [164]; ionic liquids [165, 166]; glycerol [167]; etc.). It achieves raw chemical fractionation and forms a high-value transformed route for straw that includes xylooligosaccharide (or xylitol) from straw hemicellulose, enzymatic hydrolysis and fermentation of cellulose, and lignin separation and purification. By the combination of steam explosion and wet ultrafine grinding [168], it achieves the separation of raw fibrous tissue and nonfibrous tissue, with fibrous tissue used for enzymatic hydrolysis and fermentation and nonfibrous tissue converted to levulinate. By the combination of steam explosion and hierarchical mechanical carding [169], it realizes the separation of long fibers, short fibers, and miscellaneous cells. The long fibers are used for papermaking; short fibers are used for enzymatic hydrolysis and fermentation; and cytocidal cells are thermochemically converted into nanosilica.

(2) Flexible process
A flexible process can change specific operational links in accordance with the different characteristics of materials and can change parameters or add new technologies to enrich and develop the system. For example, steam explosion treatment not only can be associated with enzyme but also can be used in conjunction with microwave extraction, ultrasonic extraction, and supercritical fluid extraction. However, for the separation and purification of the product, ultrafiltration and membrane separation technology are required.

(3) Multilevel industrial structure
Biomass resources are relatively sparse and have a limited collection radius, which causes the lignocellulose biomass to be impossibly centralized for large-scale production. Based on the fact, it is essential to establish a multi-level structural system integrated minitype with overall layout [19].

(4) Product diversification
The composition of the biomass raw material includes a variety of substances and contains hydroxyl groups, carbonyl groups, and benzene ring structures. Compared with petroleum, which contains $–CH_2$ and has a broad linear polymeric structure, biomass can provide a chance for more development of new products and is more conducive to the chemical transformation of various chemical products [1]. The biomass-based products are diversified; the diversification of products from biomass materials is determined by the multicomponent structure, throughout bio-based energy, bio-based materials, and bio-based chemicals and other areas.

(5) Wide-range application

Plant material is restricted by location and season. So, the production of bio-based products depends on site and production scale. We must consider the versatility of the device to adapt to the supply of different plant materials in the different seasons.

Take cellulose-rich *Hibiscus mutabilis,* for example, to describe the research method for the cellulose ecological industry chain development.

Hibiscus mutabilis belongs to Malvaceae, hibiscus plants. It is a kind of deciduous shrub, originating in the Yellow River Basin in China and throughout the eastern and southern parts of China. It likes a warm and humid, sunny environment; it also has drought tolerance and slight shade tolerance. The growth requirements of *Hibiscus mutabilis* is extensive, so it is distributed in almost all parts of China.

The flowers, leaves, roots, and bark of *Hibiscus mutabilis* could be added to medicine; its effects involve cooling blood, stopping bleeding, detoxification, detumescence, apocenosis, and pain relief. Its bark has a high cellulose content (40–50 %); fiber can be used as textile raw materials with flexibility and water resistance, and fiber yield reaches 45 % of the dry weight of *Hibiscus mutabilis* peel after steam explosion, degummed bleaching, and preserving. The *Hibiscus mutabilis* peel contains large amounts of flavonoids, sterols, and other medicinal ingredients with antibacterial and anti-inflammatory properties and the capability of use as raw materials for the preparation of antibacterial textiles. Peel and stem removed from *Hibiscus mutabilis* mainly contain cellulose; they are not only good raw material for ethanol fermentation, but also good raw material for production of ecosheet, which has not been better utilized yet.

Based on chemical composition and structural characteristics of *Hibiscus mutabilis*, Chen [170] established an integrated ecological industrial chain for the comprehensive utilization of *Hibiscus mutabilis*, using steam explosion as the core technology and applying the integration to the processes and engineering technologies. In this integrated ecological chain, many products can be produced by *Hibiscus mutabilis*, such as anti-inflammatory textile fibers, fuel ethanol, ecological plate and flavonoids, sterols, and so on (Fig. 11.10).

The comprehensive utilization ecological industry chain of *Hibiscus mutabilis* includes the following aspects: (1) *Hibiscus mutabilis* peel contains many medicinal ingredients, and its bast is good fiber for textile raw materials; flavonoids, sterols, and other drug ingredients can be extracted from the steam-exploded *Hibiscus mutabilis*. Antimicrobial textile fiber is formed from bast fibers. (2) *Hibiscus mutabilis* stalks have a high cellulose content and relatively loose structure, so they are used for ethanol fermentation or papermaking. (3) After the harvest of flowers, leaves, roots, and the leather part, they are directly dried as herbal medicines. (4) High-value-added flavonoids are first used to extract sterols and other medicinal ingredients from flowers, leaves, and roots. Then, the extracted residue containing mostly lignocellulose is used for ethanol fermentation or papermaking. *Hibiscus mutabilis* bark fiber yield is 42–46 % of dry weight, and flavonoids yield is 1.1–1.245 % of stem bark dry weight. The extraction rates of leaves and flowers are 2.1 % and 3.3 % of raw materials dry weight respectively; fuel ethanol yield is 13 % of raw materials dry weight after fermentation.

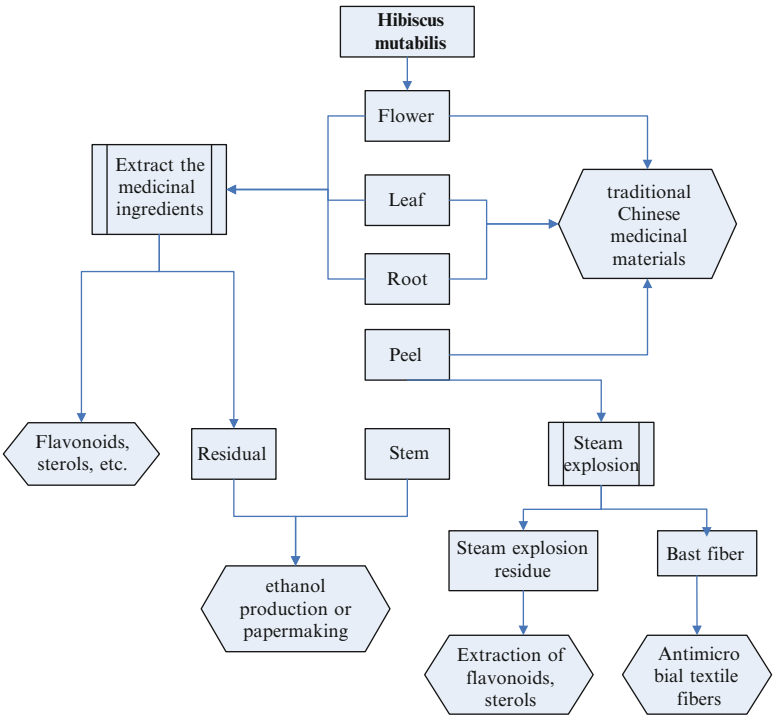


Fig. 11.10 Ecological industrial chain of *Hibiscus mutabilis* [170]

Ecological industrial chain development of *Hibiscus mutabilis* has the following advantages:

① *Hibiscus mutabilis* is low demand for soil and is suitable for large-scale cultivation in the barren hills or river edge, is easily cultivated, and grows rapidly. *Hibiscus mutabilis* has important cultivation value because it can be planted in marginal land on a large scale, does not affect food production, and can maintain soil and water.

② Antimicrobial textile materials are an important direction of the development of the textile industry. *Hibiscus mutabilis* peel has antimicrobial efficacy and is a good raw material for the production of antimicrobial textile fibers, with attractive economic value.

③ Fuel ethanol production using nonfood raw materials is the ultimate direction of ethanol production and has huge market potential. The structure of *Hibiscus mutabilis* stem is loose, so it is a good raw material for fuel ethanol production.

④ High-value-added medicinal compounds such as flavonoids and sterols can be extracted from flowers, leaves, roots, and bark of *Hibiscus mutabilis* and will generate enormous economic benefits.

11.4.5 Analysis Methods for Cellulose Biotransformation Process Technology and Economy

The success of cellulose biotransformation technology is a basic prerequisite for the industrialization development process. Cellulose biomass conversion technologies become economically viable depending on the analysis of economics. Simply, cellulose economic systems engineering is a technical success in helping cellulose biotransformation pathways achieve industrialization, namely, the analysis of establishment costs and expected economic benefits in the process of complete production plants. This economic analysis includes (1) cost estimates, such as capital cost estimates, operating cost estimates; (2) analysis of profitability and solvency; (3) the basic statements of project economic evaluation; (4) uncertainty analysis, such as sensitivity analysis, break-even analysis, and probability analysis; and (5) process design [171].

11.4.5.1 Cost Estimates

The cost estimate includes two main aspects: capital cost and operating cost. The former is a cost associated with factory establishment, such as land preparation and a production workshop. The latter costs are associated with factory operation, such as labor, raw materials, and utilities (steam, water, electricity) [172].

The estimated capital cost of a project generally includes the construction of production equipment and supporting instruments; equipment; instrumentation and pipe installation and labor costs; construction costs, such as land costs, building and project contracting costs; administrative expenses; insurance and taxes; and emergency costs.

The estimated project operating costs generally include raw materials, utilities, labor, management, research and development, patent fees, cost of sales, plant maintenance, insurance, and taxes.

11.4.5.2 Profitability Analysis and Solvency Analysis

Profitability analysis calculates the main indicators of the financial internal rate and period for recovery of investment. According to the characteristics of the project and the actual needs, the financial net present value, investment profit, investment profit rate, and capital profit margin are calculated. The solvency analysis calculates the asset-liability ratio, loan repayment period, and current ratio indicators [173].

(1) Financial internal rate of return
The project financial internal rate of return is the discount rate when cumulation of annual net cash flow equals zero during the entire calculation period. It reflects the profitability of the project occupied funds and is the main indicator for evaluating the dynamic profitability of the project.

(2) Payback period

The payback period (Pt) is the time needed when the net income compensates for the entire investment (investment in fixed assets, regulatory taxes on investment, and liquidity). It inspects the main static indicators of financial payback ability for the project. The payback period generally counts from the construction years, and it should be indicated when it counts from the beginning of production.

The payback period can be calculated according to the accumulated net cash flow in the table of financial cash flow (total investments); the formula is as follows:

$$\text{Payback period (Pt)} = \begin{pmatrix} \text{cumulative net cash} \\ \text{positive number of years} \end{pmatrix} - 1 + \left(\frac{\textit{previous year cumulative net present flow of absolute value}}{\text{year net cash flow}} \right)$$

When the calculated payback period (Pt) $\leq$ the industry benchmark payback period (Pc) in the financial evaluation, it means that the project investment can be recovered in the stipulated time.

(3) Asset-liability ratio

$$\text{Asset-liability ratio} = \frac{\text{total liabilitie}}{\text{total asset}} \times 100\ \%$$

The asset-liability ratio is an indicator that reflects the degree of project financial risk each year and the ability to repay.

(4) Loan repayment period

The loan repayment period is directly calculated by the source of funds and the use of tables and a domestic borrowing debt service table, which is expressed in years. The detailed formula is as follows:

$$\text{Loan repayment period} = \begin{pmatrix} \text{repayment of borrowings} \\ \text{late surplus year} \end{pmatrix} - (\text{start borrowings year}) + \left(\frac{\text{repayment of borrowings for the year}}{\textit{the amount of funds used for repayment}} \right)$$

When the loan repayment period meets the requirements of the lender's deadline, the project is able to repay.

(5) Current ratio

The current ratio is an indicator that reflects the annual ability to pay the current liabilities of the project.

$$\text{Current ratio} = \frac{\text{total circulating capital}}{\text{total circulating liabilitie}} \times 100\%$$

The quick ratio is the index that reflects the ability to quickly pay current liabilities of the project.

$$\text{Quick ratio} = \frac{\text{total circulating capital-stock}}{\text{total current liabilities}} \times 100\%$$

11.4.5.3 Project Economic Evaluation of the Basic Statements

The basic statements of evaluation include cash flow statement, income statement, statement of fund sources and applications, and the balance sheet [172].

(1) Statement of cash flow
The statement of cash flow reflects the cash receipts and payments for each year in the project period (cash inflow and cash outflow). It is used to analyze the profitability of project financially through calculating the FIRR and financial net present value and evaluating the payback period, for the calculation of dynamic and static evaluation.

(2) The profit and loss account
The profit and loss account reflects the project's total profit during the period, income tax and profit after tax, for calculating the profit rate of the investment and capital profit margins and other indicators.

(3) Statement of fund sources and applications
The income statement reflects annual fund surplus or shortage of the project for financing alternatives, development of suitable borrowing and repayment plans; it provides the basis for the preparation of the balance sheet.

(4) Balance sheet
The balance sheet reflects the year-end assets, liabilities, and increase or decrease of the owners' equity and the corresponding relations of the project to examine the reasonability of the assets, liabilities, and owner's equity structure for calculating the asset-liability ratio, current ratio and the quick ratio, and solvency analysis.

11.4.5.4 Uncertainty Analysis

Uncertainty analysis includes sensitivity analysis, break-even analysis, and probability analysis [174].

(1) Sensitivity analysis
The aim of sensitivity analysis is to find the sensitive factors and to determine the impact extent by analyzing and predicting the effects of main factors on the economic evaluation. Product yields, product prices, product costs or raw materials and power prices, investment in fixed assets, construction period, and other factors may change in the project period. Sensitivity analysis analyzes the effects of changes from these factors on the internal return rate. The impact of the static investment payback period and the loan repayment period are also analyzed if necessary. The evaluation magnitude of change caused by the sensitivity factor can be represented

by a certain percentage change; it can also be expressed as the evaluation index reached a critical point, such as the FIRR equals the financial benchmark rate of return or the economic internal rate of return equals the social discount rate, that is, the limit changes. The limit can be found by drawing the diagram of sensitivity analysis.

(2) Break-even analysis
The break-even analysis analyzes the balance relationship of project costs and benefits through the break-even point (BEP) balance analysis.

$$\text{BEP (capacity utilization)} = \frac{\text{Annual fixed total cost}}{\text{Annual sales income-year variable total cost-annual sales tax and surcharges}} \times 100\%$$

The BEP is usually based on calculation of the production or sales amount in a normal production year, variable costs, fixed costs, product price sales tax, and additional data, expressed in capacity utilization or production. The formula is as follows:

$$\text{BEP (yield)} = \text{(design production capacity)} \times \text{BEP (capacity utilization)}$$

A lower BEP indicates that the project has a stronger ability to adapt to changes in the market and a greater ability to resist risks.

$$\text{BEP (yield)} = \frac{\text{Year fixed total cost}}{\text{Unit price-unit variable costs-unit sales tax and surcharges}} \times 100\%$$

(3) Probability analysis
Probability analysis is used to predict the occurrence of a variety of uncertainties and risk factors for project evaluation. Generally, calculate the cumulative probability of project net present value if the expected value is greater than or equal to zero; the greater cumulative probability is, the less risk there is to undertake.

11.4.5.5 Process Design

The process should be designed to meet the requirements of the production plant. The total volume of a reactor can be estimated by total production, the product concentration in the reactor, and the production capacity of the reactor. Then, the number of reactors can be estimated so that the size of bioreactor and the number of seed and inoculum containers can be designed preliminarily. If we have known the products concentration within the reactor and the production capacity of reactor, we can determine the medium component and the energy demand and thus clarify the substrates needed. The design procedures and methods of the cellulose biotransformation process are shown in Table 11.5.

Table 11.5 Cellulose biotransformation process design procedures and methods

Step	Parameter	Parameter information
Production target	Total production	Total production depends on market demand
	Product concentration Production capacity	The reactor product concentration and the production capacity are estimated by the factory
Reactor volume	Reactor size	Good performance of large reactor
Reactor number	Substrate requirements Substrate pretreatment	Process chemical calculations The effectiveness of the medium composition, cost, and quality
Upstream operations		
Product separation	Separation of biological substances	Biomass is retained or abandoned Product concentration
Product purification	Purification requirements	Product characteristics and nature
Packaging and storage	Stability of the product Shelf life Degradability	

11.4.5.6 Technoeconomic Appraisal for a Project with an Annual Output of 120,000 t of Cellulose Ethanol

(1) Investment estimate
The total investment of the project is 450 million yuan; fixed assets investment is 42,951.68 million yuan, in addition to interest; the other capital expenditure is circulating capital.

Fixed-assets investment, including equipment, costs 247 million yuan, accounting for a construction investment of 57.51 %; installation fee of 24.7 million yuan, accounting for construction investment of 5.75 %; land use fee of 20 million yuan, accounting for construction investment of 4.66 %; construction costs 60 million yuan, share of construction investment is 13.97 %; other infrastructure costs of 7781.68 million yuan, accounting for construction investment of 18.11 %.

Circulating capital is mainly used for the purchase of the production and consumption of raw materials: cellulose raw material, wheat bran, fermentation medium, and water and gas consumption. Electricity and steam are supplied from the fermentation residue.

(2) Economic indicators estimate
Information on economic indicator estimation is presented in Table 11.6.

(3) Sensitivity analysis
The main factors that may affect project benefits include construction investment, sales price, and operating costs. The impact on project profitability is analyzed; It can be seen from the results of the sensitivity analysis that products price is most sensitive to the project financial internal revenue. When the price decreases 5 and 10 %, the project FIRR is 28.58 and 22.59 %, which is higher than the discount rate of 12 %. The project has a good ability to resist risks.

11.4.6 Cellulose Biotransformation Process Life Cycle

Life cycle assessment quantitatively describes product resources, energy consumption, and environmental emissions in the life cycle process; it is the best framework for evaluating potential environmental impact and provides a scientific method for policy formulation of production and consumption. It has been applied internationally in industry and policymaking for environmental products.

Life cycle assessment includes the following three aspects [175, 176]:

(1) Purpose and scope determination
The first step is to determine the LCA research purposes and to define the scope of the study. The purpose, scope, and intent involve the study geographical breadth, time span, and data quality; they will affect the direction and depth of the research.

Table 11.6 Overall economic indicators

Order number	Item	Unit	Indicator	Comments
1	Gross investment	Million yuan	4,500	
1.1	Construction investment	Million yuan	4,478.768	
1.1.1	Fixed-assets investment	Million yuan	4,295.168	
1.1.2	Interest during construction (dynamic part)	Million yuan	183.6	
1.2	Circulating capital	Million yuan	204.832	
2	Annual sales revenue	Million yuan/year	12,908	Date of output production
3	Selling tax	Million yuan/year	829.285	Date of output production
4	Selling profit	Million yuan/year	2,484.729	Date of output production
5	Income tax	Million yuan/year	819.961	Date of output production
6	Profit after income tax	Million yuan/year	1,664.768	Date of output production
7	Repayment period of loan	Year	3.87	From the date of construction
8	Earnings power of real assets	%	43.12	
9	Internal return rate of project financial investment	%	34.28 %	
10	Recovery period of project financial investment	Year	4.21	
11	Total capital	Million yuan	4,500	
11.1	Construction investment	Million yuan	4,295.168	
11.1.1	Static part of construction investment	Million yuan	4,111.568	
11.1.2	Interest during construction	Million yuan	183.600	
11.2	Circulating capital	Million yuan	204.832	

Research purposes should include definite reasons for LCA study and application of future results. To ensure that the research meets the stated objectives in breadth and depth, the scope of the study must be amply defined, including the system boundary, the time boundary, methods, data types, and assumptions.

The LCA process usually requires much data; the data in a time period cannot be an effective evaluation for the entire life cycle, which needs to consider whether the data is still representative in different periods. In addition, it is necessary to consider the timeliness of the LCA data.

(2) Inventory analysis

Inventory analysis is an expression of the LCA basic data and is the basis of life cycle impact assessment (LCIA). It is a quantitative analysis for products, processes, or activities of resources, energy consumption, or emissions to the environment in the entire life cycle stage. The core of inventory analysis is expressed in units of product functionality system input and output. Inventory analysis is a repeated process, generally including data collection preparation, data collection and calculation procedures, allocation methods, and results.

This analysis conducts a detailed inventory for input and output for each unit of the process and provides detailed data for the diagnostic process logistics, energy flow, and waste stream. At the same time, the inventory analysis is also the basis of the impact assessment phase.

(3) Impact assessment

Life cycle assessment is used in various decision-making processes, and it is necessary to assess the exchange potential impact on the environment and describe the relative importance of various environmental exchanges and the environmental impact of each member in each stage of production or product size. This phase is called the life cycle impact assessment. LCIA, as part of the evaluation of the entire life cycle, can be used to identify opportunities to improve the product system and help determine prioritization; to describe characteristics of the product system or unit process or to establish a frame of reference; and to provide environmental data or information support for decision makers through the establishment of series-type parameters relatively.

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