

Chapter 13

Agricultural Waste: A Suitable Source for Biofuel Production



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Abstract In current era, world is dependent on fossil fuels such as oil coal, natural gas, etc. Demand for the fossil fuels increase day by day due to increase in urbanization and industrialization. Excessive use of fossil fuels results in environment pollution especially in terms of generation of greenhouse gases. Natural sources of energy like wind, water, sun, biomass and geothermal heat can be utilized for fossil fuel production, and petroleum-based foods can be replaced by biomass-based fuels as bioethanol, biodiesel, biohydrogen, etc. Biodiesel production from food crops is no more an attractive option due to food versus fuel issue. Utilization of lignocellulosic waste from agriculture serves as better alternative looking to its lower cost, renewability and abundance. Lignocellulosic waste includes grasses, sawdust, wood chips, etc. Rice straw, wheat straw, corn straw and sugarcane bagasse are the major agricultural wastes. This chapter aims to present a brief overview of the available and accessible technologies for bioethanol production using these major lignocellulosic agro-waste.

13.1 Introduction

The International Energy Agency foresees that energy consumption will rise by 40% up to 2030, as the population growth will go beyond 10 billion by the year 2050 (Bilgen et al. 2004). Global increase in demand for fuel is mainly due to increased industrialization and urbanization. Of the total available energy resources, fossil fuel is the primary source for energy satisfying around 80% of total fuel demand. It is well known that number of problems is being attached to fossil fuel-based energy generation system including high amount of greenhouse gas (GHG) emission which ultimately results in global warming. Fossil fuels are nonrenewable source of energy

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and its consumption is rapid due to increased demand which altogether results in increase in price of crude oil.

13.2 Bioenergy and Biofuel

If we think about the simplest definition of bioenergy, then it is the energy obtained from organic matter (biomass) which can be utilized straightway as fuel or processed to generate various kinds of solid, liquid and gaseous biofuels. The number of advantages is associated with bioenergy production including environmental and economical including getting better carbon balances, extenuating global climate change, improved economical growth, decrease in energy cost, local energy safety and utilization of local technologies. Despite well-known potential for societal and economical transformation, generation and adoption of energy from organic biomass is not an easy task and has a number of challenges as a potential driver of sustainable development. The key factors affecting sustainability of biomass for energy generation systems are soil, land, water, productivity, biodiversity and energy/carbon balance.

Biofuel can be defined as the fuel derived directly from plants (i.e. energy crops), or indirectly from agricultural, commercial, domestic and/or industrial wastes. Plant and microalgae carry out photosynthesis which results in carbon fixation and ultimately results into formation of biofuel, whereas biomass can be converted into biofuels by thermal, chemical or biochemical conversion resulting in the formation of fuel in solid, liquid or gas form. This new biomass can also be utilized straightway as biofuels.

13.2.1 Generations of Biofuels

There are four generations of biofuels characterized by their sources of biomass, advantage and limitations and technological progress.

13.2.1.1 First-Generation Biofuels

It is also well known as conventional biofuel and generally manufactured from sugar, starch or vegetable oils through processes like fermentation, distillation and transesterification. For production of first-generation biofuels, sugar, starch or vegetable oil acquired from crops is transformed into biodiesel or ethanol by transesterification or yeast fermentation. Alcohols generally used as first-generation biofuels are made by fermentation of sugars and starches. The fermentation processes primarily produce ethanol followed by small amount of butanol and propanol. In many countries, ethanol is generally used as additive of gasoline. Biodiesel is manufactured

by transesterification of plant oil or animal fat by allowing reaction between oils and methanol in the presence of a catalyst followed by distillation. Biodiesel can be utilized in place of petroleum diesel in many diesel engines or in a mixture of the two. The major constraint related to first-generation biofuel is that the majority of feedstocks used to produce first-generation biofuels are food crops that raise the problem of food versus fuel. An additional problem linked with first-generation biofuels includes loss of biodiversity as there are chances of monoculturing, competition for land and water as well as non-economic production.

13.2.1.2 Second-Generation Biofuels

It is generally manufactured from non-food crops, lignocellulosic biomass or woody crops, agricultural residues or waste plant material. Second-generation biofuels are an effective answer to the food versus fuel argument as they utilize leftover portion of food crops grown on arable land or specialized non-food crops raised on land which is not appropriate for growing food crops. Non-food feedstock for second-generation biofuels includes grasses, jatropha and other crops, waste vegetable oil, municipal solid waste, etc. Ethanol production from fast-growing trees can be extracted by enzymatic hydrolysis of biomass to separate out sugars from lignin fibres of the plant, whereas straw and other forest residues undergo thermochemical pretreatment such as gasification to synthesize syngas which is a mixture of carbon monoxide, hydrogen and other hydrocarbons. Hydrogen so produced is generally used as fuel and the other hydrocarbons can be used as additives to gasoline. The constraints associated with second-generation biofuels include difficulty in extraction of sugars from the fuel crops, high capital costs and extraction of nutrients from soil by fuel crops, etc.

13.2.1.3 Third-Generation Biofuels

These biofuels are based on algae having naturally more than 50% oil content. Generally, algae can be grown in wastewater and the oil content can be extracted and processed to produce biodiesel. Moreover, the leftover portion after extraction of oil can be further processed to produce ethanol. Algae are considered to be a low-cost, high-energy renewable feedstock. It also overcomes limitations of land and water as it does not require farmland or freshwater. Limitation of the algal biofuel technology is high capital investment.

13.2.1.4 Fourth-Generation Biofuels

This type of fuel is generally produced from biomass that has absorbed carbon dioxide during their growth. The process for production of fourth-generation biofuels as the

carbon dioxide is caught using practises such as oxy-fuel combustion (Schmetz et al. 2014).

Carbon dioxide can then be geo-sequestered by its storage in old oil and gas fields or saline aquifers. The fourth-generation biofuels are produced using non-arable land and do not need to breakdown biomass. It includes electrofuel and photobiological solar fuels. The technology for development of fourth-generation biofuels is in its infancy and thus needs high capital investment and more processing time which should be improved to make it a viable biofuel option in long run.

13.3 Feedstocks for Biofuels

Generally, biomass feedstocks for production of biofuels are classified according to their sources. It includes agricultural crops, plants directly grown for energy purpose, agriculture and forestry residues and other organic wastes including processing as well as animal and human waste. They are generally falling into following broad categories.

13.3.1 Energy Crops

Energy crops include starch and sugar-rich crops like maize, sugarcane and oilseed crops like soybean, sunflower, etc. Sugar and starch crops generally are utilized as human food and animal feeds. These crops and their specific products can readily be transformed into biofuel, i.e. ethanol through a simple fermentation process which further can be used as fuel. Generally, grasses grown as hay and pasture for livestock feed or for soil safeguarding can also be included in this category. Such crops serve as feedstock for energy production as it contains higher amount of fibres, viz. cellulose, hemicellulose, lignin and lower in carbohydrates, proteins and oils. Energy can be generated from these crops by different methods which include direct burning for heat and/or power, ethanol synthesis from cellulose fermentation, thermochemical processes for fuel supplements or anaerobic digestion for methane synthesis.

Moreover, oil crops such as soybean, canola, mustard, camelina, etc. produce 15% to more than 50% oil. Plant oils can be transformed into high-value biofuels that can be utilized as an alternative to fossil fuel-based substances. Plant oils can be obtained by seed crushing followed by oil extraction. After extraction, biodiesel is produced by transesterification of oil.

13.3.2 Forest Growth

Generally, the woody plants grown in forest are considered into this category. Hardy trees and their products are generally being used directly for energy production for heating and cooking by direct combustion. Dry wood products are having higher heating value which is approximately 10% higher than herbaceous plant biomass and around two-thirds of coal. Wood and wood products are being used as fuel source through gasification and ethanol production from cellulosic waste. However, biofuel production from forest biomass may pose the risk of competition with forest products industry such as timber, boards, pulp, paper, etc.

13.3.3 Residues from Agriculture and Forestry

Biomass residues after harvesting of feed and food part of the forestry such as small branches leaves, decayed flowers and fruits and agricultural crops such as corn stover, corn cobs, wheat, small grain straw, etc. can be converted to renewable fuels. Anaerobic digestion of crop residues converts organic wastes into methane and other combustible gases that can be straightway utilized for combustion heat, fuelling gas turbines or cleaned to supplement natural gas. Moreover, residues are generated by industrial treatment of wood and food crops like black liquor from wood processing industry, molasses or press cake from food processing industry.

13.3.4 Organic Wastes

Organic wastes generated from municipal solid waste, urban activity, rural and mostly agricultural industry can be used for biofuel production. Moreover, wastewater from sewage or produced from industrial processes is conventionally disposed of by industries as waste but can be utilized as raw material to produce biohydrogen and biofuel using microalgae.

13.4 Biofuel Production from Lignocellulosic Biomass

Production of biofuels for replacement of transportation fuels from lingo-cellulosic biomass is a practical route to ensure energy security and environment safety. Biodiesel production from agricultural residues is also having environmental benefits which is more considerable as compared to its economic benefits (Hill et al. 2006). Agricultural residues generally are available throughout the year in abundance and are relatively inexpensive. As per an estimate, presently biomass is contributing 14%

of total world energy and that is how it is contributing in world economy (Parikka 2004; Antonopoulou et al. 2008).

Biofuels are generally produced from the starch-based material derived from sugarcane, corn, beet, wheat, millet and sorghum. But this will raise the problem of food versus fuel and that is why it is not sustainable strategy in long run. In contrast to this, million tonnes of agricultural remains are available (Xu et al. 1998) and in the absence of low-cost technologies for conversion of agro-waste into fuel, farmers tend to burn them in field which again cause environmental pollution (Li et al. 2008). Basically, agro-wastes are made up of lignocellulosic material such as crop residues, grasses, sawdust, wood chips, etc. Lignocellulose is a complex polymer comprising of cellulose, hemicellulose and lignin. As per an estimate, approximately 442 billion litres of bioethanol can be made from lignocellulosic biomass per year and rice straw, wheat straw, corn straw and sugarcane bagasse are the chief agricultural wastes (Kim and Dale 2004) which can contribute to the biofuel production.

13.4.1 Crop Residues

Agricultural waste can be defined as crop residues lost in handling, storage and transport of agricultural crops. It includes field residues like stalks and stubble (stems), leaves, straw and seedpods left in agricultural field after crop harvesting as well as processing residues like husks, seeds, bagasse and roots of crops (Soccol et al. 2011). Using crop residues for production of energy can also reduce greenhouse gas emission from agricultural waste burning. Moreover, it will lower down risk of air, water and soil contamination due to application of organic residues on land (Champagne 2007). Moreover, crop residues can increase and stabilize levels of organic carbon in soil, improves soil structure, minimize erosion, improves nutrient availability, neutralize soil and increase water holding capacity and soil fertility (Reijnders 2008).

Generally rice, wheat and corn straw as well as sugarcane bagasse considered as major agro-waste feedstocks for biofuel production. A very small fraction of such agro-waste is utilized and utilization pattern varies with geographic region (Kim and Dale 2004), whereas majority of the agricultural remains are disposed of as waste. For example, about 600–900 million tonnes of rice straw are formed worldwide per year (Karimi et al. 2007). Only, small part of rice straw is utilized as animal feed and large portion is disposed from field by burning. Disposal of the rice straw is the great problem as it is produced in great bulk, having slow degradation in soil and high mineral content (Xie et al. 2010).

13.4.1.1 Sugarcane Bagasse

Sugarcane generally contains stem and straw. After extraction of juice from sugarcane, the leftover portion is known as bagasse. Approximately, one metric tonne sugarcane produces 280 kg bagasse (Canilha et al. 2012). Looking to the composition

of bagasse, there is 19–24% lignin, 27–32% hemicelluloses, 32–44% cellulose and 4.5–9.0% ashes as well as small fraction of minerals, waxes and other compounds (Jacobsen and Wyman 2002). It can assist as the best source for biofuel manufacturing due to production of large amount of bagasse as industrial waste and methodologies for manufacturing of ethanol should be widely explored (Wanderley et al. 2013).

13.4.1.2 Corn Stover

Generally, corn stover and grain are made in equivalent quantities and stover can be efficiently utilized for ethanol manufacturing (Graham et al. 2007). A study suggests that full utilization of corn waste for biofuel production can provide about 35 million litres of bioethanol which could efficiently substitute approximately 25 million litres of gasoline (Kim and Dale 2004).

13.4.1.3 Rice Straw

Globally, major portion of rice is being used as human food (about 88%), around 2.6% as animal feed and 4.8% lost as waste. Looking to biochemical composition of rice straw, it comprises 32–47% cellulose, 19–27% hemicelluloses, 5–24% lignin and 19% ashes. Carbohydrate portion of rice straw contains 41–43% glucose, 15–20% xylose, 3–5% arabinose, 2% mannose and 0.4% galactose (Roberto et al. 2003). Each year approximately 205 billion litres of bioethanol may be made from rice straw that contributes about 5% of the total global ethanol utilization (Belal 2013).

13.4.1.4 Wheat Straw

Generally, bioethanol can be made from lignocellulosic wheat waste like wheat bran and wheat straw considering it as a key source for bioethanol manufacturing. Wheat straw comprises 33–40% cellulose, 20–25% hemicellulose and 15–20% lignin.

13.4.2 Wood Waste Biomass

Wood waste produced by forest activities is the largest biomass available in the world (Dan et al. 2015). There are two major classes of wood waste biomass, i.e. softwood and hardwood based on the difference in processing and ultimately affecting ethanol production. Generally, hardwood comprises more xylan and less mannan and that is why resistant to recalcitration (Zhu et al. 2010). Wood waste generated by construction and demolition contains wood content of about 20–30% (Cho et al. 2011). Construction wood waste represents an effective natural resource for manufacturing of cellulosic ethanol. Wood waste biomass from forests, plantations and trees

grown outside the forest as well as wood logging and processing residues could have potential to produce high amount of ethanol without deforestation.

13.5 Biofuel Production from Agro-waste

Basically, lignocellulose is a complex carbohydrate polymer of cellulose, hemicellulose and lignin. Cellulose is long, linear polymer made up of glucose sugar joined together by β -1,4glycosidic linkages, whereas hemicellulose is a highly branched heteropolymer of D-xylose, D-arabinose, D-glucose, D-galactose and D-mannose. Lignin is strongly attached to these two carbohydrate polymers, thereby protecting lignocellulosic material from microbial attack (Peiji et al. 1997).

Globally, peoples are interested to produce bioethanol from the agro-wastes. Lignocellulosic biomass can be processed to produce bioethanol through three major operations: release of cellulose and hemicellulose through pretreatment for delignification followed by hydrolysis of cellulose and hemicellulose to yield fermentable sugars like glucose, xylose, arabinose, galactose, mannose and fermentation of sugars. Ethanol can be manufactured from greatly plentiful lignocellulosic sugars in crop wastes (Kabel et al. 2007). Lignocellulosic biomass needs specific pretreatment followed by enzymatic hydrolysis and fermentation to be converted into bioethanol.

13.5.1 Pretreatment of Lignocellulosic Biomass

The lignocellulosic biomass is composed of a matrix of cellulose and lignin bound by hemicellulose chains. Pretreatment is carried out to liberate components of lignocellulosic biomass by decreasing crystallinity, thereby making cellulose available and remove lignin (Sun and Cheng 2002). Pretreatment is done to change macroscopic and microscopic size and structure of biomass, submicroscopic structure as well as chemical conformation. This process makes lignocellulosic biomass more vulnerable to hydrolysis and improves production of monomeric sugars (Mosier et al. 2005a, b).

In pretreatment lignocellulosic structure is destroyed to reduce the extent of crystallinity of cellulose which makes it more accessible for enzymatic hydrolysis (Sanchez and Cardona 2008). The aim of pretreatment is the formation of sugars directly or subsequently by hydrolysis, limit of loss of sugars, reduce formation of inhibitory products and decrease energy burdens which ultimately minimize costs. Due to complex structure of lignocellulose, simple pretreatment process is not feasible. Various types of pretreatment methods are used, based on properties of substrate.

Physical, chemical, physicochemical and biological treatments are four major types of pretreatment methods used.

Pretreatment methods to be utilized commercially should fit into norms mentioned below.

1. It should concentrate pretreated biomass without adding any other binding agents, e.g. AFEX, wet oxidation and extrusion at raised temperature.
2. It should generate less amount of by-products which hampers downstream processing.
3. It should be scaled up and can process 2000 tonnes per day or more.
4. It should be energy-efficient and cost-effective.

13.5.2 Pretreatment of Lignocellulosic Biomass by Physical Methods

13.5.2.1 Mechanical Size Reduction

The preliminary step in pretreatment of lignocellulosic biomass is mechanical size reduction through milling, grinding or chipping. Aim of mechanical size reduction is to decrease particle size and to increase surface area. This step is critical for reducing crystallinity of cellulose, thereby reducing complications in downstream processing (Sun and Cheng 2002). Disk milling/grinding produce particle sizes of 0.2–2 mm and chipping generates particle sizes of 10–30 mm (Sun and Cheng 2002). Mechanical size reduction of the lignocellulosic biomass is usually done by wet milling, dry milling, vibratory ball milling and compression milling. Reduction of size by mechanical means will provide better results as far as ethanol production is concerned (Bjerre et al. 1996; Pandey 2009), but very small particle size may generate clumps during subsequent processing which may lead to channelling. It is advised to employ hammer mill or ball mill for hardwood and cutter mill for softwood.

13.5.2.2 Pyrolysis

Pyrolysis process can be considered as endothermic process requiring less amount of energy. In pyrolysis, lignocellulosic biomass is heated at more than 300 °C which results in rapid degradation of cellulose to generate gases like hydrogen and carbon monoxide. Here, disintegration is somewhat slow and small quantity of volatiles are produced at low temperature (Sanchez and Cardona 2008; Mtui 2009). The leachate came out of the processing contains carbon which supports growth of microbes for bioethanol manufacturing. Glucose is primary constituent of water leachate, and it is assumed that approximately 55% of total biomass is removed by water leaching (Das et al. 2004).

Pretreatment by microwave oven and electron beam irradiation: This method uses thermal and non-thermal effects of microwaves in aqueous environments. In thermal process, heat is produced in biomass through microwave radiation and hotspot is

generated within heterogenous matter which ultimately results in a burst amongst particles and increases the commotion of lignocellulose structure (Hu and Wen 2008). Thermal pretreatment releases acetic acid from the lignocellulosic biomass. High-energy radiations bring about added alteration in cellulosic biomass comprising higher specific surface area, reduction in amount of polymerization and crystallinity of cellulose, hydrolysis of hemicellulose and partial depolymerization of lignin. Research outcomes showed that reducing sugar formation from rice straw and sugarcane bagasse can be improved by a factor of 1.6 and 3.2 when it is irradiated by microwaves followed by lignin extraction which seemed to produce 43–55% of total available reducing sugars (Kitchaiya et al. 2003).

13.5.3 Physicochemical Pretreatment

13.5.3.1 Steam Explosion or Auto-hydrolysis

Auto-hydrolysis by steam burst is a favourable method for pretreatment of lignocellulosic biomass making biomass further reachable to cellulase hydrolysis (Neves et al. 2007). In this process of pretreatment, lignocellulosic biomass is converted into levulinic acid, xylitol and alcohols (Balat et al. 2008) without using any catalyst and heated by more pressurized steam (20–50 bar, 160–290 °C) for a few minutes followed by stopping reaction with unexpected degradation to atmospheric pressure (Sanchez and Cardona 2008; Neves et al. 2007). As steam expands within lignocellulosic matrix, it separates out individual fibres of the matrix (Balat et al. 2008). By pretreatment of lignocellulosic biomass by steam explosion, 45–65% of xylose is being recovered which makes it economically striking (Neves et al. 2007; Hamelinck et al. 2005).

13.5.3.2 Liquid Hot Water Method

This hydrothermal pretreatment method employs compressed hot liquid water to hydrolyze the hemicellulose (Neves et al. 2007) that liberate major part of oligomeric sugars from hemicellulose and happens at 170–230 °C and pressures more than 5 MPa for 20 min. This method is environmentally and economically attractive as no acid or chemical is needed (Neves et al. 2007). Yu et al. (2010) recovered 86.4% xylose by two-step liquid hot water treatment of eucalyptus grandis. From 80% xylan recovered from soybean straw, maximum 70–76% glucose can be obtained by combining liquid hot water and alkaline treatment (Wana et al. 2011).

13.5.3.3 Ammonia Fibre Explosion (AFEX)

In this method, high temperature and pressure are employed followed by rapid pressure release to explore lignocellulosic materials. In this method, inhibitors of downstream processing are not liberated as well as it does not require small particle size (Mosier et al. 2005a, b; Sun and Cheng 2002). Drawbacks of process include less efficiency for biomass comprising high amount of lignin and ability to solubilize only a very small amount of solid material especially hemicellulose (Sun and Cheng 2002; Talebnia et al. 2010). Advantages of this method include simplicity and less time-consuming. In this system, direct release of sugars will not occur; instead, it permits enzymatic hydrolysis of polymers (hemicellulose and cellulose) to produce sugars. The major limiting factor affecting procedure comprises ammonia loading, temperature, high pressure and moisture content of biomass as well as residence time (Talebnia et al. 2010). Temperature is 60–100 °C, and residence time varies from 5 to 10 min to 30 min relying on degree of saturation of biomass. At optimal conditions, 90% cellulose and hemicellulose transformations could be obtained as well as less amount of enzymes are required in comparison to other pretreatment processes (Wyman et al. 2005).

13.5.4 Chemical Pretreatment

In chemical pretreatment, methods are easy and have better transformation efficiency in limited time. It is easy in operation and involves the usage of dilute acid, alkali, ammonia, organic solvent, sulphur dioxide, carbon dioxide and other chemicals. Chemical pretreatments are practiced at acidic, neutral or basic conditions. Under acidic conditions (using mineral acids such as H_2SO_4 , HCl, H_3PO_4 and HNO_3 or organic acids like fumaric, maleic and acetic acid), hemicellulose is converted into monomeric xylose and cellulose as well as lignin remains behind.

13.5.4.1 Acid Pretreatment

Acid pretreatment done by 0.2–2.5% w/w acids at temperatures between 130 and 210 °C brings about hydrolysis and yields higher amount of sugars. Sulphuric acid is preferred for acid pretreatment as it hydrolyse hemicellulose (Cardona et al. 2009). Generally, by-products of the acid pretreatment are acetic acid, furfural and 5 hydroxymethylfurfural which acts as inhibitors of microbial growth, so that hydrolysates obtained after acid pretreatment need to be detoxified before fermentation.

13.5.4.2 Alkaline Pretreatment

Alkali treatment of lignocellulose breakdown the cell wall by solubilising hemicelluloses, lignin and silica. During alkaline pretreatment, crystallinity of cellulose is decreased. The remainder (mainly cellulose) left after can be utilized for manufacturing of paper (Mosier et al. 2005a, b). Hydroxides of sodium, potassium, calcium and ammonium are utilized in this method. Alkaline pretreatment method uses low temperature and pressure as compared to other pretreatment techniques (Sanchez and Cardona 2008). Sun et al. (1995) evaluated efficiency of various alkaline solutions for delignification as well as solubilisation of hemicellulose within wheat straw. They reported use of 1.5% NaOH for 144 h at 20 °C, could liberate 60% and 80% lignin and hemicellulose, respectively, and considered to be optimum. NaOH can reduce lignin content of hardwood from 24–55 to 20%, thereby improves its digestibility from 14 to 55% (Kumar and Wyman 2009).

13.5.4.3 Wet Oxidation

In wet oxidation, raw material is acted upon by water and either by air or oxygen at temperatures above 120 °C (Martín et al. 2007). In this technique, addition of water in biomass at the rate of 1 L per 6 g of biomass promotes conversion of solid phase hemicelluloses into liquid phase. Here, hydrolysis of liberated hemicellulose does not occur. The output obtained in this method is sugar oligomers (Cardona et al. 2009).

13.5.4.4 Organic Solvent-Based Pretreatment

Organic solvent can be used for delignification of lignocellulosic biomass. Organic solvent/water mixture enables extraction of lignin (by distillation of organic solvent). Methanol, ethanol, acetic acid, performic acid, peracetic acid, acetone, etc. can also be used as organic solvents for delignification (Zhao et al. 2009). Combination of pretreatment processes like ammonia fibre extraction and ionic liquid pretreatments yield 97% transformation of cellulose to glucose (Nguyen et al. 2010).

Catalyst Recovery: Majority of catalysts (either acid or base) utilized for pretreatment of lignocellulosic biomass are water soluble, so they will be lost with wastewater after completion of the process. Catalyst recovery from wastewater is costly and high-energy demanding process as it can be accomplished by chemical precipitation or ultrafiltration. In certain processes, very low concentration of catalyst is utilized like diluted sulfuric acid, diluted ammonium hydroxide, etc. which does not require to be recovered. Generally, acid or base is used to neutralize wastewater that ultimately results in salt production which adds up cost during water recycling consequent processing steps. The above concerns are not needed for pretreatment techniques which utilize ammonia (Balan et al. 2009; Chundawat et al. 2013) as ammonia being a

volatile alkali can be reutilized same way as in AFEX process. Organic solvents like ethanol used in pretreatment processes such as organosolv method can be recovered by distillation which is high-energy demanding process. Whereas pretreatment processes like mechanical processing, microwave processing, wet oxidation, ozonolysis, hot water, supercritical water or carbon dioxide pretreatment do not involve any catalyst, so do not require subsequent costly catalyst recovery steps but they need expensive reactor systems. Phosphoric acid pretreatment of lignocellulosic biomass generates highly degradable amorphous cellulose but it also involves recovery of phosphoric acid from water during downstream processing which is a costly process (Zhang et al. 2007).

13.5.5 Biological Pretreatment

Cellulose can be liberated from lignocellulose complex by microorganisms like brown-rot attacking cellulose, white-rot fungi attacking both cellulose and lignin. Cellulose mutant of white-rot fungal strains has been developed to ensure release of lignin and preventing loss of cellulose. Mutants which cannot produce cellulase were produced that can only digest lignin, thereby preventing loss of cellulose. Biological pretreatment is not adopted at commercial scale just because of the low hydrolysis rates and low yields (Balat et al. 2008; Hamelinck et al. 2005). Biological pretreatment especially delignification requires long time.

13.6 Enzymatic Hydrolysis

Conversion of complex carbohydrates into monomeric units is by saccharification process in the critical step in bioethanol production. Enzymatic hydrolysis of sugars is been preferred over acid and alkali hydrolysis due to its low energy requirement, less toxicity, no toxic by-product formation and low corrosion (Sun and Cheng 2002; Ferreira et al. 2009; Taherzadeh and Karimi 2007). Cellulase enzyme works optimally at temperature of 40–50 °C and pH 4–5 (Neves et al. 2007) as well as xylanase works best at 50 °C temperature and pH 4–5 (Park et al. 2002). Cellulase and hemicellulose enzymes breakdown bonds of cellulose and hemicellulose in enzymatic hydrolysis of lignocellulosic biomass. Cellulosic enzymes include endo- and exo-glucanase and β -glucosidase. Cellulose basically contains glucans and endo 1,4-D glucanhydrolase (endoglucanase) attacks low crystalline regions of cellulose fibres and 4- β -D glucan cellobiohydrolase (exo-glucanase) removes cellobiose units which ultimately be transformed into glucose by β -glucosidase (Banerjee et al. 2010; Taherzadeh and Karimi 2007). Hemicellulose comprises various sugar units like mannan, xylan, glucan, galactan and arabinan. Hemicellulase enzymes are extra complex and comprise a combination of eight enzymes like endo-1,4- β -D-xylanases, exo-1,4- β -D xylocuronidases, α -L-arabinofuranosidases, endo-

1,4- β -D mannanases, β -mannosidases, acetyl xylan esterases, α -glucuronidases and α -galactosidases (Jorgensen et al. 2003). Bacterial of genus *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Bacillus*, *Bacteroides*, *Ruminococcus*, *Erwinia*, *Acetovibrio*, *Microbispora*, *Streptomyces* as well as fungi belonging to genera *Trichoderma*, *Penicillium*, *Fusarium*, *Phanerochaete*, *Humicola*, *Schizophillum* sp. reported to synthesize cellulase enzyme (Sun and Cheng 2002; Rabinovich et al. 2002). Of all different cellulolytic microbial strains, *Trichoderma* can be considered as one of the best studied cellulase and hemicellulose producing microorganisms (Xu et al. 1998). *Trichoderma* can produce two cellobiohydrolases and five endoglucanases and three endoxylanases (Xu et al. 1998; Sandgren et al. 2001). *Aspergillus* is an efficient producer of β -glucosidase (Taherzadeh and Karimi 2007). Up to 81.2% cellulose hydrolysis and improved cellobiase activity up to 10 CBU/g of substrate can be achieved by combining cellulose of *T.reesei* ZU-02 and cellobiase of *Aspergillus niger* ZU-07.

Cellulase enzyme is the costlier component in lignocellulosic biomass-based bioethanol production technology. And thus it is needed to design a pretreatment technology that can decrease crystallinity of cellulose and removes lignin to maximum extent so that cellulose loading can be reduced (Eggman and Elander 2005). Generally, use of surfactants adsorbs lignin, thereby modifying cellulose surface which prevents unfruitful binding with lignin and ultimately results in reduced enzyme requirement (Eriksson et al. 2002). Belkacemi and Hamoudi (2003) reported that hydrolysis of hemicellulose from corn stalk at 30 °C and pH 5 could release 90% sugar after 10 h. Chen et al. (2008) reported that adding Tween 80 at the rate of 5 g L⁻¹ during enzymatic hydrolysis of maize straw by cellulase of *T. reesei* ZU-02 and cellobiase of *A. niger* ZU-07 can increase 7.5% rate of saccharification. *T. reesei* degrades 68.21% of alkali pretreated rice straw. Alkali-assisted photocatalysis of rice straw yield 73.96% decomposition after enzymatic hydrolysis (Xu et al. 1998). Alkaline peroxide pretreated wheat straw provided 96.75% decomposition after enzymatic hydrolysis and atmospheric autocatalytic organic solvent pretreated wet wheat straw provided more than 75% yield (Saha and Cotta 2006).

13.7 Fermentation

The sugar released after hydrolysis is being subjected to fermentation by several microorganisms. There is a lack of knowledge about the best microorganisms that can effectively ferment sugars (Talebnia et al. 2010). For a commercially acceptable technology of ethanol manufacturing process, best microorganism should have a wide range of substrate usage efficiency, more ethanol production and throughput, should have capability to survive under elevated quantity of ethanol as well as high temperature and should be resistant to inhibitors prevailing in hydrolysate with cellulolytic activity. Commercially, genetically modified organisms are preferred to utilize more sugar in hydrolysate and enhanced manufacturing benefits. Preferred fermentation methods include fermentation including concurrent saccharification and

fermentation and separate hydrolysis and fermentation. Usually, separate hydrolysis and fermentation process is being employed as there is no limitation to neutralize alteration in ideal temperature requirement for hydrolysis and fermentation. Simultaneous saccharification and fermentation are superior as it can overcome limitation of end product inhibition and less resource requirement. The shortcoming of temperature optimization can be eliminated with thermotolerant microorganisms like *Kluyveromyces marxianus* that can tolerate more temperatures required for enzymatic hydrolysis (Bjerre et al. 1996), in latest method, i.e. direct microbial conversion, within single reactor cellulose synthesis and biomass hydrolysis as well as fermentation of ethanol being carried out (Bjerre et al. 1996). Monocultures of mixed cultures are commonly utilized for conversion of cellulose into ethanol directly and having benefit of low cost as there is no need purchase enzyme to produce it separately (Hamelinck et al. 2005; Lynd et al. 2005). *Clostridium thermocellum* (bacteria) and few fungi comprising *Neurospora crassa*, *Fusarium oxysporum* and *Paecilomyces* sp. have been efficiently utilized for direct microbial conversion method. Drawback of the method includes low ethanol production and extensive fermentation time. If one wants to utilize mixed culture of microorganisms for direct microbial conversion method, the mixing should be done after checking compatibility of the bacterial strains as well as equal requirement for operating temperature and pH (Kitchaiya et al. 2003). Successive fermentation with two dissimilar microbes at two altered stages of bioethanol production for enhanced consumption of sugar was accomplished with hexose sugar fermentation in the first phase by *S. cerevisiae* and pentose utilization by *C. shehatae* in successive phase but ethanol production was not more (Sanchez and Cardona 2008).

Some native or wild-type microbes utilized in fermentation are *S. cerevisiae*, *Escherichia coli*, *Zymomonas mobilis*, *Pachysolen tannophilus*, *C. shehatae*, *Pichia stipitis*, *Candida brassicae*, *Mucor indicus*, etc. Amongst all yeast and bacteria utilized, *S. cerevisiae* and *Z. mobilis* are found to be best for ethanol synthesis from hexose sugars (Talebniya et al. 2010). A number of genetically modified microorganisms such as *P. stipitis* BCC15191 (Buaban et al. 2010), *P. stipitis* NRRLY-7124 (Moniruzzaman 1995; Nigam 2001), recombinant *E. coli* KO11 (Takahashi et al. 2000), *C. shehatae* NCL-3501 (Abbi et al. 1996) and *S. cerevisiae* ATCC 26603 (Moniruzzaman 1995) have been established. Strict anaerobic haemophilic bacteria such as *Clostridium* sp. and *Thermoanaerobacter* sp. have been projected (Sanchez and Cardona 2008; Talebniya et al. 2010) to provide benefit of fermentation at high temperatures. Some other thermo-resistant microbes developed are *K. marxianus*, *Candida lusitanae* and *Z. mobilis* (Bjerre et al. 1996).

13.7.1 Separation of Biofuels from Fermentation Broth

Conventional methods of separation of biofuels include distillation to separate out alcohol from water in fermented broth which ensures recovery of around 95% of pure ethanol. Molecular sieves or additives are required to breakdown azeotrope

to acquire pure ethanol. Distillation process requires high energy and work only with 4% initial ethanol concentration to be more economically viable (Ubersax and Platt 2010). Generally, grains/extracted sugars are utilized for the synthesis of first-generation biofuels, so the substrate does not contain any inhibitors which can reduce activity of microorganisms or enzymes. Hence, more than 10% ethanol concentration can be simply attained permitting cost-effective distillation practise. Researchers are considering various biofuels that are not solubilized in water so that one can escape distillation process (Dien et al. 2003).

13.8 Conclusion and Future Prospect

To fulfil increasing demand for transportation fuels, biofuel is the best option to be explored at large scale. Starch-based biofuels are greatest option of bioethanol manufacturing, but it cannot be utilized for large-scale manufacturing looking to the demand for starch grains for food and feed purpose. Agricultural residues or lignocellulosic biomass are potential feedstock for bioethanol manufacturing looking to its abundance in nature as well as it does not require separate land, water and energy. Technologies for converting crop residues to biodiesel are under development. The numerous problems encountered for development of biofuel production technologies should be overcome by technological advancement to develop efficient and economic process to satisfy needs of bioethanol, thereby providing solution to existing energy crisis due to exhausting oil and gas.

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