Chapter 15
Bioconversion of Biomass to Biofuel Using Fungal Consortium

Pavana Jyothi Cherukuri and Rajani Chowdary Akkina

15.1 Introduction

The global economy is highly influenced by fuel energy. Global pollution and energy consumption expanded tremendously during the last two decades which led to exhaustion of fossil fuels, resulting in emerging of energy crisis. Therefore, there is a need to search for alternative new energy sources and technologies which have increased logistically in recent years. The entire globe depends on petroleum as a sole energy source. The high usage of petroleum has led to adverse impact on environmental issues such as catastrophic emission of greenhouse gases (Hill et al. 2006). In India, the required petroleum is imported from Middle East; these high imports of petroleum significantly influence the Indian economy. Many attempts are being done to search for alternative fuel sources in transportation sector, such as diesel, gasoline and natural gas (Tabassum Ansari and Choube 2012). But no fuel exhibits unique feature like petroleum such as high energy density, compatibility with vehicles, and being in liquid state. Developed countries like the United States, Europe, Brazil, and China invented new technologies such as solar, hydro, and wind energy usage as an alternative to petroleum or alternative liquid fuels such as butanol, ethanol, methane, and CNG. Among all the liquid fuels, ethanol occupies the first place by its unique properties.

P. J. Cherukuri (✉) · R. C. Akkina
Department of Microbiology & Food Science and Technology, Institute of Science, GITAM (Deed to be University), Visakhapatnam, Andhra Pradesh, India

A. N. Yadav et al. (eds.), *Recent Advancement in White Biotechnology Through Fungi*, Fungal Biology, https://doi.org/10.1007/978-3-030-25506-0_15

15.2 Ethanol Production

Ethanol exhibits almost similar properties with petroleum. It can mix with gasoline and reduce emission of smog by unburned hydrocarbons and CO (carbon monoxide) from vehicles. Ethanol blending with gasoline enhances octane number and oxygen content of fuel (Limayem and Ricke 2012). The specific properties of ethanol like high octane number, water free, low flame temperature, high gas volume change, high heat of vaporization, and total combustibility make it convenient to use as automotive fuel (Balan et al. 2013). Ethanol is a transparent liquid, with mild odor boiling temperature at 78 °C and freezing temperature at −112 °C. Energy Policy Act of 2005 in the United States, demands the blending of 7.5 billion gallons of alternative fuels by 2012. Global rapid depletion of fossil fuels has increased the demand for alternate fuels. The usage and production of ethanol have to be increased markedly (Haghighi et al. 2013).

The production of ethanol is not sufficient to meet today's demand. Industrial ethanol production is obtained from coarse grains (56%), cane (32%), molasses (4%), wheat (3%), nonagricultural substrates (3%), and sugar beet (2%) (Tabassum Ansari and Choube 2012). Economically feasible ethanol production from cellulosic wastes is one of the best ways (Rastegari et al. 2019). Nowadays, integrated biomass system is being used for production of biofuel through biotechnological process. Lignocellulosic biomass provides competitive renewable resource for generation of ethanol in an eco-friendly manner. This technology ensures increased energy security and economic progress and eliminates problems of solid waste management (Das and Singh 2004). According to the studies by Sanchez and Cardona (2008), 73.9 Tg of dry crop waste and 1×10^{10} MT weed biomass is produced annually.

Numerous edible crops like cane, corn, beet, and sorghum are used in ethanol commercial production. In this technology, the raw material cost is very expensive. This leads to food insecurity and very high cost of food products. Therefore, plant biomass (lignocelluloses waste) has become an inexpensive carbon source for the production of ethanol. The lignocellulosic wastes such as agricultural residues like corn stover, corncobs, rice straw, wheat straw, sugarcane bagasse, rice husk, wheat husk, and weed biomass like Lantana camara, Prosopis juliflora, Saccharum spontaneum, Eichhornia crassipes, etc. are promising substrates for the production of ethanol.

15.2.1 Demand for Ethanol Production

It was estimated that there was 90% increase from the existing levels on the India's dependency on oil imports by 2020 AD. In order to reduce the oil imports, India proposed an EBP (ethanol blending program report 2014–2015) during the year 2002 with 5% blending of ethanol in petrol which is mandatory in nine major sugar-producing states (Tamil Nadu, Gujarat, Andhra Pradesh, Karnataka, Uttar Pradesh,

Maharashtra, and Union Territories). In the year 2008, Indian Government introduced a "National Biofuel Policy" for blending of petrol with 20% ethanol (Tabassum Ansari and Choube 2012). To fulfill the supply of bioethanol, it is highly essential to search for new cost-effective raw materials like agricultural residues and weeds. Global ethanol production is around 30 billion liters per year. Global ethnaol production is around 30 billion litters per year, in this, the United States occupies a major share (53%), followed by Brazil (21%), Europe (6%), China (7%) and India (3%). In India, the main source of ethanol production is by molasses (80%) and grains (20%). The production of bioethanol in India is around 2.4 billion liters per annum.

The industrial production of bioethanol by biotechnological process using microorganisms and gross productivity ranges from 75% to 90%, and the remaining small fraction of production is from chemical technology by ethylene hydration reactions (Mc. Millan 1997). Currently, ethanol production is by sugarcane and corn in developed countries like the United States and Brazil (Limayem and Ricke 2012). The first-generation biofuels are the results of bioconversion of edible food crops (sugarcane, corn), the second-generation biofuels are from nonedible sources like agricultural and nonagricultural residues, and third-generation biofuels are from algal biomass (Bacovsky et al. 2010; Yewale et al. 2016; Kim and Dale 2004; Chen et al. 2010).

15.3 Biomass for Biofuels

Hard wood angiosperms such as a poplar, eucalyptus, and beech wood are rich in cellulose, hemicellulose, and lignin. Efficient conversion of ethanol production from hard wood spent sulfite liquor (HSSL) using *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* was reported (Jeffries et al. 2007). HSSL contains high amount of microbial inhibitors. Biofuel production from renewable plant sources is an attractive and alternative process in many countries. Mainly, three substrates like sugars, starches, and cellulose are used for ethanol production in a cost-effective manner. Among these, cellulose materials are renewable and plenty. The agroresidues such as sugarcane bagasse, corncobs, corn fiber, corn stover, wheat straw, rice straw, forestry, paper pulp, weed plants, sawdust, sorghum straw, cotton seeds, sunflower seed coats, kitchen waste, and fruit and vegetable waste are collectively known as organic biomass (Lin and Tanaka 2006). The plant biomass is an attractive feedstock for ethanol production consisting of rich carbohydrate composition in various polymeric complex forms (lignin, cellulose, and hemicellulose). Pretreatment process is necessary to use available carbohydrates in biomass. The current technologies are not cost-effective process for commercialization of bioethanol. Elaborate investigations have been done from the past two decades by many researchers for value addition of lignocellulosic biomass (Zhang et al. 2014; Yewale et al. 2016). Weed, starch, and by-products of paper industry were used for ethanol production which includes spent sulfite (Pereira et al. 2013).

15.4 Composition of Biomass

In the present chapter, focus is on biofuel production from lignocellulosic biomass. According to the plant taxonomy, lignocelluloses are classified into soft wood (belongs to gymnosperms), hard wood (belongs to woody angiosperms), and annual plants (herbaceous angiosperms, crops). Biomass of lignocelluloses is a heterogenous mixture consisting of hemicellulose, cellulose, and lignin, and these compositions vary from plant to plant (Yadav et al. 2019a, b). Generally, cellulosic fractions of biomass comprise 40–60% by weight, and long-chain polymers of glucose are bonded together which appear as fiber bundles. Hemicellulose comprises 20–40% by weight and contains short-chain polymers of heterogenous sugars (glucose, galactose, mannose, arabinose, and xylose) and co-jointly binds the cellulose fibers (Bobleter 1994). Lignin consists of 10–30% by weight and contains three-dimensional propyl-phenol polymers and contributes rigidity to the entire structure. Lignin is derived from the dehydrogenated products of lignin monomers (p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol). These main aromatic phenol rings of monomer residues of p-hydroxy phenyl, guaiacyl, and syringyl and their composition vary from plant to plant (Campbell and Laherrere 1998).

Lignocellulosic biomass shows resistance toward degradation and hydrolysis because of structural firmness. The presence of cross-linkages exists between the cellulose, hemicellulose, and lignin components in biomass. Hemicellulose and lignin are compactly packed cellulosic fraction. For hydrolysis of cellulose, initially digest the hemicellulose and lignin fractions. Cellulosic fraction is protected and occupies the central position in the three-dimensional structure of lignin and hemicellulosic fraction (Balan et al. 2013). For exposure of cellulose by hydrolysis, it is essential to remove the lignin seal. The degradation of cellulosic and hemicellulosic fractions of plant biomass are carried by acid hydrolysis or enzymatic hydrolysis or enzyme producing microrganisms. From these methods, acid hydrolysis requires high temperatures and energy, and this process releases various non-eco-friendly inhibitors (furfurals, hydroxyl furfurals, acids) during the hydrolysis process (Sreenivas et al. 2006). Secondly, enzymes used for the hydrolysis of biomass are eco-friendly and expensive. Among these, bioconversion of biomass using fungal consortium is cost-effective and eco-friendly process (Dirk et al. 2003).

The bioconversion of biomass to ethanol requires more processing steps, i.e., pretreatment of lignocellulosic biomass, hydrolysis, and conversion (fermentation/transformation) of sugars into (biofuel) ethanol. An extensive work has been carried out by utilization of microorganisms (yeast, bacteria, and fungi) for ethanol production. Commercial production of ethanol by fermentation process is highly influenced by fungal and yeast strains. In pretreatment process, initially digest the hemicellulose and lignin crystalline structure and then expose it to cellulose. In the pretreatment process of lignocellulosic matrix, major sugar released from hemicellulosic fraction is xylose, and small amounts of arabinose, galactose, and glucose are also released by the laccase and xylanase enzymes using fungal consortium. Then, cellulose is broken down to monomeric sugars by cellulase-producing fungal

strains and release of hexose (glucose) sugars. These sugars are converted to ethanol by fermentation process using yeast strains such as *Saccharomyces* and *Candida* sp. The yeast Saccharomyces cerevisiae is used for ethanol production from glucose as carbon source. This strain is unable to utilize xylose as carbon source. The *Candida* and *Pichia* sp. of yeast are used to convert pentose sugars to ethanol by biotechnological process (Steven and Lee 1990).

15.5 Pretreatment of Lignocellulose Materials

The lignocellulosic biomass derived from plant biomass is initially treated by chemical or enzymatic or by both methods. By this degradation, polymeric forms of biomass such as lignin, cellulose, and hemicelluloses are digested to release monomeric sugars. These monomeric sugars are further fermented by microorganisms to produce bioethanol (Claudio et al. 2011).

The pretreatment methods are classified into three types:

1. *Physical process*: Physical process such as steam explosion or auto-hydrolysis, carbon dioxide explosion, ammonium fiber/freeze explosion, and liquid hot water.
2. *Chemical process*: Chemical process consists of wet oxidation ozonolysis, acid pretreatment, alkaline pretreatment, and organosolv.
3. *Biological process employed by microorganism or their enzymes* (Buruiana et al. 2013).

The pretreatment of starch substrates includes digestion by gasification of lignocellulosic materials by acid or enzymatic hydrolysis for solubilization of cellulose. Novel pretreatment methods such as ultra-sonication, nano-technological methods, and microwave digestion processes are used for pretreatment of lignocellulosic biomass in order to improve the bioethanol production and reduce inhibitors. After this treatment process, the obtained slurry contains two different types of fractions: one is liquid fraction consisting xylose and small amounts of glucose, galactose, and arabinose, and another is solid fraction containing lignin and cellulose material. The potential utilization of these two (hemicellulosic and cellulosic) fractions of biomass is one of the cost-effective method for ethanol production (Agbogbo and Wenger 2007).

15.5.1 Parameters Affecting Pretreatment of Biomass

Optimization by screening the efficient strains and maintaining the culture conditions can make this process more effective and reduce the treatment time. The governing process parameters include biomass type, nature, and composition, and physical parameters include incubation temperatures, pH, incubation time, moisture

content, and aeration rate. Biological parameters such as type of microorganism, culture conditions, growth characteristics, optimum conditions for growth, and enzyme production are the key factors for this process.

Lignocellulosic biomass is available abundantly and consists of various proportions of lignin, cellulose, and hemicelluloses. The selection of microorganism for biological treatment is based on the nature and composition of the biomass. The optimum temperature varies with fungal strain such as ascomycetes with optimum temperature at 39 °C while basidiomycetes at 25–30 °C. Incubation time also varies depending on strain and nature of biomass. Long incubation time is required for pretreatment and delignification process. Moisture content plays a key role in solid-state fermentations and is essential for growth establishment of microorganisms. White-rot fungi or brown-rot fungi are used for efficient enzymatic saccharification. Pretreatment with fungal strains could enhance enzymatic hydrolysis of lignin. Several studies have revealed that fungal consortium has faster degradation ability of lignocellulosic biomass when compared with single strain. Lignin degradation is an oxidative process, and aeration is a critical factor for production of lignolytic enzymes such as lignin peroxidase and manganese peroxidase (Millati et al. 2011). pH is another important factor for microbial growth; majority of fungal strains grow tremendously in acidic pH range between 4.0 and 5.0. Inoculum level and cell biomass are also critical factors and influence the treatment time of biomass. In SSF (solid-state fermentation), particle size plays a crucial role in biological treatment. Penetration limitations are large with particle size, when compared to small particle size (Kuijk et al. 2015).

15.6 Limitations by Chemical Pretreatment

Inhibitors were produced during the digestion of hemicellulosic fraction. These inhibitors are classified into three types: (a) organic acids (acetic acid, formic acid, levulinic acid, ferulic acid, and p-coumaric acid), (b) furan derivatives (furfural and 5-hydroxy furfural), and (c) phenolic compounds (4-hydroxybenzoic acid and ferulic acid) (Jonsson and Martin 2016). All these inhibitors influence the growth and ethanol production efficiency of microorganism (Cragg et al. 2015). Advanced treatment methods (ultrasonication, microwave digestion) are used to reduce the inhibitor formation and increase the fermentation efficiency of organism. Therefore, detoxification steps are required for removal of inhibitory compounds from hydrolysate before the fermentation process. Detoxification strategies include active charcoal treatment, ion exchange resins, alkali treatment, overliming using calcium hydroxide, change in the fermentation methodologies, and treatment with soft-rot fungi *Trichoderma reesei* to degrade inhibitors (Yu et al. 2011).

The need for efficient pretreatment or hydrolysis process for the recovery of maximum amount of fermentable sugars with minimum toxic chemicals is a major challenge. WRF (white-rot fungi) is one of the promising organisms to convert biomass to bioethanol production with eco-friendly and cost-effective manner. This

process has ample advantages over chemical process such as simpler technique, less energy utilization, less wastage, and absence of inhibitors. In this process, various strains of WRF that include *Pleurotus ostreatus*, *Cyathus stercoreus*, *Ceriporiopsis subvermispora*, *Trametes versicolor*, and *Phanerochaete chrysosporium* are used for conversion of biomass to ethanol production (Nigam and Pandey 2009).

15.7 Fugal Consortium Used for Biofuel Production from Plant Biomass

Several studies have proved that co-culture studies are promising processing methods for production of ethanol from lignocellulosic biomass. Development of fungal consortium has played a significant role for the conversion of polymeric fractions of lignin, cellulose, and hemicellulose into sugars. This consortium includes lignin-, cellulose-, hemicellulose-degrading white-rot fungi and ethanol producing efficient strains. Examples including *P. chrysosporium*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus* and *Cyathus stercoreus* are able to produce lignin-degrading enzymes. Laccases are involved in degradation of lignin and show activity with lignin peroxidase and manganese peroxidase (Binod et al. 2010). Cellulose-degrading enzymes endoglucanases, cellobiohydrolase, and β-glucosidease are produced by a number of fungal species. Hemicellulose-degrading enzymes are xylanases and β-xylosidases and produced by *Aspergillus niger*, *Trichoderma reesei* fungal strains (Zhang et al. 2012; Kour et al. 2019; Rana et al. 2019a, b).

Currently, genetic engineering techniques are widely used for bioconversion of value-added products from lignocelluloses biomass. Transfer of genes encodes xylose reductase and xylitol dehydrogenase from *Pichia stipitis* to S. cerevisiae (wild strain) for utilization of xylose for enhanced production of ethanol (Agbogbo and Wenger 2007). In industrial scale, it is expensive to maintain the biochemical and fermentative characters of recombinant strains. Due to this disadvantage, a co-culture process (both glucose- and xylose-utilizing strains) is cost-effective method for ethanol production along with enzyme-producing white-rot fungi (Cheng et al. 2010). Various factors influencing the production efficiency of the strain include oxygen, aeration, agitation, pH, temperature, concentration of carbon source, inhibitor presence in hydrolysate, and medium components (Sreenivas Rao et al. 2006).

15.8 Enzymes Produced by White-Rot Fungi

White-rot fungi produce various extracellular oxidases such as laccase, Mn peroxidase, and lignin peroxidase (LiP) including lignin-modifying enzymes (LME). These enzymes effectively degrade the lignin content in lignocellulosic biomass. Lignin degradation is a key process for biofuel production from lignocellulosic biomass. White-rot fungi (WRF) *Phanerochaete chrysosporium* produce multiple

isoenzymes (LiP, MnP) but not laccase. Other WRF were able to produce laccases. Based on the enzyme production, WRF are categorized into three main groups: (1) lignin-manganese peroxidase (*P. chrysosporium*, *Phlebia radiata*), (2) manganese peroxidase *(Dichomitus squalens*, *Rigidoporus lignosus*), and (3) lignin peroxidase (*Phlebia ochraceofulva* and *Junghuhnia separabilima*). These enzymes are able to degrade various types of plant polymers (Heinzkill 1998; Yadav et al. 2016) (Table 15.1).

Table 15.1 Enzymes released by WRF

Biomass	Microorganism	Enzyme	Reference
Corn stover	Gloeophyllum trabeum KU-41	Cellulase	Gao et al. (2012)
Cotton stalks	*Phanerochaete chrysosporium*	Cellulase	Jian et al. (2008)
Milled tree leaves, banana peel, apple peel, mandarin peel	*Pleurotus* spp., *Lentinus edodes*	Laccase	Songulashvili et al. (2005)
Sugarcane trash	*Aspergillus terreus*	Cellulases	Singh et al. (2008)
Sorghum husk	*Phanerochaete chrysosporium*	Lignin peroxidase and manganese peroxidase	Pankajkumar et al. (2018)
Young plant leaves (from Aster genus), lamella from oats and maize plants	*Fusarium oxysporum*	Endopolygalacturonases galactosidase	Mikan and Castellanos (2004)
Sugarcane bagasse	*Aspergillus niger*	Xylanases, cellulases	Park et al. (2002)
Clavel leaves, young plant leaves (from Aster genus), lamella from oats and maize plants	*Fusarium merismoides*	Endo-xylanase, cellulases, arabinofuranosidase, acetylesterase	Fernández-Martín et al. (2007)
Sugi wood	*Strobilurus ohshimae*	Lignin peroxidase and manganese peroxidase	Homma et al. (2007)
Bagasse of cane maize straw	*Pleurotus ostreatus*	Xylanases, cellulases, laccase, manganese peroxidase	Márquez et al. (2007), Okamoto et al. (2002)
Grape seeds, barley bran, and wood shavings	*Phanerochaete chrysosporium*	Lignin peroxidase and manganese peroxidase	Rodríguez et al. (1997), Srinivasan et al. (1995), Kersten and Cullen (2007), Quintero et al. (2006)
Clavel leaves, young plant leaves (from Aster genus), lamella from oats and maize plants	*Clonostachys rosea*	Endopolygalacturonases galactosidase endo-xylanase, cellulases, arabinofuranosidase, acetyl	Mikan and Castellanos (2004), Rezácová et al. (2006)

(continued)

Table 15.1 (continued)

Biomass	Microorganism	Enzyme	Reference
Coffee pulp, used nappy, grass residues, cleaned coffee (substrates analyzed separately and in mixture), wheat straw, industrial cotton fiber	*P. ostreatus, P. pulmonarius*	Endoglucanase, cellobiohydrolase, laccases, manganese peroxidase	Marnyye et al. (2002), Delfin and Duran de bazúa (2003), Okamoto et al. (2002)
Clavel leaves, young plant leaves (from Aster genus), lamella from oats and maize plants	*Streptomyces*	Cellulases, xylanases, arabinofuranosidase xylosidase, acetylesterase	Mikan and Castellanos (2004), Benimelia et al. (2007)
Wood shaving, carozo maize, and compost of gardening wheat straw	*Trametes versicolor*	Laccases	Moredo et al. (2003), Márquez et al. (2007), Dumonceaux et al. (2001), Villagran and Renan (1991), Cabuk et al. (2006), Tong et al. (2007)
Oat husk	*Cerrena unicolor*	Laccases, manganese peroxidase	Moilanel et al. (2015)
Saw dust	*Coriolopsis gallica*	Laccases	Daassi et al. (2016)

15.9 Ligninolytic Enzymes from White-Rot Fungi

Ligninolytic enzymes from WRF are used in industrial biotechnological process. Free enzyme applications were limited in industrial scale due to instability and lack of reusability. Immobilization techniques were used to improve stability and can be reusable. Voberkova et al. (2018) have reported that immobilization methods are desirable, operational stability and cost-effective process.

The delignification is a key task for proper utilization of lignocelluloses biomass. The first demonstration of commercial plant for the ethanol production from lignocellulosic biomass is in operation in Canada since 2004 (Tampier et al. 2004). White-rot fungi is a filamentous fungi, which can produce ligninase, cellulase enzymes for degradation of lignocellulosic plant material. *Pleurotus cystidiosus, P. ostreatus, Phlebia, Ganoderma lucidum,* and *Flammulina velutipes* are able to produce ethanol from biomass. Various WRF are used for production of enzymes, which degrade the lignocellulosic material. Integrated production of ethanol by WRF is influenced by factors like moisture content, temperature, pH, and chemical nature. Various reports of biological pretreatment of lignin are presented in Table 15.2.

Table 15.2 Lignin degradation by fungal strains

Microorganism	Biomass	Effect	Reference
Merulius tremellosus	Aspen wood	0.26–0.37 mg/ml as compared to 0.15–0.16 mg/ml of control	Bradley et al. (1989)
Phanerochaete chrysosporium	Cotton stalks	0.027 g/g	Jian et al. (2008)
Trichoderma viride	Rice straw	56% of lignin reduction	Ghorbani et al. (2015)
Trichoderma reesei	Wheat straw	Sugar Yield -270 Reduction (mg/g dry substrate)	Barakat and Rouau (2014)
Ceriporiopsis subvermispora	Corn stove	2- to 3-fold increase in reducing sugar yield	Wan and Li (2011)
Irpex lacteus	Corn stalks	82% of hydrolysis yield	Du et al. (2011)
Phanerochaete chrysosporium	Corn stover silage	Improved degradation of substrate cell wall components 39% lignin removal of initial substrate	Liu et al. (2014)
Pleurotus ostreatus	Wheat straw	35% of lignin reduction	(1983)
Pleurotus ostreatus	Rice straw	33% lignin removal	Mustafa et al. (2016)
Ceriporiopsis subvermispora	Corn stover	31.59% lignin loss	Wan and Li (2010)
Fusarium spp.	Paddy straw	17.1% decrease in lignin content, 10.8% decrease in silica content compared with controls	Phutela and Sahni (2012)

15.10 Biochemical Pathway of Ethanol by Fungi

The yeast cells have the sense to identify the sugar-rich environment. This intensity by the yeast can affect the enzyme activity during biochemical processes, change of translation by mRNA, stability of protein degradation, and concentration of metabolites (Yadav et al. 2017, 2018). After glucose uptake then enters the glycolytic pathway and is converted to pyruvate and produces ATP and then coupled to intermediate products and reducing power through NADH for biosynthetic pathway. Pyruvate in glycolysis enters TCA cycle or fermentative pathway. In alcoholic fermentation, decarboxylation of the pyruvate gives acetaldehyde by pyruvate decarboxylase enzyme. This enzyme converts acetaldehyde to ethanol by reduction of NADH to NAD^+, from one molecule of glucose to two CO_2 molecules and ethanol is formed.

The second abundant sugar, from hemicellulosic fraction of biomass, is xylose. Xylose is a five-carbon sugar molecule, utilized through pentose phosphate pathway in fungi. The xylose transportation in fungi is followed by two different mechanisms. *Pichia stipitis* and *Candida* spp. follow proton symport (PS) mechanism, whereas Saccharomyces cerevisiae follows facilitated diffusion system (FDS) for transport of xylose. The PS transport is for pentose sugars and FDS transport for both hexose and pentose sugars. The medium consisting of low quantities of hex-

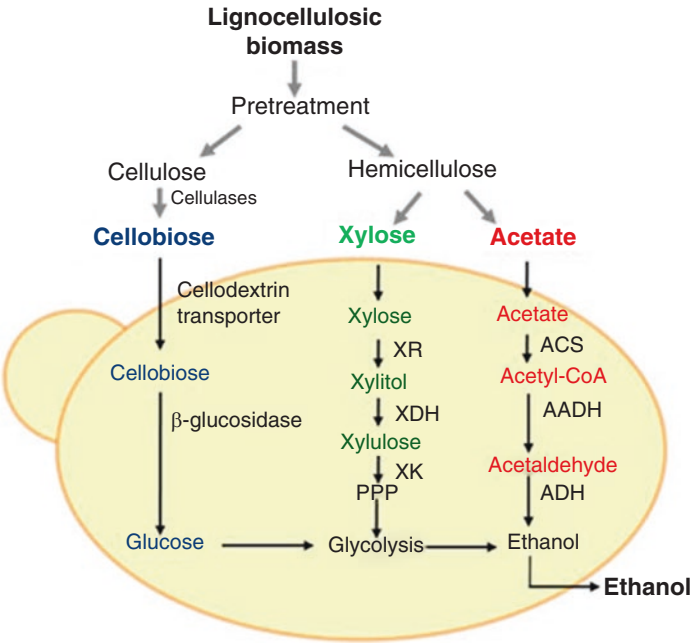

Fig. 15.1 Biochemical pathway of ethanol production by fungi

oses will inhibit xylose transport by FDS mechanism. In fungi xylose metabolism, xylose is converted to xylulose through xylitol by xylitol dehydrogenase, and then xylulose is phosphorylated and enters to pentose phosphate cycle. Conversion of xylose to xylitol in the presence of xylose reductase utilizes NADH or NADPH as a cofactor. In anaerobic or microaerophilic conditions, yeast utilizes NADH for conversion. High expression of xylose reductase and xylitol dehydrogenase will tend to enhance the ethanol production. Co-current isomerization of xylose and co-fermentation of xylose and glucose increase the production of ethanol (Fig. 15.1).

15.11 Conclusion and Future Prospects

Overcoming the challenges of fossil fuels through the biotechnological route by fungal consortium is one of the promising approaches to reach global demand. The lignocellulosic biomass is a significant substrate for bioethanol production using fungal consortium for commercial production of ethanol throughout the year in a cost-effective process. This biotechnological approach of ethanol production is an eco-friendly process through enhancing the yield and reduction of the greenhouse gas emission. Saccharifications of lignocellulosic biomass by white-rot fungi have overcome the challenges with chemical digestive methods such as gasification and acidification. The chemical process using high temperatures, acids, and high-energy

input lead to release of inhibitors and pollutants. The development of fungal consortium consists of lignin-, cellulose-, and hemicellulose-degrading strains in combination with ethanol-producing strains (from hexoses and pentoses) which can achieve the global demand for ethanol production. The enzymes like laccases, cellulases, and xylanases play a significant role for the digestion of lignocellulosic plant biomass by eco-friendly manner. Several advantages were reported by fungal consortia which include high adaptability, productivity, and efficiency of the production. This process is also considered as inexpensive and eco-friendly. The future prospect for ethanol production using fungal consortium is a need to development of unique fungal consortia with noncompetitive synergistic fungal strains for production of lignocellulosic digestive enzymes along with efficient ethanol-producing strains. Standardization of co-culture studies for optimization of digestive enzymes and ethanol production, optimized conditions for microbial growth, and metabolite production (enzymes) were varying from one strain to another. In co-culture studies (development of fungal consortium), physical factors (temperature, pH, agitation, moisture levels, surface area, and SSF) and chemical factors such as carbon source, nitrogen sources, minerals, salts, and their concentrations need to standardize for commercial production of ethanol. Simultaneous saccharification and fermentation have enhanced the yield.

Acknowledgments Authors sincerely acknowledge University Grant Commission (UGC), Government of India, for providing financial support for major research project entitled "Overcoming fossil fuel challenges: Co-culture fermentations for biofuel production using agro-industrial waste materials."

References

Agbogbo FK, Wenger KS (2007) Production of ethanol from corn Stover hemicellulose hydrolyzate using *Pichia stipitis*. J Ind Microbiol Biotechnol 34:723–727

Balan V, Chiaramonti D, Kumar S (2013) Review of US and EU initiatives toward development, demonstration, and commercialization of lignocellulosic biofuels. Biofuels Bioprod Biorefin 7:732–759

Bacovsky D, Mabee W, Worgetter M (2010) How close are second-generation biofuels. Biofuels Bioprod Bioref 4:249–252

Barakat A, Rouau X (2014) New dry technology of environmentally friendly biomass refinery: glucose yield and energy efficiency. Biotechnol Biofuels 7:138

Benimelia CS, Castroa GR, Chailec AP, Amoroso MJ (2007) Lindane uptake and degradation by aquatic *Streptomyces* sp. strain M7. Int Biodeterior Biodegradation 59:148–155

Binod P, Janu KU, Sindhu R, Padey A (2010) Hydrolysis of lignocellulosic biomass for bioethanol production. In: Ashok P, Christian L, Ricke SC (eds) Biofuels: alternative Feedstock's and conversion processes. Elsevier Inc, pp 229–250

Bobleter O (1994) Hydrothermal degradation of polymers derived from plants. Prog Polym Sci 19:797–841

Bradley C, Wood P, Kearns R, Black B (1989) Biological delignification of wood and straw for ethanol production via solid state culture. Final report, Montana Department of Natural Resources and Conservation, Montana

Buruiana CT, Garrote G, Vizireanu C (2013) Bioethanol production from residual lignocellulosic materials: a review-Part-1. Food Technol 37:9–24
Cabuk A, Unal AT, Kolankaya N (2006) Biodegradation of cyanide by a white rot fungus, Trametes versicolor. Biotechnol Lett 28:1313–1317
Campbell CJ, Laherrere JH (1998) The end of cheap oil. Sci Am 3:78–83
Cheng KK, Zhang JA, Chave ZE, Li JP (2010) Integrated production of xylitol and ethanol using corn cob. Appl Microbiol Biotechnol 87:411–417
Chen S, Zhang X, Singh D, Yu H, Yang X (2010) Biological pre-treatment of lignocellulosics: potential, progress and challenges. Biofuelss 1:177–199
Claudio M, Jaime B, Juanita F, Regis T (2011) Mendonca bioethanol production from tension and opposite wood of *Eucalyptus globulus* using organosolv pretreatment. J Ind Microbiol Biotechnol 38:1861–1866
Cragg SM, Beckham GT, Bruce NC, Bugg TDH, Distel DL, Dupree P, Etxabe AG et al (2015) Lignocellulose degradation mechanism across the tree of life. Curr Opin Chem Biol 29:108–119
Daassi D, Zouri-Mechichi H, Frikha F, Rodriguez-Couto S, Nasri M, Mechichi T (2016) Sawdust waste as a low-cost support substrate for laccases production and adsorbent for azo dyes decolorization. J Environ Health Sci Eng 14:1–12
Das H, Singh SK (2004) Useful by products from cellulosic wastes of agriculture and food industry-a critical appraisal. Crit Rev Food Sci Nutr 44:77–89
Delfin AI, Duran de bazúa C (2003) Biodegradación de residuos urbanos lignocelulósicos por Pleurotus. Rev Int Contam Ambient 19:37–45
Du W, Yu H, Song L, Zhang J, Weng C, Ma F, Zhang X (2011) The promising effects of by-products from Irpex lacteus on subsequent enzymatic hydrolysis of bio-pretreated corn stalks. Biotechnol Biofuels 4:37
Dumonceaux T, Bartholomew K, Valeanu L, Charles T, Archibald F (2001) Cellobiose dehydrogenase is essential for wood invasion by nonessential for Kraft pulp delignification and Trametes versicolor. Enzym Microb Technol 29:478–489
Fernández-Martín R, Domenech C, Cerdá-Olmedo E, Avalos J (2007) Ent-Kaurene and squalene synthesis in Fusarium fujikuroi cell-free extracts. Phytochemistry 54:723–728
Gao Z, Mori T, Kondo R (2012) The pretreatment of corn Stover with Gloeophyllum trabeum KU-41 for enzymatic hydrolysis. Biotechnol Biofuels 5:28
Ghorbani F, Karimi M, Biria D, Kariminia HR, Jeihanipour A (2015) Enhancement of fungal delignification of Rice Straw by *Trichoderma viride sp*. to improve its saccharification. Biochem Eng J 101:77–84
Haghighi Mood S, Hossein Golfeshan A, Tabatabaei M, Salehi Jouzani G, Najafi GH, Gholami M, Ardjmand M (2013) Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew Sust Energ Rev 27:77–93
Hatakka AI (1983) Pretreatment of wheat straw by white-rot fungi for enzymic saccharification of cellulose. Appl Microbiol Biotechnol 18:350–357
Heinzkill M (1998) Characterization of laccases and peroxidases from wood rotting fungi. Appl Envion Microbiol 64:1601–1606
Hill J, Nelson E, Tilman D, Polasky S, Tiffany D (2006) Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuels. Proc Natl Acad Sci U S A 103(30):11206–11210
Homma H, Shinoyama H, Nobuta Y, Terashima Y, Amachi S, Fujii T (2007) Lignin-degrading activity of edible mushroom Strobilurus ohshimae that forms fruiting bodies on buried soil (Cryptomeria japonica) twigs. J Wood Sci 53:80–84
Jeffries TW, Grigoriev IV, Grim Wood J, Laplaza JM, Aerts A, Salamov A (2007) Genome sequence of the lignocellulose-bioconverting and xylose fermenting yeast *Pichia stipitis*. Nat Biotechnol 25:319–326
Jian S, Ratna R, Sharma-Shivappa CM, Howell N (2008) Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production. Biomass Bioenergy 33:88–96

Jonsson LJ, Martin C (2016) Pretreatment of lignocelluloses: formation of inhibitory by products and strategies for minimizing their effects. Bioresour Technol 199:103–112

Kim S and Dale BE (2004) Global potential bioethanol production form wasted crops and crop residues. Biomass Bioenergy 26:361–375

Kersten P, Cullen D (2007) Extracellular oxidative systems of the lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. Forest Genet Biol 44:77–87

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA, Saxena AK (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Volume 2: perspective for value-added products and environments. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kuijk SJA, Sonnenberg ASM, Baars JJP, Hendriks WH, Cone JW (2015) Fungal treated lignocellulosic biomass as ruminant feed ingredient: a review. Biotechnol Adv 33:191–202

Limayem A, Ricke SC (2012) Lignocellulosic biomass for bioethanol production: current prospectives, potential issues and future prospects. Prog Energy Combust Sci 38:449–467

Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 69:627–642

Liu S, Li X, Wu S, He J, Pang C, Deng Y, Dong R (2014) Fungal pre-treatment by *Phanerochaete chrysosporium* for enhancement of biogas production from corn Stover silage. Appl Biochem Biotechnol 174:1907–1918

Marnyye A, Velásquez C, Mata G, Michel SJ (2002) Waste-reducing cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* on coffee pulp: changes in the production of some lignocellulolytic enzymes. World J Microbiol Biotechnol 18:201–207

Márquez ATA, Mendoza MGD, González MSS (2007) Actividad fibrolitica de enzimas producidas por Trametes sp. EUM1, Pleurotus ostreatus IE8 y Aspergillus niger AD96.4 en fermentation solida. Interciencia 32:780–785

Mc. Millan JD (1997) Biomass conversion bioethanol production: status and prospects. Renew Energy 10(2):295–302

Mikan VJF, Castellanos SDE (2004) Screening for isolation and characterisation of microorganisms and enzymes with useful potential for degradation of cellulose and hemicellulose. Rev Colomb Biotechnol 6:58–67

Millati IR, Syamisiah S, Nikalasson C, Cahyanto MN, Lundquist K, Taherzadeh MJ (2011) Biological pretreatment of lignocelluloses with white rot fungi and its applications: a review. Bioresources 6:5224

Moilanen U, Winquist E, Mattila T, Hatakka A, Eerikainen T (2015) Production of manganese peroxidase and laccase in solid state bioreactor and modeling of enzyme production kinetics. Bioprocess Biosyst Eng 28:57–68

Moredo N, Lorenzo M, Domínguez A, Moldes D, Cameselle C, Sanroman A (2003) Enhanced ligninolytic enzyme production and degrading capability of Phanerochaete chrysosporium and Trametes versicolor. World J Microbiol Biotechnol 19:665–669

Mustafa AM, Poulsen TG, Sheng K (2016) Fungal pretreatment of rice straw with *Pleurotus ostreatus* and *Trichoderma reesei* to enhance methane production under solid state anaerobic digestion. Appl Energy 180:661–671

Nigam P, Pandey A (2009) Solid-state fermentation technology for bioconversion of biomass and agricultural residues. In: Biotechnology for agro-industrial residues utilization. Springer Netherlands, pp 197–221

Okamoto K, Narayama S, Katsuo A, Shigemat sui I, Yanase H (2002) Biosynthesis of p-anisaldehyde by the white-rot basidiomycete Pleurotus ostreatus. J Biosci Bioeng 93:207–210

Park YS, Kang SW, Lee JS, Hong SI, Kim SW (2002) Xylanase production in solid state fermentation by Aspergillus Niger mutant using statistical experimental design. Appl Microbiol Biotechnol 58:762–766

Pankajkumar RW, Rahul VK, Byong-Hun Jeon, Sanjay PG (2018) Enzymatic hydrolysis of biologically pretreated sorghum husk for bioethanol production. Biofuel Res J 5(3):846–853

Pereira SR, Portugal-Nunes DJ, Evtuguin DV, Serafim LS, Xavier AMRB (2013) Advances in ethanol production from hard wood spent sulphite liquors. Process Biochem 48:272–282

Phutela UG, Sahni N (2012) Effect of Fusarium sp. on Paddy Straw digestibility and biogas production. J Adv Lab Res Biol 3:9–12

Quintero DJC, Gumersindo FEJOOC, Lemar RJM (2006) Production of ligninolytic enzymes from basidiomycete fungi on lignocellulosic materials. Rev Facult Quim Farmaceut 13:61–67

Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN, Rastegari AA, Singh K, Saxena AK (2019a) Endophytic fungi: biodiversity, ecological significance, and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi: Volume 1: diversity and enzymes perspectives. Springer International Publishing, Cham, pp 1–62. https://doi.org/10.1007/978-3-030-10480-1_1

Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V, Singh BP, Dhaliwal HS, Saxena AK (2019b) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer International Publishing, Cham, pp 105–144. https://doi.org/10.1007/978-3-030-03589-1_6

Rastegari AA, Yadav AN, Gupta A (2019) Prospects of renewable bioprocessing in future energy systems. Springer International Publishing, Cham

Rezacova V, Hrselova H, Gryndlerová H, Mikšık I, Gryndler M (2006) Modifications of degradation-resistant soil organic matter by soil saprobic microfungi. Soil Biol Biochem 38:2292–2299

Rodriguez J, Ferraz A, Nogueira FPR, Ferrer I, Esposito E, Duran N (1997) Lignin biodegradation by the ascomycete Chrysonilia sitophila. Appl Biochem Biotechnol 63:233–242

Sanchez O, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feed stocks. Bioresour Technol 3:5270–5295

Singh P, Suman A, Tiwari P, Arya N, Gaur A, Shrivastava AK (2008) Biological pretreatment of sugar cane trash for its conversion to fermentable sugars. World J Microbiol Biotechnol 24:667–673. https://doi.org/10.1007/s11274-007-9522-4

Songulashvili G, Elisashvili V, Penninckx M, Metreveli E, Hadar Y, Aladashvili N, Asatiani M (2005) Bioconversion of plant raw materials in value added products by *Lentinus edodes* (Berk.) Singer and *Pleurotus* spp. Int J Med Mushrooms 7(3):467–468

Sreenivas Rao R, Pavana Jyothi C, Prakasham RS, Sarma PN, Venkatestwar Rao L (2006) Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis*. Bioresour Technol 97:1974–1978

Srinivasan C, Dsouza TM, Boominathan K, Reddy CA (1995) Demonstration of laccase in white rot basidiomycete *Phanerochaete chrysosporium* BKM-F1767. Appl Environ Microbiol 6:4274–4277

Steven RW, Lee H (1990) Regulation of D-xylose utilization by hexoses in pentose fermenting yeasts. Biotechnol Adv 8(4):685–697

Tabassum Ansari F, Choube A (2012) Impact of biofuel in petrol engine-a review. Int J Thermal Technol 2(2):ISSN2277-4114

Tampier M, Smith D, Bibeau E, Beauchemin PA (2004) Identifying environmental preferable uses for biomass resources, http://www.cec.org/giles/PDF/ECONOMY/Biomass-Stage-I-II_en.pdf

Tong P, Hong Y, Xiao Y, Zhang M, Tu X, Cui T (2007) High production of laccase by a new basidiomycete, *Trametes* sp. Biotechnol Lett 29:295–301

Villagran F, Renan J (1991) Simulación y modelo matemático de la delignification selective de la madera por hongos blancos en ambient natural. Temuco Universidad de la Frontera 24:465–487

Voberkova S, Solcany V, Vrsanska M, Adam V (2018) Immobilization of ligninolytic enzymes from white-rot fungi in cross-linked aggregates. Chemosphere 202:694

Wan C, Li Y (2010) Microbial pre treatment of corn Stover with *Ceriporiopsis subvermispora* for enzymatic hydrolysis and ethanol production. Bioresour Technol 101:6398–6403

Wan C, Li Y (2011) Effectiveness of microbial pretreatment by *Ceriporiopsis subvermispora* on different biomass feed stocks. Bioresour Technol 102:7507–7512

Wesenberg D, Kyriakides I, Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 22:161–187

Yadav AN, Sachan SG, Verma P, Kaushik R, Saxena AK (2016) Cold active hydrolytic enzymes production by psychrotrophic Bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. J Basic Microbiol 56:294–307

Yadav A, Verma P, Kumar R, Kumar V, Kumar K (2017) Current applications and future prospects of eco-friendly microbes. EU Voice 3:21–22

Yadav AN, Verma P, Kumar V, Sangwan P, Mishra S, Panjiar N, Gupta VK, Saxena AK (2018) Biodiversity of the genus *Penicillium* in different habitats. In: Gupta VK, Rodriguez-Couto S (eds) New and future developments in microbial biotechnology and bioengineering, *Penicillium* system properties and applications. Elsevier, Amsterdam, pp 3–18. https://doi.org/10.1016/B978-0-444-63501-3.00001-6

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi Volume 1: diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Mishra S, Singh S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 2: perspective for value-added products and environments. Springer International Publishing, Cham

Yewale T, Panchwagh S, Rajagopalan S, Dhamole PB, Jain R (2016) Enhanced xylitol production using immobilized *Candida tropicalis* with non-detoxified corn cob hemicellulosic hydrolysate. 3 Biotech 6:75

Yu Y, Feng Y, Xu C, Liu J, Li D (2011) Onsite biodetoxification of steam exploded corn Stover for cellulosic ethanol production. Bioresour Technol. https://doi.org/10.1016/j.biortech.2011.01.067

Zhang Z, Donaldson AA, Ma X (2012) Advancements and future directions in enzyme technology for biomass conversion. Biotechnol Adv 35:367–375

Zhang LY, Xia L, Liu Z, Pu Y (2014) Enhanced xylitol production from statistically optimized fermentation of corn stalk hydrolysate by immobilized *Candida tropicalis*. Chem Biochem Eng 28:87–93