

# 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  $1.5 \times 10^{11}$  people, more than 28 times the current world population [3].

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  $1.8 \times 10^{11}$  tons of biomass aboveground,  $4.0 \times 10^7$  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  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . 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  $7 \times 10^8$  tons, with wood consumption for fuel of  $2.13 \times 10^8$  tons. Annual resources of about  $1.36 \times 10^8$  tons of dry human and animal feces exist, with about  $3.7 \times 10^5$  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.

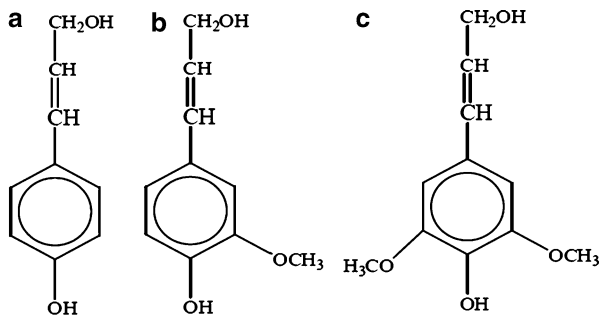
Multiform utilization modes for straw exist in China, including utilization as energy, fertilizer, industrial raw materials, and substrates for edible mushrooms. Actually, the utilization mode of straw is differed from the economic development degree and industrial structure. According to field investigation 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  $\beta(1-4)$ -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-glucuronxyllans. 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  $\alpha$ -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, enveloping 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
Corn cob	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]

Each organ	Length (mm)			Width ( $\mu\text{m}$ )			L/W		
	Average	Maximum	Minimum	General	Average	Maximum		Minimum	
	General	General	General	General	General	General		General	
Whole	1.32	2.94	0.61	1.03–1.60	12.9	24.5	7.4	9.3–15.7	102
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 ( $\mu\text{m}$ )				L/W
	Average	Maximum	Minimum	General	Average	Maximum	Minimum	General	
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	<i>Populus diversifolia</i>	<i>Aspen wood</i>	<i>Populus tomentosa</i>	<i>Populus ussuriensis</i>	<i>Lombardy poplar</i>	<i>Populus × canadensis Moench cv. I-214</i>	<i>Populus euramericana San Martino-72</i>	<i>Populus derides 'Harvard'</i>	<i>Populus derides 'Lux'</i>	<i>Populus dakanensis</i>
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]

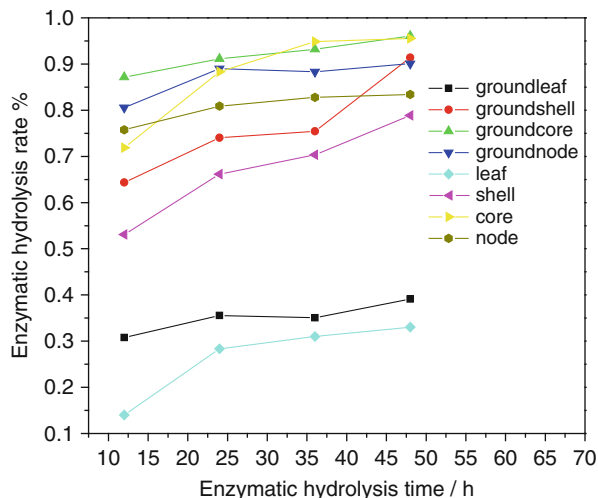
		Wheat straw	Wood
Lignin	Phenolic hydroxyl (C9 phenyl propane unit)	0.4–0.45/C9	0.1–0.3/C9
	Phenolic hydroxyl in lignin	44/100 units	9–13/100 units of birch
	Carboxyl in lignin	13.8/100 units	
	Carboxyl in lignin	0.1–0.2/C9	–
	Proportion of ester in lignin	2.60 % ester bond 1–2.8 %	Almost absence of ester bond
	Ferulic acid in lignin	$\alpha$ -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 <sub>2</sub> )	Less than 1 % (mainly contains CaO, K <sub>2</sub> O, Na <sub>2</sub> O)
	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<sub>2</sub>, but more than 80 % of CO<sub>2</sub> 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<sub>2</sub> 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 obtain 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  $\text{H}_2\text{SO}_4$  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<sub>2</sub> through photosynthesis, and the production and utilization process constitutes closed circulation of CO<sub>2</sub> [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 to 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. cellulosa*.

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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