

Chapter 16

Role of Microbes in the Synthesis of Industrial Products from Lignocellulosic Materials



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Abstract Agricultural residues are a major renewable source available on earth. In developing countries, food, agriculture, and forestry and industries produce high amounts of lignocellulosic wastes that cause disposal problems because these wastes are not easily degraded. Here we review the microbial synthesis of industrial products from lignocellulosic wastes. Lignocellulose is a complex compound of plant cell wall, composed of cellulose, hemicellulose, lignin, pectin and inorganics. As a consequence, feedstock pretreatments are required before the microbial production of industrial products through microbial intervention. Pretreatment techniques include physical, chemical, and biological methods. We discuss the microbial synthesis of industrial products such as enzymes, bioethanol, xylitol, and vinegar.

Keywords Lignocellulosic biomass · Cellulose · Hemicellulose · Lignin · Agricultural waste · Pretreatment · Hydrolysis · Industrial products · Xylitol · Bioethanol · Microorganisms

16.1 Introduction

Lignocellulose, mainly consists of cellulose, hemicellulose and lignin, is one of the key components of woody and non-woody plants. Lignocellulosic materials are obtained from three sources namely primary sources like short rotation energy plantations, sugar cane etc., secondary sources like corn cob, rice husk, straw, bagasse, and tertiary sources like sewage treatment sludge, municipal solid waste, and wood trimmings (Fischer and Schratzenholzer 2001). Primary sources have economic and social barriers for their utilization; hence, secondary and tertiary sources are

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commonly used as potential feed stocks for synthesis of industrial products. Lignocellulosic materials are produced in large amount annually by developing, and agricultural countries like India from food, agricultural, and forestry industries. These wastes cause serious disposal problems and cause harmful effects on environment because these wastes are often disposed of by burning in many developing countries (Demirbas 2008). Lignocellulose acts as major substrate for the production of enormous products of biotechnological value due to its chemical properties (Malherbe and Cloete 2003). The current focus of researchers is towards utilization of such renewable sources for microbial synthesis of industrial important products.

From the past few decades, considerable improvements have been made in green biotechnology by utilizing these lignocellulosic materials. These wastes are promising feedstocks for the production of numerous value-added products like animal feeds, amino acids, biofuels, chemicals, cheap energy sources, enzymes, organic acids, polyols, and human foods (Anwar et al. 2014; Sooch et al. 2019; Kauldhar et al. 2021; Lugani et al. 2019, 2021a, b). The global market of these bio-based products is enhancing at a very fast pace due to environmental concerns and continuous depletion of fossil fuels including petrol and diesel.

The major steps involved in production of industrial products using biomass as feedstocks are pretreatment, hydrolysis/saccharification, fermentation and purification. The schematic representation for biosynthesis of value-added industrial products from lignocellulosic biomass is shown in Fig. 16.1. The further steps vary with the type of product and form of its availability in the market. Pretreatment, hydrolysis, and purification are three major steps which regulate overall product market cost (Amiri and Karimi 2018). One of the major obstacles for using lignocellulosic materials is their recalcitrant nature due to their complex geometry, and hence, different pretreatment and hydrolysis methods are used to release fermentable sugars from these complex polysaccharides (Kumar and Sharma 2017). Different physical, chemical, biological, physico-chemical, thermo-chemical and thermo-physical methods are available for pretreatment of various feedstocks (Lugani et al. 2019, 2021b; Sooch et al. 2019; Mann and Sooch 2020; Kauldhar et al. 2021). The selection of pretreatment method relies on type of substrate, type of hydrolysis method, and type of microbial strain and fermentation mode. Hydrolysis can be achieved either by chemical or enzymatic methods but enzymatic methods appear to be more promising due to environmental and safety concerns.

Fermentation is an attractive approach for currently used green biotechnology in which microbial (bacteria, yeast, fungi and algae) strains are used for the synthesis of bio-products in the form of primary and secondary products (Box 16.1). Due to less productivity with the use of wild microbial strains, some improved approaches such as synthesis of genetically modified strains and use of computational models have been developed to produce industrial important strains in the past. Immobilization of biocatalyst is one of the alternative methods for development of continuous systems with reusability of enzymes.

Separation and purification of product is generally carried out on the basis of the type of compounds being separated using various chromatographic techniques such as affinity chromatography, dye-ligand chromatography, gel permeation

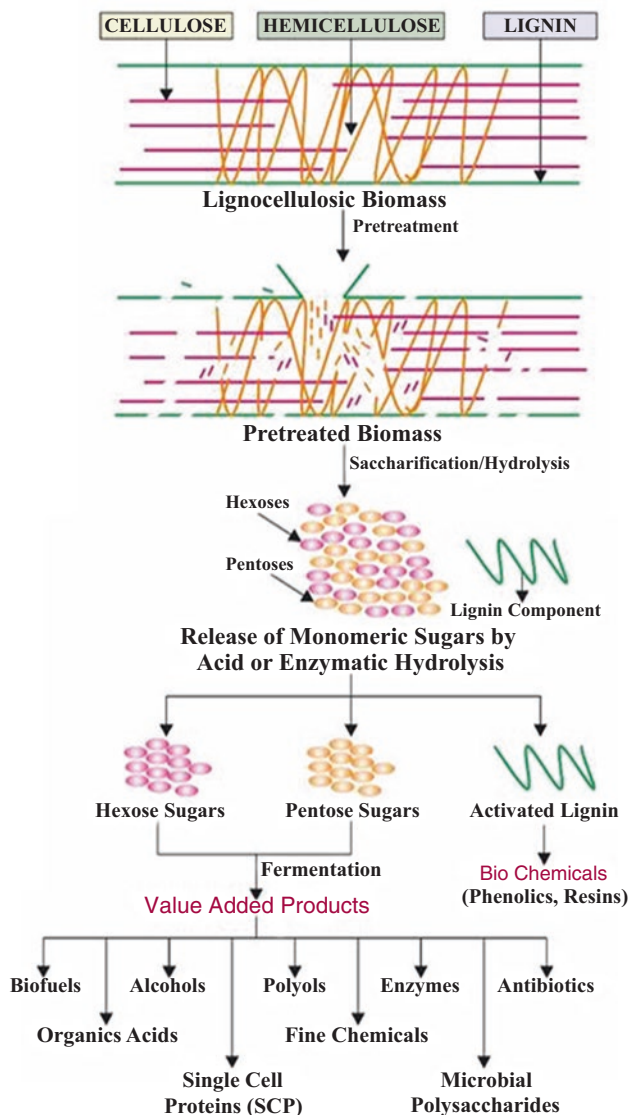


Fig. 16.1 Biosynthesis of value-added industrial products from lignocellulosic biomass

chromatography, high pressure liquid chromatography, hydrophobic interaction chromatography, ion exchange chromatography and gas chromatography (Coskun 2016). Deep eutectic solvents are used for the extraction of value-added components like flavones (apigenin, luteolin), lignans (acetoxypinoresinol), phenolic acids, phenolic alcohols, secoiridoid derivative (aglycone, ligstroside), tocopherols and tocotrienols (Jablonsky et al. 2018). The current chapter enlightens the significance of lignocellulose biotechnology with emphasis on different pretreatment and

Box 16.1 Primary and Secondary Metabolites Produced by Microorganisms

Primary metabolites	Examples
Amino acids	L-alanine, L-arginine, L-glutamic acid, L-glutamine, L-histidine, L-proline, L-serine, L-valine, L-tyrosine, L-tryptophan, L-threonine, L-phenylalanine, L-leucine, L-isoleucine
Organic acids	Acetic acid, butyric acid, citric acid, lactic acid, succinic acid, fumaric acid, Itaconic acid, Gluconic acid, Xyloic acid
Alcohols	Ethanol, 1,3-Propanediol, 2,3-Butanediol
Enzymes	Cellulase, protease, pectinase, lipase, xylanase, xylose reductase, laccase, peroxidase, arabinase, mannase
Proteins	Single cell protein, Phycobiliproteins
Vitamins	Riboflavin, ascorbic acid
Trace elements	Ca, Fe, Mg, Zn, P, K, S, N, Mo, Cl, Zn
Sugars	Xylitol, mannitol, sorbitol
Secondary metabolites	Examples
Antibiotics	Penicillin, cephalosporin, cyclosporine A, neomycin, Oxytetracycline, Rifamycin A, tetracycline
Phenolic compounds	Benzene, biphenyls, catechol, cyclohexane, toluene, xylene, lutein, Astaxanthin, zeaxanthin, β -carotene
Essential oils	Docosahexanoic acid, Eicosapentanoic acid, terpenes, lactones, aldehydes, ketones, paraffin, monoterpenoid alcohols
Chemicals	Furfural, ethylene, propylene, phenazines, quinolines, vanillin
Polymers	Poly-3-hydroxybutyrate

hydrolysis steps, production of various value-added products and their current market status. Some other aspects like improved strategies for production of industrial products including development of genetically modified microbial strains, immobilization and computation techniques have also been reviewed in this chapter.

16.2 Types and Composition of Lignocellulosic Biomass

Lignocellulosic biomass is typically nonedible plant materials mainly comprises of cellulose (40–50%), hemicellulose (25–30%), lignin (15–20%), and traces of inorganic and nitrogen compounds along with pectin (Mori et al. 2015). Cellulose is the plentiful compound on earth with special features such as hydrophobicity, biocompatibility, and stereoregularity, and the framework of cell wall is determined by its structure (Tayyab et al. 2018). It is a linear syndiotactic, rigid, crystalline homopolymer in which D-glucose residues are linked together by β -1,4-glycosidic

linkage. The non-covalent interactions (hydrogen bond and van der Waals forces) present help in packing of cellulose polymers into microfibrils. The hydrolysis of cellulose into monomeric sugars is influenced by its crystallinity and polymerization, and it varies with plant species (Gray et al. 2006). Hemicellulose is an amorphous structure which provides structural strength through linkage of cellulose fibers into microfibrils, and their crosslinkage with lignin. It comprises of short, linear, heterogenous, highly branched chains, and it is a mixture of polysaccharides including pentose sugars (D-xylose, D-arabinose) and hexose sugars (D-glucose, D-galactose, and D-mannose) and uronic acids (Saha and Cotta 2007; Saha et al. 2017). Hemicellulosic component require a great variety of enzymes for complete hydrolysis into fermentable sugars due to diversity of hemicellulosic sugars (Limayem and Ricke 2012). This polysaccharide does not possess crystalline regions, and it has low degree of polymerization, therefore, it can be easily hydrolysed into monomeric units (Karimi and Taherzadeh 2016).

Lignin is a non-sugar based complex polymer of phenyl propanic alcohol (p-coumaryl, coniferyl, and sinapyl alcohol), which provides impermeability and structural support to plants. It also confers resistance against oxidative stress and microbial attack due to its distinctive properties such as water insolubility, optical inactivity, and amorphous nature (Howard et al. 2003). A great amount of energy is produced by burning lignin; hence, it is used in biorefinery for combined heat and power production in environmental-friendly manner (Bonawitz and Chapple 2010). Along with cellulose, hemicellulose, and lignin, the lignocellulosic materials contain small amount of ash, extractives (chlorophyll, nitrogenous materials, and waxes), proteins, and pectin (Kumar et al. 2009). There is great variation in composition of biomass constituents among different plant sources based on species, tissue, maturity of plant cell wall, variety, soil fertility, climate, and species. The composition of some lignocellulosic materials is shown in Table 16.1.

The methods for determining chemical composition of biomass have been developed by some global organizations like Technical Association of the Pulp and Paper Industry, National Renewable Energy and Laboratory, and American Society for Testing and Materials. The purest sources of cellulose are cotton (80–95%), flax and chemical pulp (60–80%), however, approximately 45% cellulose is present in hardwoods, and softwoods (Demirbas 2005). Monterey pine is a preferred substrate for production of industrial products due to its rapid growth, high cellulose content (48%), and compositional uniformity (Dong et al. 2018). Hemicellulosic content is found maximum in crop residues like wheat straw, rice straw, and corn stover (Foody and Foody 1991). The main hemicelluloses in hardwood species are glucuronoxylans (O-acetyl-4-O-methyl-D-glucuronoxylan), and in softwood species are galactoglucomannans (O-acetyl-galactoglucomannans). Hardwood species have higher xylan and lower mannan content than softwood; therefore, softwood species are more recalcitrant to enzymatic action (Alvarez et al. 2016). Lignin content is highest in softwood barks (30–60%), followed by hardwood barks (30–55%), whereas, minimum lignin content is found in grasses (10–30%), and agricultural

Table 16.1 Composition of lignocellulosic materials

Lignocellulose material	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	References
<i>Eichhornia crassipes</i>	18.2	48.7	3.50	NA	Nigam (2002)
Switchgrass	5–20	30–50	10–40	5–6	McKendry (2002)
Wheat straw	30	50	15	NA	Sun and Cheng (2002)
Sorted refuse	32.1	24	18	NA	Howard et al. (2003)
Waste paper from chemical pulp	60–70	10–20	5–10	NA	Howard et al. (2003)
Primary wastewater solid	8–15	NA	24–29	NA	Howard et al. (2003)
Swine waste	6	28	NA	NA	Howard et al. (2003)
Spruce	43.0	29.4	27.6	0.6	Demirbas (2005)
Banana waste	13.2	14.8	14	NA	John et al. (2006)
Rice straw	32.1	24.0	18.0	NA	Prasad et al. (2007)
Hardwood	45–47	25–40	20–25	0.80	Swart et al. (2008)
Softwood	40–45	25–29	30–60	0.50	Swart et al. (2008)
Sponge gourd fibers	66.59	17.44	15.46	NA	Guimaraes et al. (2009)
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7	NA	Singh et al. (2011)
Nut shells	25–30	25–30	30–40	NA	Singh et al. (2011)
Water hyacinth	18.4	49.2	3.55	NA	Singh et al. (2011)
Sweet sorghum	45	27	21	NA	Kim and Day (2011)
Miscanthus	38–40	18–24	24–25	5.5	Brosse et al. (2010) and Rabemanolontsoa and Saka (2013)
Corn leaves	26.93	13.27	15.18	10.95	Rabemanolontsoa and Saka (2013)
Corn cob	42–45	35–39	14–15	3.53	Rabemanolontsoa and Saka (2013)
Sugarcane bagasse	42–48	19–25	20–42	NA	Saini et al. (2015)
Rice straw	28–36	23–28	12–14	19.8	Saini et al. (2015)
Jatropha waste	56.3	17.5	23.9	NA	Nikolic et al. (2016)
Grasses	25–40	25–50	10–30	NA	Nikolic et al. (2016)
Switch grass	45	31.4	12	NA	Kumar and Sharma (2017)
Newspaper	12	40–45	25–40	NA	Kumar and Sharma (2017)
Cotton seed hairs	80–95	5–20	0	NA	Kumar and Sharma (2017)
Garlic and onion	41–50	16–26	26–39	NA	Reddy and Rhim (2018)
Rice straw	41.94	25.58	0.80	NA	Wang et al. (2021)

NA data not available

residues (3–15%) (Demirbas 2005). The complex chemical structure of lignocellulosic biomass is the major challenge for their utilization to develop and commercialize value-added industrial products (Tayyab et al. 2018). Hence, it is necessary to select suitable, cost-effective and economic pretreatment method for their utilization as raw materials with minimum formation of by-products.

16.3 Pretreatment and Hydrolysis of Lignocellulosic Biomass

Pretreatment and saccharification/ hydrolysis are two crucial steps used for the release of monomeric sugars from polysaccharides. Pretreatment causes alteration in the structure, and chemical composition of biomass resulting in improved substrate porosity by decreasing cellulose crystallinity, increasing surface area, size reduction, and lignin removal (Zhu et al. 2009). The fermentable sugars are released from pretreated biomass either by enzymatic hydrolysis or acid hydrolysis (Azhar et al. 2017).

16.3.1 Methods of Pretreatment

There are many physical, chemical, biological, electrical, physico-chemical, thermo-physical, and thermo-chemical methods available for pretreatment of lignocellulosic materials, and each method has some merits and demerits. Figure 16.2 shows different methods available for pretreatment of lignocellulosic biomass but none of these methods could be adapted successfully at industrial scale, and researchers are still focusing on development of some cost and energy efficient pretreatment method. The physical methods used for the disruption of recalcitrant biomass are size reduction (Maurya et al. 2015), microwave irradiation (Amin et al. 2017), and pyrolysis (Den et al. 2018). These methods do not use chemicals and disrupt the biomass by reducing its size by mechanical forces (milling, grinding, and chipping), generating heat from magnetic and electrical components, and rapid decomposition by producing gaseous products (Amin et al. 2017). However, physical pretreatment methods are not economical for commercial use due to high power consumption, and slow rate of reaction.

Different chemicals such as acids (Wyman et al. 2005), alkali (Kumar et al. 2009), ionic liquids (Capolupo and Faraco 2016), organic solvents (Monavari et al. 2009), surfactants (Qing et al. 2010), and lime (Sierra et al. 2009) are used in chemical pretreatment. The most conventional chemical pretreatment method is acid

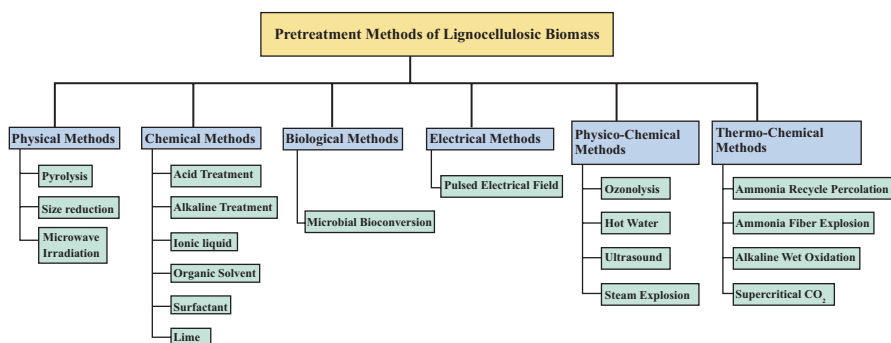


Fig. 16.2 Methods for the pretreatment of lignocellulosic biomass

hydrolysis in which acids like H_2SO_4 , HNO_3 , HCl , and H_3PO_4 are used for disrupting covalent and non-covalent interactions. Dilute acid treatment methods are simple with less generation of toxic products (Wyman et al. 2005). Alternatively, the pretreatment method using concentrated acids result in rapid extraction of reducing sugars from diverse feedstocks; however, reducing sugars are easily degraded by this method (Zhu et al. 2009). Hemicellulose fraction is rapidly hydrolysed by alkaline agents like KOH , NaOH , NH_4OH , and $\text{Ca}(\text{OH})_2$ (Mosier et al. 2005). Simultaneous hydrolysis and delignification for hardwood and softwood can be achieved by using organic solvents (ethanol, methanol, tetrahydrofurfuryl alcohol, acetone, ethylene glycol) (Pan et al. 2005), however, this method is expensive, and leads to synthesis of toxic inhibitors (Eggeman and Elander 2005). Surfactants possess both hydrophilic and hydrophobic properties, and the most commonly used surfactants are polyethylene glycol 4000, polyethylene glycol 6000, Tween 20, and Tween 80 (Zhang et al. 2016a).

Biological pretreatment method is energy-saving and environment friendly. It is conducted at mild temperature and pressure conditions using microbial cells or enzymes as biocatalyst (Tayyab et al. 2018). The major drawbacks for using this method at industrial scale are requirement of chemical mediators, large space, and long residence time with optimum growth conditions. Pulsed electrical field is electrical pretreatment method which needs simple equipment, and ambient conditions (Ammar et al. 2011). Ozonolysis is a physico-chemical method which is conducted at mild temperature, and pressure, and effective for lignin removal, however, this method is expensive and leads to production of toxic inhibitors (Cubero et al. 2009). Hot water (Banerjee et al. 2009), ultrasound (Ivetic et al. 2017), and steam explosion (Pielhop et al. 2016) are thermo-physical methods in which physical and chemical forces cause loosening of lignocellulose materials which become highly susceptible to hydrolysis (Den et al. 2018). The methods used in thermo-chemical pretreatment are alkaline wet oxidation (Monavari et al. 2009), supercritical CO_2 (Kumar et al. 2009), ammonia recycle percolation (Chaturvedi and Verma 2013), and ammonia fiber explosion (Kim 2018), and these methods are reported to be more effective for biomass containing high lipid fraction (Kumar et al. 2009).

16.3.2 Factors Affecting the Pretreatment

There are various factors affecting the pretreatment of lignocellulosic materials and particle size is an important factor of size reduction pretreatment process because it affects the power requirement, and high-power consumption can make the process economically non-feasible (Hendriks and Zeeman 2009). High decomposition rate of biomass can be achieved using pyrolysis at low temperature in the presence of sodium carbonate or zinc chloride as catalyst (Singh et al. 2011). Dielectric properties of lignocellulosic material influence the performance of microwave irradiation (Amin et al. 2017). Dilute acid pretreatment is an economical method and used with a broad range of feedstocks like agricultural waste materials, hardwood, herbaceous

crops, municipal solid waste, and softwood for complete removal of hemicelluloses. The temperature-time combination is very crucial during selection of method for pretreatment, this can be carried out at high temperature (180 °C) for short time or low temperature (120 °C) for long time (Myat and Ryu 2016). The efficiency of pulsed electric field pretreatment method relies on various parameters such as electric field strength, treatment time, and pulse parameters, and moisture distribution of plant tissue (Barba et al. 2015). The major factors like temperature, particle size, and residence time affect the efficacy of pretreatment by steam explosion method.

There is enhanced removal of hemicellulose from solid fraction and cellulose digestibility at higher temperature (Oliva et al. 2003). High frequency ultrasonic waves are used in ultrasound pretreatment, and the effectiveness of this method is governed by ultrasonic frequency, reactor geometry, and type of solvent used (Den et al. 2018). In organosolv process, different organic acids like salicylic, acetyl salicylic, and oxalic acid are used as catalyst, however, the use of catalyst is insignificant for satisfactory delignification at high temperature i.e., above 185 °C (Aziz and Sarkanen 1989). Steam explosion pretreatment method is economic and environment friendly which used high pressure saturated steam for explosive decompression of hemicelluloses.

Ammonia fiber expansion is an ammonia-based pretreatment method aimed to improve the susceptibility of lignocellulosic biomass for enzymatic action. In this process, ammonia load and residence time are the critical factors affecting the economics of the process. The conditions optimized for this method are like temperature of 90 °C, liquid ammonia dosage of 1–2 Kg ammonia/Kg dry biomass, and residence time of 30 min (Sun and Cheng 2002). The energy consumption by steam explosion method affects the energy efficiency ratio. The energy efficiency ratio is less (0.26 Kg sugar/MJ) for steam explosion than organosolv (0.31–0.40 Kg sugar/MJ) (Zhu et al. 2010), hence steam explosion pretreatment method is a method of choice for wide variety of feedstocks including industrial hump (Sipos et al. 2010), wheat straw (Erdei et al. 2012), wood chips (Pielhop et al. 2016), and corn stover (Walker et al. 2018; Sulzenbacher et al. 2021).

16.3.3 Methods for the Hydrolysis of Pretreated Feedstock

The fermentable sugars are released from pretreated biomass using saccharification/hydrolysis, which is generally conducted by acid or enzymatic treatment (Azhar et al. 2017). Acid hydrolysis is carried out through dilute or concentrated acids (Kim et al. 2005). Organic acids, phosphoric acid, nitric acid, sulfuric acid and hydrochloric acid are commonly used for acid hydrolysis (Zhou et al. 2013). Enzyme hydrolysis, also known as biological hydrolysis, is another method for hydrolysis of pretreated materials in which biocatalysts (cellulases, and hemicellulases) are used, and biological method is observed to be more promising over acid hydrolysis for commercial applications due to less chemical and energy requirement, less generation of toxic by-products, and high product yield (Madadi et al. 2017). The factors

affecting enzymatic hydrolysis involve enzyme mediated factors such as source of enzymes, combined action of two or more enzymes and adsorption of enzymes in addition to lignin content, and substrate related factors such as accessible surface area, cellulose crystallinity, hemicellulose content, particle size and specific surface area ratio and degree of polymerization (Lukajtis et al. 2018). Other physical and chemical parameters like temperature, pH, substrate concentration, enzyme dosage, and treatment time also affect the enzyme mediated hydrolysis (Kamzon et al. 2016).

Bacteria and fungi can be easily grown under laboratory conditions due to their rapid growth and less generation time, and species of *Aspergillus*, *Clostridium*, *Cellulomonas*, *Fusarium*, *Neurospora*, *Penicillium* and *Trichoderma* possess the ability for production of cellulases and hemicellulases (Chandel et al. 2007). Cellulase mediated hydrolysis has been conducted by synergistic action of three enzymes namely, endo-glucanase, exo-glucanase and β -glucosidase, and three major steps are involved in this process for production of monomeric sugars from polysaccharide cellulose (Madadi et al. 2017). A complex group of hemicellulases involving endo- β -1,4-xylanase (EC 3.2.1.8), α -D-galactosidase (EC 3.2.1.22), acetyl xylanesterase (EC 3.1.1.72), β -xylosidase (EC 3.2.1.37), α -L-arabinofuranosidase (EC 3.2.1.55), α -D-glucuronidase (EC 3.2.1.139), and ferulic acid esterase (EC 3.1.1.73) was employed for degradation of complex hemicellulose structure (Ivetic et al. 2017).

16.4 Production of Industrial Products from Lignocellulosic Biomass

Lignocellulosic biomass is found to be one of the primary raw materials for renewable fuels, chemicals, and different value-added products (Liu et al. 2014), and the major sources of lignocellulosic biomass are agricultural sources like crop residues, perennial grasses and woody crops, logging and wood processing mill waste, and biomass from forest waste (Wahlstrom and Suurnakki 2015). It has been reported that more than 200 value-added compounds have been obtained from lignocellulosic biomass by development of novel cost-effective technique (Kumar et al. 2018a). There are different microbial factories which have been involved in bio-transformation of lignocellulosic biomass into value-added products. The major factors influencing rate of microbial growth and production of different primary and secondary metabolites are temperature, pH, inoculum size, inoculum age, aeration rate, type and concentration of carbohydrate, concentration of salt, osmolarity, and ethanol concentration (Sooch and Lugani 2017). The three major steps involved in transformation of lignocellulosic biomass into high value compounds are pre-treatment, hydrolysis and fermentation (Kumar et al. 2018b). Different value-added industrial products produced by bioconversion of lignocellulosic biomass have been described in Table 16.2.

Table 16.2 Biosynthesis of value-added industrial products from lignocellulosic biomass

Type of products	Products	Ligno-cellulosic waste	Pretreatment and hydrolysis methods	Microorganisms	Operation conditions	Product yield/titre	References
Industrial enzymes	Xylose reductase	Sugarcane bagasse	Hydrolysis with 98% H ₂ SO ₄ followed by detoxification with CaO and activated charcoal	<i>Candida guilliermondii</i> FTI 20037	Xylose: 30 g/L, pH of medium: 5.5, inoculum size: 1.0 g/L, operated at 30 °C under 1.0 vvm (aeration rate) for 12 h (batch bioreactor)	2.5 U/mg of protein	Milessi et al. (2011)
	α-amylase	Banana peel	Homogenization in blender with addition of water 10% (w/v)	<i>Aspergillus niger</i> NCIM 616	Fermentation media with NH ₄ NO ₃ (10 g/L), CaCl ₂ (5 g/L), water (30%), operated at 37 °C for 72 h (solid state fermentation)	1350 U/mg of protein/g peel	Krishna et al. (2012)
	Polygalacturonase	Wheat bran	Particle size reduction to 100–250 µm	<i>Aspergillus sojae</i> ATCC20235	Inoculum size (10 ⁷ spores/g substrate) at 37 °C for 96 h (solid state fermentation)	535.4 U/g substrate	Demir and Tari (2014)
	Cellulase	Rice bran and wheat bran	NA	<i>Aspergillus niger</i>	Substrate (10 g/L), inoculum size of 10 ⁸ spores at 30 °C under 180 rpm for 168 h (solid state fermentation)	29.81 U/g FPase activity, 25.2 U/g CMCase activity	Reddy et al. (2015)
	Lipase	Coconut dregs	Size reduction (1 mm), H ₂ SO ₄ (1.5% v/v) treatment, pH adjustment, pretreatment with cellulase (30,000 U/g of cellulysin) at 40 °C for 96 h	<i>Bacillus stratosphericus</i> PW3	Basal media containing MgSO ₄ ·7H ₂ O (0.1 g/L), KH ₂ PO ₄ (0.1 g/L), peptone (5 g/L), inoculum size (5%, v/v), pH:7, temperature: 37 °C at 200 rpm for 32 h (submerged fermentation)	140 U/mL	Zin et al. (2017)

(continued)

Table 16.2 (continued)

Type of products	Products	Ligno-cellulosic waste	Pretreatment and hydrolysis methods	Microorganisms	Operation conditions	Product yield/titre	References
	Protease	Organic municipal solid waste	NA	<i>Bacillus subtilis</i> AKAL7	Basal media containing solid waste (30 g/L), glucose (10 g/L), yeast extract (5 g/L), peptone (5 g/L), pH of 9.0, temperature 30 °C under stirring conditions (120 rpm) for 24 h (batch bioreactor)	141.8 U/mL	Hakim et al. (2018)
	Xylose reductase	Rice straw hydrolysate	Size reduction (1 cm), H ₂ SO ₄ treatment and enzymatic pretreatment	<i>Pseudomonas putida</i> BSX-46	Xylose (30 g/L) from pretreated rice straw, urea (10 g/L), malt extract (10 g/L), ZnSO ₄ (1 mg/100 mL), inoculum size (5.0%, v/v), inoculum age (6 h), agitation rate (200 rpm), aeration rate (0.4 vvm), pH (6.5), temperature (30 °C), fermentation time (24 h)	213.14 ± 0.47 IU/mg of cells	Lugani et al. (2021b)

Biofuels	Bioethanol	Soft wood (<i>Moringa oleifera</i>)	Chipping and milling, drying at 60 °C for 4 h, pretreatment with H ₂ SO ₄ (2%, w/v)	<i>Clostridium</i> sp. MTCC 1349	Fermentation media having beef heart (196 g/L), peptone (40 g/L), dextrose (4 g/L); NaCl (10 g/L) under anaerobic conditions at 37 °C for 168 h	12.8 g/L	Bharathiraja et al. (2015)
	Bioethanol	Rice straw	Pretreatment with aqueous ammonia with solid: liquid ratio of 1:12, hydrolysis with cellulase (15 FPU/g substrate) and xylanase (100 XU/g substrate) at 50 °C for 16 h	<i>Saccharomyces cerevisiae</i>	Fermentation media containing yeast extract (10 g/L), peptone (20 g/L) at 37 °C under agitation conditions (200 rpm) for 48 h (semi-solid-state fermentation)	24.6 g/L	Phitsuwan et al. (2017)
	Bioethanol	Monterey pine slurry	Pretreatment with dilute sulfuric acid (2.2%, w/w) and sodium bisulfite (10%, w/w), hydrolysis with cellulase (15 FPU/g of substrate) at 50 °C, 200 rpm for 24 h	<i>S. cerevisiae</i>	Fermentation media containing glucose (200 g/L), yeast extract (1 g/L), peptone (2 g/L) incubated at 35 °C for 24 h (fed-batch, semi-solid-state fermentation)	82.1 g/L	Dong et al. (2018)

(continued)

Table 16.2 (continued)

Type of products	Products	Ligno-cellulosic waste	Pretreatment and hydrolysis methods	Microorganisms	Operation conditions	Product yield/titre	References
Polyols	Xylitol	<i>Eucalyptus</i> leaves	Pretreatment with steam explosion, H ₂ SO ₄ (5%, v/v) hydrolysis, pH adjustment	<i>Aspergillus niger</i> NCIM 1015	Hydrolysate containing xylose (1.030 g/L), peptone (20 g/L), yeast extract (10 g/L), (NH ₄) ₂ SO ₄ (2 g/L), initial pH adjusted to 5.0, inoculum size (0.2%, v/v) under 120 rpm for 96 h (batch)	0.300 g/L	Mudaliyar et al. (2011)
	Sorbitol	Rice bran	NA	<i>Lactobacillus plantarum</i> NCIM 2912	Substrate (75%, w/v), K ₂ HPO ₄ (10 g/L), tween 80 (5 g/L), MnCl ₂ (0.3 g/L), ammonium citrate (10 g/L) incubated at 37 °C under 150 rpm for 72 h (solid state fermentation)	5.3 g/L	Jan et al. (2017)
	Xylitol	Cocoa pod husk	Oven drying (until 3% humidity) and size reduction, pretreatment with H ₂ SO ₄ (3.49%, v/v), pH adjustment, ion exchange resin with hydrolysate volume: resin mass of 2:1	<i>Candida boidinii</i> XM02G	Hydrolysate containing xylose (11 g/L), peptone (5 g/L), yeast extract (5 g/L), (NH ₄) ₂ SO ₄ (2 g/L), inoculum size (0.05 g/L) for 96 h (batch)	0.52 g/g, 11.34 g/L	Santana et al. (2018)

Organic acids	Succinic acid	<i>Arundo donax</i>	Cellulase (140 U/g of wet biomass) hydrolysis	<i>Basfia succiniciproducens</i> BPP7	Hydrolysate with xylose (13–22 g/L), glucose (25–37 g/L), acetic acid (6–10 g/L), fur (20–50 mg/L), hydroxymethylfurfural (40–70 mg/L), vanillin (10–30 mg/L), 4-hydroxy benzoic acid (10–30 mg/L), yeast extract (5 g/L) with pH 7.5 at 37 °C for 48 h (bioreactor)	17 g/L	Cimini et al. (2016)
	Lactic acid	<i>Phragmites australis</i> straw	Grinding to 20 mesh, pretreatment with 2% (w/v) H ₂ SO ₄ , hydrolysis with cellulase (20 FPU/g)	<i>Bacillus coagulans</i> IPE22	Inoculum size (5%, v/v), simultaneous saccharification and co-fermentation at 50 °C and 150 rpm	0.41 g/g	Zhang et al. (2018)

(continued)

Table 16.2 (continued)

Type of products	Products	Ligno-cellulosic waste	Pretreatment and hydrolysis methods	Microorganisms	Operation conditions	Product yield/titre	References
	Acetic acid	Pine apples waste	–	<i>S. cerevisiae</i> and <i>Acetobacter acetii</i>	17 g pine apple peel +20 g sugar + ammonium phosphate 6 g + 800 ml H ₂ O, pH 4.0, incubated at 25–28 °C for ethanolic fermentation with <i>S. cerevisiae</i> for 2 days and then for acetic acid fermentation with <i>A. acetii</i> for 11 days	4.77% (v/v)	Raji et al. (2012)
		Papaya peel	8 g papaya peel treated by steam at 121 °C for 20 min; 200 ml H ₂ O addition and boiled at 80 °C for 30 min + acid hydrolysis with 1% HCl v/v for 30 min. pH adjusted with CaO to 6–6.8	<i>S. cerevisiae</i> and <i>Acetobacter acetii</i>	100 ml papaya hydrolysate +1% glucose+1 gm dry yeast culture, incubated for ethanolic fermentation at 28 °C, 300 rpm for 2 h initially and then for anaerobic fermentation in dark for 48 hrs at room temperature. For acetic acid fermentation incubated with <i>A. acetii</i> for 3 days at 37 °C	5.23% (v/v)	Vikas and Mridul (2014)
					30 g pulp+70 ml H ₂ O, pH 6.5, ethanolic fermentation by <i>Saccharomyces cerevisiae</i> in 52 hours at 36 °C producing 3.16% (v/v) ethanol; acetic acid fermentation by <i>A. acetii</i> in 48 hours at 34 °C	3.91% (w/v)	Chakraborty et al. (2015)

						Fibrous bed reactor	48% (w/w)/20.9 g/L	Wei et al. (2012)
	Corn husk	Pretreated with acid hydrolysis (0.4 M H ₂ SO ₄ at 110 °C for 6 h)		<i>Clostridium tyrobutyricum</i>		Fibrous bed reactor at 37 °C for 2 days	39% (w/w)/21.8 g/L	Fu et al. (2017)
Others	Wheat bran	NA		<i>Candida utilis</i> , <i>Rhizopus oligosporus</i>		Substrate (1 kg/L), chloramphenicol (0.4 g/L), (NH ₄) ₂ PO ₄ (40 g/L), inoculum size (10%, v/v), inoculum age (48 h) incubated at 30 °C for 96 h (solid state fermentation)	41.02%	Yunus et al. (2015)
	Aleppo pine cones	Pretreatment with conc. H ₂ SO ₄ of pH 2.0, enzymatic hydrolysis with cellulase (30.37 U/mg)		<i>S. cerevisiae</i>		Fermentation hydrolysate (15 mL) containing yeast extract (200 g/L), peptone (400 g/L) pH of 5.5 at 30 °C under 100 rpm for 96 h	162 µg/mL	Messaoudi et al. (2017)
	Hemicellulose	NA		<i>Rhodotorula toruloides</i> CBS 14		Crude glycerol (55 g/L), hemicellulose hydrolysate (100 g/L)	10.6 g/L	Chmielarz et al. (2021)
	Microbial lipids							

Note: *vvm* volume of gas per volume of liquid per minute, *FPUse* Filter-Paper cellulase, *CMCase* Carboxymethyl cellulase, *FPU* Filter Paper Units, *XU* Xylanase Units

16.4.1 Production of Industrial Enzymes

Enzymes are proteinaceous biomolecules which enhances the rate of reaction by lowering the activation energy. Enzyme production is one of the central parts of global industrial biotechnology, and utilization of low-cost lignocellulosic biomass as substrate is an appropriate approach for production of proficient enzymes. Different bacteria (*Acidothermus*, *Acidobacterium*, *Bacillus*, *Clostridium*, *Cellulomonas*, *Corynebacterium*, *Enterobacter*, *Pseudomonas*, *Staphylococcus*), fungi (*Aspergillus*, *Cephalosporium*, *Fusarium*, *Humicola*, *Mucor*, *Neurospora*, *Penicillium*, *Rhizopus*, *Trichoderma*), and yeasts (*Candida*, *Cryptococcus*, *Debaryomyces*, *Hansenula*, *Kloeckera*, *Kluyveromyces*, *Monilia*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Torulopsis*, *Trichosporon*) are involved in the production of industrially important enzymes (Shahriarinnour et al. 2011; Lugani et al. 2015; Sooch et al. 2019).

Actinomycetes constitute an important group of microbial population for production of novel enzymes having commercial potential due to their extreme stability, ability to decompose various materials, and unusual substrate specificity. Many commercially relevant enzymes like amylase, cellulase, chitinase, glucose oxidase, lipase, lipoxygenase, pectinase, phytase, peroxidase, protease, and xylanase have been reported to be produced from lignocellulosic biomass (Table 16.2) by different genera of actinomycetes including *Actinomadura*, *Cellulomonas*, *Microbispora*, *Nocardiopsis*, *Streptomyces*, *Thermoactinomyces*, *Thermobifida*, *Thermomyces*, *Thermomonospora* (Prakash et al. 2013). Among all the enzymes, cellulases and hemicellulases have tremendous applications in different industrial sectors like agriculture, animal feed, brewery and wine, chemicals, food, fuel, laundry, paper, pulp, and textiles (Beauchemin et al. 2003).

The two major fermentation strategies used for production of enzymes are solid-state fermentation and submerged fermentation, and there is different genetic expression of microbes under different fermentation modes (Gonzalez 2012; Sirohi et al. 2018). Solid state fermentation leads to utilization of solid substrate for the production of industrial enzymes from different fungal species like ascomycetes, basidiomycetes, and deuteromycetes (Batche et al. 2014) and lignocellulosic materials are found suitable for production of many industrial enzymes. This method promises less downstream processing, minimum effluent production, high volumetric productivity, and increased product concentration (Singhania et al. 2010). However, there are several limitations also associated with this method like there is limited reproducibility of results, and it is not able to standardize process parameters such as temperature and aeration which may lead to enzyme inactivation (Holker and Lenz 2005).

The second fermentation strategy is submerged fermentation which utilizes aqueous medium to maintain uniform temperature, pH, aeration and agitation conditions within the vessel, and this method is commonly used for large scale enzyme production (Ravindran and Jaiswal 2016). There is strict control of different parameters within this system; hence, there are minimum chances of contamination, and less byproducts production like metal ions, butylated hydroxytoluene and hydrogen

peroxide (Roukas 2016). Hence, submerged fermentation is commonly used for the fermentative production of different enzymes and bioactive compounds from bacteria, and filamentous fungi using lignocellulosic biomass. However, there are many reports which clearly revealed that submerged fermentation is not an economical mode of enzyme production at large scale because there is a need of sophisticated and large equipment in this mode. Previously, Zhang et al. (2012) showed that capital investment for submerged fermentation is 78% more than solid state fermentation. Research work of various enzyme producing industries is now focusing towards isolation of novel extremophilic and recombinant microbial strains with enhanced enzyme production from lignocellulosic biomass.

Different agricultural lignocellulosic materials like wheat straw (Sumantha et al. 2005), rice straw, rice bran (Virupakshi et al. 2005), coffee by-products (Murthy and Naidu 2010), oil palm empty fruit bunch (Shahriarinnour et al. 2011), chocory Dahlia (El-Hersh et al. 2011), wheat bran (Kumar and Sushma 2012), pine apple (Thangaratham and Manimegalai 2014), banana waste (Dabhi et al. 2014), sugarcane bagasse (Kaur et al. 2015), corn cob (Barathikannan et al. 2016), sugarcane industrial waste (Ellila et al. 2017), and wastewater (Zouaoui and Bouziane 2018) were used as feedstocks for production of industrial important enzymes. Different lignocellulosic materials like bajra straw, *Trifolium* hay, sorghum straw, maize straw, oat hay, and wheat straw were screened for production of thermostable xylanase production from *Trichoderma viride*, and the maximum enzyme production was achieved with maize straw under submerged fermentation (Goyal et al. 2008). Detoxified sugarcane bagasse hydrolysate was used for production of xylose reductase from *Candida guilliermondii* FTI 20037, and the maximum enzyme production (2.5 U/mg of protein) was found under microaerobic conditions (Milessi et al. 2011).

Lignocellulosic feedstocks like date seeds, grasses and palm leaves were also tested for the production of different hydrolytic enzymes (α -amylase, pectinase, xylanase) under solid state fermentation from *Bacillus megatherium*, and wheat bran was found to be the best among all the substrates used for production of different hydrolytic enzymes (El-Shishtawy et al. 2014). In another study, carrot peel was used for pectinase production by *Bacillus mojavensis* I4 in submerged fermentation using two statistical methods i.e., Taguchi design (for key ingredients) and Box-Behnken design for optimizing different parameters, and maximum pectinase production of 64.8 U/mL were obtained under optimized conditions with 6.5% (w/v) carrot peel powder (Ghazala et al. 2015). In another study, among all the seven fungal strains tested for lipase production, the maximum enzyme activity of 5.12 ± 0.0059 U/mL was attained with *Aspergillus niger* at 30 °C after fermentation period of 72 h using olive oil as substrate (Mukhtar et al. 2015).

Different forms of cellulases i.e., endoglucanase, exoglucanase and β -glucosidase were produced by *Aspergillus flavus* Linn after utilizing freshly ripe tomato fruit under solid state fermentation (Damato et al. 2010), and by *Bacillus subtilis* from molasses sawdust and bagasse under submerged fermentation (Singh et al. 2016). Response surface methodology was adopted for production of protease from different substrates (corn cob and coffee pulp waste) using Box-Behnken design and the

maximum enzyme yield of 920 U/mL was attained with 3 g/L and 2 g/L of coffee pulp waste and corncob, respectively at 37 °C after 60 h of fermentation (Kandasamy et al. 2016). Extracellular lipase activity of 140 U/mL was also obtained from novel *Bacillus stratosphericus* using coconut dregs as feedstock in submerged fermentation, and the resultant enzyme was observed to be active under wide range of pH and temperature conditions in the presence of detergents (Zin et al. 2017).

16.4.2 Production of Bioethanol

Bioethanol is a clean, environment-friendly, and alternate fuel for future generation, which results in less emission of green house gases, and hence considered as an alternative strategy for reducing global warming (Joshi et al. 2011). Based on type of raw material, biofuels are classified into four generations i.e., first generation biofuels from edible agricultural crops, second generation biofuels from non-edible lignocellulosic feedstocks, third generation biofuels from algal biomass, and fourth generation biofuels from metabolically engineered algal strains (Meneses et al. 2017). Currently, sucrose and starch-based grains are utilized for industrial level bioethanol production (Asgher et al. 2013); whereas it may generate a competition between food production and fuel ethanol for future generations (Gnansounou 2011). Hence, to avoid such situation, one of the alternative potential approaches is utilization of different agricultural lignocellulosic residual materials for low-cost fermentation production (Iqbal et al. 2013; Lugani et al. 2019). The major steps involved in biofuel production are pretreatment, hydrolysis, fermentation and distillation (Mosier et al. 2005).

Different fermentation modes for bioethanol production are batch, fed-batch and continuous mode. The most traditional method of ethanol production is batch fermentation in which high initial substrate concentration is used (Olsson and Hagerdal 1996). In a continuous system, there is constant addition of nutrients and substrate with removal of biomass and metabolites, and the merits associated with this mode of fermentation are easy to control, less labor intensive, high productivity, and elimination of undesired products (Sanchez and Cardona 2008). Fed-batch system is a combination of batch and continuous process in which substrate is added intermittently, which results in shorter fermentation time, higher ethanol productivity, high dissolved oxygen and low toxicity of media components (Cheng et al. 2009). In another study, ethanol productivity of 0.44 g/L/h, and ethanol concentration of 53.3 g/L was obtained from wheat meal and wheat straw in fed batch system at 32 °C for 120 h under agitation conditions (300 rpm) using *Saccharomyces cerevisiae* TMB3400 (Erdei et al. 2012). The ethanol productivity of 3.8 g/L/h was obtained in a continuous system with a dilution rate of 0.131/h using nonlinear model predictive controller algorithm (Ajbar and Ali 2017).

Various previous reports have been published on ethanol production by utilizing different lignocellulosic feedstocks such as water hyacinth (Kumar et al. 2009), cassava powder (Choi et al. 2010), grass silage (Sieker et al. 2011), spent coffee grounds

(Mussatto et al. 2012), coffee processing waste (Kefale et al. 2012), poplar (Wang et al. 2012), grasses (Scordia et al. 2014), douglas fir (Inoue et al. 2016), rice straw (Wi et al. 2013; Phitsuwan et al. 2017), waste paper (Nishimura et al. 2017), pine needle (Vaid et al. 2018), and pine slurry (Dong et al. 2018). In one previous study, ethanol was produced from cotton hydrolysate using *Saccharomyces cerevisiae* var. *ellipsoideus* at 30 °C under agitation conditions (Nikolic et al. 2016). In a similar study, maximum ethanol production of 33.7 g/L was observed after 96 h of fermentation at 34 °C from corn stover pretreated with ethylenediamine using simultaneous saccharification and co-fermentation strategy (Qin et al. 2018). The ethanol yield is very less with wild microbial strains; hence many recombinant strains have been developed for improved production of ethanol (Cavalheiro and Monteiro 2013; Sar et al. 2017; Ko et al. 2018; Lopez-Hidalgo et al. 2021).

There are some fermentation integrated strategies such as simultaneous saccharification and fermentation, simultaneous saccharification and co-fermentation and consolidated bioprocessing, which are adopted for bioethanol production to overcome the drawbacks of traditional fermentation systems. Simultaneous saccharification and fermentation system allows use of a single vessel for simultaneous hydrolysis and fermentation, which allows ease of process operation, less chances of contamination, and economical ethanol production in short duration. Simultaneous saccharification and co-fermentation method is one step complete simultaneous hydrolysis and fermentation of both pentoses and hexoses into ethanol in short duration (Nikolic et al. 2016). The most upgraded highly integrated approach is consolidated bioprocessing in which single microbial community brings all the processes in a single step including sugar production, enzyme hydrolysis and fermentation (Lynd et al. 2005).

Previously, waste newspaper was used as substrate for ethanol production in simultaneous saccharification and fermentation using thermotolerant yeast *Saccharomyces cerevisiae* KNU5377 at 50 °C, and the maximum ethanol production of 8.4% was attained after 72 h of fermentation (Park et al. 2010). 82.1 g/L ethanol was produced from 25% (w/w) undetoxified pile slurry in simultaneous saccharification and fermentation system (Dong et al. 2018). Simultaneous saccharification and co-fermentation system was used previously by many researchers for the production of ethanol (Liu and Chen 2016; Sharma et al. 2018; Qin et al. 2018). In a recent study, the maximum ethanol yield of 0.148 g/g after 72 h of fermentation was obtained from pine needle biomass using *Saccharomyces cerevisiae* and *Pichia stipitis* through consolidated bioprocessing (Vaid et al. 2018).

16.4.3 Production of Xylitol

Xylitol is an industrially important polyol sugar having tremendous applications in different industrial sectors such as food, bakery, confectionery, cosmetic, odontological, pharmaceutical, and medical sectors (Lugani and Sooch 2017, 2018, 2020; Lugani et al. 2017, 2020; Baptista et al. 2018). The unique properties of xylitol such

as negative heat of dissolution, improvement of taste and color of food, and bakery products, absence of Maillard reaction, low calorie sugar, insulin independent metabolism, enhanced remineralization of carious lesions, and ability to retain moisture make this alcoholic sugar an attractive choice for industrial applications (Albuquerque et al. 2014), and the global market of xylitol is supposed to be USD 6.30 billion by 2022 (Markets and Markets 2016). Biotechnological methods (fermentative and enzymatic), being environment friendly, are currently explored to produce xylitol to meet its increasing global demand. Fermentation method, also known as microbial method, uses whole microbial (bacterial, yeast, and fungal) cells for xylitol production at ambient temperature, and pressure conditions with enhanced product yield and productivity, and there is minimum synthesis of by-products during this process (Tran et al. 2004; Lugani et al. 2021a).

Yeasts are the primary candidate of choice for xylitol production due to improved yield and productivity compared to bacteria and fungal strains, and *Candida boidinii*, *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, *C. boidinni*, *Pichia* sp., *Pachysolen* sp., *Kluyveromyces marxianus*, *Kloeckera* sp., *Saccharomyces* sp., *Rhodotorula* sp., *Hansenula* sp., *Torulopsis* sp., *Trichosporon* sp., *Cryptococcus* sp., and *Debaromyces hansenii* have been extensively utilized by many researchers for xylitol production from various agricultural waste materials (Cortez and Roberto 2014; Tamburini et al. 2015; Zahed et al. 2016; Dasgupta et al. 2016; Xavier et al. 2018). Various genera of bacteria such as *Acetobacter*, *Gluconobacter*, *Achromobacter*, *Actinomadura*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Brevibacterium*, *Corynebacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Nocardia*, *Planococcus*, *Pseudomonas*, *Rhodococcus*, *Morganella*, *Streptomyces*, and *Gluconobacter* (Takeuchi et al. 2001; Sugiyama et al. 2001; Lugani and Sooch 2020), and fungi *Neurospora*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Gliocladium*, *Byssoschlamys*, *Myrothecium* (Sampaio et al. 2003; Mudaliyar et al. 2011) are also reported for xylitol production.

Different agricultural wastes such as walnut shell (Tran et al. 2004), sugarcane bagasse (Santos et al. 2005), spent brewing grain (Carvalho et al. 2005), rice straw (Zeid et al. 2008), sorghum straw (Sene et al. 2011), groundnut shells, glass straw (Mudaliyar et al. 2011), rice bran (Martinez and Santos 2012), coffee husks, mung-bean hull, oat hull, peanut hull, corncob (Jeevan et al. 2011; Tada et al. 2012), sago trunk (Mohamad et al. 2013), coconut husk (Neeru et al. 2013), banana peel (Rehman et al. 2013), pomegranate peel (Barathikannan et al. 2016), oil palm empty fruit bunch (Kresnowati et al. 2016), beech wood, and cocoa pod husk (Santana et al. 2018) have been used as raw material of xylose for xylitol production. Two agricultural wastes such as beech wood and walnut shells were screened for xylitol production by *Candida tropicalis* IFO0618, and the maximum xylitol yield (50%) was obtained with beech wood hydrolysed solution mixed with glucose (1%, w/v) (Tran et al. 2004).

Agricultural wastes such as coconut husks, groundnut shells, grass straw, *Eucalyptus* leaves, *Eucalyptus* wood, and *Jambulina* leaves have been tested for production of xylitol by *Aspergillus niger*, and the maximum xylitol production (0.300 g/L) has been attained with *Eucalyptus* leaves (Mudaliyar et al. 2011). The

maximum xylitol production of 35 g/L was obtained from corn cob hydrolysate (containing 40 g/L xylose) by *Pichia* sp. under stirring conditions (150 rpm) at temperature of 28 °C and pH of 6.0 after 72 h of fermentation (Jeevan et al. 2011). Xylitol was produced from cocoa pod husk hemicellulose hydrolysate by *Candida boidinii* XM02G, and the highest xylitol yield of 0.52 g/g was observed at the end of fermentation and the yeast strain was also observed to tolerate phenolic compounds present in hemicellulose hydrolysate up to 6 g/L (Santana et al. 2018). Recently, Lugani et al. (2021b) have utilized rice straw as feedstock for the production of xylose reductase (a xylitol producing enzyme), from novel isolated *Pseudomonas putida* BSX-46. Some attempts have been made for improved xylitol production using industrially efficient recombinant microbial strains produced by mutagenesis and recombination DNA technology (Peng et al. 2012; Pal et al. 2013; Zhang et al. 2015; Kogje and Ghosalkar 2017; Baptista et al. 2018). Other polyol like sorbitol is also produced fermentatively using rice bran from *Lactobacillus plantarum* NCIM 2912 with 5.3 g/L yield (Jan et al. 2017).

16.4.4 Production of Vinegar

Vinegar is a sharp and sour liquid produced by double fermentation of sugar containing solution and is used as condiment and preservation of food. According to Food and Drug Administration, USA, vinegar is produced by two step fermentation process i.e., splitting of sugar into alcohol followed by bioconversion of alcohol into acetic acid. Different processes which have been adapted for production of acetic acid from past decades are Orleans/generator process, submerged fermentation and quick process, and the last two processes are used presently for commercial production of vinegar. Different steps involved in commercial vinegar production are fermentation, filtration, clarification, distillation, pasteurization, and bottling (Oyetoro et al. 2017). Traditionally, different types of raw materials like apples, berries, fruit juices, grapes, grains, honey, plant extracts, rice, sugars, whey have been utilized for production of different types of vinegar. However, in the recent past, different agricultural materials like banana peels (Bazirake et al. 2014), decomposed fruits (Diba et al. 2015), pineapple wastes (Raji et al. 2012; Krusong and Vichitraka 2010; Roda et al. 2014), star fruit juice (Minh 2014), and wood (Donald et al. 2009) have been utilized as substrate for vinegar production.

16.4.5 Other Products

Synthesis of various value-added products like vanillin and gallic acid from lignin-based materials by *Phanerochaete chrysosporium* was reported in literature (Ribbons 1987). Vanillin has potential applications in different industrial products like anti-foaming agents, drugs, herbicides, and household products (Priefert et al.

2001). Furfural is another value-added product produced by bioconversion of xylose containing lignocellulosic feedstock, and it is used for manufacturing of furfural-phenol plastics, pesticides and varnishes (Peleteiro et al. 2016).

The other products produced by lignocellulosic materials are antibiotics, methane, phenolic compounds, polysaccharides, and single cell proteins (Howard et al. 2003). In a recent study, a novel yeast strain *Candida intermedia* FL021 was reported to produce single cell protein by consuming lignocellulosic hydrolysate (Wu et al. 2018). Some organic chemicals such as benzene, ethylene, propylene, toluene, and xylene are also produced by lignocellulosic materials (Pothiraj et al. 2006), and these chemicals are in turn required for the synthesis of different chemical products like resins and polymers (Coombs 1987). Lignocellulosic waste is also utilized for the synthesis of high value byproducts like amino acids, organic acids, vitamins, bacterial and fungal polysaccharides such as xanthan (Pothiraj et al. 2006). In a previous study, L-lysine was produced by both wild and engineered strains of *Corynebacterium glutamicum* using acid pretreated detoxified biorefinery waste stream (Christopher et al. 2016). Chemicals such as 2,3-butanediol (2,3-BD) (Okonkwo et al. 2017), 1,3-propanediol (1,3-PD) (Vivek et al. 2018), and organic acids like lactic acid (Zhang et al. 2018; Juturu and Wu 2018), fumaric acid (Liu et al. 2017), itaconic acid (Saha et al. 2017), butyric acid (Wei et al. 2012; Fu et al. 2017; Xiao et al. 2018) gluconic acid, xylonic acid (Zhang et al. 2016b) and succinic acid (Cimini et al. 2016; Alexandri et al. 2016) have been reported to be produced by microbial fermentation by utilizing lignocellulosic feedstocks. The biodegradable polymer poly-3-hydroxybutyrate was also produced previously by utilizing xylose rich lignocellulosic wastes (Raposo et al. 2017).

Various valuable components such as polysaccharides, vitamins and trace elements like Ca, Fe, Mg, Zn, produced by lignocellulosic materials, improved digestibility of feedstuffs in animals (Zhu et al. 2012). Some previous authors have utilized lignocellulosic materials like corncob, corn stover, eucalyptus, rice straw, spent grain, and sugarcane bagasse for the synthesis of renewable and marketable biochemicals like benzene, biphenyls, catechol, cyclohexane, guaiacols, phenols, syringaldehyde, vanillin and vanillic acid (Messaoudi et al. 2017; Ji et al. 2012; Varanasi et al. 2013). Apart from bioethanol, other environment friendly clean bio-fuels, such as bio-butanol (Nilsson et al. 2015; Maiti et al. 2018) and bio-hydrogen (Abdul et al. 2013; Sen et al. 2016) are also produced by lignocellulosic biomass. During the past few years, some research has been directed towards development of bio-based composites with different functionalities of interest from lignocellulosic waste residual materials (Bajpai et al. 2013), and production of medium-density fiber board is one of the successful attempts in this research (Li et al. 2013).

Agricultural lignocellulosic wastes like cassava peel, coconut oil cake, corn cob, corn husk, corn steep waste, peanut shells, rice husk, rice straw, sugarcane bagasse, sugarcane molasses, and wheat bran flour have been utilized previously for the production of various antibiotics like cephalosporin, cyclosporine A, neomycin, oxytetracycline, rifamycin A, and tetracycline using solid state fermentation (Kaur et al. 2014). In a previous study, corn stover was utilized to produce succinic acid from *Basfia succiniproducens* and a final productivity of 0.43 g/L/h was obtained

(Salvachua et al. 2016). In another study, *Actinobacillus succinogenes* and *Basfia succiniproducens* was used for biotransformation of spent sulphite liquor into succinic acid production and 39 g/L yield was achieved. Along with succinic acid, lignosulphonates (32.4 g/L), and phenolic rich extract (1.15 g) was also produced after fractionation (Alexandri et al. 2016). Many industrial valuable products such as astaxanthin, biohydrogen, β -carotene, docosahexanoic acid, eicosapentanoic acid, lutein, lycopene, phycobiliproteins, and zeaxanthin are produced by microalgal biomass (Bhalamurugan et al. 2018).

16.5 Improved Strategies for the Production of Industrial Products

The yield and productivity of industrially important valuable products is very less with wild microbial strains, and hence these methods can't be adapted at commercial level for production of energy and cost-effective bio-based products. Hence, the focus of researchers is towards development of different strategies like genetic and metabolic engineering, immobilization methods and *in silico* computational techniques to reduce the cost of products produced by utilization of lignocellulosic biomass.

16.5.1 Development of Genetically-Modified Strains

Major techniques used to produce recombinant microorganisms are genome-based strain reconstruction, metabolic engineering, protein engineering, genome-wide transcript expression analysis, molecular breeding, genome mining, recombination DNA technology, whole genome shuffling and genome mining (Adrio and Demain 2010; Yang et al. 2017; Yang et al. 2018). One of the emerging fields of recombinant DNA technology is metabolic engineering to improve the production of novel products, accelerating rate determining step, engineer enzyme activities and shift metabolic flux towards synthesis of desired product by modulating transport of sugar, gene regulation and enzyme expression. In this process, there is introduction of heterologous genes or regulatory elements for developing novel metabolic configuration (Joshi et al. 2011). Various attempts have been made for adopting genetic engineering techniques for development of industrially efficient microbial strains which can show enhanced gene expression, produce improved product yield and tolerate high temperature and low pH.

The genetically engineered strains of *S. cerevisiae* and *Zymomonas mobilis* have been employed by DuPont Dannisco Cellulosic Ethanol and Butalco for commercial production of ethanol (Weber et al. 2010). Protein engineering is another advance in biotechnology for construction of tailored biocatalysts with desired functions using directed evolution or rational design strategies. The other techniques

of protein engineering are semi-rational design (Lutz 2010), truncation and fusion (Yang et al. 2014) and structure-based designs using site-specific or non-specific chemical modifications (Davids et al. 2013). Various genetically modified fungal strains have been developed which possess the ability for production of large-scale hydrolytic enzymes such as cellulose, hemicellulase and xylanase and fermentable sugars from agricultural waste materials such as corn stover, straw, sugarcane bagasse, and switchgrass (Deswal et al. 2014).

However, genetically modified microorganisms possess several environmental and public health risks when used in large-scale fermentation systems (Limayem and Ricke 2012). Hence, to avoid such risks associated by exposure of genetically modified microorganisms, microbial risk assessment modeling approach is used for risk assessment of microbial dissemination in four steps namely hazard identification, hazard characterization, exposure assessment and risk characterization (Haas et al. 2004). Nanotechnology is one of the emerging fields of industrial biotechnology for generation of recombinant microbial strains by site-specific insertion of desired gene (Chekol and Gebreyohannes 2018).

16.5.2 Immobilization Strategies

Biocatalysts show tremendous applications to produce industrial important products from lignocellulosic materials by accelerating the rate of reaction at optimum conditions. However, there are some limitations for their use at commercial scale like easy solubility in aqueous media, high cost, less stability, and availability in small amounts (Sarrouh et al. 2012). Immobilization is one of the attractive approaches as an alternate which makes the enzyme immobilize onto insoluble matrix for retaining its geometry and economic reuse under stabilized conditions. Enzymes can be immobilized on supports either reversibly using adsorption, affinity, ionic and metal binding or irreversibly using entrapment and covalent binding. Different immobilization techniques used to bind the enzymes are shown in Fig. 16.3.

Enzymes bind to different carrier support materials through various functional groups (amine, alcoholic, carboxylic, guanidine, imidazole, phenol, thioester and thiol) by physical (hydrophobic and van der Waals interactions), covalent or ionic interactions. Covalent interactions are observed to be strongest and ionic interactions are weakest among different types of enzyme-carrier interactions (Ali et al. 2017; Kauldhar et al. 2016). Adsorption, cross-linking, encapsulation and entrapment are traditional immobilization strategies, whereas, protein engineering, nanotechniques and affinity tags are considered as modern strategies for biocatalyst immobilization (Ali et al. 2017). Protein engineering for enzyme immobilization is done by site-directed protein modification techniques such as enzymatic modification, native chemical ligation, auxotrophic expression and nonsense suppression.

The tags used for affinity immobilization are His (Histidine), GST (glutathione-S-transferase), FLAG (epitope tag), HA (hemagglutinin), myc, biotin and DNA

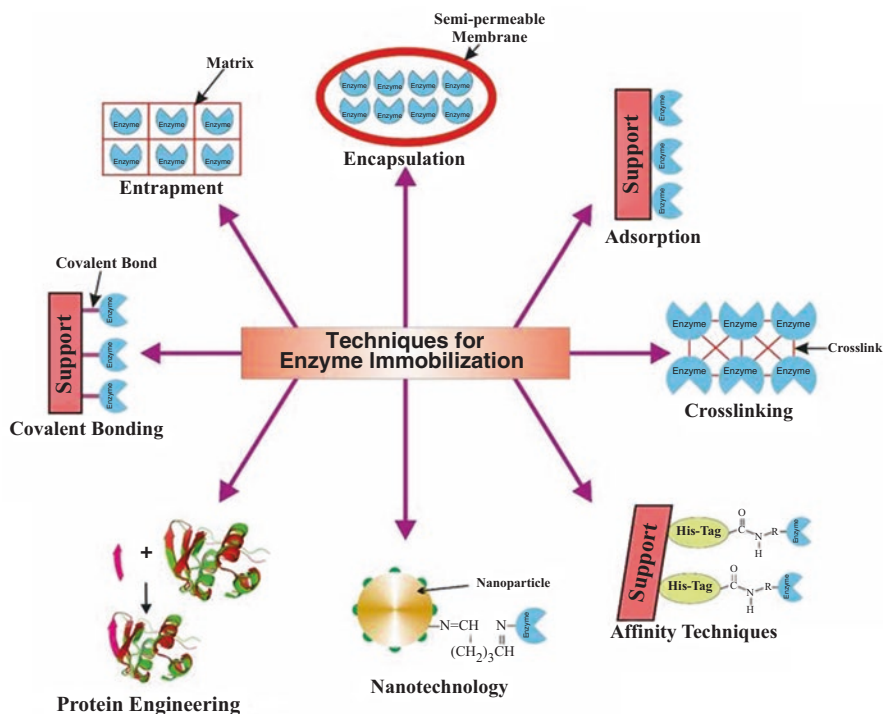


Fig. 16.3 Techniques for enzyme immobilization

mediated tags (Redeker et al. 2013). Some attempts have already been made by previous authors for development of cost-effective immobilization system for production of various bio-based products from lignocellulosic biomass; still minimum success has been achieved from previous studies for generation of commercial immobilization system. Development of multi-enzyme immobilization system is one of the promising alternate strategies which can be utilized for production of valuable products from lignocellulosic biomass. Hence, further studies and research is required to develop enzyme-based immobilization systems by understanding properties of proteins, their stability, conformational changes under different conditions and interactions involved in their immobilization.

16.5.3 Computational Strategies

Several genome sequence databases such as GenBank, European Nucleotide archive and *Saccharomyces* genome database, and protein sequence databases such as Swiss-Prot, Universal Protein Resource, TrEMBL, Protein Information Resource, worldwide Protein Data Bank are available for *in silico* computational studies. Other commonly used databases for protein-protein interactions, membrane

transporters and carbohydrate-modifying enzymes are Molecular Interaction database, Transporters Classification Database and Carbohydrate-Active enzyme database. Molecular interactions are studied using Docking, AUTODOCK, High Ambiguity Driven Protein-Protein Docking and GRID (Mehmood et al. 2014). COMPOSER (Sutcliffe et al. 1987), 3D-IIGSAW (Bates and Sternberg 1999) and MODELLER (Sali and Blundell 1994) are used for molecular modelling of proteins.

For evaluation of potential stabilizing mutations, computational high-throughput screening methods have been observed to attract more attention (Goldenzweig et al. 2016). Molecular dynamics and quantum mechanics studies are used to understand the effect of every single amino acid on protein structure and function (Kaushik et al. 2016). Computational approaches have been integrated with metabolic engineering for developing models for improved prediction of metabolic fluxes and their regulation by metabolite concentration, gene expression and protein expression (Strauer et al. 2009). Different methods such as pathway-based approaches, optimization-based approaches, kinetic modeling approaches and many other bioinformatics tools are being used for the production of desired products from microorganisms (Reed et al. 2010; Sooch et al. 2016). Computational tools have also been used for development of engineered enzymes having novel and improved activities (Damborsky and Brezovsky 2014). Machine learning is one of the advanced techniques of metagenome analysis and this technique can be used in future for the selection of efficient wild and genetically modified microbial strains producing large amount of industrial important compounds in considerable good amount from lignocellulosic biomass.

16.6 Current Status of the Green Technology

The global market of industrial important enzymes like amylase, cellulase, protease, lipase and phytase is found to be increased by 6.30 billion USD by 2022 (Industrial Enzymes Market 2018). The major players in global market for production of industrial enzymes are Advanced Enzyme Technologies Ltd., Aumgene Biosciences, PAC Bio Fungbact Pvt. Ltd. (India), Amano Enzyme Inc., Asahi Kasei Pharma Corporation, Hayashibara Company (Japan), AB Enzyme GmbH, BASF SE, Direvo Biotech AG (Germany), BioResource International, Inc., Codexis, Inc., DuPont Nutrition and Health, Enzymatic Deinking Technologies LLC, Enzyme Innovation, Thermo Fischer Scientific, Inc. (USA), Chr. Hansen A/S, Novozymes A/S (Denmark), Royal DSM (The Netherlands) (Research and Markets 2018).

The major companies producing bioethanol in India from biomass are Aatmiya Biofuels Pvt. Ltd., D1 Oil Plc, Godrej Agrovet, Emami Group, Gujarat Oelo Chem Ltd., Jain Irrigation System Ltd., Nova Bio Fuels Pvt. Ltd., Reliance Industries Ltd., Sagar Jatropa Oil Extraction Pvt. Ltd. The main focus of their research work is towards establishment of cost-effective biofuels (butanol, ethanol, dimethylether, hydrogen, biodiesel and hydrocarbons). Different research centers which have been developed in India with prime interest towards biofuel generation using renewable

resources are located in Delhi (International Centre of Genetic Engineering, Biotechnology Centre for Advanced Bioenergy Research), Faridabad (Centre for Advanced Bioenergy Research: Indian Oil Corporation Ltd. and Department of Biotechnology Institute), Mumbai (Institute of Chemical Technology) and Indian Institute of Technology located at Mumbai, Kharagpur, Guwahati, Roorkee and Jodhpur (Lali 2016).

The market of petroleum and natural gas sector was found to be USD 7 billion in June 2018 (IBEF 2018). Xylitol is one of the important industrial products which has shown tremendous applications in various industrial sectors and its global market is expected to be USD1 billion by 2020. The major companies of xylitol production are CSPC Shengxue Glucose Company Limited, Roquette Freres, Cargill Incorporation, Novagreen Incorporation, DFI Corporation, S2G Biochem, Dupont Nutrition and Health, Ingredion Incorporation, Shandong Futaste Company Limited, O'Laughlin Industries Company Limited, Xylitol Canada Incorporation, Mitsubishi Shoji Foodtech Company Limited, Shandong Longlive Bio-Technology Company Limited, Shandong Lujian Biological Technology Company Limited, Thomson Biotech Company Limited, Zichem Incorporation, Zhejiang Huakang Pharmaceuticals Company Limited. DuPont Dansico is one of the global leading players of xylitol production with three production plants in USA, China and Finland with their sweetener business of Danisco USA Incorporation, Danisco Sw. Anyang Company Limited and Danisco Sweeteners Oy (Markets and Markets 2016).

The key global manufacturers of vinegar are AcetificiItaliani Modena, Australian Vinegar, Bizen Chemical, Fleischmann's Vinegar, Krafet Heinz, Mizkan and Shanxi Shuita Vinegar (Marketers Media 2018). Deloitte Touche Tohmatsu Ltd. has published a report in 2017 entitled 'The chemical multiverse 4.0' on market size of chemical industry, and in this report ethylene capacity is observed to be enhanced in 2018 by US and China. The maximum chemical mergers and acquisitions activity is found in US followed by China, UK, Germany, India, France, Brazil and Switzerland. In the same report *in silico* experimentation and machine learning are found to be two major computational techniques for development of novel chemicals in this competitive market (Deloitte 2017). United States Department of Agriculture has given a report on bio-based chemicals containing commodity chemicals (6–10%), polymers (10%) and fine chemicals (45–50%) in 2025 (USDA 2008).

The sales of DuPont's industrial biotechnology products increased to USD 1 billion in 2015 from USD 200 million in 2009. A joint venture was developed between DuPont and Tate & Lyle which was focused on production of propanediol from corn and other feedstocks by fermentation (www.duponttateandlyle.com). The global players of single cell proteins market are NOW Food Health LLC., Willows Ingredients, Devenish Nutrition Ltd., BIOMIN Holding GmbH, PRO SOLO SPA, Aumgene Biosciences, BIO-CAT, Novozymes, Alltecch Inc., Nutreco N.V. (Market Research 2018), and the market size of single cell proteins is expected to be USD 8.7 billion by 2023 with commercial success of *Chlorella* and *Spirulina* (P & S Market Research 2018).

16.7 Conclusion

It has been concluded from the present studies that lignocellulosic biomass can be exploited to produce numerous industrial important value-added products namely ethanol, vinegar, xylitol, industrial enzymes and chemicals (organic solvents, olefins and plastics), drink softener solvents and fermentable sugars. Lignocellulosic biomass is an important renewable source present in abundance in nature and its use for production of value-added products provides various environmental benefits. The major bottleneck for production of value-added chemicals at commercial scale is chemical complexity of lignocellulosic biomass, complex metabolic pathways of microorganisms, less product yield from wild strains and presence of inhibitors in lignocellulosic biomass hydrolysate. Development of improved pretreatment strategies, fermentation processes and recovery of value-added products by efficient methods may reduce their capital and operating costs.

Media and process engineering through statistical tools is one of the alternate methods to improve economic production of different products. System biology combines experimental and computational research, and it involves integration of many disciplines such as biology, engineering, computer science, chemistry and physics for understanding complex biological systems and network behavior between gene, protein and informational pathways. Hence, system biology can be adapted in future to understand microbial metabolic pathways for development of commercial biological systems which can carry out all the required steps (pretreatment, hydrolysis and fermentation of both pentose and hexose sugars) in a single pot.

Solid state fermentation is also a promising approach over submerged fermentation to use lignocellulosic biomass for the production of industrial products. Therefore, by adapting combinational advanced techniques like biodiversity studies, meta-genomics, system biology, protein engineering, developing research for regulating different media and process parameters, high automation for regulation of parameters during operation, it will become a reality in near future to produce renewable and pollution free value-added fuels and chemicals by utilizing cost effective lignocellulosic materials.

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