Chapter 2
Role of Systematic Biology in Biorefining of Lignocellulosic Residues for Biofuels and Chemicals Production

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Abstract World has witnessed most unprecedented economic/industrial growth during past few decades. But this resulted in massive depletion in the fossil fuel reserves, and grave environmental concerns like green house gas emissions, climate change etc. Keeping in view the serious consideration there is a paradigm shift towards the exploration of renewable energy resources, and development of processes/products that are green, clean and ecobenign. Lignocellulosic biomass, being an inexpensive and abundant energy source could be exploited for the production of bioenergy and other oleochemicals. But due to recalcitrant nature of lignocellulosic biomass, which is attributed to presence of lignin and hemicelluloses making the substrate inaccessible to hydrolytic enzymes. Therefore, the major challenge in biomass to biofuel/bioactives is conversion delignification of lignocellulosic biomass. With the application of appropriate pretreatment technique, the complex biomass can be partially loosened and made accessible for hydrolysis. Environment friendly and cost effective biological pretreatment method using microorganisms offers advantages in getting the desired results in energy efficient manner. Appropriate combination of hydrolytic enzymes is required for complete degradation of cellulose and hemicelluloses into simpler sugars which served as raw material for further transformation. Successful saccharification of lignocellulosic biomass results in release of fermentable sugars which could act as starting material for production of bioenergy (Bioethanol, biobutanol, biohydrogen, biogas etc.) and other value-added products (Bioplastic, animal feed, composites, enzymes, xylooligosaccharides etc.). With the advancement in technology (green biotechnology), the conversion costs of lignocellulosic biomass could be lowered and product yields could be enhanced making the production processes more economical and alleviating the deleterious effects of harsh chemicals and fossil fuels on environment.

Keywords Biofuel · Lignocellulosic biomass · Pretreatment
Xylooligosaccharides · Polyhydroxybutyrate · Biohydrogen · Biobutanol
Saccharification

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2.1 Introduction

The current global energy appetite relies upon the use of fossil fuels which accomplish the needs of industrial and automobile sector. The massive use of fossil fuels as a major source of energy has not only left shortage of fuels but has also created alarming environmental concerns viz. green house gas emission, global warming etc., economic and social concerns (Nargotra et al. 2016). This has motivated the researchers to work intensively for finding out renewable sources of energy. Numerous renewable energy resources like wind energy, hydrothermal energy, oceaothermal energy, tidal energy, solar energy etc. have been explored as alternate sources, but suffer with their individual limitations. Among various renewable sources of energy, abundantly present lignocellulosic biomass serves as the most potential alternative to overcome the energy crisis (Vaid and Bajaj 2017).

Lignocellulosic biomass (LB) is an inexpensive feedstock and available on the earth's crust in plenty. It is easily accessible as agricultural and forest residues, municipal wastes etc. (Saini et al. 2015) and may act as a valuable substitute for fossil fuels (oil, natural gas and coal), which are finite and mostly non renewable (Mohr and Raman 2013). LB generally composed of 40–50% cellulose, 20–30% hemicellulose and 10–25% lignin (Saini et al. 2015). About 50–80% of polysaccharides available in LB can be subjected to microbial fermentation for production of several useful products. Cellulose is a highly stable polymer which is majorly composed of (1, 4)-D-glucopyranose units attached by β-1,4 linkages (Sindhu et al. 2016). The LB contains cellulose molecules which are held together by intermolecular hydrogen bonds in native state, but they have a strong tendency to form intra-molecular and intermolecular hydrogen bonds and this tendency increases the rigidity of cellulose making it crystalline, insoluble and highly resistant to most organic solvents. The major bottleneck in bioconversion of LB into biofuels is the recalcitrant nature of LB (Sharma and Bajaj 2014).

Pretreatment of the LB is imperative for efficient conversion of LB into useful based products. The process of pretreatment is essential for the modification of the plant cells so as to reduce the recalcitrance of the cell wall. An ideal pretreatment process should not only be cost effective, energy efficient and having a high performance rate but should also lead to the yield of high fermentable sugars and least inhibitor formation (Vaid and Bajaj 2017). Pretreatment removes the physical and chemical barriers that make native biomass amorphous and accessible to enzymatic hydrolysis (Sun et al. 2016). Several pretreatment approaches (physical, chemical and biological) have been developed for generating suitable raw material for saccharification.

Pretreatment plays an important role in increasing the permeability of the LB but efficiency of biomass to biofuel conversion can only be valued with complete utilization of reducing sugars. Saccharification of biomass after an appropriate pretreatment determines the viability of process (Khare et al. 2015). Saccharification is an important step but still remain as one of the bottleneck in LB-biofuel conversion strategy. Saccharification can be done by various carbohydrases like cellulases, xylanases and

other hydrolases but cellulases and xylanases are primarily the predominant ones for hydrolysis of biomass (Sartori et al. 2015). Cellulases act on cellulose part of LB which comprises of three predominant activities viz. exo-1,4-glucanase, endo-1,4-glucanase and cellobiase. Xylanases like endo-1-4,-β-xylanase, β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase, as well as acetylxylan esterases are required for the degradation of hemicellulosic component of biomass (Sadhu and Maiti 2013). Both enzymes help in determining the efficiency of saccharification when applied in synergism. Saccharifying enzymes may be used that are available commercially or produced *in-house* from microorganisms like bacteria/fungi. Commercial enzymes like Celluclast (cellulase), Novozyme 188 (xylanse) etc. have been very effective (Khare et al. 2015) but fewer studies showed that *in-house* produced enzyme complex gave better saccharification results (Sartori et al. 2015). Effective saccharification in turn may lead to enhanced LB-biofuel product generation.

Systematic biology has developed strongly during the last decades, partly due to improved molecular techniques and more advent of computer sciences. Systematic biology has enabled understanding of how life has developed and all the flora and fauna are on earth are related to one another in an evolutionary manner (Systematic Biology-Editorial Board 2014). The classification of the flora and fauna is carried out by studying morphological, embryology, physiological, molecular, behavioral, ecological and geographic characters (Hecht and Steere 1970). Plants have been classified and evolved as one as sole contributor to enable the sustenance of all other life forms. Plant based biomaterial have a wide range of application particularly lignocellulosic biomass (LB). Plant LB has emerged as a very novel application for biorefinery products. But plant cell walls have evolved to be recalcitrant to degradation as walls contribute extensively to the strength and structural integrity of the entire plant (Vaid et al. 2017). There is an immense structural diversity within the walls of different plant species and cell types within a single plant as well. Depending on diverse nature of LB used in biorefinery, various steps involved in pretreatment (such as chemical/enzymatic reactions) and subsequent fermentation of different sugar components to viable biofuel production needs to be studied and enhanced in terms of various process parameters and product yield (Foster et al. 2010).

The lignocellulosic biomass can be classified into woody biomass (hardwood and softwoods) and the agricultural crops. Woody biomass is structurally stronger and denser, and has higher lignin content than agricultural biomass. As a result, woody biomass is more recalcitrant to microbial and enzymatic actions than non-woody biomass. On the other side the agricultural crops contain less lignin content which make them easily accessible to the microbial enzymes for the production of biofuels like ethanol (Zhu and Pan 2010). Lignin is a complex heteropolymer of three *p*-hydroxycinnamoyl alcohol monomers i.e. *p*-coumaryl, coniferyl and sinapyl alcohols that are called as *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively, when incorporated into the polymeric form. Their contribution to lignin composition varies significantly among cell types, taxa and between tissues in the same plant. Among woody biomass, the angiosperms (hardwood) contain less amount of lignin which majorly contain G and S units with a little traces of H units whereas lycophytes, ferns and gymnosperms (softwoods) contain G units with

insignificant amounts of H units in their lignin (Cesarino et al. 2012). Hence, both agricultural crops and the woody biomass present as diverse plant forms that may be efficiently utilized in the modern concept of biorefineries.

Utilization of plant based renewable feed stock as a source for fuels and other commodity chemicals is the need of the hour and the concept of developing alternatives to the volatile petroleum market has been widely embraced by governments and scientific community alike. The potential for exploitation of LB is enormous as it comprises the non-edible portion of the plant and therefore, does not compete with food supplies. Moreover LB is extremely eco-friendly as it is renewable and possesses properties like biocompatibility and biodegradability (Almeida et al. 2012). Even though biofuels produced from LB presents a promising alternative, but the major bottleneck encountered in this approach is the high production costs making the overall process unprofitable. Therefore in order to overcome this challenge biorefining of LB for producing renewable oil as well as valuable co-products is being undertaken (Sun et al. 2016). Biorefineries focus on the economical production of value-added chemicals at high selectivities and yields. On one hand cellulosic ethanol, is considered as a strong substitution of petroleum-based polymers, where as hemicellulose could be converted to xylitole, levelinic acid, xylooligosaccharides among other. Lignin, the other principle component, could be marketed as a chemical (Kim et al. 2016).

Considering the enormous potential of LB, the present chapter deals with the strategies for biorefining and valorization of biomass to value added products. Recent advancements in the fields of pretreatment, metabolic engineering, enzyme production and fermentation would help in developing a suitable technology which could replace the deteriorating effects of fossil fuels.

2.2 Concept of Biorefineries

Lignocellulosic biomass (LB) from agriculture and forestry, which includes agro-industrial residues, forest-industrial residues, energy crops, municipal solid waste, and other materials, has emerged as an excellent feedstock for biorefineries that complement oil refineries as sources of fuels and other value added chemicals (Arevalo-Gallegos et al. 2017). Biorefinery in general terms imply the conversion of biomass into a number of useful products ranging from bulk products (bioenergy) up to specialty chemicals. It is a collection of processes that utilizes renewable grain, lignocellulosic or high moisture content biomass to produce a final useful product or a variety of products, in such a way that no waste is left behind. This concept is analogous to today's petroleum refinery, which produces multiple fuels and chemicals from petroleum (Moncada et al. 2016). A biorefinery is a network of facilities that integrates biomass conversion processes and equipment to produce biofuels, energy and chemicals from biomass. Many authors define the biorefinery as the analogy to current oil refineries, which produce multiple fuels and chemicals from petroleum (Morais and Bogel-Lukasik 2013).

The oil refineries and biorefineries are different from each other in two ways. The first is the raw material (biomass) that is used in biorefineries has not undergone the biodegradation of crude oil over millions of years. Biomass is organic matter derived from living organisms (Moncada et al. 2016). The second is the complexity after application of different existing and emerging technologies in order to obtain bioproducts integrally and simultaneously. In addition to this a biorefinery involves assessing and using a wide range of technologies to separate biomass into its principal constituents (carbohydrates, protein, triglycerides etc.), which can subsequently be transformed into value-added products. Lignocellulose is one of the most abundant bioresource in the world that is considered as a best cheap source of carbohydrates and has been applied as a potential substrate for the production of high value products including biofuels such as bioethanol, biodiesel and biogas. Apart from that it is easily available and highly abundant due to the fact that 75% of its composition is contributed by polysaccharide makes it a purely suitable raw material for the biofuel production (Saini et al. 2015).

2.3 Biofuel as Renewable Energy Source

The shortage of the fossil fuels and the increasing pollution rates has shifted the focus to the bioenergy. Biomass energy is a promising source of renewable energy, but the feedstock used for producing it should come from non-food crops or agricultural waste (second generation feedstock), to avoid competition with food sources and arable land. A number of biomass energy resources are found all over the world that mainly includes wood product industry wastes, municipal solid waste, agriculture residues and the energy crops (Saini et al. 2015). These biomass resources have an excellent potential to be used as the future substrate for biofuel production.

First generation (1G) biofuels are produced primarily from foods crops such as grains, sugar cane and vegetable oils. But the limitation in using the 1G ethanol is that it creates food versus fuel conflict (Mohr and Raman 2013). The second generation (2G) biofuels are generally obtained from non-edible LB, including residues of crops or forestry production (corn cobs, rice husks, sugarcane bagasse, forest thinning, sawdust, etc.), and whole plant biomass (energy crops such as switchgrass, poplar and other grasses). Biofuels obtained from vegetable oils produced from sources that do not directly compete with crops for high quality land (jatropha and microalgae) can also be labeled as second generation biofuels. The emergence of 2G biofuels is widely seen as a sustainable response to the increasing controversy surrounding the 1G biofuels as 2G biofuels possesses excellent quality features, and can be better controlled and maintained in time. LBs such as wood, grass, agricultural and forestry residues such as straw, firewood, sawdust, rice husks, coconut shell, groundnut shell, pine needles, bamboo, sugarcane baggase, cotton and chilli stalks etc. are the potential substrates for bioethanol and biogas production (British petroleum 2013). At present the production of the second generation ethanol is still in infancy and is produced only in few demo plants around the world that are not yet commercially feasible. At the

moment, Borregaard company located in Norway declares to be the largest producer of second generation ethanol with an annual production of 20,000 m^3 (Lennartsson et al. 2014).

It has been observed that the first-generation biofuels have increasingly been adopted, and these are projected to steadily increase in production. However, growing interest is being paid to second generation and third generation biofuels, which are not food competitors, and which might have better environmental performance, particularly in terms of lower green house gas emissions. Microalgae are currently being promoted as an ideal third generation biofuel feedstock because of their rapid growth rate, greenhouse gas fixation ability and high production capacity of lipids (Alam et al. 2015). The major advantage of using them is that they don't compete with food or feed crops, can be grown on non-arable land and saline water and a very short harvesting cycle (Harun et al. 2010).

2.4 Lignocellulosic Biomass for Bioethanol Production

Lignocellulosic biomass refers to crops, crops residues or forestry biomass. These include wood, grass, agricultural and forestry residues such as straw, firewood, sawdust, rice husks, coconut shell, groundnut shell, pine needles, bamboo, sugarcane baggase, cotton and chilli stalks, etc. that are considered as potential substrates for bioethanol production (Vaid and Bajaj 2017). LB mainly consists of 40–50% cellulose, 25–30% hemicellulose, 15–20% lignin, and traces of pectin, nitrogen compounds, and inorganic ingredients (Table 2.1, Mori et al. 2015). Cellulose and hemicelluloses together constitute approximately 70% of the entire biomass and are tightly linked to the lignin component through covalent and hydrogen bonds that make the structure highly recalcitrant to any treatment.

Cellulose represents the main constituent of LB, and is a polysaccharide that consists of a linear chain of D-glucose linked by β-(1, 4)-glycosidic bonds to each other. In a plant cell wall, cellulose exists in crystalline (organized) structure as well as in amorphous structure can be easily digested by enzymes (Kulasinski et al. 2014). Hemicelluloses located in secondary cell walls, are heterogeneous branched biopolymers containing pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids). Hemicelluloses are linked to cellulose by hydrogen bonds and to lignin by covalent bonds. Lignin is a complex hydrophobic, cross-linked aromatic polymer that is susceptible to microbial attack. It is a polyphenolic aromatic compound synthesized from phenylpropanoid precursors. Generally, the plant cell wall microstructure is regarded to be a matrix of lignin and polysaccharides intimately associating with each other that make the cell wall of the plant cell rigid and stable. Thus in order to make the components of lignocellulose (cellulose and hemicellulose) accessible to the microbial enzymes, a suitable pretreatment strategy is required (Behera et al. 2014; Chaula et al. 2014).

Table 2.1 Lignocellulose composition of different biomass

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Barley straw	31–45	27–38	14–19	Saini et al. (2015)
	33.3	20.4–28	17.1	Tye et al. (2016)
Corn stover	42.6	21.3	8.2	Sarkar et al. (2012)
	44	25.1	9	Danish et al. (2015)
	35–42.6	17–35	7–21	Tye et al. (2016)
Banana peels	18.2	10.5	14.8	Wadhwa and Bakshi (2013)
Rice straw	32–47	18–28	5.5–24	Tye et al. (2016)
	32	8.4	30.3	Danish et al. (2015)
	28–36	23–28	12–14	Saini et al. (2015)
Sorghum straw	32.4	27	7	Tye et al. (2016)
	32	24	13	Saini et al. (2015)
Wheat straw	35–45	20–30	12–15	Sarkar et al. (2012)
	37–42	27–32	13–15	Saini et al. (2015)
	33–45	20–32	8–20	Tye et al. (2016)
	45.1	9.2	37.4	Danish et al. (2015)
Sugarcane bagasse	65	–	18.4	Sarkar et al. (2012)
	32–48	19–24	23–32	Saini et al. (2015)
	43.9	21.5	17	Danish et al. (2015)
	45.4	28.7	23.4	Tye et al. (2016)
Oat straw	37.6	23.3	12.9	Tye et al. (2016)
Rye grass	34	20.6	24.4	Zheng et al. (2008)
Tomato pomace	12	12	39	Wadhwa and Bakshi (2013)
Algae (green)	20–40	20–50	–	Saini et al. (2015)
Grasses	25–40	25–50	10–30	Li et al. (2010)

2.5 Pretreatment of the Lignocellulosic Biomass

Lignocellulosics are abundantly available in nature, relatively distributed worldwide and also act as an alternate source of energy. Bioconversion of LB to liquid and gases is one of the prospective approaches for sustainable biofuels, biochemical and biomaterials as combined in a concept called biorefinery. The traditional microorganisms used to produce the valuable products such as ethanol cannot directly ferment the complex LB. Thus, a suitable pretreatment strategy is necessary and essential to hydrolyze the lignocellulosics into fermentable sugars. Due to highly recalcitrant nature LB needs pretreatment (chemical, physical and biological) prior to enzymatic hydrolysis (Sharma and Bajaj 2014).

Pretreatment removes the physical and chemical barriers that make native biomass recalcitrant and makes cellulose amenable to enzymatic hydrolysis, which is a key step in biochemical processing of lignocellulose based on the sugar platform concept. This effect is achieved by increasing the accessible cellulose surface area through solubilization of hemicelluloses and/or lignin, which are coating the cellulose of the native biomass (Jönsson and Martin 2016). Several factors have been reported to play instrumental role for developing optimal cost and energy-effective pretreatment process (Maurya et al. 2015). The aim of the effective pretreatment of lignocellulosic biomass should be focused on to increase the accessible surface area and decrystallize cellulose, along with partial depolymerization of cellulose and hemicellulose, to solubilize hemicelluloses and lignin. It should also maximize the enzymatic digestibility and minimize the loss of sugars, of the pretreated material, to minimize capital and operating costs and finally must also preserve the pentose (hemicellulose) fractions that limit the formation of toxic components which inhibit growth of fermentative microorganism.

A number of pretreatment techniques are available that increase the accessibility the fermentable sugars to the hydrolyzing enzymes.

2.5.1 Physical Pretreatment

The main purpose of physical pretreatment such as milling, grinding, chipping, freezing, radiation is to increase surface area and reduce particle size of lignocellulosic materials. Moreover, it leads to decrease degree of polymerization and decrystallization of feedstock. Combination of physical and other pretreatment method is usually used.

2.5.2 Chemical Pretreatment

Chemical pretreatment for LB involves different chemicals such as acids, alkalis, and oxidizing agents e.g. peroxide and ozone, dilute acid pretreatment using H_2SO_4/HCl are the most widely used methods. Pretreatment could have different effects on structural components of lignocellulose, based on the type of chemical used. Alkaline pretreatment, ozonolysis, peroxide and wet oxidation pretreatments are more effective in lignin removal whereas dilute acid pretreatment is more efficient in hemicellulose solubilization.

Acid pretreatment is one of the most popular methods to attain high sugar yields from LB (Lee et al. 2015). The main objective of acid pretreatment is to increase the accessibility of the enzymes to the cellulosic fractions by solubilizing the hemicellulosic fraction of the biomass. Alkaline pretreatment as compared to other chemical pretreatments, can be conducted at lower temperature and pressure causing less sugar degradation than acid pretreatment but the reaction times take several hours or days, or even weeks for softwood. Alkaline pretreatment of lignocellulosic materials causes swelling leading to an increase in internal surface area, decrease in the degree of polymerization and crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure making cellulose and hemicellulose available for the enzymatic degradation.

Ionic liquids have also been recognized as one of the potent pretreatment methods for the effective dissolution of cellulose and hemicellulose sugars. Ionic liquids (ILs) are thermally stable organic salts with potential application as 'green solvents' and ILs exhibit excellent physical characteristics including the ability to dissolve polar and non-polar organic, inorganic and polymeric compounds. Use of ILs is emerging as an efficient strategy for pretreating recalcitrant LB. Xu et al. (2015) isolated a novel ionic liquid-tolerant microorganism, *Fusarium oxysporum* BN secreting ionic liquid stable cellulase and reported that *F. oxysporum* BN directly converted IL pretreated rice straw to bioethanol yielding 0.125 g ethanol g^{-1} of rice straw.

2.5.3 Biological Pretreatment

A variety of bacteria and fungi can hydrolyze cellulose and hemicellulose into corresponding mono-sugars like glucose, arabinose, xylose, etc. (Table 2.2). Fungi such as brown, white, and soft-rot fungi are widely used for selective degradation of lignin and hemicellulose among which white-rot fungi seems to be the most effective ones. Biological pretreatments unlike physical and chemical pretreatment methods do not involve high temperature and pressure and does not require acids, alkali or any reactive species. This pretreatment is environmental friendly because of its low energy use and mild environmental conditions.

Table 2.2 Culture conditions and performances of various cellulolytic, hemicellulolytic and/or ligninolytic microorganisms

Microorganism	Activity	pH	Temperature (°C)	Treatment time (days)	Degradation (%)	References
Clostridium thermocellum	C, H	6.1–7.8	60	4–5	85–100	Rabemanolontsoa et al. (2015)
Clostridium cellulolyticum	C, H	7.2	32–34	3–6	20–75	Desvaux et al. (2001) as reviewed by Dionisi et al. (2015)
Ruminococcus albus	C, H	6.7–7.1	37	0.5–2	30–70	Pavlostathis et al. (1988) as reviewed by Dionisi et al. (2015)
Ruminococcus flavefaciens	C, H	6.5–6.8	39	2–7	54–87	Shi and Weimer (1992) as reviewed by Dionisi et al. (2015)
Actinotalea fermentans	C, H	6.5	30–55	28	60	Bagnara et al. (1987)
Trichoderma viride	C, H	5	30	0.5–1.2	50–75	Peitersen (1977) as reviewed by Dionisi et al. (2015)
Trichoderma reesei	C, H	4.8	28	7	100	Velkovska et al. (1997) as reviewed by Dionisi et al. (2015)
Pseudomonas spp.	L	5.3–7.8	30	7–60	20–52	Sørensen (1962) as reviewed by Dionisi et al. (2015)
Xanthomonas spp.	L	–	30	7–30	39–48	Odier et al. (1981) as reviewed by Dionisi et al. (2015)
Acinetobacter spp.	L	–	30	30	47–57	Odier et al. (1981) as reviewed by Dionisi et al. (2015)
Streptomyces cyaneus	C, H, L	–	28–37	21–28	29–52	Berrocal et al. (2000) as reviewed by Dionisi et al. (2015)
Phanerochaete Chrysosporium	L	–	39	14–30	28–60	Shi et al. (2008) as reviewed by Dionisi et al. (2015)
Echinodontium taxodii 2538	L	–	25	28	24	Zhang et al. (2007) as reviewed by Dionisi et al. (2015)
Pleurotus ostreatus	L	–	25–30	30–60	40–41	Kerem et al. (1992) as reviewed by Dionisi et al. (2015)

2.6 Enzymatic Saccharification of Pretreated Lignocellulosic Biomass

Lignocellulose can be hydrolytically broken down into simple sugars either enzymatically by cellulolytic enzymes or chemically by sulfuric or other acids (Zhang et al. 2012). However, enzymatic hydrolysis is becoming a suitable way because it requires less energy and mild environment conditions, while fewer fermentation inhibiting products are generated (Brummer et al. 2014). Enzymatic hydrolysis includes the processing steps that convert the carbohydrate polymers into monomeric sugars. The various potential factors that contribute to the resistance of biomass to enzymatic hydrolysis include cellulose crystallinity, accessible surface area and protection by lignin and cellulose sheathing by hemicelluloses. Enzymatic hydrolysis is carried out with cellulases at mild conditions of pH and temperature, 4.5 and 50 °C, respectively. Some proteins like swollenin play an important role in non-hydrolytically loosening the cellulosic fibril network and do not act on β-1,4 glycosidic bonds in cellulose. Swollenin increases the accessibility of cellulases to cellulose chains by dispersion of cellulose aggregations and thereby exposing individual cellulose chains to the enzyme (Santos et al. 2017). Enzyme related factors which affects hydrolysis includes enzyme concentration, enzyme adsorption, end-product inhibition, thermal inactivation and unproductive binding to lignin.

2.7 Ethanol Fermentation

Ethanol fermentation is a biological process in which sugars are converted by microorganisms to produce ethanol and CO_2. An important factor which prevents industrial utilization of lignocelluloses for bioethanol production is the lack of microorganisms able to efficiently ferment both pentoses and hexoses released during pretreatment and hydrolysis (Kang et al. 2014).

In recent years, the concept of consolidate bioprocessing (CBP) has emerged as an efficient method for the saccharification and fermentation of the sugars to produce ethanol and other organic acids. It involves depolymerization of the lignocellulosic matrix with simultaneous production of enzymes and ethanol in one single step. In CBP, the ethanol and all the required enzymes are produced by a single microorganism strain in a single bioreactor. CBP reduces the ethanol production cost by eliminating the operating costs and capital investment associated with purchasing or producing the enzymes. In CBP, mono or co-cultures of microorganisms can be used that directly ferment cellulose to ethanol. Khuong et al. (2014) optimized the alkaline pretreatment (NaOH) of sugarcane bagasse for consolidated bioprocessing fermentation by the cellulose fermenting fungus *Phlebia* sp. MG-60 and got an ethanol yield of 4.5 from 20 g l^{-1} of sugarcane bagasse. Brethauer and Studer (2014) successfully achieved 67% ethanol yield from pretreated wheat straw (dilute acid) using three naturally occurring strains: *Trichoderma reesei*, *Saccharomyces cerevisiae* and

Scheffersomyces stipites. Okamoto et al. (2014) isolated and characterized a strain of the white rot basidiomycete *Trametes versicolor* that was capable of efficiently fermenting xylose and found that strain, designated KT9427, was capable of assimilating and converting xylose to ethanol under anaerobic conditions with a yield of 0.44 g ethanol per 1 g of sugar consumed. So as a whole CBP serves as the most economically viable and less time consuming process that converts LB directly into ethanol in a single step appropriately.

2.8 Biohydrogen as Biofuel

Biohydrogen is considered as the fuel of future as its combustion generates huge energy and results only in the production of water as an end product making it a clean fuel (Singh et al. 2015a). Hydrogen possesses the gravimetric energy density at 141 MJ Kg^{-1} i.e. the highest in comparison to other biofuels. The second generation of biofuels mainly utilizes lignocellulosic materials for the production of liquid (ethanol, butanol) or gaseous (biohydrogen or biogas) fuels (Cheng et al. 2011; Datar et al. 2007). The third generation feedstock in the form of microalgae has received more attention in the biofuel production. Biofuel production from microalgae has gained positive ground because of their high carbohydrate and lipid content.

2.9 Microalgae for Biohydrogen Production

Algae are considered as the potential feedstocks for the production of third generation biofuels as biomass can be converted directly into energy. Microalgae are the photosynthetic organisms and are known as the primary producers in any ecosystem. Microalgae include dinoflagellates, green algae (chlorophyceae), golden algae (chryosophyceae) and diatoms (bacillariophyceae) (Jambo et al. 2016). They have relatively simple requirements for growth when compared to other sources of LB. Some algae may contain a huge amount of cellulose and hemicellulose content in their cell walls with accumulated starch as the main carbohydrate source (Domozych et al. 2012). In addition to this they also contain least amount of lignin which otherwise limits the accessibility of cellulose and hemicellulose to cellulolytic enzymes (Park et al. 2011). Both starch and most cell wall polysaccharides can be converted into fermentable sugars for subsequent biofuel production via microbial fermentation (Wang et al. 2011). The cultivation of microalgae shall not only reduce the need of arable land but may also channelize the waste water, saline and brackish waters for their growth and could be harvested nearly on daily basis (John et al. 2011).

Biohydrogen can also be directly produced by microalgae by photofermentation process. It is an anaerobic process that uses the hydrogenase enzyme for the oxidation of the ferredoxin. But all the hydrogenases produced by microalgae are not efficient enough and compete with many metabolic processes. So looking into the

insights of the hydrogenase action on the ferredoxin and their engineering may lead to generation of efficient hydrogenases in order to form biohydrogen in large amount (Yacoby et al. 2011). For an ideal production of biohydrogen co-culture of micro and macro algae can employed as some micro algae (*Arthrospiraplatensis*) may have a low C/N ratio that is not feasible for hydrogen production. Xia et al. (2016) co-cultured *Laminaria digitata* (macroalgae) and *A. platensis* (microalgae) pre-treated with 2.5% dilute H_2SO_4 at 135 °C for 15 min, with a total yield of carbohydrate monomers of 0.268 g g^{-1} volatile solids (VS) and an optimal specific hydrogen yield of 85.0 ml g^{-1} VS at an algal C/N ratio of 26.2 and an algal concentration of 20 g VS l^{-1}. Ding et al. (2016a) co-fermented *Laminaria digitata* (macroalgae) and *Chlorella pyrenoidosa* and *Nannochloropsis oceanic* (microalgae) that facilitated hydrolysis and acidogenesis, resulting in hydrogen yields of 94.5–97.0 ml per gVS which was 15.5–18.5% higher than mono-fermentation using *L. digitata*. Although hydrogen production from algae still seems years away from commercial viability, continued progress in this area shows its ultimate potential.

Pretreatment of lignocellulosic biomass is must for biohydrogen production. The selection of an effective and suitable pretreatment method is a prerequisite for the biohydrogen production from lignocellulosic biomass. Biological pretreatment method show some unique advantages such as low energy requirement, least inhibitors production and operability at room temperature. This pretreatment involves the microorganisms like white rot fungi that secretes the lignin degrading enzymes and hence increases the accessibility of cellulose to the hydrolyzing enzymes (Ren et al. 2009). After pretreatment, the lignin and hemicellulose are dissolved in the prehydrolysate. The free hemicellulose is then subjected to further hydrolysis that releases pentoses and hexoses like xylan, xylose, mannose, arabinose, galactose and glucose. The detailed pretreatment methods employed for various LB substrates for the production of biohydrogen are enlisted in Table 2.3.

Pretreatment of LB is followed by enzymatic saccharification of the complex sugars. Lignocellulose can be hydrolytically broken down into simple sugars either enzymatically by cellulolytic enzymes or chemically by sulfuric or other acids (Zhang et al. 2012). However, enzymatic hydrolysis is becoming a suitable way because it requires less energy and mild environment conditions, while fewer fermentation inhibitor products are generated (Brummer et al. 2014). Enzymatic hydrolysis is one of the most common and effective methods employed to generate fermentable sugars due to the high yields of sugars that can be obtained, with subsequent fermentation of these sugars to produce biohydrogen. The effectiveness of hydrolysis in the polysaccharides present in the lignocellulose substrates, therefore, is determined by an appropriate pretreatment, good selection of enzymatic complexes and cellulose accessibility (Meng and Ragauskas 2014). It depends on optimized conditions for maximum efficiency like hydrolysis temperature, time, pH, enzyme loading, and substrate concentration (Milagres et al. 2011).

Various microorganisms may be employed for biohydrogen production. Biological processes are carried out largely at ambient temperatures and pressures, and hence, are less energy intensive than chemical or electrochemical ones. A number of microorganisms have been found to produce hydrogen from the fermentable sug-

Table 2.3 Different pretreatment strategies and lignocellulosic biomass (LB) composition for the hydrogen production

Lignocellulose biomass	Cellulose (wt%)	Hemicellulose (wt%)	Lignin (wt%)	Pretreatment	Hydrogen production index	References
Corn stover	37.6	21.5	19.1	NaOH + Enzymatic	12.9 mmol l h^{-1}	Ren et al. (2010)
	N.R	N.R	19.1	H_2SO_4	3305 ml H_2 l^{-1}	Cao et al. (2009)
	36.5	31.3	11.9	H_2SO_4 + Microwave	182.2 ml	Liu and Cheng (2010)
Corn cob	38.9	42.2	10.9	HCl	107.9 ml H_2 g^{-1} TVS	Pan et al. (2010)
Corn stalk	33.64	24.4	8.65	Bio pretreatment	176 ml H_2 g^{-1} TS	Fan et al. (2008)
	38.92	20.87	21.52	HCl	149.69 ml H_2 g^{-1} TVS	Zhang et al. (2007)
Miscanthus	38.2	24.3	25	NaOH + Enzymatic	82.2 mmol H_2	De Vrije et al. (2001)
Rice straw	41.4	19.6	22.8	NH_4OH + H_2SO_4	2.7 mmol H_2 g^{-1} straw	Nguyen et al. (2010)
	33.1	26.7	35.9	NaOH	0.76 mol H_2 mol^{-1} xylose	Lo et al. (2010)
Soybean straw	39.6	14.6	23.4	HCl	60.2 ml H_2 g^{-1} dry straw	Han et al. (2012)
Sugarcane bagasse	33.63	23.88	4.31	H_2SO_4	1.73 mol H_2 mol^{-1} sugar	Pattra et al. (2008)
Sweet sorghum bagasse	38.50%	21.4	17.6	NaOH	2.6 mol H_2 mol^{-1} C6 sugar	Panagiotopoulos et al. (2010)
Wheat bran	8.27	33.7	N.R	HCl + Microwave	128.2 ml H_2 g^{-1} TVS	Pan et al. (2010)
Wheat straw	22.5	21.5	N.R	HCl	68.1 ml H_2 g^{-1} TVS	Fan et al. (2006)
	N.R	N.R	N.R	H_2SO_4	168.4 ml H_2 g^{-1} VS	Nasirian et al. (2011)

ars. Biological processes use the enzyme hydrogenase or nitrogenase as hydrogen producing protein. This enzyme regulates the hydrogen metabolism of uncountable prokaryotes and some eukaryotic organisms including green algae. The function of nitrogenase as well as hydrogenase is linked with the utilization of the products of photosynthetic reactions that generate reductants from water. Recent research studies on dark fermentative organisms have been intensively developed and new bacterial species have been isolated for this purpose. For effective production of biohydrogen, it is necessary to identify suitable fermentative microorganisms that can ferment pentose and hexose sugars from lignocellulosic biomass. The pentoses mainly consist of xylose as the main fermenting sugar.

A number of studies of hydrogen production from xylose have been reported using functional microorganisms and mixed cultures. Abdeshahian et al. (2014) successfully produced the fermentative hydrogen by *Clostridium* sp. YM1 with the cumulative hydrogen volume of 1294 ml l^{-1} with a hydrogen yield of 0.82 mol H_2 mol^{-1} xylose consumed. As the lignocellulosic biomass is a mixture of pentose and hexoses, studies have reported the use of both C_5 (pentose) and C_6 (hexose) fermenting microorganisms simultaneously. Ren et al. (2008) reported a hydrogen yield of up to 2.37 mol H_2 mol^{-1} substrate from a thermophilic strain of *T. thermosaccharolyticum* W16 that simultaneously fermented the mixture of glucose and xylose.

Anaerobic bacteria like those of *Clostridium* sp. have been found to ferment sugars due to its high production rate and the ability to use a wide range of carbohydrates including wastewater. *Clostridium* sp. is a typical acid and hydrogen producer which ferments carbohydrate to acetate, butyrate, hydrogen, carbon dioxide and organic solvent. Chong et al. (2009) isolated *C. butyricum* from palm oil mill effluent sludge with optimum hydrogen production at pH 5.5 with POME as substrate and a potential hydrogen yield of 3.2 l H_2 l^{-1} palm oil mill effluent. Some studies have also been reported where the crystalline cellulose was directly converted into hydrogen. Wang et al. (2008) reported that *Clostridium acetobutylicum* X9 generated the maximum hydrogen production and cellulose hydrolysis rate of 6.4 mmol H_2 h^{-1} g^{-1} dry cell and 68.3%, respectively, using microcrystalline cellulose as the substrate.

Dark fermentation may be employed for production of biohydrogen. Biohydrogen can be produced through thermochemical and biological technologies but biological techniques are preferred over thermochemical processes because of their ecological benefits and lower energy requirements (Bundhoo and Mohee 2016). Biohydrogen can be produced via biological processes from technologies such as dark fermentation (DF), photo fermentation, direct and indirect biophotolysis and water-gas shift reactions. However, the DF process is considered to be an efficient method that can have commercial value and importance in the future (Hallenbeck et al. 2012). DF is a process of degradation of organic substrates by anaerobic bacteria in absence of light and oxygen to produce biohydrogen. A series of biochemical reactions are involved in the breakdown and conversion of complex sugars into biohydrogen (Karthic and Shiny 2012). The complex polysaccharides are initially hydrolyzed into simple sugars by biological methods or different pretreatments. The simple sugars then undergo a chain of biochemical reactions in pathways like glycolysis, pyruvate formate lysate pathway, pyruvate ferridoxinoxidoreductase pathway, etc. In addition metal ions also

play an important role in the dark fermentation process as they assist in bacterial metabolism, cell growth, enzyme and co-enzyme activation and functioning and biohydrogen production (Sinha and Pandey 2011). Trchounian et al. (2017) pointed out the importance of Ni^{2+}, Fe^{2+}, Fe^{3+} and Mo^{6+} and some of their combinations for *E. coli* bacterial growth and H_2 production and their experiments found a 2.7 fold increase of hydrogen production by using different combinations of these metal ions.

2.10 Biobutanol as Biofuel

Amongst the production of biofuels acetone–butanol–ethanol (ABE) fermentation ranks the second largest bioprocess. The major product of ABE fermentation is biobutanol. Biobutanol is used as a solvent in the formation of various valued products like hormones, drugs, antibiotics, cosmetics, hydraulic fluids, vitamins etc. (Ding et al. 2016b). Recently, biobutanol is gaining interest as a direct replacement of gasoline. Besides being renewable it has similar properties to gasoline (Gottumukkala et al. 2013). It is an important alternative to bioethanol due to its superior chemical and physical features like lower viscosity, higher energy density, lower affinity to water, better blending capacities, lower hygroscopicity and less corrosive for certain motor parts (Liu et al. 2015a). It can be produced by two ways viz. petrochemically and through fermentation (Gottumukkala et al. 2013; Su et al. 2015).

Lignocellulosic biomass may be exploited for biobutanol production. Biobutanol production through fermentation of sugars using fermenting organism like *Clostridium* species is an industrially active process (Ding et al. 2016b). *Clostridia* strains produce acetone, butanol and ethanol (ABE) at a mole ratio of 3:6:1 during the biobutanol fermentation process (Plaza et al. 2017). However, high substrate cost, low product yield, and high recovery cost hinders its large-scale productivity. Recently, there have been resurging interests in producing biobutanol using inexpensive substrates like low-cost lignocellulosic biomass, developing microbial hyper producing strains, and optimized fermentation conditions (Ding et al. 2016b; Xue et al. 2016). But the process still suffers from low titer and productivity due to recalcitrant LB. The pretreatment of LB increases the feasibility of the biobutanol fermentation process (Xue et al. 2016). Various pretreatment methods have been executed for the biobutanol production from various lignocellulosic feedstocks like sugarcane bagasse, cassava, rice straw etc. as shown in Table 2.4.

A fermentation-pervaporation (PV) coupled process was investigated for the production of ABE from cassava. Glucose consumption rate and ABE productivity increased by 15 and 21%, respectively, in batch fermentation–PV coupled process as compared to batch fermentation without PV. In a continuous fermentation–PV coupled process, the substrate consumption rate, solvent productivity and yield increased by 58, 81 and 15% after 304 h respectively. Thus, the fermentation–PV coupled process helps in decreasing the cost in ABE production (Li et al. 2014). Batch fermentation of sugar hydrolysate (41 g l^{-1} total sugars) obtained after microwave-alkali pretreatment of sugarcane bagasse in assistance with gamma-valerolactone yielded

Table 2.4 Lignocellulosic biomass for biobutanol production

Lignocellulosic biomass	Pretreatment method	Biobutanol production	References
Cornstalk	Alkali-catalyzed organosolv	9.9 g l^{-1}	Tang et al. (2017)
Corn stover	Ionic liquid, 1-butyl-3-methylimidazolium chloride [Bmim][Cl]	7.4 g l^{-1}	Ding et al. (2016b)
Rice straw	Sulphuric acid	3.43 g l^{-1}	Gottumukkala et al. (2013)
Apple pomace	Autohydrolysis, acids, alkalis, organic solvents and surfactants	9.11 g l^{-1}	Hijosa-Valsero et al. (2017)
Sugarcane bagasse	Gamma-valerolactone	9.3 g l^{-1}	Kong et al. (2016)
Birch kraft black liquor	Acid-hydrolysis followed by CO_2 acidification	7.3 g l^{-1}	Kudahettige-Nilsson et al. (2015)
Cassava	Fermentation–pervaporation (PV) coupled process	122.4 g l^{-1}	Li et al. (2014)
Eastern redcedar	Sulfuric acid, sodium bisulfate	13 g l^{-1}	Liu et al. (2015a)
Switchgrass	Hydrothermolysis	11 g l^{-1}	Liu et al. (2015b)
Brewer's spent grain	Sulfuric acid	75 g kg^{-1}	Plaza et al. (2017)
Rice straw	Sodium hydroxide	0.16 g g^{-1}	Rahnama et al. (2014)
Sugarcane bagasse	Liquid hot water, microwave	6.4 g l^{-1}	Su et al. (2015)
Corn stover	Sodium hydroxide	11.2 g l^{-1}	Xue et al. (2016)
Jerusalem artichoke stalk	Sodium hydroxide or/and hydrogen peroxide	11.8 g l^{-1}	Xue et al. (2017)

a high acetone-butanol-ethanol concentration of 14.26 g l^{-1}, including 4.1 g l^{-1} acetone, 9.3 g l^{-1} butanol and 0.86 g l^{-1} ethanol (Kong et al. 2016).

Acetone does not qualify as biofuel. ABE fermentation with known *Clostridium* species produces acetone in addition to butanol and ethanol which results in net low yield of biofuel solvents. *Clostridium sporogenes*, a non neurotoxigenic counterpart of group 1 *C. botulinum* produces ethanol and butanol without producing acetone in the final mixture, which is advantageous in converting biomass to alcoholic biofuels (Gottumukkala et al. 2013). Gottumukkala et al. (2013) evaluated biobutanol yield of 3.43 g l^{-1} and a total solvent yield of 5.32 g l^{-1} using *Clostridium sporogenes* BE01 from enzymatic hydrolysate of acid pretreated rice straw. Brewer's spent grain (BSG) is a promising lignocellulosic industrial waste, available throughout the year in large amounts at very low cost, for ABE fermentation. Plaza et al. (2017) investigated sulfuric acid pretreatment of BSG at pH 1, 121 °C and different solid loadings (5–15% w/w) followed by enzymatic hydrolysis and ABE fermentation by *Clostrid-*

ium beijerinckii DSM 6422 of non-washed and washed pretreated BSG. A higher titre of biobutanol (75 g biobutanol kg^{-1} BSG) and ABE (95 g ABE kg^{-1} BSG) was obtained when 15% w/w pretreated unwashed BSG was utilized. Fermentation of washed pretreated BSG yielded 6.0 ± 0.5 g l^{-1} of butanol which was lower than that obtained in case of control (7.5 ± 0.6 g l^{-1} butanol, Plaza et al. 2017).

In a study conducted by Rahnama et al. (2014) rice straw was used for its bioconversion to biofuels such as biobutanol. Sodium hydroxide (2%, w/v) pretreatment of rice straw was executed and resulted in 29.87 g l^{-1} reducing sugar after saccharification using *T. harzianum* SNRS3. The sugar hydrolysate was fermented using *Clostridium acetobutylicum* ATCC 824 and yielded 0.27 g ABE yield g^{-1} reducing sugar and 0.16 g biobutanol g^{-1} reducing sugar (Rahnama et al. 2014). A sequential, combinatorial lignocellulose pretreatment procedure for microbial biofuel (ABE) fermentation from sugarcane bagasse was designed to increase sugar yields and reduced generation of microbial growth inhibitors (Su et al. 2015). A series of methods including microwave decomposition, enzyme hydrolysis, ammonia immersion, microbial decomposition, and liquid hot water pretreatment were performed so as to obtain high sugar yields and limited inhibitor production. To assess the effectiveness of sequential, combinatorial lignocellulose pretreatment procedure for biobutanol production through microbial *Clostridium beijerinckii* NCIMB 8052 conversion, two schemes viz. simultaneous saccharification fermentation and separate hydrolysis fermentation were used of which simultaneous saccharification fermentation revealed the highest concentrations of butanol (6.4 g l^{-1}) and total ABE (11.9 g l^{-1}) as compared to that with the separate hydrolysis fermentation method (Su et al. 2015).

The feasibility of producing biobutanol from any LB feedstock also depends on other factors like mild pretreatment approach, detoxification of sugar hydrolysate etc. The detoxification process is an important step need to be carried after pretreatment because most of conventional pretreatment approaches like acid hydrolysis often produce inhibitors like furfural and 5-hydroxymethylfurfural that greatly affects the efficacy of the whole biobutanol fermentation process (Su et al. 2015; Kudahettige-Nilsson et al. 2015).

Kudahettige-Nilsson et al. (2015) studied ABE fermentation of acid-hydrolyzed xylan recovered from precipitate of hardwood kraft black liquor obtained by CO_2 acidification. Activated carbon was used for the detoxification of hydrolysate in order to evaluate the impact of inhibitor removal and fermentation. Mini scale fermentation of semi-defined P2 media and batch fermentation of the hydrolysate using *Clostridium acetobutylicum* ATCC 824 resulted in a total solvent yield of 0.34 and 0.12–0.13 g g^{-1}, respectively, of which 7.3 and 1.8–2.1 g l^{-1} of butanol concentration was obtained. Kudahettige-Nilsson et al. (2015) for the first time studied the process for the production of a biologically-derived butanol-biofuel from xylan recovered directly from industrial kraft pulping liquors as a feedstock and also demonstrates the feasibility of the process. Liu et al. (2015b) evaluated hydrothermolysis pretreatment based butanol production from switchgrass. Non-detoxified hydrolysate showed poor butanol production (1 g l^{-1}) due to the presence of inhibitors. After detoxification with activated carbon the butanol titer increased up to 11 g l^{-1} with a total (ABE) concentration of 17 g l^{-1}.

2.11 Strategical Improvements for Biobutanol Production

Adoption of IL based pretreatment of LB for biobutanol production has received an immense interest during recent years. ILs are the greener solvents which are able to reduce cellulose crystallinity, hemicelluloses and lignin content of biomass (Vaid and Bajaj 2017). ILs increases the surface area of biomass which in turn increasing the enzymatic hydrolysis kinetics, and the yield of fermentable sugars. In addition, low melting points, wide liquid temperature range, high thermal and chemical stability, non-flammability, negligible vapor pressure, consisting of ions (cations and anions) and good solvating properties makes ILs an eminent pretreatment solvent. Ding et al. (2016b) reported fresh and recycled IL [Bmim][Cl] based pretreatment of corn stover hydrolysate for biobutanol fermentation using *Clostridium saccharobutylicum* DSM 13864. A 18.7 and 24.2 g l^{-1} of sugar hydrolysate was produced from pretreated corn stover using ten times recycled and fresh [Bmim][Cl]. Fermentation of corn stover-sugar hydrolysate obtained by fresh [Bmim][Cl] resulted in 7.4 g l^{-1} biobutanol, while 7.9 g l^{-1} biobutanol was achieved in fermentation using hydrolysate pretreated by ten times recycled IL with similar levels of acetone and ethanol (Ding et al. 2016b).

Type of buffer plays an important role in biobutanol production. Eastern redcedar, an invasive softwood feedstock was targeted for butanol production using *Clostridium acetobutylicum* ATCC 824 and *Clostridium beijerinckii* NCIMB 8052. In the acetate buffer medium, both the strains grew well and yielded 3–4 g l^{-1} biobutanol from redcedar hydrolysate as compared to that in citrate buffer medium. After detoxification of inhibitors by activated carbon from redcedar hydrolysate, butanol and total ABE concentration reached up to 13 and 19 g l^{-1} (Liu et al. 2015a). The strength of buffer also has a significant impact on lignocellulosic butanol fermentation (Xue et al. 2016). The effect of various strengths (20–100 mM) of citrate buffer on enzymatic hydrolysis and corn stover feedstock based ABE fermentation was investigated (Xue et al. 2016). The enzymatic hydrolysis is not affected by varied strength of citrate buffer but greatly influenced the production of ABE fermentation using corn stover hydrolysate. With 30 mM citrate buffer the maximum butanol and ABE concentrations of 11.2 and 19.8 g l^{-1} respectively, which was concentrated to 100.4 g l^{-1} butanol and 153.5 g l^{-1} ABE by vapor stripping–vapor permeation process (Xue et al. 2016).

Optimization is an important component for the efficiency of any bioprocess. Optimizing various process variables may help in reducing the cost of whole bioprocess and increasing the product yield (Vaid et al. 2017). Hijosa-Valsero et al. (2017) studied the production of biobutanol from apple pomace after pretreating it using five different soft physicochemical pretreatments viz. autohydrolysis, acids, alkalis, organic solvents and surfactants followed by saccharification. These pretreatments were compared and optimized in a high-pressure reactor using working parameters like temperature, time and reagent concentration. The surfactant polyethylene glycol 6000 (1.96% w/w) based pretreatment of apple pomace released 42 g l^{-1} sugars at relatively mild conditions (100 °C, 5 min) with less production of inhibitors and without detoxification yielded 3.55 g l^{-1} acetone, 9.11 g l^{-1} butanol, 0.26 g l^{-1} ethanol

after fermentation using *Clostridium beijerinckii* CECT 508 in 96 h (Hijosa-Valsero et al. 2017).

Optimized pretreatment protocol was developed for efficient biobutanol production from cornstalks (Tang et al. 2017). Alkali-catalysed organosolv pretreatment of biomass was performed. After optimization of process parameters, about 80% of the total lignin was removed at 110 °C, 4% (w/w dry cornstalk) NaOH, 90 min reaction time, and 60% v/v ethanol, with minimal hemicellulose degradation. A total of 83.7% monosaccharide was obtained after enzymatic hydrolysis which released an ABE concentration of 11.9 g l^{-1} after fermentation with 9.9 g l^{-1} concentration of biobutanol (Tang et al. 2017).

2.12 Lignocellulosic Biomass as Source of Prebiotics

Prebiotics are non-digestible carbohydrates which are capable of mediating alteration in the gut microflora by selectively stimulating the growth and/or activity of certain health benefiting bacteria. Prebiotic supplementation basically involves using carbohydrates of varying chain lengths from diverse sources including polysaccharides from plant cell wall material that resist getting digested in the upper gastrointestinal tract and on reaching the colon are metabolized to form short chain fatty acids comprising mainly of propionic acid, acetic acid, butyric acid. Short chain fatty acids apart from creating a hostile environment for the survival of gut pathogens and can also act as a source of energy for the host (Geigerová et al. 2017).

Annually tons of LB is generated in the form of plant waste from various post-harvest processing activities (Jeske et al. 2017). Thus high yield and rich carbohydrate contents make lignocellulosic biomass an attractive option for production of bioactives, having nutritional and functional value, which could be incorporated into foods. The hemicellulose component consist mainly of xylan, which is a polysaccharide made up of backbone of xylose linked by β-1,4-xylosidic linkages (Biely et al. 2016). Xylan is one of the major structural components of woody tissues of dicots, monocots, some grasses and tissues of cereal grains and could also act as an inexpensive source for retrieving xylan based-xylooligosaccharide (XOS) prebiotics that selectively stimulates the growth of beneficial bacteria (Lin et al. 2016).

Currently non-digestible oligosaccharides falling in the range of di-, oligo-, and poly-oligosaccharides are known to possess prebiotic properties. Plant material like corncobs, straws, bagasse, rice hulls, malt cakes and bran, which are abundant, inexpensive, and renewable biomass, being naturally rich in resistant sugars and polysaccharides like xylan and therefore, can also act as an inexpensive source for retrieving fructan and xylan based prebiotics by applying simple hydrolysis techniques (Moniz et al. 2016). Therefore an extensive research directed at conversion of agro-residues into functional food ingredients from underutilized agro-wastes becomes pertinent and is the need of the hour (Samanta et al. 2015).

Application of probiotics is one of the most promising approaches for averting dysbiosis, and restoring normal gut microbiota. These microorganisms are live micro-

bial feed supplements that improve the intestinal microbial population of the host. The benefits of probiotics include their capacity in controlling intestinal infection, reducing elevated serum cholesterol levels, beneficially influencing the immune system, improving lactose utilization, and having anticarcinogenic activity (Bajaj et al. 2015). The survival of probiotics is suppressed from the environmental stress, such as oxygen, acids and environment of digestive system. Constant efforts are being made to increase the number and/or the activity of beneficial probiotic bacteria in the gut. Therefore, to overcome this challenge prebiotic approach is being employed, which essentially involves the administration of non-viable entity (Noori et al. 2017).

Inulin, fructooligosaccharides, galactooligosaccharides, lactulose and polydextrose are established prebiotics where as isomaltooligosaccharides, xylooligosaccharides and lactitol are emerging ones. Inulin and fructooligosaccharides, are the most dominating prebiotics due to low-calorie, fat-replacement ability, overall texture, mouth-feel and flavor. The most known prebiotics, with the exception of inulin, which is a mixture of fructooligo and polysaccharides, are indigestible oligosaccharides having of 3–10 carbohydrate monomers (Flores et al. 2016). The health benefits associated with the administration of prebiotics are mainly due to an increase in the production of short chain fatty acids. They act as a source of energy and a signaling molecule on the G-protein coupled receptor. Short chain fatty acids play important role in regulating glucose metabolism and energy homeostasis. Metabolism of acetate in human occurs mainly in brain, kidney muscle and heart whereas. Butyrate exerts prodifferentiation, anti-proliferation and anti-angiogenic effects on colonocytes. On the other hand propionate suppresses cholesterol synthesis by acting as a possible gluconeogenic precursor (Valdés-Varela et al. 2017).

2.12.1 Xylooligosaccharides as Prebiotics

Xylooligosaccharides (XOS) are oligomers of two to ten xylose molecules linked by β-1–4 bonds and have substitution of acetyl, phenolic, and uronic acid. They are naturally present in fruits, vegetables, bamboo, honey, milk, onions, garlic, artichoke, chicory etc. (Singh et al. 2015b). XOS are commercially available in the form of a white powder with degree of polymerization (DP) ≤ 20. For food industry applications, XOS chains of 2–4 units are considered. They have been shown to possess pH stability over a wide range (2–8) and withstand low gastric pH. Moreover, XOS show heat resistance and remain stable sterilization. Furthermore, they have low calorie content and are able to achieve appreciable biological effects at a low dietary dose (Singh et al. 2015b).

XOS prebiotics are capable of stimulating the growth of intestinal beneficial bacteria Bifidobacteria. The health benefits associated with XOS are mainly due to their effects on the gastrointestinal flora (Belorkar and Gupta 2016). Results obtained from in vitro as well as in vivo assays have proved that *Bifidobacterium* spp. (*B. adolescentis*, *B. infantis*, *B. longum*, *B. bifidum* etc.) and most *Lactobacillus* spp. are capable of utilizing XOS. Bacteroides can also utilize XOS but to a lesser

extent. Whereas common gut enteropathogens like *Staphylococcus*, *Escherichia coli* and *Clostridium* spp. cannot do the same (Nieto-Dominguez et al. 2017).

The prebiotic potential of XOS is being extensively studied. Nieto-Dominguez et al. (2017) demonstrated the prebiotic potential of XOS mixture produced from birchwood xylan. There was short chain fatty acid production, an increase bifidobacteria population, and beneficial commensals and a decrease in potentially pathogenic bacteria, confirming the prebiotic nature. In another study, XOS produced from sugarcane bagasse supported the growth of bifidobacterial strains, with simultaneous production of short chain fatty acids, under anaerobic conditions (Reddy and Krishnan 2016). Similarly, prebiotic XOS from corn straw resulted in an increased bifidobacteria populations and high short chain fatty acids production (Moniz et al. 2016).

Of all the known oligomeric prebiotics, XOS have garnered much interest in the recent years due to its numerous positive effects viz. increased mineral absorption, immune stimulation, promoting pro-carcinogenic enzymes and have antiallergy, antiinfection, antiinflammatory and antioxidant properties. XOS also stimulates increased levels of bifidobacteria to a greater extent than does fructooligosacchardies and other oligosaccharides are required at lower doses than fructooligosacchardies (De Figueiredo et al. 2017)

2.13 Delignification of Biomass for XOS Production

Lignocellulosic agro-residues, from which XOS are produced by various chemical, biological, or by combination of various processes have xylan as xylan-lignin complex in the biomass and is therefore, resistant to hydrolysis. Therefore, XOS production is carried out in a sequential manner starting from removal of lignin, followed by extraction of xylan, and finally followed by enzymatic hydrolysis for the production of XOS (Rabemanolontsoa and Saka 2016) (Fig. 2.1). Lignin is closely attached to the polysaccharide component through non-covalent and covalent linkages structural linkages thus obstructing the detachment of xylan from biomass and drastically reduces the yield of xylan. The presence of lignin largely affects the efficiency of various xylan extraction techniques by hindering the contact of xylan in raw materials with various xylan solubilizing chemicals making the overall process non-productive. Therefore, delignification of the biomass becomes pertinent lignin removal increases the pore size and makes the surface of the lignocellulosic materials more accessible (Samanta et al. 2015). Furthermore delignification reduces the recalcitrance of the biomass thus making it more amiable to subsequent processing.

Various pretreatment techniques have been employed for delignification. Reddy and Krishnan (2016) employed aqueous ammonia for delignification of sugarcane bagasse and achieved a higher production of XOS from the pretreated biomass. Cassava peel and waste were successfully delignified after pretreatment with 0.5% (w/v) sodium hypochlorite solution for 5 h (Ratnadewi et al. 2016). In another study, various pretreatment strategies such as Fenton, sonocatalytic, and sonocatalytic–synergistic

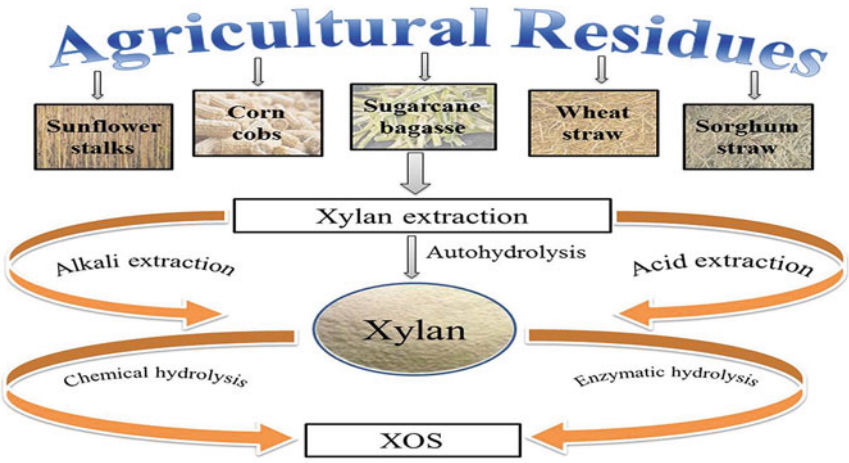

Fig. 2.1 Processes involved in the production of xylooligosaccharides in agro-waste

Fenton were employed to expose lignin content in corncob and enhance the enzymatic XOS production (Kaweeai et al. 2016). A two-stage delignification adopted using calcium hydroxide and peracetic acid was successfully used for kenaf wherein there was appreciable delignification and a high amount of hemicellulose was maintained in the pretreated biomass (Azelee et al. 2014). Bian et al. (2013) employed acidic sodium chlorite solution for delignification of sugarcane bagasse. Sodium hypochlorite pretreatment effectively removes lignin from corncob (Chapla et al. 2012). *Miscanthus* biomass was delignified using a combination of sodium chlorite and acetic acid (Li et al. 2016). Therefore, delignification of biomass is necessary in order to expose the complex structure of lignocellulosic biomass and for making the subsequent treatment steps more effective.

2.14 Xylan Extraction from Lignocellulosic Biomass

The surrounding lignocellulosic components as well as substituents on the xylan backbone restrict the access to xylosic linkages. Moreover, ether bonds are instrumental in linking the hemicellulose with the lignin components via single bonds to an oxygen atom in the biomass. Therefore, for manufacturing XOS from a suitable xylan-rich biomass, the ether bonds of the xylan backbone are targeted, using different approachs, to produce compounds of lower polymerization degree (Rabemanolontsoa and Saka 2016). Xylooligosaccharide production from various feedstocks is a systematic process involving: delignification of native, xylan-containing LB, solubilization and extractiot of xylan from delignified biomass and hydrolysis

of xylan to XOs by steam, dilute solutions of mineral acids, enzymes, etc. (Sun et al. 2016). Therefore, the raw lignocellulosic materials are pretreated with alkali, acids, high temperature autohydrolysis, among others, for the extraction of xylan; the extracted xylan is than subjected to hydrolysis for XOS production.

2.14.1 Alkaline Extraction

Alkaline pretreatment involves dissolution of hemicellulose and saponification of ester and ether bonds as major chemical reactions during extraction process. Alkaline method is an effective method for xylan extraction as it separates structural linkages between hemicellulose and cellulose, avoiding fragmentation of the hemicellulose polymer. The main targets of alkali treatment are ester and ether linkages in hemicelluloses, thus cleaving them down, promotes solubilization of hemicelluloses (Rajagopalan et al. 2017). Furthermore, it causes swelling of LB, which increase its internal surface area decreases the degree of polymerization and crystallinity, thus making hemicellulose more accessible. It also removes acetyl and various uronic acid substitutions on hemicellulose that further, increase the accessibility of hemicelluloses (Kim et al. 2016). Most commonly used alkaline reagents for xylan extraction are NaOH, $Ca(OH)_2$, KOH and Na_2CO_3. Alkaline hydrolysis is carried out at lower temperature and pressure resulting in less sugar degradation. Moreover, as alkali extraction is carried out under ambient conditions, it eliminates the need of specially designed reactors in order to cope up with the severity of the reaction. Recovery of reagents is also possible in some of the alkaline pretreatment methods (Sun et al. 2016).

Rajagopalan et al. (2017) solubilize xylan from pretreated mahogany and mango wood sawdust with NaOH solution (15% w/v) for 24 h. Similarly, Li et al. (2016) extracted xylan from *Miscanthus* biomass with 10% v/v KOH at 25 °C for 16 h. Cassava peel and waste (Ratnadewi et al. 2016) and sugarcane bagasse (Bian et al. 2013) gave a xylan yield of 4.83 and 6.23 and 30% w/v respectively, by carrying out xylan extraction with 10% w/v NaOH for 24 h. Yadav and Hicks (2015) employed an alkaline sodium hydroxide-hydrogen peroxide extraction to obtain arabinoxylans from barley hulls and straws by followed by ethanol precipitation. A yield of 20.51% and 7.41–12.94% was obtained from barley hulls and barley straws, respectively. A xylan yield of 85% w/v was obtained using 12% NaOH in combination with steam application from sugarcane bagasse (Jayapal et al. 2013). Similarly alkaline treatment coupled with steam treatment has been used in other studies (Li et al. 2013; Samanta et al. 2012). Chapla et al. (2012) obtained a xylan yield of 30% w/v from wheat straw and rice straw using dilute alkali treatment (1.25 M NaOH) for 3 h. Similar to the present studies, garlic straw (Kallel et al. 2015) and corncob (Driss et al. 2014; Haddar et al. 2012) were subjected to mild alkali treatment and a high amount of xylan could be extracted from the biomass. The targeted nature of alkaline extraction and high yield of xylan that can be achieved makes it a method of choice for xylan extraction.

2.14.2 Acid Extraction

In acid extraction the susceptibility of the glucosidic bonds of hemicelluloses is exploited to solubilize hemicelluloses from lignocellulosic materials (Sun et al. 2016). Concentrated as well as diluted acids are used to extract xylan from various lignocellulosic materials. Various mineral acids, such as H_2SO_4, HCl, H_3PO_4 and HNO_3 have been commonly used in the process. Concentrated acid pretreatment is less attractive due to the severe degradation of hemicellulose, formation of inhibitors, high toxicity and corrosiveness of the process (Singh et al. 2015c). On the other hand, dilute acid pretreatment promotes hydrolysis of hemicelluloses, resulting in high recovery of hemicelluloses in the liquid fraction and high cellulose content in the solid fraction. However, higher temperature (200 °C) and strong reaction conditions are required to increase the yield of xylan from biomass, thus causing degradation of the amorphous hemicelluloses (Singh et al. 2015c).

Gowdhaman and Ponnusami (2015) subjected corncobs to 0.1% w/v H_2SO_4 treatment at a temperature of 121 °C for 1 h. A xylan yield of 14.7% w/v was achieved with dilute acid treatment. Similar pretreatment with dilute acid (0.1% w/v H_2SO_4) followed by autoclaving for 1 h gave 26.57 g of xylan (Chapla et al. 2012) and 39.2% of xylan (Aachary and Prapulla 2009) from raw dried corncobs. Ruiz et al. (2013) carried out xylan extraction from sunflower stalks by dilute sulfuric acid (1.25% w/v) treatment at 175 °C and 5 min. Liquid fractions showed up to 33 g xylan per 100 g raw material. In another study, Otieno and Ahring (2012) treated lignocellulosic biomasses with 0.1% H_2SO_4 at 145 °C for 1 h. Xylan comprised of more than 20 g dry matter per 100 g of the biomasses. The excessive degradation of biomass associated with using acid makes it a less preferred method for xylan extraction.

2.14.3 Autohydrolysis

Autohydrolysis or hydothermolysis is a non-chemical process of xylan extraction. In this process, xylan is deacetylated in an aqueous medium. Autohydrolysis occurs in presence of hydronium ions [H^+] generated from water and acetic groups released from hemicelluloses. H^+ ions produced by water ionization act as catalysts in higher concentrations and high temperatures thus, providing an effective medium for extraction (Surek and Buyukkileci 2017). The physical disruption of the lignocellulose structure also takes place, since high pressure is involved. This results in decreased crystallinity as well as the degree of polymerization. The hydrolysis liquor obtained thereafter is rich in hemicelluloses or hemicelluloses derived sugars and can therefore be further converted into high value products. Hydothermolysis has become a popular technique for xylan extraction as there is no catalyst required and low inhibitor formation. Moreover low reactor cost is involved thus making the overall process economical (Rabemanolontsoa and Saka 2016). The major drawback of autohydrolysis is the occurrence of several undesirable side-processes resulting in

the accumulation of unwanted compounds like monosaccharides, furfural, and others, thereby making purification necessary and thus increasing the overall cost of the process.

Surek and Buyukkileci (2017) carried out autohydrolysis of hazelnut (*Corylus avellana* L.) shell for obtaining high amount of xylan. Moniz et al. (2016) performed autohydrolysis of corn straw at a temperature of 215 °C and the autohydrolysis liquor obtained afterwards was rich in xylan. *Miscanthus* × *giganteus* hybrids were subjected to autohydrolysis and the hydrolysate obtained after treatment was rich in xylan content (Chen et al. 2016). Hydrothermal pretreatment given to sweet sorghum stems at a high temperature of 170 °C for 0.5 h resulted in high yield of hemicellulose with a relatively low level of xylose and other degraded products (Sun et al. 2015). In another study, water soluble fibers separated from ground corn flour and distillers dried grains with soluble were subjected to autohydrolysis at 180 °C temperature and 20 min hold time resulted in high xylan yield (Samala et al. 2015). Wheat bran samples were subjected to coupled aqueous extraction followed hydrothermal treatment to evaluate their potential as a raw material for obtaining of xylan-derived prebiotics. Hemicellulose rich liquid was obtained after second stage of treatment (Gullon et al. 2014). Rice straw subjected to autohydrolysis at 210 °C yielding a maximum of 40.1 g per 100 g of initial xylan (Moniz et al. 2014). A high yield of hemicellulose was obtained from bamboo culm when it was autohydrolyzed at 180 °C for 30 min (Xiao et al. 2013). Therefore autohydrolysis is a promising technique for conversion of agricultural by-products into useful, high value products, such as prebiotic oligosaccharides.

2.15 Enzymatic Production of Xylooligosaccharides

The current trend of sustainable development has encouraged efforts towards development of environment-friendly techniques for utilization and conversion of xylan component of plants into XOS. Therefore, enzymatic production of XOS, due to its highly specific nature and comparatively lesser amounts of impurities in the products in comparison to thermo-chemical processes, is the method of choice for industries engaged in food and pharmaceutical production (Sun et al. 2016). Enzymatic hydrolysis is preferred over acid hydrolysis for XOS production as acid hydrolysis involves high temperature and always forms xylose, accompanied by toxins, which should be removed leading to high process cost due to downstream processing to obtain highly pure XOS. The enzymatic hydrolysis involves low temperature, and high specificity preventing toxic byproducts formation (Morgan et al. 2017).

Xylanase is the key enzyme for the hydrolysis of xylan producing XOS of variable length ranging from 2 to 10. For XOS production, First endo-β-1,4-xylanase hydrolyze the xylan backbone followed by enzyme like β-xylosidase amd glycosidases for cleaving side chain groups. Xylanase used should have high endo-xylanase activity and low or negligible exoxylanase or β-xylosidase activity as it produces high amount of xylose causing inhibitory effects on the production of XOS. These

enzymes are capable of operating under a wide range of temperature, pH, water activity, and redox potential (Biely et al. 2016).

Liu et al. (2017) produced xylooligosaccharides using *Bacillus amyloliquefaciens* xylanase and xylobiose and xylotriose were the major products, respectively. In another study, xylanase from *Bacillus subtilis* Lucky9 produced xylobiose and xylotriose from beechwood xylan and corncob, respectively (Chang et al. 2017). Reddy and Krishnan (2016) produced high-pure XOS from sugarcane bagasse delignified with aqueous ammonia using a crude xylosidase free xylanase of *Bacillus subtilis*. Presence of XOS including xylobiose, xylotriose and xylotetraose with negligible amount of xylose (0.4%), was confirmed by MALDI-TOF-MS and HPLC analysis. Ratnadewi et al. (2016) used endoxylanase (2.21 U ml^{-1}) from *Bacillus subtilis* of soil termite abdomen for production of XOS. TLC as well as HPLC chromatography confirmed that xylopentose, xylotriose and xylotetrose were present as the major end products, with no xylotriose. A purified alkaline xylanase from *Bacillus mojavensis* A21 was employed for the production of xylooligosaccharides from garlic straw. Xylobiose and xylotriose were the main hydrolysis products yielded from garlic straw, as confirmed by TLC analysis (Kallel et al. 2015). Faryar et al. (2015) utilized an alkali-tolerant endoxylanase for production of XOS. Xylobiose was the predominant oligosaccharide after hydrolysis.

2.16 Strategical Improvements for Production of XOS

Microbes are quite versatile in nature and are capable of producing several enzymes suitable for industrial applications. Xylanases are produced by a variety of organisms, including *Streptomycetes*, *Aspergillus*, *Phanerochaetes*, *Chytridiomycetes*, *Trichoderma*, *Bacillus*, *Fibrobacter*, *Clostridium*, *Ruminococus*, *Thermoascus*, etc. by utilizing various agro-residues (Moreira 2016). Even though the demand for xylanases compatible at industrial level is ever increase, however, low yields and high production costs are the main bottlenecks which are being faced at the industrial level. Therefore statistical optimization is being done to optimized process parameters in order to maximize XOS yields with minimum impurities and unwanted by-products. Moreover incorporation of inexpensive sources which mostly comprises of agricultural refuse in the culture media for enzyme production further help to decrease the production costs (Gupta et al. 2015).

Reddy and Krishnan (2016) optimized culture conditions for production of β-xylosidase-free endo-xylanase from *Bacillus subtilis* KCX006 under solid state fermentation (SSF). Highest xylanase product was supported by wheat bran and groundnut oil-cake at 2158 IU gdw^{-1} and 24.92 mg gdw^{-1} respectively improving xylanase production by 1.5 fold. In another study, Box–Behnken design was used to optimize xylanase production from *Aspergillus candidus*. Parameters optimized were nitrogen source, time of incubation, temperature and moisture content giving maximum xylanase activity of 770 U gds^{-1} (Garai and Kumar 2013). Xylanase production from a newly isolated *Bacillus aerophilus* KGJ2 was statistically optimized using

Plackett–Burman fractional factorial design and Box–Behnken method. Substrate concentration, nitrogen source, moisture content and $MgSO_4 \cdot 7H_2O$ were the significant variables studied, giving a xylanase yield of 45.9 U gds^{-1} (Gowdhaman et al. 2014). Similarly, Plackett–Burman design and Box–Behnken design were used for enhancing xylanases production by *Bacillus mojavensis* A21. Barley bran, NaCl, speed of agitation and cultivation time were the significant variables and statistical optimization resulted in a 6.83 fold increase in xylanase production (Haddar et al. 2012).

Efficiency of XOS production through enzymatic means can also be increased by using immobilized enzymes. Immobilized enzyme can be reused in subsequent batches, as a result of which the amount of enzyme required for the reaction and the reaction time could be effectively reduced. Therefore enzyme immobilization could make commercial production of XOS more affordable (Sun et al. 2016). Sukri and Sakinah (2017) used immobilised xylanase for the production of XOS and obtained xylobiose and xylotriose as the major products of xylan degradation by xylanase. Driss et al. (2014) immobilize *Penicillium occitanis* xylanase on chitosan with glutaraldehyde by covalent coupling reaction. The immobilizated xylanase retained 94.45 ± 3.5% of its activity. In another study, xylanase from *Aspergillus versicolor* were immobilized on glyoxyl-agarose supports. 85% of its catalytic activity was maintained by the immobilized enzyme (Aragon et al. 2013). In another study, an anionic exchange resin via the ionic linkage was used to immobilize an endo-xylanase secreted by the alkaliphilic *Bacillus halodurans,* retaining 80.9% of its activity (Lin et al. 2011).

2.17 Lignocellulosic Biomass for Polyhydroxybutyrate (PHB) Production

Plastic also known as synthetic polymer has become significant because of its properties like durability, mechanical and thermal stability, and resistance to degradation. It is replacing glass, wood and other constructional materials, and also possesses application in industries (Tripathi et al. 2013). Inspite of its widespread usage in day today life, it possess serious drawbacks which includes persistence in the environment leads to deleterious effects on wild life, waterways, quickly fill up landfills and natural areas and greatly affects aesthetic quality of the area (Santimano et al. 2009). The potential hazards generate from synthetic plastic waste incineration and the economy of disposal process makes waste management a problem (Webb et al. 2012). With the increase in population and depletion of non-renewable resources (petroleum products) develops concern not only for energy industry, but also changes the chemical industry. For example, plastic produced annually of 200 million tons and it is predominately derived from petroleum (Du et al. 2012). The shortage of crude oil resources make the production of conventional plastics expensive. It leads to the immense need of using environment friendly substitute and cost effective raw

materials to replace fossil resources. Biopolymer produced using starch, sugars, or cellulose is biodegradable and derived from sustainable biomaterials making it an environmental benign process (Du et al. 2012).

Biodegradable plastics represent a solution to environmental problems generated by the utilization of plastics from petrochemical sources, which have many undesirable properties such as durability and resistance to biodegradation. Biodegradable plastics are plastics that will decompose in natural aerobic and anaerobic environments (Emadian et al. 2017). It can be achieved by enabling microorganisms in the environment to metabolize the molecular structure of plastic films to produce inert humus like material that is less harmful to the environment and allows composting as an additional way for waste disposal (Gouda et al. 2001). Wide range of microorganisms produced polyhydroxyalkanoates (PHAs)—family of biodegradable polymers. During nutrient starving conditions bacteria such as *Ralstonia eutropha* and *Alcaligenes latus* could synthesize PHAs using nutrient non-limiting media (Ojumu et al. 2004). They are usually accumulated as an intracellular energy reserve. According to the chain length of the branching polymers PHAs family can be classified as shortchain-length PHAs possess 3–5 carbon atoms, while medium-chain-length and longchain-length composed of 6–14 and 15 or more carbon atoms, respectively (Altaee et al. 2016).

Polyhydroxybutyrate (PHB) is the most extensively studied member of the PHA family. It was in the mid 1920s, the presence of PHB in *Bacillus megaterium* was first identified (Yu and Stahl 2008). PHB is the oldest known biopolymer used as a biodegradable and biocompatible material for production of bioplastic. It possesses high tensile strength, inertness, high melting point and thermoplastic like properties (Azizi et al. 2017). These features make them suitable for application in the packaging industry and as substitute for hydrocarbon-based plastics. It has wide applications in different areas such as packaging material, long term dosage of drugs, medicines, insecticides, herbicides, fertilizers cosmetic world, disposable items such as razors, utensils, diapers, feminine hygiene products, cosmetics containers, shampoo bottles, cups etc. (Gowda and Shivakumar 2014; Rehm 2006). Studies are progressing for its relevance in medical field for bone replacements and plates, surgical pins, sutures, wound dressings, and blood vessel replacements (Chen and Wang 2013).

In response to imbalanced nutritional conditions, such as an excess of carbon source combined with nutrient limitations, such as oxygen, nitrogen or phosphorus, PHB is synthesized and accumulated by several bacterial species including *Azohydromonas lata*, *Cupriavidus necator* (formerly known as *Ralstonia eutropha*), *Pseudomonas* sp., *Bacillus megaterium*, *Paracoccus denitrificans* and *Protomonas extorquens*, *Aeromonas hydrophila*, *Pseudomonas putida* and recombinant *Escherichia coli* (Castillo et al. 2017). Several microorganisms studied for PHB production utilizing cost effective substrates are shown in Table 2.5.

The major obstruction in the commercialization of PHA and its polymers is their high production cost as compared to synthetic plastic. The process viability of microbial production of PHB is dependent on the development of a low cost process that produces biodegradable plastics with properties close to or surpassing petrochemical plastics (Du et al. 2012). Process economics reveal that the use of renewable carbon

Table 2.5 Organisms producing PHB and other biodegradable polymers using cost effective substrates

Microorganism	Biomass used	Product	Yield	References
Bacillus megaterium B2	Raw glycerol	PHB	1.20 g l^{-1}	Moreno et al. (2015)
Cupriavidus necator	CO_2	PHB	0.26 g g^{-1} cell H^{-1}	Mozumder et al. (2015)
Bacillus megaterium	Glycerol	PHB	4.8 g l^{-1}	Naranjo et al. (2013)
Rhodococcus equi	Crude palm kernel oil	PHB	38%	Altaee et al. (2016)
Bacillus sp.	Soy molasses oligosaccharides	PHAs	90% of CDW	Full et al. (2006)
Bacillus megaterium	Sugarcane molasses and corn steep liquor	PHB	46.2% mg^{-1} CDW	Gouda et al. (2001)
Bacillus megaterium	Sugarcane molasses, urea and trace elements	PHB	1.27 g l^{-1} h^{-1}	Kulpreecha et al. (2009)
Bacillus megaterium R11	Oil palm empty fruit bunch	PHB	9.32 g l^{-1}	Zhang et al. (2013)
Cupriavidus necator PTCC 1615	Brown sea weed *Sargassum* sp.	PHB	3.93 ± 0.24 g l^{-1}	Azizi et al. (2017)
Ralstonia eutropha MTCC 8320 sp.	*P. hysterophorus* and *E. crassipes*	PHB	8.1–21.6% CDW	Pradhan et al. (2017)
Bacillus cereus PS 10	Rice straw hydrolysate	PHB	10.61 g l^{-1}	Sharma and Bajaj (2015c)
Bacillus cereus PS 10	Molasses	PHB	57.50%	Sharma and Bajaj (2015b)
Alcaligenes sp.	Cane molasses and urea	PHB	8.8 ± 0.4 g l^{-1}	Tripathi et al. (2013)
Pseudomonas corrugate	Soy molasses	PHAs	5–17%	Solaiman et al. (2006)
Methylobacterium sp. ZP24	Whey	PHA	2.6–5.9 g l^{-1}	Nath et al. (2008)
Burkholderia cepacia ATCC 17759	Hemicellulosic hydrolysates	PHB	2.0 g l^{-1}	Keenan et al. (2006)
Pseudomonas sp. strain DR2	Waste vegetable oil	PHA	23.5% CDW	Song et al. (2008)

substrates and lignocellulosic biomass in PHA production can cause 40–50% reduction in the overall production cost (Gowda and Shivakumar 2014). Therefore the use of waste residues like starch, whey, molasses, bagasse, soyameal etc., and dairy waste can significantly reduce the substrate cost and in turn downsize the production costs (Du et al. 2012). Hence, the use of waste for production of value adding products not only provides value to the waste but also solve the problem of waste disposal. Other factors which also affect the total production costs are bacterial strains, fermentation strategies and recovery processes (Santimano et al. 2009).

Different biomass and biomass derived products are used for the production of PHB and other polymers to make the process economical. A brown seaweed *Sargassum* sp. biomass was used for production of PHB by *Cupriavidus necator* PTCC 1615. Biomass was pretreated with acid and then enzymatically hydrolysed to release monomeric sugars. Ammonium sulphate (nitrogen source) with hydrolysate resulted in PHB yield of 0.54 ± 0.01 g g^{-1} reducing sugar. NaCl, an external stress factor show positive impact on PHB yield, but increasing concentration of NaCl to 16 g l^{-1} was found to inhibit the PHB production. The highest cell dry weight and PHB concentration were 5.36 ± 0.22 and 3.93 ± 0.24 g l^{-1} respectively of 20 g l^{-1} reducing sugars (Azizi et al. 2017). Furthermore two invasive weeds, viz. *P. hyysterophorus* and *E. crassipes* were used for the production of biodegradable PHB by *Ralstonia eutropha* MTCC 8320 sp. Both the biomass were pretreated with acid and then enzymatically hydrolysed to produce pentose and hexose rich hydrolysates. Sonication was used for the extraction of PHB. Yield of PHB produced was 6.85×10^{-3}–36.41×10^{-3}% of w w^{-1} raw biomass (Pradhan et al. 2017).

Saccharophagus degradans (ATCC 43961) degrade the major components of plant cell walls by readily attaching to cellulosic fibers, and utilize this as the primary carbon source. The minimal media containing glucose, cellobiose, avicel, and bagasse was used for the growth of *S. degradans* to support growth. Lignin in media alone did not support growth, but on addition of glucose support growth. When nitrogen gets depleted, PHA production commences and continues for at least 48 h. This work reveals for the first time, that a single organism can utilize insoluble cellulose and produce PHA (Munoz and Riley 2008).

2.18 *Bacillus* spp. for PHB Production

Bacillus spp. produces variety of enzymes and has been explored for wide range of industrial products including PHB. *Bacillus* spp. comparatively grows faster and has potential to exploit vast range of agro-industrial wastes as substrates (Masood et al. 2012). Furthermore, *Bacillus* spp. acts a model system for heterologus expression of foreign genes including PHA production and other chemicals (Law et al. 2003; Schallmey et al. 2004). Numerous reports are available on the production of PHB by *Bacillus* spp. For the production of PHB granules in cells of *Bacillus megaterium* sugarcane molasses and corn steep liquor were used as sole carbon and nitrogen source respectively. Maximum yield of PHB obtained was 46.2% mg^{-1} cell dry

matter with 2% molasses (Gouda et al. 2001). In another study, Zhang et al. (2013) studied the use of oil palm empty fruit bunch collected from Malaysia palm oil refinery (rich in cellulose and hemicelluloses) for production of PHB by *B. megaterium* R11. From the overall oil palm empty fruit bunch sugar concentration of 45 g l^{-1}, 58.5% of PHB content obtained. On increasing the hydrolysate content to 60 g l^{-1} productivity increases to 0.260 g l^{-1} h^{-1}, reaching the PHB content to 51.6%.

Furthermore, Kulpreecha et al. (2009) studied the homopolymer PHB production by *B. megaterium* BA-019 using renewable and inexpensive substrate sugarcane molasses by fed batch cultivation and urea as a nitrogen source. The optimal feeding conditions require sugar concentration of 400 g l^{-1} and C/N molar ratio of 10 mol mol^{-1} and attained PHB content of 42% of cell dry weight in a short time of 24 h. A maximum of 90% of cell dry mass as PHA was obtained by *Bacillus* sp. strain CL1 isolated from nature capable of fermenting soy molasses and other waste carbohydrates produced. It produced PHA without requiring a nutritional limitation (Full et al. 2006). Albuquerque et al. (2007) described an interesting work of three-step fermentation strategy by producing PHAs from cane molasses. Firstly, molasses were fermented to organic acids. Then, triggered to PHA accumulation and finally, in batch fermentation using the fermented molasses and the PHA-accumulating cultures, PHAs were produced. In another study, PHAs synthesis from fermented molasses was obtained by using a consortium of microorganisms (Pisco et al. 2009 and Bengtsson et al. 2010).

2.19 Strategies for PHB Production

2.19.1 Process Optimization for PHB Production

Many reports were available on production of PHB using crude resources (Du et al. 2012). But to make process efficient and economical, optimization of process parameters is of utmost importance (Singh et al. 2016). Conventional one-variable-at-a-time method was used for the screening of various substrates affecting production. But it is time consuming, laborious and ignores the combined interaction among various variables (Singh and Bajaj 2015). Plackett–Burman design and the central composite design of response surface methodology (RSM) are the statistical tools used for efficient medium optimization and for studying the interaction of various variables (Vaid et al. 2017).

PHB producing strain *B. thuringiensis* IAM 12077 used agro wastes substrates like rice husk, wheat bran, vagi husk, jowar husk, jackfruit seed powder, mango peel, potato peel, bagasse and straw for production. Among all substrates, mango peel yielded the highest PHB of 4.03 g l^{-1}; 51.3% (Gowda and Shivakumar 2014). Also, bacterial isolate *Bacillus cereus* PS10 grow on low cost agro-based residues viz. maize bran, rice husk, wood waste, molasses, whey, walnut shell powder, almond shell powder, corn steep liquor, soy bean bran, mustard cake etc. and accumulated

appreciable amount of PHB. Carbon source molasses support maximum PHB production of 9.5 g l^{-1} after 48 h of fermentation at pH 7 (Sharma and Bajaj 2015a). The isolate *Bacillus cereus* PS10 was then used for statistical optimization of PHB production with crude source molasses. Variables first identified through Plackett-Burman design were molasses, pH and NH_4Cl and then process was optimized through RSM approach resulting in enhancement of PHB yield by 57.5% (Sharma and Bajaj 2015b).

Sharma and Bajaj (2015c) produced bioplastic using the same isolate *Bacillus cereus* PS10. In this study, biphasic-acid-treated rice straw produced hydrolysate was used for fermentation by isolate. Rice straw hydrolysate (RSH) produced more PHB than the refined carbon source glucose. Then the process was optimised using RSM for various process variables were the amount of RSH, NH_4Cl and medium pH and enhanced yield of 23%.

B. megaterium B2 has the ability to accumulate PHB using raw glycerol from biodiesel production as the carbon source. PHB production was statistically optimized to establish key variables and optimal culture conditions by Plackett-Burman and central composite designs. Experimental variables influencing PHB production are temperature, glycerol concentration and Na_2HPO_4 in shake flask with optimized medium produced 0.43 g l^{-1} of PHB with a 34% accumulation in the cells after 14 h of fermentation. The maximum PHB concentration of 1.20 g l^{-1} was reached at 11 h under the same conditions. It corresponds to a 48% and 314% increase in PHB production compared to the initial culture conditions (Moreno et al. 2015). Tripathi et al. (2013) did the optimization by central composite rotatable design for three physical process variables viz; pH, temperature and agitation speed for enhancing PHB production by *Alcaligenes* sp. Cane molasses and urea were used as carbon and nitrogen source. The optimum physical conditions resulted in PHB mass fraction yield of 76.80% on dry molasses substrate. On scale up studies of same optimized media produce maximum yield and productivity of 0.78 and 0.19 g l^{-1} h^{-1}, which was higher than previous reports.

2.19.2 Application of Genetic Engineering Tools

Genetical engineering approaches were also used for maximizing the PHB production. PHA, a bio-based plastic was produced by successful engineering of biomass crop switchgrass (*Panicum virgatum* L.). Engineered crop was able to grow and produce polymer in vitro and glass house conditions. Transformants produce 3.72% dry weight of PHB in leaf tissues and 1.23% dry weight of PHB in whole tillers. First generation of transformants obtained from controlled crosses of transgenic plants also accumulate polymer (Somleva et al. 2008). Halophiles also have potential to produce PHB using agro-industrial wastes. Halophilic bacteria allow the PHA production under continuous mode and unsterile conditions. They are easily manipulated genetically and allow the construction of a hyper-producing strain. For example, both recombinant and wild type *Halomonas campaniensis* LS21 were allowed to grow on

mixed substrates (kitchen wastes) in the presence of NaCl (26.7 g l^{-1}), at pH 10 and temperature of 37 °C continuously, for 65 days, without any contamination. Recombinant produced higher PHB content (70%) than the wild type strains (Kourmentza et al. 2017).

2.19.3 Pretreatment of Biomass

Different methods were used for the pretreatment of biomass to release simple sugars which were easily fermented by microorganisms. Various pretreatment includes mechanical comminution, acid and alkaline hydrolysis, ozonolysis and biological pretreatments. For the pretreatment of biomass switchgrass, radio frequency assisted heating was used to generate hydrolysates and then enzymatically hydrolysed to fermentation with recombinant *Escherichia coli*. Results clearly indicated that hydrolysates obtained through radio frequency pretreatment produced consistently better PHB production. Supplementation of media with yeast extract enhances production under all conditions. In comparison to traditional heating pretreatment process, radio frequency creates harsher conditions for unwinding of biomass structure more and generates more nutrients for fermentation (Wang et al. 2016)

2.19.4 Structural Modifications of PHB

Composition of the PHA during the biosynthesis changes the applications of bioplastic. The representative member of PHA family, namely the homopolyester PHB, possesses high degree of crystallinity and restricted processability of this material. Due to the small difference between the decomposition temperature and melting point provides a little space for processability. This composition can be changed by alternating the building blocks such as (R)-3-hydroxyvalerate or the achiral building blocks 4-hydroxybutyrate and 5-hydroxyvalerate (Koller et al. 2009). Varied techniques were used for the determination of structural and physcio-chemical properties of biopolymers. Thermal properties were determined by TGA, DTG and DSC. ^{1}H NMR results revealed the molecular weight and polydispersity index value. PHB with low polydispersity index value can be used for nanoparticle formation (Sathiyanarayanan et al. 2013). With the advent of genetic engineering techniques, PHA with different compositions and higher productivity has been possible to design (Tsuge 2002). By altering the physical and genetical properties, biopolymers can be produced according to the necessity.

In response to the ever increasing demand, biodegradable plastic may serve as substitute for petroleum derived plastics because of its biodegradable and biocompatible nature, and production from sustainable and agro-waste raw materials which provide independence from fossil fuels. Even though, their manufacturing cost is too high to compare with petrochemical derived plastics but advances in the production

processes using inexpensive substrates makes possible the broad use of bioplastic in future.

2.20 Lignocellulose Biomass for Production of Industrial Enzymes

Enzymes are of great importance for various industries due to their action on specific substrate, resulting in high yield production. They are indispensable ingredients in various processes that are involved in product development. But lot of hurdles is encountered in production of enzyme (Ravindran and Jaiswal 2016) and the major ones are their high production cost and low product yields. The LB is a cheap source for production of enzymes and other valuable products such as bioethanol, organic acids etc. Hydrolytic enzymes likes cellulases, xylanase and pectinase are of utmost importance in valorization of food industry waste (Meng and Ragauskas 2014). Different species of bacteria (*Clostridium*, *Cellulomonas*, *Bacillus, Pseudomonas, Fibribacter*, *Ruminococcus*, *Butyrivibrio*, etc.), fungi (*Aspergillus*, *Rhizopus*, *Trichoderma, Fusarium, Neurospora, Penicillium* etc.), and actinomycetes (*Hermomonospora, Hermoactinomyces* etc.) are involved in the degradation of lignocelluloses due to their extracellular enzyme production attribute (Sajith et al. 2016).

Enzymes are biological catalysts found in all living systems and are proteinaceous in nature with potential to catalyze diverse reactions (Ravindran and Jaiswal 2016). There is a long history of enzyme use for the commercial production of various metabolites, and have been documented to be efficient for industrial scale production. Enzymes are now being genetically manipulated in order to enhance their ability for better results in fermentation media. Such modifications have enabled researchers to use several simple microorganisms with no history of industrial use for production of native enzymes, such as *Escherichia coli* K-12, *Fusarium venenatum* and *Pseudomonas fluorescens* to be successfully utilized as source for expression of industrially important enzymes (Olempska-Beer et al. 2006). Hemicellulase production from filamentous fungi are being developed as one of the best enzyme production systems due to their ability to secrete high quantities of enzymes suitable for industrial applications such as development and commercialization of new products (Gudynaite-Savitch and White 2016).

Since hydrolysis of biomass is essential for generation of fermentable sugars which are then converted to ethanol by microbial action. Thus, alternate enzyme production method using cheaper and abundantly available substrates with higher yield is need of the hour (Ang et al. 2013). Cellulase and hemicellulase production from *Aspergillus niger* KK2 was studied on SSF using different ratios of rice straw and wheat bran biomass. Maximum FPase activity was 19.5 IU g^{-1} in 4 days was found on rice straw. Also, CMCase (129 IU g^{-1}), β glucosidase (100 IU g^{-1}), xylanase (5070 IU g^{-1}) and β-xylosidase (193 IU g^{-1}) activities were concurrently obtained after 5–6 days of fermentation and such enzyme activities are critical for practical

saccharification reaction during bioethanol production (Kang et al. 2004). Ang et al. (2013) utilized untreated oil palm trunk for cellulases and xylanase production by *Aspergillus fumigatus* SK1 under SSF. The cellulases and xylanase activities obtained were 54.27, 3.36, 4.54 and 418.70 U g^{-1} substrates for endoglucanase (CMCase), exoglucanase (FPase), β-glucosidase and xylanase respectively. To bring down the cost of cellulases production, a multifaceted approach using cheap lignocellulosic substrates such as sugar cane bagasse, rice straw and water hyacinth biomass under SSF was utilized. Cellulolytic and β-galactosidase enzymes were produced using SSF on wheat bran as substrate using fungi *Trichoderma reesei* RUT C30 and *A. niger* MTCC 7956, respectively. Kshirsagar et al. (2015) produced cellulases and xylanases from *Amycolatopsis* sp. GDS which were thermostable and active up to 70 °C and were able to function at higher NaCl (2.5 mol l^{-1}) and ionic liquid (10%) concentrations during the pretreatment of biomass. Crude enzymes also resulted in comparable saccharification (60%) of wheat straw as compared to commercial enzymes (64%). The copper dependent lytic polysaccharide mono-oxygenases has been patented by biotech company Novozymes A/S holds patents on the use of these enzymes for the conversion of steam-pretreated plant residues such as straw to free sugars. lytic polysaccharide mono-oxygenases show striking synergistic effect when combined with canonical cellulases enzyme for efficient performance in several large-scale plants for the industrial production of lignocellulosic ethanol (Johansen 2016).

Saratale et al. (2017) isolated lignocellulolytic enzymes using *Streptomyces* sp. MDS cultivated in various agricultural wastes under SSF. The harvested enzyme exhibited good stability at a range of pH (5–8) and temperature (50–80 °C) and efficient activity in the presence of organic solvents, surfactants, and commercial detergents. Novel extracellular endoxylanase and endoglucanase from halo- and thermotolerant *Actinomadura geliboluensis* with molecular mass of 30 and 38 kDa were produced with optimum pH and temperature values of pH 6.0 and 60 °C respectively. These enzymes were strongly inhibited by Hg^{2+} and reducing sugar content was 265.12 mg g^{-1} biomass after incubation with alkali pretreated wheat straw (Adıgüzel and Tunçer 2017). Production of amylolytic enzymes by solid state or submerged fermentations (SmF), followed by purification and evaluation of enzymatic hydrolysis of the polysaccharides of *Spirulina* was carried out. Microfiltration of the crude extracts resulted in an increase in their specific activity and thermal stability at 40 and 50 °C for 24 h, as compared to extracts obtained by SSF and SmF (Rodrigues et al. 2017).

Likewise many other lignocellulosic biomasses have been utilized for commercially important enzyme production. Various microorganism including both bacteria and fungi were used as source of enzyme production in different reaction conditions. A detailed account for such enzymes from various biomasses is given in Table 2.6.

2.21 Conclusion

It may be concluded that though lignocellulosic biomass from agro/forestry and other sources, may have immense potential as a renewable feedstock for production of energy, materials and varierty of other products of commercial importance. But hurdles like pretreatment, apt enzymes for saccharification, efficient fermentation process organisms, and down stream processing need more research attention.

2.22 Future Prospects

The important criterion for the long-term feasibility of any bioprocess is its cost effectiveness. Though LB represents an efficient and abundantly available renewable energy feedstock but its recalcitrance is a big hurdle. It is the need of the hour that effective pretreatment approaches must be developed for LB along with apt enzyme cocktail for saccharification. The fermentation organism must be capable of utilizing the sugars and generate high product yield and concentration. Modified/engineered hydrolases may be developed for efficient saccharification. There is a need to find more innovative methodologies in which the reuse and recycle of pretreatment agents/saccharifying enzymes as well as fermenting organisms can be practiced in an effective manner. The consolidated bioprocesses may be developed in which pretreatment, saccharification and fermentation can be executed in a single vessel using balanced and appropriate combo of biomass, pretreatment agents, enzymes and fermenting organisms that functions synergistically.

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Table 2.6 Different lignocellulosic biomass for commercially important enzymes

Microorganism	Enzyme produced	Substrate used	Mol. mass, pH and temperature	References
Trichoderma reesei RUT C30 and *Aspergillus niger* MTCC 7956	Cellulase and β- glucosidase	Wheat bran	pH 4.8 Temperature 70 °C 43 kDa and 124.0 kDa	Rajeev et al. (2009)
Geobacillus sp. strain WSUCF1	Xylanases	Prairie cord grass and corn stover	pH 5.5–7 Temperature 80 °C 39.5 kDa	Bhalla et al. (2015)
Penicillium echinulatum 9A02S1	Cellulase and xylanase	Elephant grass	pH 4.8 Temperature 50 °C 50 kDa and 80 kDa	Menego et al. (2016)
Aspergillus oryzae P21C3	Xylanase	Sugarcane bagasse	pH 4.8 Temperature 50 °C 35.402 kDa	Braga et al. (2014)
Trichoderma reesei NRRL-6156	Exocellulase, endocellulase, and xylanase	Soybean bran	pH 5.0 Temperature 50 °C 110 kDa	Gasparotto et al. (2015)
Geobacillus sp. strain WSUCF1	Thermostable xylanase	Prairie cord grass and corn stover	pH 6.5 Temperature 70 °C 17 kDa	Bhalla et al. (2015)
Aspergillus niger	Cellulase	Paper and timber sawmill industrial wastes	pH 4–7 Temperature 20–50 °C 17 kDa	Devi and Kumar (2012)
Scytalidium thermophilum	Endoglucanase	Lentil bran and sunflower seed bagasse	pH 4.6 Temperature 45 °C 23 kDa	Ögel et al. (2001)
Coculture of *Trichoderma reesei* and *Aspergillus oryzae*	Cellulolytic Enzyme-β-glucosidase, CBH I, CBH II, EG I and xylanase	Soybean hulls supplemented with wheat bran	pH 4.8 Temperature 30–32 °C 40 kDa	Brijwani et al. (2010)
Gracilibacillus sp. SK1	Alkali-stable cellulase	Corn stover and rice straw	pH 8 Temperature 60 °C 37 kDa	Yu and Li (2015)

(continued)

Table 2.6 (continued)

Microorganism	Enzyme produced	Substrate used	Mol. mass, pH and temperature	References
Aspergillus terreus D34	Exo- and endoglucanases, β-glucosidases, and xylanases	Rice straw and sugarcane bagasse	pH 4.5 Temperature 45 °C 23 kDa	Kumar and Parikh (2015)
Trichoderma reesei Rut C-30	Cellulase	Paper sludge and wood	pH 4.8 Temperature 27 °C 43 kDa	Shin et al. (2000)
Streptomyces viridobrunneus SCPE-09	Cellulase	Wheat bran and corn steep liquid	pH 4.9 Temperature 50 °C 37 and 119 kDa	Da Vinha et al. (2011)
Aspergillus sydowii	Endoglucanase, exoglucanase and β-glucosidase	Lactose	pH 5.5 Temperature 40 °C 95 kDa	Matkar et al. (2013)
Thermoascus aurantiacus RBB1	Cellulase	Wheat bran	pH 4.0 Temperature 70–80 °C 35 kDa	Davea et al. (2013)
Amycolatopsis sp. GDS	Cellulase and xylanase	Paddy straw, sugarcane barboja, corn straw, sorghum husk, water hyacinth and sugarcane bagasse	pH 4.0 Temperature 70 °C 30 kDa	Kshirsagar et al. (2015)
Talaromyces Cellulolyticus	Cellulase	Corn stover	pH 5.0 Temperature 45 °C cellulase III-B (49 kDa), I (61 kDa), and III-A (58 kDa)	Inoue et al. (2014)
Aspergillus awamori	Xylanase, α-L-arabinofuranosidase, β-xylosidase, and β-glucosidase	Sugarcane bagasse	pH 4.0 Temperature 70 °C 30 kDa	De Sousa et al. (2015)
Chrysoporthe cubensis	Cellulolytic cocktails	Sugarcane bagasse	pH 3–4.0 Temperature 55–80 °C 110 and ~38–40 kDa	Dutra et al. (2017)

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