

Chapter 8

Lignocellulosic Biomass: As Future Alternative for Bioethanol Production

Tanveer Bilal Pirzadah, Bisma Malik, Manoj Kumar, and Reiaz Ul Rehman

Contents

8.1	Introduction.....	146
8.2	Background.....	148
8.3	Biofuel: Solution to Sustainable Energy.....	149
8.4	Lignocellulosic Feedstock.....	149
8.5	Technologies Involved in Cellulosic Ethanol Production.....	150
8.5.1	Pretreatment.....	150
8.5.2	Hydrolysis.....	154
8.5.3	Fermentation.....	155
8.6	Conclusion and Future Perspective.....	158
	References.....	159

Abstract Biofuels provide a potential and promising green alternative to avoid the global political instability and environmental crises that arise from dependence on petroleum. It has an important role to mitigate global warming and to conserve fossil fuels. Currently, starchy crops such as corn are utilized as a source of raw material for the production of bioethanol but it cannot meet global fuel requirements. Besides, due to their food value these conventional crops are not able to cater the demand of biofuel production. Therefore, lignocellulosic biomass seems to be an attractive alternative for inexorable supplies of biofuels, cutting down the credence on fossil fuel resources. Lignocellulosic biomass feedstock is abundant, recyclable, cheap, and

T.B. Pirzadah • B. Malik • R.U. Rehman (✉)
Department of Bioresources, University of Kashmir,
Srinagar, Jammu & Kashmir 190006, India
e-mail: pztanveer@gmail.com; bisma767@gmail.com; reiazrehman@yahoo.co.in

M. Kumar
Amity Institute of Microbial Technology, Amity University Uttar Pradesh,
Noida, Uttar Pradesh 201303, India
e-mail: manojjnu@gmail.com

is evenly distributed in nature. However, lignocellulosic bioethanol production is not commercialized at a large scale due to certain economic and technical barriers which make ethanol production exorbitant. Therefore, research should be focussed to develop commercially profitable processes (green technology) for bioethanol production. Moreover, current approach is focussed on enzyme-based conversion of lignocellulosic biomass to bioethanol. The assurance of highly dynamic conversion coupled to a “Green” technology is now universally appealing. Therefore, the main aim of this chapter is to critically analyze the current situation and future needs for technological developments in the area of producing liquid biofuels from lignocellulosic biomass. It primarily covers distinct lignocellulosic biomass conversion technologies, challenges, and future research targets.

Keywords Lignocellulosic biomass • Agriculture residue • Bioethanol • Conversion technologies

8.1 Introduction

Currently, the economy of the nation is solely dependent upon the nonrenewable energy resources such as coal, petroleum, natural gas, etc. that are being used for the generation of electricity, fuel, and other goods (Uihlein and Schbek 2009). In the new era of industrialization, the demand for the petroleum resources has gained a steep rise in energy sector and today these fossil energy resources contribute about 80 % of the primary energy consumption in the world, of which about 58 % alone is consumed by the automobile sector (Agrawal 2007; Escobar et al. 2009). Because of such huge consumption of these fossil fuels especially in urban areas the pollution level increases at a very high pace. Therefore, the concentration of GHG-emissions in the earth’s atmosphere has immensely enhanced (Ballesteros et al. 2006). Besides, other factors that are responsible for high energy consumption include population explosion, industrial prosperity that ultimately lead to deleterious effects to the ecosystem such as global warming, biodiversity loss, etc. (Gullison et al. 2007). Further, it is predicted that the total global oil production will begin to decline within near future due to the limited resources of fossil fuels (Campbell and Laherrere 1998). Besides, due the ill effects associated with these conventional energy resources, scientists all over the globe switched on towards green energy resources that are renewable, sustainable, cost-effective, and eco-friendly (Prasad et al. 2007; Singh et al. 2010). Among the various green energy resources biofuels (bioethanol, biodiesel) gained a world-wide importance due to eco-friendly nature. To boost the lignocellulosic bioethanol industry, various developed countries directed state policies towards the improvement and economic utilization of biomass feedstock for meeting their future energy crises in order to meet GHG reduction targets as specified in the Kyoto Protocol as well as to decline reliance and dependence on the supply of nonrenewable energy resources. Biomass being abundantly available in nature is commonly used in a combined heat and power programme (CHP)

to generate heat and electricity. However, at present it is also being used to generate liquid fuels such as bioethanol for automobiles (Demirbas 2005). Currently, it has gained a worldwide interest both at international and national levels. The world market for bioethanol has entered a phase of rapid and transitional growth. With the result, many nations are shifting their research focus towards sustainable and green sources for bioethanol production because of depleting conventional energy reserves.

Although bioethanol has tremendous potential in transport sector to replace gasoline but at present it is not cost-effective when compared to nonrenewable energy resources. However, during the last few decades bioethanol production shows an increasing trend with 31 billion liters during 2001 to 39 billion liters in 2006 and is expected to reach about 100 billion liters in 2015 (Taherzadeh and Karimi 2007a). Currently, Brazil and the USA are the leading countries using sugarcane and corn respectively as raw material for bioethanol production contributing about 62 % of the world bioethanol production (Kim and Dale 2004). However, the bioethanol production from these food crops possess certain limitations as it cause food crisis especially in developing countries. Besides, the cost that is involved in biofuel production is also an important factor to commercialize this technology. In order to overcome these challenges, lignocellulosic biomass that is considered as a green gold is a promising alternative to avoid the existing competition of food versus fuel caused by grain-based bioethanol production (Bjerre et al. 1996). Lignocellulosic biomass typically contains 50–80 % (dry basis) carbohydrates that are generally composed of pentose and hexose sugars. Moreover, lignocellulosic biomass has a potential to produce about 442 billion liters of bioethanol. Kim and Dale (2004) reported that these green energy sources have the potential to produce about 491 billion liters of bioethanol/annum which is about 16 times greater than the actual global bioethanol production. It has been revealed that an average individual in the United States produces about 1.8 kg of municipal solid waste (MSW)/day which mainly contains about 75 % cellulosic biomass (wastepaper, wood wastes and cardboard). Therefore, a city with one million people generates about 1,800 tonnes of MSW/day, or about 1,300 tonnes/day of organic material. About 330 L of bioethanol is produced/ton of biomass waste. Thus, the total biomass waste generated/day from a city with one million individual produces approximately about 430,000 L of ethanol/day that is sufficient fuel to cater the needs of more than 58,000 people in the US; 360,000 people in France, or nearly 2.6 million people in China at current rates/capita fuel use. This green gold is renewable, cost effective, abundant and evenly distributed throughout the nature. Lignocellulosic biomass includes grasses, agricultural waste, forest residues, etc. Comprehensive research has been carried out on bioethanol production from lignocellulosic biomass during the last two decades (Cadoche and Lopez 1989; Binod et al. 2010). Hence bioethanol generation could be the sustainable and green route to the effective utilization of biomass feedstock. This chapter presents a brief overview of the applicable technologies for bioethanol production using lignocellulosic biomass as a substrate source.

8.2 Background

Henry Ford in 1925 considered grain alcohol or ethanol as future fuel. He further quoted a well-known statement, “The fuel of the future is going to come from apples, weeds, sawdust almost anything. There is fuel in every bit of vegetable matter that can be fermented. It remains for someone to find out how this fuel can be produced commercially better fuel at a cheaper price than we know now.” Today Henry Ford’s futuristic vision significance can be easily understood. Biofuels the important component of “Green Technology” is a promising alternative to replace fossil fuels. Biofuels is a promising alternative that reduces dependence on petroleum resources and has gained a widespread attention throughout the world especially in developing nations (Pickett et al. 2008). In early 1860s little attention was paid towards bioethanol production due to low prices of fossil energy resources but during 1908 Henry Ford developed a Ford Model T that has the ability to operate on ethanol, gasoline, or blends of both (Bernton et al. 1982). However, bioethanol gained importance during 1970s when abruptly the price of petroleum resources arose besides one of the constituent (methyl tertiary butyl ether (MTBE) used in gasoline was declared as a toxic pollutant (Kovarik and Kettering 1982). The government of United States (US) focus on novel R&D programs directed toward the development of more ecofriendly, sustainable and promising alternative fuels generated from cheap renewable sources. Initially, during 1980 and 1990, there was great effort from the government sector to boost industrial efforts toward production of bioethanol from biomass feedstock by providing subsidies to farmers, tax-exemptions, and support bioethanol research programs. In recent years biofuel production exhibits an increasing trend from about 4.4 billion litres during 1980 to 50.1 billion litres in 2005 (Murray 2005), with further dramatic increases in future. Now-a-days US become the leading producer of bioethanol with about 13.5 billion gallon capacity. Currently, about 200 operational corn-based ethanol plants are operating in 29 states (RFA 2010) most of them are placed in the “corn belt” in the US Midwest (Gnansounou 2010). Despite of the global economic burden during 2010, bioethanol production continues to expand rapidly and contributes significantly to the economic development of rural communities in the US (RFA 2010). Biofuels seems to be a potential alternative to expel the use of fossil fuels as an automotive fuel and lower toxic emissions. In particular, cellulosic bioethanol is believed to possess tremendous potential in this regard, even though there are currently no commercial scale plants in the United States (Raneses et al. 1998). Although lot of R&D work has been done on lignocellulosic bioethanol production, until 1995 not even a single plant capable of converting lignocellulosic biomass to bioethanol via biological route on the commercial scale has been put into operation (Szczo drak and Fiedurek 1996). However, during World War II, when wartime conditions altered the economic growth, few cellulosic-bioethanol plants were constructed and regulated in distinct countries (Russia, Korea, China, Germany, Switzerland, and the USA) to provide an alternative energy resource. However, many of these plants were closed at the end of the war due to competition with synthetically produced petroleum products (Badger 2002). The first demonstration plant based on lignocellulosic

feedstock was developed in Canada during 2004 (Tampier et al. 2004). Further, novel green technologies have been developed to enhance the bioethanol production (Natural Resources Canada's Management Team 2005). Recently, in US cost-effective technologies utilizing lignocellulosic feedstock for the production of bioethanol have started to emerge (Badger 2002). In Canada, commercial scale cellulosic-bioethanol plant using proprietary enzymatic hydrolysis technology was developed by Iogen Corporation limited. During 1997, they partnered with Petro-Canada to generate cellulose-bioethanol inaugurated with one million gallon/year ethanol demonstration facility using corn stover and switchgrass as bio-feedstocks. Another bioethanol plant was developed in Sweden during 2005 using sawdust as a raw material. However, the plant is still in infancy stage, but the optimism is high. In near future, Sweden could become competent of bioethanol from forest residues especially wood and wood residues, which would be a much more green way of supplying bioethanol to the Swedish market (Advanced course in LCA 2005).

8.3 Biofuel: Solution to Sustainable Energy

Biofuels generally include liquid, gas, and solid fuels predominantly generated from bio-renewables or combustible renewable feedstock. Currently wide range of fuels (ethanol, butanol, biodiesel, and bio-hydrogen) can be produced from these green energy sources (Demirbas 2008). Contemporary much attention has been dedicated to the transformation of biomass feedstock into bioethanol, considered the cheapest and cleanest liquid fuel alternative to petroleum resources. Due to ill effects of the global warming, scientists all over the world had paid much attention towards the latest cost-effective technologies responsible for bioethanol production. Biofuel sector has gained a momentum across the world because of following reasons: (1) Sustainable and renewable. (2) Eco-friendly in nature thus mitigates GHG-emissions. (3) Abundant and evenly distributed in nature. (4) Provide an economic boost to rural communities (5) Alleviate dependence on foreign oil, promising alternative to petroleum reserves (Farrell et al. 2006). Moreover, in the current scenario the production from huge oil reserves is declining at a faster pace of 4–5 % annually, finally the world production of oil is expected to peak in near future. Hence, as an alternative to nonrenewable energy resources, biofuels have been characterized as an inexorable preeminent supplier of green energy sources that have the capability to enhance the security of supply, lower the GHG emissions, and contribute to the steady income for farmers.

8.4 Lignocellulosic Feedstock

Lignocellulosic biomass constitutes the world's largest renewable and sustainable resource for bioethanol production. Monserrate et al. (2001) reported that an estimated 7.5×10^{10} tons of cellulose is annually synthesized through photosynthetic processes. In the world there are distinct biomass resources which are generally

categorized into four categories viz. wood residues (sawdust and other organic matter) constitutes the largest green energy source for bioenergy production. The other biomass reserves include municipal solid waste (MSW), agriculture waste, and potential energy crops. Among them, energy crops seem to be the largest, attractive, and future resource of biomass because of short harvesting period and less energy inputs (Monique et al. 2003). The agro-wastes (rice straw and wheat straw) are mainly produced by Asian countries and each year a major portion of agricultural residues is disposed of as waste. Karimi et al. (2006) estimated that about 600–900 million tons/year rice straw is generated globally. In some regions only a small fraction of these agro-wastes are exploited as animal feed while rest is destroyed by burning which ultimately affects the environment (Wati et al. 2007). In United States, majority of the corn straw approximately about 90 % is left in the fields (Glassner et al. 1999). In recent years, many European countries posed a ban on open field burning of these agro-wastes. In Brazil, the sugarcane by-products (bagasse) are utilized in combined heat and power program or cogeneration technology to generate heat and electricity (Banerjee et al. 2010). Now-a-days these agro-wastes are diverted towards bioethanol production by using latest technologies. It is estimated that rice straw has the potential to produce about 205 billion liters of bioethanol/annum. Besides, these potential biofeedstock possess significant advantages over the first generation biofeedstock for bioethanol production as they are not consumed as food. Lignocellulosic feedstock typically constitutes about 50–80 % carbohydrates which are polymers of C₅ and C₆ sugars. Currently, these carbohydrates are either chemically or biologically converted to yield bioethanol production. Perlack et al. (2005) estimated that United States alone produces about 1.4 billion tons of biomass (dry)/annum of which forests alone contribute about 30 %. Due to energy crises the world energy demand will extend to increase by 45 % during 2008–2030, an average rate of increase in 1.6 %/year. Conventional biomass conversion technologies are inefficient to meet the energy demand therefore, world is focusing on more innovative and novel green technologies for biofuel production using wide range of green energy resources.

8.5 Technologies Involved in Cellulosic Ethanol Production

Bioethanol production from lignocellulosic feedstock involves three main processes, viz. pretreatment, hydrolysis, and fermentation.

8.5.1 Pretreatment

Pretreatment process is generally used to alter chemical composition, size, and structure of biomass so that it becomes amenable to hydrolysis or biological treatment with increased yield of monomeric sugars (Moiser et al. 2005). It is considered as an essential step in the production of bioethanol. The lignocellulose biomass is composed

of different polysaccharides (cellulose and lignin bound by hemicellulose chains). The aim of the pretreatment process is to make these polysaccharides amenable to enzymatic hydrolysis by decreasing the degree of crystallinity of the cellulose (Sanchez and Cardona 2008). Currently, numerous pretreatment technologies have been extensively studied to process biomass feedstock for bioethanol production. But, none of them can be declared as an efficient technology because each pretreatment technology has its own merits and demerits. The criteria for an efficient pretreatment technology should be: (1) to avoid size reduction and preserving hemicellulose fractions (2) to prevent loss or degradation of sugars formed (3) Avoid generation of inhibitors during degradation of biomass feedstock (4) to reduce energy inputs and (5) to reduce cost (Zheng et al. 2009). Besides, several other criteria are also taken into consideration such as, recovery of value-added coproducts (lignin) for bio-power production. In addition, pretreatment results must be weighed against their impact on the ease of operation and cost of the downstream processes and the trade-off between several costs (capital costs/operating costs and biomass costs (Palmqvist and Hahn-Hagerdal 2000). Currently, this technology is very expensive with cost as high as 30 cents/gal ethanol produced (Moiser et al. 2005). Recently techno-economic analysis has been made in order to assess the cost and performance of various pretreatment technologies (Eggeman and Elander 2005). Scientists all over the world are focusing to cut down the costs of pretreatment processes through extensive R&D programs. This step is currently a major challenge in the biofuel sector due to the crystalline nature of cellulose as it is recalcitrant to enzymatic hydrolysis. Therefore, various thermochemical techniques have been developed to promote its digestibility (Wyman et al. 2005). Kim and Holtzapple (2006) reported that there exists a direct correlation between lignin removal and cellulose digestibility. The various pretreatment technologies for biomass processing involve physical, chemical, and biological approaches. Sometimes for effective pretreatment of biomass a combination of either technique is being applied (Hsu 1996).

8.5.1.1 Physical Pretreatment Technique

Physical pretreatment does not involve the chemical agents to process the biomass but it involves liquid hot water pretreatment (LHW), steam explosion, mechanical comminution, and high energy radiation. The former two techniques are more common than the latter. Steam explosion is one of the effective approaches that decreases the degree of crystallinity of lignocellulosic biomass and makes it amenable to cellulase attack. During this method the biomass is heated utilizing high pressure steam (20–50 bar, 210–290 °C) for few minutes followed by sudden and rapid decompression to atmospheric pressure. Most steam treatment yields high semi-cellulose solubility and low lignin solubility. Without any catalyst, xylose sugars recovers between 45 and 65 %. This option seems viable if modified to increase the yield and reduce the cost. Another approach of pretreatment processes involves liquid hot water (LHW) in which compressed hot liquid water at pressure above saturation point is used to hydrolyze the matrix of hemicellulose. Pentose sugar (xylose) recovery is quit high (88–98 %) and no acid or chemical catalyst is required.

Although it is an interesting approach, but is still in infancy stage. In one experimental analysis, LHW was used to treat wheat straw and the results revealed that the optimum hemicellulose-derived sugar recovery was approximately about 53 % and enzymatic hydrolysis yield of 96 % (Perez et al. 2007). Besides, biomass size reduction is not needed as the particles are broken apart during pretreatment process that is why LHW appears promising and attractive pretreatment process for commercial scales (Weil et al. 1997). Moreover, some acids like acetic acid and other organic acids are also released from hemicellulose due to the cleavage of o-acetyl and uronic acid substitutions helps to catalyze the hydrolysis of polysaccharides (hemicellulose) into soluble sugars (oligosaccharides) first and then monomeric forms. However, under acidic conditions the monomeric sugars are frequently degraded into aldehydes (furfural, 5-HMA) which act as hindrances/inhibitors to fermenting microorganism. Besides, due to the high dielectric constant of hot water it has the ability to degrade major portion of hemicellulose and few content of lignin, depending upon the temperature thus LHW plays a significant role just like an acid to degrade hemicellulosic biomass into soluble sugars and acids (Antal and Water 1996).

8.5.1.2 Chemical Pretreatment

This pretreatment approach involves the use of various chemicals such as ammonia/dilutes acid, organic solvents, sulfur dioxide, carbondioxide and other chemicals. The most common chemicals studied are as follows: Acid catalyzed hydrolysis uses dilute sulfuric/hydrochloric or nitric acids. It has been reported that the appropriate concentration of dilute sulfuric acid ranges between 0.5 and 1.5 % and temperature above 160 °C has been found the most favored for industrial applications. Under these conditions high sugar yields from hemicellulose, at least 75–90 % yields of xylose (Sun and Cheng 2002). The acid should be recycled to reduce solid waste. It is regarded as an important pretreatment technique to produce high amount of sugars from hemicellulose. Hemicellulose is more amenable to dilute acid hydrolysis compared to cellulose (Cardona et al. 2009). However, one of the limitations of this technique is the production of various inhibitors (furfural, 5-hydroxymethylfurfural and acetic acid) which retards the growth of microorganisms. Therefore, some hydrolysates are being used for fermentation in order to detoxify such chemical inhibitors. Alkaline pretreatment uses bases like sodium or calcium hydroxide. During this process main portion of lignin polymer is removed and thus enhances the hydrolysis reaction. Although reactor costs are lower than those acid technologies but at the same time it involves expensive and toxic chemicals that have negative impact on the environment. Alkali pretreatment degrades cell wall by disrupting lignin, hemicellulose, and silica by either hydrolyzing uronic acid or acetic esters besides swelling cellulose. This swelling of cellulose in turn reduces its crystallinity. This pretreatment process involves lower temperature and pressure compared to other techniques (Moiser et al. 2005). Sun et al. (1995) carried out an experimental

analysis to study the effectiveness of various alkaline solutions (1.5 % NaOH for 144 h; at 20 °C) using wheat straw as a substrate source and he found that it releases about 60 and 80 % lignin and hemicellulose respectively. Further it has also been reported that sodium hydroxide enhances hardwood digestibility from 14 to 55 % by decreasing lignin content from 24–55 to 20 % (Kumar and Wyman 2009).

8.5.1.3 Biological Pretreatment

Biological pretreatment involves the use of microorganism that possesses the ability to hydrolyze lignocellulosic biomass. Bacteria and fungi are the important sources of these extracellular enzymes (cellulases/hemicellulases/xylanases) that could be utilized for the hydrolysis of pretreated lignocellulosic biomass (Singh et al. 2008). Among the various cellulolytic microorganisms *Trichoderma* species which is considered as an industrial horse for biomass hydrolytic enzymes has been well studied (Xu et al. 1998). Sandgren et al. (2001) reported that *Trichoderma* has the ability to produce at least two cellobiohydrolases, five endoglucanases, and three endoxylanases but it possess less β -glucosidase activity that plays a pivotal role in polymer conversion (cellobiose to glucose) (Kovács et al. 2009). Another microorganism *Aspergillus* is very efficient in producing β -glucosidase (Taherzadeh and Karimi 2007b). Experimental analysis carried out by different researchers in which *Trichoderma* cellulase was supplemented with extra β -glucosidase and the results showed positive effects in degrading lignocellulosic biomass (Krishna et al. 2001). The efficiency of biological degradation of biomass depends upon the number of parameters like temperature, pH, substrate concentration, surfactant addition, and enzyme specificity and efficiency (Olsson and Hahn-Hägerdal 1996; Börjesson et al. 2007). Currently, the major bottleneck associated with lignocellulosic biofuel technology is the cost of enzymes (Banerjee et al. 2010). Therefore, research should be focussed to develop potent microbial strains that possess the ability to produce enzymes with high specificity and activity (Eggman and Elander 2005). Besides, some adjuvants like surfactants should also be used as they prevent the enzyme from unproductive binding with lignin polymer and thus lowers the enzyme loading (Eriksson et al. 2002). The enzymatic cocktails are usually blends of various hydrolytic enzymes (cellulases/xylanases/hemicellulases) that act synergistically on biomass. This method is economical, green, and cost-effective due to less mechanical support (Talebnia et al. 2010). It does not involve any chemical but low hydrolysis rates and low yields impede its implementation (Balat et al. 2008). Besides, it also involves some inhibitory molecules that impede its efficiency (Chandra et al. 2007). Shi et al. (2008) also reported that some microorganisms possess the ability to solubilize all the three types of biopolymers. Therefore, at the commercial scale, the biological pretreatment pathway faces many hindrances. Current research must be focussed to explore novel sources of enzymes with high potency. Moreover, genetic engineering plays a pivotal role to develop super strains that are highly capable of hydrolyzing cellulose and xylan along with simultaneous fermentation of glucose and xylose to ethanol (Lin and Tanaka 2006).

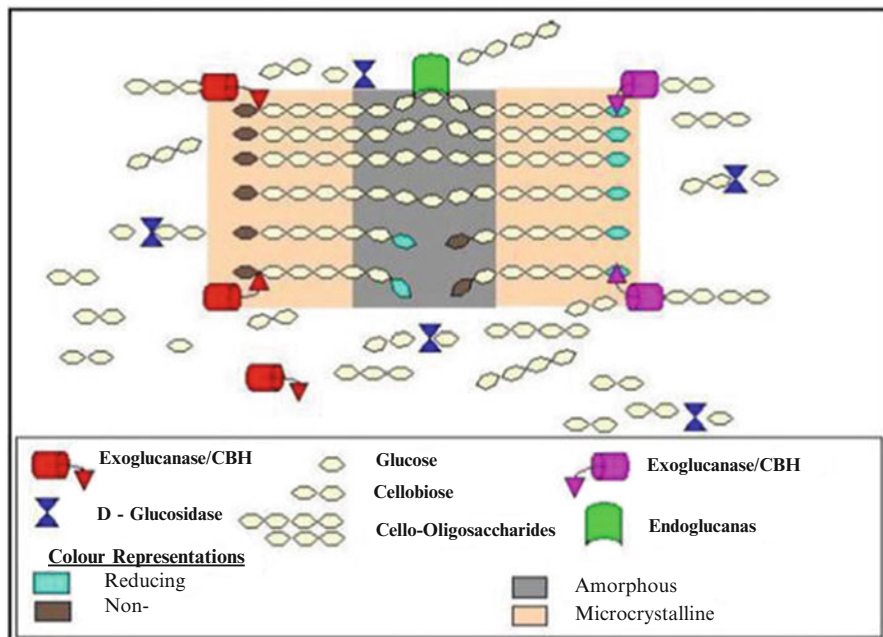


Fig. 8.1 Mechanism of *cellulase* action (Lynd et al. 2002)

8.5.2 Hydrolysis

Hydrolysis process is considered as an essential step to the effectiveness of a pretreatment operation (Gamage et al. 2010). During this phenomenon, the released polysaccharides like cellulose and hemicellulose are hydrolyzed into free monomeric sugars that are easily accessible for fermentation into bioethanol production (Chandel et al. 2007). Ferreira et al. (2009) reported that enzymatic hydrolysis is a dynamic approach compared to acid or alkaline hydrolysis as it is eco-friendly, cost-effective, and is energy efficient (Taherzadeh and Karimi 2007a). Besides, it also does not involve the formation of inhibitory or toxic by-products. Enzymatic hydrolysis is carried out by a complex of cellulase and hemicellulase enzymes that are extremely substrate specific (Banerjee et al. 2010). These enzymes are responsible for degrading the lignocellulosic biomass into simple sugars (Fig. 8.1). It has been reported that the optimum conditions for cellulase activity involves temperature of 40–50 °C and pH 4–5 (Neves et al. 2007). Similarly the optimum parameters for xylanase activity have also been reported to be 50 °C and pH 4–5 (Park et al. 2002). These hydrolytic enzymes are produced by various microorganisms including bacteria and fungi however; *Trichoderma* species is one of the most potent fungi to produce industrial grade cellulolytic enzymes. It produces a complete set of

cellulase enzymes (endoglucanase, exoglucanase, and β -glucosidase) that cause cleavage of β -1, 4-glycosidic linkage (Eggeman and Elander 2005). These enzymes act synergistically to hydrolyze the biomass. Enzymatic hydrolysis was carried out using corn stalk as substrate source at 30 °C and pH 5 Belkacemi and Hamoudi (2003). The results revealed that the saccharification was 90 % and sugar was released after 10 h. Similar study was carried out by combination of *T. reesei* ZU-02 cellulase and cellobiase from *A. niger* ZU-07 improved the hydrolysis yield to 81.2 % with cellobiase activity enhanced to 10 CBU/g substrate (Chen et al. 2008). Moreover, there are certain adjuvants that enhance the hydrolysis process. In an experimental analysis, Börjesson et al. (2007) reported that addition of polyethylene glycol enhances the enzymatic transformation of soft lignocellulose from 42 to 78 % at 16 h at an optimum temperature of 50 °C. On addition of Tween 80 (5 g/L) boost hydrolysis yield by 7.5 %. Xu et al. (1998) reported that *T. reesei* degrade 68.21 % of alkali pretreated rice straw whereas upon enzymatic hydrolysis 73.96 % conversion was obtained from alkali assisted photocatalysis of rice straw. Wheat straw when pretreated with alkaline peroxide showed 96.75 % yield after enzymatic hydrolysis whereas atmospheric autocatalytic organosolv pretreated wet wheat straw produce above 75 % yield (Saha and Cotta 2006). Further, it has also been demonstrated that some metals such as, Ca (II) and Mg (II) substantially enhanced enzymatic hydrolysis via lignin-metal complexation (Liu et al. 2010). Hydrolysis of biomass can also be enhanced by using some additives (surfactants and bovine serum albumin) in order to block lignin interaction with cellulases (Eriksson et al. 2002). Similarly, Sewalt et al. (1997) have described that the detrimental effect of lignin on cellulases can be conquered by ammonization and distinct nitrogen compounds. Moreover, for efficient hydrolysis simultaneous approach of enzymatic treatment accomplished with the engineered co-fermentation microbial process known as simultaneous saccharification and fermentation (SSF) was developed (Cao et al. 1996). This phenomenon has gained importance during the late 1970s for its capability to diminish toxic by-product and subsequently enhance bioethanol production (Bisaria and Ghose 1981). Another approach for biomass hydrolysis involves separate hydrolysis and fermentation (SHF) processes but it possess certain limitations such as it involves the blockage of the hydrolytic enzymes (cellulases) by saccharide products like glucose and cellobiose. Unlike SHF, the SSF process carried out hydrolysis and fermentation simultaneously and thus keeps the sugar level too low to cause any noticeable cellulase inhibition (Kumar et al. 2009).

8.5.3 Fermentation

Biomass processing (pretreatment and hydrolysis) is an essential step to optimize the fermentation process (Gamage et al. 2010). These pretreatment techniques results in the formation of saccharified biomass that is now readily available for fermentation process by using several potent microorganisms. However, the major bottleneck in lignocellulosic biofuel technology involves the lack of potent

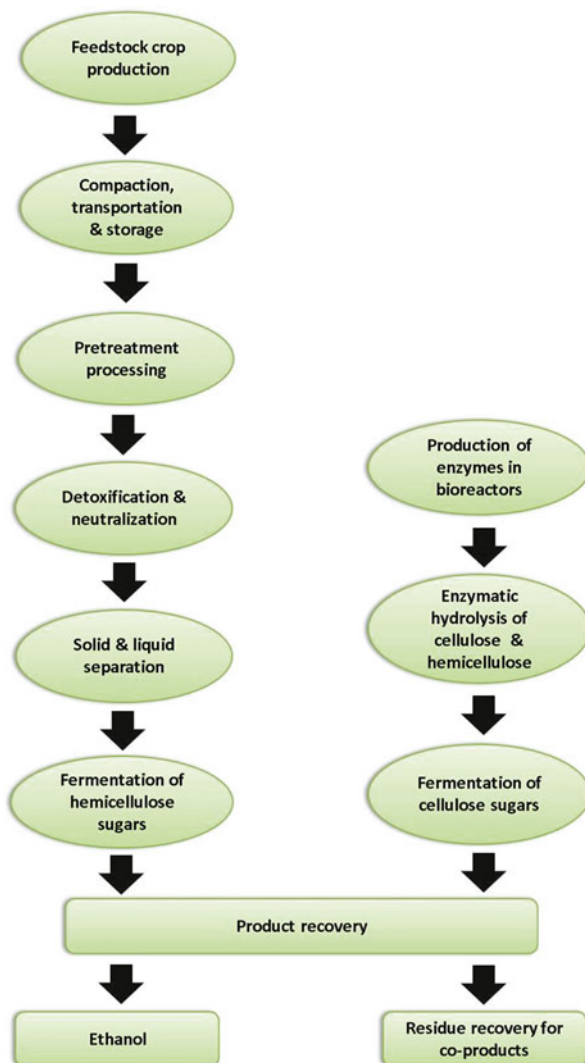
Table 8.1 Comparison between the two main fermentation techniques

Fermentation process	Features and advantages	Limitations	References
Simultaneous saccharification and fermentation	Low costs Higher ethanol yields due to removal of end product inhibition of saccharification step	Difference in optimum temperature conditions of enzyme for hydrolysis and fermentation.	Balat et al. (2008)
Separate hydrolysis and fermentation	Reduces the number of reactors required Each step can be processed at its optimal operating conditions Separate steps minimize interaction between the steps	End product inhibition minimizes the yield of ethanol. Chance of contamination due to long period process	Sanchez and Cardona (2008)

microorganisms that possess the ability to ferment both pentose as well as hexose sugars (Talebnia et al. 2010). To commercialize the lignocellulosic biofuel technology, an ideal microorganism must fulfill the following criteria. These include: (1) wide substrate utilization (2) high ethanol yield (3) capable to bear high titre of ethanol and temperature (4) ability to withstand inhibitors or toxic by-products present in hydrolysate and have cellulolytic activity. Currently, C₆ sugars (hexoses) are readily fermented into bioethanol but bioconversion of C₅ (pentoses) sugars into bioethanol is a recent approach. Fermentation of pentoses along with hexoses is not common among microorganisms (Toivolla et al. 1984). The well-known microorganism *S. cerevisiae* possesses the ability to ferment only hexose sugar into bioethanol. Recently, novel microorganisms such as *Pichiastipitis*, *Candida shehatae*, and *Pachysolantannophilus* have been screened and characterized that possess the ability to ferment both C₅ as well as C₆ sugars into bioethanol. Thermophilic microorganisms are the ideal candidates for bioethanol production at the industrial level because they possess the ability to tolerate high temperature and toxic by-products formed during fermentation process. Currently, the main techniques that are usually involved in the fermentation of biomass hydrolysates are separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). SSF is considered as a superior, cost-effective, and efficient technique for the production of bioethanol than SHF because it prevents the formation of inhibitory by-products and does not involve the use of separate reactors. However, any fluctuation in optimum temperature conditions of enzymes for hydrolysis and fermentation diminishes its efficiency (Neves et al. 2007). Buaban et al. (2010) reported that ethanol yield coefficient is higher under SSF than SHF because of more conversion of pentose sugar (xylose) into xylitol. Comparative study among these two techniques (SHF and SSF) is presented in Table 8.1. Apart from SSF or SHF, there are other alternative techniques which include consolidated

bioprocessing (CBP) and simultaneous saccharification and co-fermentation (SSCF) (Cardona et al. 2009). The CBP technique is carried out inside a single reactor and the various steps involved in this process (cellulase enzyme production, biomass hydrolysis, and ethanol fermentation) are carried out simultaneously (Bjerre et al. 1996). This phenomenon is also termed as direct microbial conversion (DMC) and involves either mono- or co-culture of microorganisms to degrade cellulose polysaccharide into bioethanol. Moreover, CBF technique is cheap, easily available, and does not involve any costly inputs (Hamelinck et al. 2005). The various microorganisms used for the conversion of biomass into bioethanol during CBF technique include *Clostridium thermocellum*, *Neurospora crassa*, *Fusarium oxysporum* and *Paecilomyces* species. One of the important limitations related to this technique is that its efficiency is very low because of poor ethanol yield coefficient and long fermentation periods (3–12 days) (Szczo drak and Fiedurek 1996). In an experimental analysis, Sree et al. (1999) employ the use of some thermotolerant microorganisms (*S. cerevisiae* strain-VS3) under solid state fermentation for the bioethanol production using potato and sweet sorghum as a substrate source. Recent approaches are focusing on development recombination yeast which can metabolize all forms of sugars to improve ethanol production and reduce operation costs. In this concern, two approaches are addressed. The first approach involves modifying the genetic makeup of particular microorganisms and their metabolic pathways (ethanologens additional pentose metabolic pathways). Secondly, with the help of genetic engineering, microorganisms should be engineered in such a way so that they are able to ferment both pentose as well as hexose sugars (Dien et al. 2003). Currently, a number of genetically modified microorganisms such as *P. stipitis* BCC15191 (Buaban et al. 2010), *P. stipitis* NRRLY-7124 (Nigam 2001), recombinant such as *E. coli* KO11 (Takahashi et al. 2000), *C. shehatae* NCL-3501 (Abbi et al. 1996), *S. cerevisiae* ATCC 26603 (Moniruzzaman 1995) have been developed. Although various technologies have been developed to enhance bioethanol production from lignocellulosic biomass but there are still some hindrances that are needed to be addressed. These involve: (1) maintaining a stable performance of genetically engineered yeast in commercial scale fermentation operation (Ho et al. 1999) (2) developing more potent pretreatment technique for lignocellulosic biomass, (3) integrating optimal components into economic ethanol production system (Dien et al. 2003). Fermentation process involves either batch, fed batch, or continuous process depending upon various parameters like enzyme kinetics, kind of lignocellulosic hydrolysate, and economic inputs. Use of immobilized cell fermenter is found successful for ethanol production from hexoses (Godia et al. 1987). Talebnia et al. (2010) has been proposed certain anaerobic hemophilic bacteria (*Clostridium* sp. and *Thermoanaerobacter* sp.) to analyze the prosperity of fermentation at higher temperatures. Other genetically engineered thermotolerant microorganisms included are *K. marxianus*, *Candida lusitanaeae*, and *Z. mobilis* (Bjerre et al. 1996) (Fig. 8.2).

Fig. 8.2 Flowchart of cellulosic ethanol production from lignocellulosic biomass



8.6 Conclusion and Future Perspective

Lignocellulosic biomass is considered as an important green energy resources for economically attractive bioethanol production. These biomass feedstocks does not possess any food value besides, they don't require inputs like land, water, and other energy requirements. The second generation biofuels (lignocellulosic biofuel) possess a great advantage over the first generation biofuels (starchy crops) being cheap, renewable, and abundantly available source of sugar for fermentation into bioethanol production. Biofuels not only reduce the dependence on petroleum

resources but also contribute to sustainable development by decreasing GHG-emissions. Currently, bioethanol production from lignocellulosic biomass is not cost-effective because of some barriers that are needed to be addressed. These include biomass feedstock, conversion technology, hydrolysis process, and fermentation. With respect to biomass feedstock major hindrances are supply, cost, handling, and harvesting. The major obstacles regarding conversion technology involve processing of biomass, convenient and cost-effective pretreatment process to detach hemicellulose and cellulose from their complex with lignin. Another objection is to develop an adequate and economical hydrolysis process for depolymerization of cellulose and hemicellulose to generate higher levels of simple sugars (Glucose). Enzymatic hydrolysis is considered as a potent phenomenon for saccharification of complex polymer. Currently, research has been focussed on cellulase engineering in order to cut down the cost of bioethanol production. Lastly, the challenges associated with fermentation process are pentose (xylose) and hexose (glucose) sugar co-fermentation besides the use of potent and efficiently engineered microbial strains. Future work should also be focussed to inhibit the generation of inhibitors (furfural, acetic acid) during biomass processing that acts as main barriers to decline the efficiency of hydrolysis and fermentation of biomass. In order to combat the challenges associated with lignocellulosic biofuel technology, novel approaches like genetic engineering, cellulase engineering should be employed so that production of bioethanol from lignocellulosic biomass may be successfully developed, optimized, and commercialized in the near future.

References

- Abbi M, Kuhad RC, Singh A (1996) Fermentation of xylose and rice straw hydrolysate to ethanol by *Candida shehatae* NCL-3501. *J Ind Microbiol* 17:20–23
- Agrawal AK (2007) Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Prog Energy Combust* 33(3):233–271
- Antal MJ, Water (1996) A traditional solvent pregnant with new applications. In: White HJ (ed) *Proceedings of the 12nd International Conference on the Properties of Water and Steam*. Begell House, New York, pp 24–32
- Badger PC (2002) Ethanol from cellulose: a general review. In: Janick J, Whipkey A (eds) *Trends in new crops and new uses*. American Society for Horticultural Science (ASHS) Press, Alexandria, VA
- Balat M, Balat H, Oz C (2008) Progress in bioethanol processing. *Prog Energy Combust* 34:551–573
- Ballesteros I, Negro MJ, Oliva JM, Cabanas A, Manzanares P, Ballesteros M (2006) Ethanol production from steam-explosion pretreated wheat straw. *Appl Biochem Biotechnol* 130: 496–508
- Banerjee S, Mudliar S, Sen R, Giri B, Satpute D, Chakrabarti T et al (2010) Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. *Biofuel Bioprod Bioref* 4:77–93
- Belkacemi K, Hamoudi S (2003) Enzymatic hydrolysis of dissolved corn stalk hemicelluloses: reaction kinetics and modeling. *J Chem Technol Biotechnol* 78:802–808
- Bernton H, Kovarik B, Sklar S (1982) *The forbidden fuel: power alcohol in the 20th century*. W.B. Griffin, New Haven, CT, p 274, Bibl. Index 81- 85112. ISBN 19.95 ISBN 0-941726-00-2

- Binod P, Sindhu R, Singhanian RR, Vikram S, Devi L, Nagalakshmi S et al (2010) Bioethanol production from rice straw: an overview. *Bioresour Technol* 101:4767–4774
- Bisaria VS, Ghose TK (1981) Biodegradation of cellulosic materials: substrate, microorganisms, enzymes and products. *Enzyme Microb Technol* 3:90–104
- Bjerre AB, Olesen AB, Fernqvist T (1996) Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. *Biotechnol Bioenergy* 49:568–577
- Börjesson J, Peterson R, Tjerneld F (2007) Enhanced enzymatic conversion of softwood lignocellulose by poly (ethylene glycol) addition. *Enzyme Microbiol Technol* 40:754–762
- Buaban B, Inoue H, Yano S, Tanapongpipat S, Ruanglek V, Champreda V et al (2010) Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting *Pichia stipitis*. *J Biosci Bioeng* 110(1):18–25
- Cadoche L, Lopez GD (1989) Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. *Biol Waste* 30:153–157
- Campbell CH, Laherrere JH (1998) The end of cheap oil. *Sci Am* 278(3):78–83
- Cao NJ, Krishnan MS, Du JX, Gong CS, Ho NWY (1996) Ethanol production from corn cob pretreated by the ammonia steeping process using genetically engineered yeast. *Biotechnol Lett* 118:1013–1018
- Cardona CA, Quintero JA, Paz IC (2009) Production of bioethanol from sugarcane bagasse: status and perspectives. *Bioresour Technol* 101(13):4754–4766
- Chandel AK, Chan E, Rudravaram R, Narasu ML, Rao LV, Ravindra P (2007) Economics and environmental impact of bioethanol production technologies: an appraisal. *Biotechnol Mol Biol Rev* 2:14–32
- Chandra RP, Bura R, Mabee WE et al (2007) Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocelluloses? *Adv Biochem Eng Biotechnol* 108:67–93
- Chen M, Zhao J, Xia L (2008) Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. *Carbohydr Polym* 71:411–415
- Demirbas A (2005) Bioethanol from cellulosic materials: a renewable motor fuel from biomass. *Energy Source* 27:327–333
- Demirbas A (2008) Comparison of transesterification methods for production of biodiesel from vegetable oils and fats. *Energy Convers Manage* 49:125–130
- Dien BS, Cotta MA, Jeffries TW (2003) Bacteria engineered for fuel ethanol production current status. *Appl Microbiol Biotechnol* 63:258–266
- Eggeman T, Elander TR (2005) Process and economic analysis of pretreatment technologies. *Bioresour Technol* 8:2019–2025
- Eggman T, Elander RT (2005) Process and economic analysis of pretreatment technologies. *Bioresour Technol* 96:2019–2025
- Eriksson T, Börjesson J, Tjerneld F (2002) Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. *Enzyme Microbiol Technol* 31:353–364
- Escobar JC, Lora ES, Venturini OJ, Yanez EE, Castillo EF, Almazan O (2009) Biofuels: environment, technology and food security. *Renew Sustain Energy Rev* 13(6–7):1275–1287
- Farrell AE, Plevin RJ, Turner BT, Jones AD, O’Hare M, Kammen DM (2006) Ethanol can contribute to energy and environmental goals. *Science* 311:506–508
- Ferreira S, Durate AP, Ribeiro MHL, Queiroz JA, Domingues FC (2009) Response surface optimization of enzymatic hydrolysis of *Cistus ladanifer* and *Cytisus striatus* for bioethanol production. *Biochem Eng J* 45:192–200
- Gamage J, Howard L, Zisheng Z (2010) Bioethanol production from lignocellulosic biomass. *J Biobased Mater Bioenergy* 4:3–11
- Glassner D, Hettenhaus J, Schechinger T (1999) Corn stover potential: recasting the corn sweetener industry. In: Janick J (ed) *Perspectives on new crops and new uses*. ASHS Press, Alexandria, VA, pp 74–82
- Gnansounou E (2010) Production and use of lignocellulosic bioethanol in Europe: current situation and perspectives. *Bioresour Technol* 101:4842–4850

- Godia F, Casas C, Sola C (1987) A survey of continuous systems using immobilized cells. *Process Biochem* 22:43–48
- Gullison RE, Frumhoff PC, Canadell JG, Field CB, Nepstad DC, Hayhoe K et al (2007) Tropical forests and climate policy. *Science* 316:985–986
- Hamelinck CN, Hooijdonk GV, Faaij APC (2005) Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. *Biomass Bioenergy* 28:384–410
- Ho NWY, Chen Z, Brainard A, Sealak M (1999) Successful design and development of genetically Engineered *Saccharomyces* yeasts for effective co-fermentation of glucose and xylose from cellulosic biomass to fuel ethanol. *Adv Biochem Eng Biotechnol* 65:164–192
- Hsu TA (1996) Pretreatment of biomass. In: Wyman CE (ed) *Handbook on bioethanol, production and utilization*. Taylor & Francis, Washington, DC, pp 179–212
- Advanced course in LCA (2005) How to decrease environmental impact by choice of car fuel. <http://www.infra.kth.se/fms/utbildning/lca/project%20reports/Group%201%20-%20E85.pdf>
- Karimi K, Kheradmandinia S, Taherzadeh MJ (2006) Conversion of rice straw to sugars by dilute acid hydrolysis. *Biomass Bioenergy* 30:247–253
- Kim S, Dale BE (2004) Global potential bioethanol production from wasted crops and crop residues. *Biomass Bioenergy* 26:361–375
- Kim S, Holtzapple MT (2006) Lime pretreatment and enzymatic hydrolysis of corn stover. *Bioresour Technol* 96:1994–2006
- Kovács K, Szakacs G, Zacchi G (2009) Comparative enzymatic hydrolysis of pretreated spruce by supernatants, whole fermentation broths and washed mycelia of *Trichoderma reesei* and *Trichoderma atroviride*. *Bioresour Technol* 100:1350–1357
- Kovarik B, Kettering CF (1982) Fuel alcohol: energy and environment in a hungry world. London: International Institute for Environment and Development. The Development of Tetraethyl Lead in the Context of Technological Alternatives, Society of Automotive Engineers, Fuels & Lubricants Division, Historical Colloquium, Baltimore, MD 1994
- Krishna SH, Reddy TJ, Chowdary GV (2001) Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast. *Bioresour Technol* 77(2): 193–196
- Kumar R, Wyman CE (2009) Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnol Prog* 25:302–314
- Kumar S, Singh SP, Mishra IM, Adhikari DK (2009) Recent advances in production of bioethanol from lignocellulosic biomass. *Chem Eng Technol* 32:517–526
- Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. *Appl Microbiol Biotechnol* 69:627–642
- Liu H, Zhu JY, Fu S (2010) Effects of lignin-metal complexation on enzymatic hydrolysis of cellulose. *J Agric Food Chem* 58:7233–7238
- Lynd RL, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev* 66:506–577
- Moiser N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour Technol* 96:673–686
- Monique H, Faaij A, van den Broek R, Berndes G, Gielen D, Turkenburg W (2003) Exploration of the ranges of the global potential of biomass for energy. *Biomass Bioenergy* 25:119–133
- Moniruzzaman M (1995) Alcohol fermentation of enzymatic hydrolysate of exploded rice straw by *Pichiastipitis*. *World J Microbiol Biotechnol* 11:646
- Monserrate E, Leschine SB, Canale-Parola E (2001) *Clostridium hungatei* sp. Nov., a mesophilic, N₂-fixing cellulolytic bacterium isolated from soil. *Int J Syst Evol Microbiol* 51:123–132
- Murray D (2005) Ethanol's potential: looking beyond corn. Earth Policy Institute, Washington, DC, <http://www.earthpolicy.org/Updates/2005/Update49>
- Natural Resources Canada's Management Team (2005) <http://www2.nrcan.gc.ca/dmo/aeb/English/ReportDetail.asp?x=265&type=rpt>
- Neves MA, Kimura T, Shimizu N, Nakajima M (2007) State of the art and future trends of bioethanol production, dynamic biochemistry, process biotechnology and molecular biology. *Global Science Books*. p 1–13

- Nigam JN (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichiastipitis*. *J Biotechnol* 87:17–27
- Olsson L, Hahn-Hägerdal B (1996) Fermentation of lignocellulosic hydrolysates for ethanol production. *Enzyme Microbiol Technol* 18(5):312–331
- Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolyzates. II: inhibitors and mechanisms of inhibition. *Bioresour Technol* 74:25–33
- Park YS, Kang SW, Lee JS, Hong SI, Kim SW (2002) Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs. *Appl Microbiol Biotechnol* 58:761–766. doi:10.1007/s00253-002-0965-0
- Perez JA, Gonzalez A, Oliva JM et al (2007) Effect of process variables on liquid hot water pretreatment of wheat straw for bioconversion to fuel-ethanol in a batch reactor. *J Chem Technol Biotechnol* 82:929–938
- Perlack RD, Wright L, Turhollow LA, Graham RL, Stokes B, Erbach DC (2005) Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. Oak Ridge National Laboratory Report ORNL/TM-2005/66. Oak Ridge: US Dept. of Energy
- Pickett J, Anderson D, Bowles D, Bridgwater T, Jarvis P, Mortimer N, Poliakoff M, Woods J (2008) Sustainable biofuels: prospects and challenges. The Royal Society, London, UK, <http://royalsociety.org/document.asp?id¼ 7366>
- Prasad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Resour Conserv Recycl* 50:1–39
- Raneses A, Hanson K, Shapouri H (1998) Economic impacts from shifting cropland use from food to fuel. *Biomass Bioenergy* 15(6):417–422
- RFA (2010) Ethanol industry outlook: climate of opportunity. http://www.ethanolrfa.org/page/-/objects/pdf/outlook/RFAoutlook2010_fin.pdf?nocdn¼1
- Saha BC, Cotta MA (2006) Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. *Biotechnol Prog* 22:449–453
- Sanchez ÓJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresour Technol* 99:5270–5295
- Sandgren M, Shaw A, Ropp TH, Wu S, Bott R, Cameron AD et al (2001) The X-ray crystal structure of the *Trichoderma reesei* family12 endoglucanase 3, Cell2A, at 1.9 Å resolution. *J Mol Biol* 308(2):295–310
- Sewalt VJH, Glasser WG, Beauchemin KA (1997) Lignin impact on fiber degradation 3. Reversal of inhibition of enzymatic hydrolysis by chemical modification of lignin and by additives. *J Agric Food Chem* 45:1823–1828
- Shi J, Chinn MS, Sharma-Shivappa RR (2008) Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. *Bioresour Technol* 99:6556–6564
- Singh P, Suman A, Tiwari P et al (2008) Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. *World J Microbiol Biotechnol* 24:667–673
- Singh A, Pant D, Korres NE, Nizami AS, Prasad S, Murphy JD (2010) Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: challenges and perspectives. *Bioresour Technol* 101(13):5003–5012
- Sree NK, Sridhar M, Suresh K, Rao LV, Pandey A (1999) Ethanol production in solid substrate fermentation using thermotolerant yeast. *Proc Biochem* 34:115–119
- Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour Technol* 83(1):1–11
- Sun RC, Lawther JM, Banks WB (1995) Influence of alkaline pretreatments on the cell-wall components of wheat-straw. *Ind Crop Prod* 4(2):127–145
- Szczodrak J, Fiedurek J (1996) Technology for conversion of lignocellulosic biomass to ethanol. *Biomass Bioenergy* 10(5):367–375
- Taherzadeh MJ, Karimi K (2007a) Acid based hydrolysis processes for ethanol from lignocellulosic materials: a review. *BioResources* 2(3):472–499

- Taherzadeh MJ, Karimi K (2007b) Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. *BioResources* 2(4):707–738
- Takahashi CM, Lima KGC, Takahashi DF, Alterthum F (2000) Fermentation of sugarcane bagasse hemicellulosic hydrolysate and sugar mixtures to ethanol by recombinant *Escherichia coli* KO11. *World J Microbiol Biotechnol* 16:829–834
- Talebnia F, Karakashev D, Angelidaki I (2010) Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation. *Bioresour Technol* 101(13):4744–4753
- Tampier M, Smith D, Bibeau E, Beauchemin PA (2004) Identifying environmentally preferable uses for biomass resources. http://www.cec.org/files/PDF/ECONOMY/Biomass-Stage-I-II_en.pdf
- Toivolla A, Yarrow D, Van-den-bosch E, Van-dijken JP, Sheffers WA (1984) Alcoholic fermentation of D-xylose by yeasts. *Appl Microbiol Biotechnol* 47:1221–1223
- Uihlein A, Schbek L (2009) Environmental impacts of a lignocellulosic feedstock biorefinery system: an assessment. *Biomass Bioenergy* 33:793–802
- Wati L, Kumari S, Kundu BS (2007) Paddy straw as substrate for ethanol production. *Indian J Microbiol* 47:26–29
- Weil JR, Sarikaya A, Rau SL et al (1997) Pretreatment of yellow poplar sawdust by pressure cooking in water. *Appl Biochem Biotechnol* 68:21–40
- Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY (2005) Comparative sugar recovery data from laboratory scale application of leading pretreatment technologies to corn Stover. *Bioresour Technol* 96(18):2026–2032
- Xu J, Takakuwa N, Nogawa M, Okada H, Morikawa Y (1998) A third xylanase from *Trichoderma reesei* PC-3-7. *Appl Microbiol Biotechnol* 49:18–724
- Zheng Y, Pan Z, Zhang R (2009) Overview of biomass pretreatment for cellulosic ethanol production. *Int J Agr Biol Eng* 2(3):51–68