The Pharma Innovation Journal 2023; SP-12(11): 2264-2271

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 2264-2271

www.thepharmajournal.com
Received: 02-09-2023
Accepted: 11-10-2023

Aditya Shukla
Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Sandeep Kumar Verma
Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

Akanksha Shukla
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Aditya Shukla
Department of Agronomy, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India

A review of production of bioethanol from agricultural wastes

Aditya Shukla, Sandeep Kumar Verma and Akanksha Shukla

Abstract
The demand for ethanol is steadily rising worldwide as a result of the world's growing industrialization and population expansion. Because their primary purpose is for food and feed, conventional crops like corn and sugarcane cannot produce enough bioethanol to meet the world's need. For this reason, lignocellulosic materials like agricultural wastes are good feed stocks for the synthesis of bioethanol. Agricultural wastes are plentiful, renewable, and inexpensive. Though the process has a number of difficulties and restrictions, including the processing and transportation of biomass and the need for effective pre-treatment techniques for the complete delignification of Ligno-cellulosics, bioethanol from agricultural waste has the potential to be a promising technology. The efficiency of the entire process can be increased by using appropriate pre-treatment techniques to raise the amounts of fermentable sugars following enzymatic saccharification. To make the entire process economically viable, new fermentation technologies are required for the conversion of both xylose and glucose to ethanol. This article discusses the various technologies that can be used to produce bioethanol from agricultural waste.

Keywords: Lignocellulosic biomass, agricultural wastes, bioethanol, pre-treatment

1. Introduction
The current global economy is heavily reliant on fossil fuels, including coal, oil, natural gas, and others. These are employed in the manufacturing of items like as gasoline and electricity [1]. Over the past few decades, there has been a significant increase in pollution due to the overuse of fossil fuels, especially in large urban centers. The earth's atmosphere now contains many more greenhouse gases than before [2]. Global energy consumption has gradually increased along with the growth of the human population and economic prosperity. The restricted supply of fossil fuels has an impact on the import of transportation fuel. The amount of oil produced annually worldwide will start to decrease soon [3]. Renewable resources could be used as a substitute in this case.

For the energy sector, renewable resources including wind, water, sun, biomass, and geothermal heat can be used; however, in the near future, the chemical and fuel industries may rely on biomass as a substitute source [4]. Renewable biomass fuels made from sugarcane, corn, switchgrass, algae, etc., such as bioethanol, biodiesel, and biohydrogen, can take the place of all petroleum-based fuels. Electricity requirements can be met via wind and solar farms. Each person's portion of the fuel and power used to produce food and goods as well as for transportation is included in the energy consumption rate. In the short and medium term, biogas has also been suggested as a potential motor fuel on organic farms. Anaerobic digestion of organic material yields biogas. The gaseous fuel can be held at high pressure and its energy content is increased when CO_2 is extracted from it for use as biofuel. In rural areas, biogas can be used as a fuel alternative to natural gas or propane for boilers and the production of power. In India, agro-wastes have the capacity to produce 1281 megawatts of biogas [5]. About 38 PJ of methane are produced annually in Sweden from organic waste, which accounted for 11% of the country's transportation energy needs in 2007 and is expected to be enough to meet the EU objective by 2020 [6]. In order to meet the Kyoto Protocol's carbon dioxide reduction targets and lessen their reliance on the supply of fossil fuels, nations all over the world have taken into consideration and directed state policies toward the increased and economical utilization of biomass for meeting their future energy demands.

Despite its potential as a major source of bioethanol and other transportation fuels, biomass is primarily burned to provide heat and power. Currently, the most popular liquid biofuel for automobiles is ethanol [7, 8]. Climate change and global warming are two factors contributing to the growing significance of ethanol.

There has been a lot of interest in bioethanol on a global, national, and local scale. But the cost of producing bioethanol is higher than that of fossil fuels. 31 billion liters of bioethanol were produced worldwide in 2001 [19]. It increased to 39 billion liters in 2006, and by 2015, it is anticipated to reach 100 billion liters [9]. With 62% of global ethanol output coming from these two countries, Brazil and the USA, are the leading producers [18]. The major feed stocks used in the large-scale manufacture of gasoline ethanol are starch (mostly from corn in the USA) or sucrose from sugarcane in Brazil. The current method of producing ethanol using corn, starch, and sugar materials may not be ideal because of how well they work as food and feed.

The market for the by product of distillers' dried grains with solubles (DDGS), which is used as animal feed, determines how economically viable the process of producing ethanol from grains is. In the future, the DDGS market might not grow as quickly as the ethanol market [9]. An significant consideration for the large-scale expansion of bioethanol production is cost. The current fuel versus food competition brought on by the manufacture of bioethanol from grains is circumvented by the green gold fuel made from lignocellulosic wastes [20]. Based on estimates, 442 billion liters of bioethanol can be made from lignocellulosic biomass, and 491 billion liters of bioethanol can be produced annually from all crop wastes and wasted crops, which is almost 16 times more than the amount of bioethanol produced globally [18]. Lignocellulosic materials are cheap, readily available, and renewable. It consists of grasses, sawdust, wood chips, crop leftovers, etc.

2. Raw material

Because they are available year-round, the four main agro-wastes listed in the previous section make the best feed stocks for producing bioethanol. While maize straw and bagasse are primarily generated in America, rice straw and wheat straw are primarily produced in Asia. Their chemical compositions also differ, with cellulose being the predominant component. These agricultural wastes are also used as boiler fuel, home fuel, and animal feed. The percentage of wheat, rice, and corn straw that is utilized varies depending on the location [18]. It is far too low. A significant amount of agricultural residue is disposed away as waste annually. For example, the annual production of rice straw worldwide is estimated to be between 600 and 900 million tons [13]. The large bulk of the material, delayed soil decomposition, presence of rice stem diseases, and high mineral content restrict the alternatives for disposing of rice straw.

The majority of rice straw generated worldwide is burned to remove it from the field, a practice that is widespread and negatively impacts human health and air pollution [14–17]. Only a small percentage of this straw is used as animal feed. Many Western European nations have already outlawed open field burning, and a few more have given it serious consideration. Of maize straw, less than 1% is gathered for industrial processing, and the remaining 5% is utilized for bedding and animal feed. In the US, more than 90% of corn straw is still in the fields [22].

One common usage for sugarcane bagasse is as a fuel for boilers and for cogeneration of electricity [23]. The generation of bioethanol from rice, wheat, corn, and sugarcane bagasse is currently of interest on a global scale. Among the four agricultural wastes stated, rice straw has the highest potential production of bioethanol, with 205 billion liters produced annually. It is also the most abundant waste. A complex polymer of carbohydrates made of cellulose, hemicellulose, and lignin is called lignocellulosic acid. Cellulose is crystalline and linear. It is a homopolymer of glucose units that repeat and are joined by b-1,4 glycosidic linkages. The polymer hemicellulose is short and heavily branched. D-xylose, D-arabinose, D-glucose, D-galactose, and D-mannose are the constituents of this heteropolymer. Lignin is firmly bonded to these two carbohydrate polymers and is hydrophobic by nature. As a result, it shields these polymers from microbial damage [24]. It is an aromatic polymer with three dimensions made up of p, hydroxyphenylpropionic units joined by C=C and C=O=C bonds.

In order to produce bioethanol from lignocellulosic materials, three main processes must occur: first, lignocellulosic materials must be pre-treated to release cellulose and hemicellulose prior to hydrolysis; second, the cellulose and hemicellulose must be hydrolyzed to produce fermentable sugars such as glucose, xylose, arabinose, galactose, and mannose; and third, reducing sugars must be fermented. There are further uses for lignin's non-carbohydrate components [21].

3. Pre-treatment

Pre-treating the biomass is the biggest processing obstacle in the creation of biofuel. Hemicellulosic biomass is mostly made up of three components: cellulose, lignin, and hemicellulose. The solubilization and separation of one or more of these biomass constituents are referred to as pre-treatment techniques. It facilitates easier access for additional chemical or biological processing of the residual solid biomass [7]. Hemicellulose chains bind a matrix of cellulose and lignin to form the lignocellulosic complex. In order to decrease the amount of cellulose that has crystallinity and increase the portion of cellulose that is amorphous - the form best suited for enzymatic attack – pre-treatment is performed to break the matrix [26]. Pre-treatment is undertaken to bring about a change in the macroscopic and microscopic size and structure of biomass as well as sub-microscopic structure and chemical composition. It increases the yields of monomeric sugars from the rapid hydrolysis of lignocellulosic biomass [27]. An efficient pre-treatment procedure aims to: (i) form sugars either directly or indirectly through hydrolysis; (ii) prevent loss and/or degradation of the sugars formed; (iii) limit the creation of inhibitory compounds; (iv) lower energy requirements; and (v) decrease expenses.

The four main categories of pre-treatment methods used are physical, chemical, physicochemical, and biological treatments. Typically, the pre-treatment stage involves a mix of these procedures.

3.1 Physical pre-treatment

3.1.1 Mechanical size reduction

Crushing, grinding, or chipping agricultural solid waste is the initial stage in the manufacturing of ethanol from it. This lowers the crystallinity of cellulose [28] and raises downstream processing efficiency. Typically, compression, wet, dry, and vibratory ball milling are carried out. Depending on the type of waste (hardwood, softwood, fibrous, etc.) being treated as well as the beginning and final particle sizes, moisture content, and power input, mechanical commination of agricultural materials is necessary [28, 29]. Although size reduction may yield better results, very tiny particle sizes may have detrimental impacts on enzymatic hydrolysis and pre-treatment during later processing [20, 30]. It could cause

channeling and produce clumps in the liquid-related processes that follow. Additionally, specific energy consumption rises. With hammer mill screen sizes of 0.8 and 3.2 mm, the specific energy consumptions for grinding wheat straw were 51.6 and 11.4 kWh, respectively [29]. For hardwood, a hammer mill or ball mill should be used, and for softwood, a cutter mill. Wet disk milling (WDM) and ball milling (BM) are two further commination techniques [31].

3.1.2 Pyrolysis
Pyrolysis is an endothermic process that requires less energy to operate. The materials are treated at a temperature higher than 300 ℃ during this process, which causes the cellulose to break down quickly and release gaseous compounds like CO and H_2 as well as leftover char. Lower temperatures cause the breakdown to occur significantly more slowly and result in the formation of less volatile compounds [26, 32, 33]. Additional treatment for the remaining char involves leaching with either mild acid or water. Enough carbon source is present in the water leachate to encourage microbial growth necessary for the synthesis of bioethanol. The primary ingredient in water leachate is glucose. Water leaching results in the loss of 55% of the biomass's total weight on average [87]. Fan *et al*. [34] have shown 80-85% conversion of cellulose to reducing sugars with more than 50% glucose through mild acid leaching (1 N H_2SO_4, 95 ℃, 1 h).

3.2 Physicochemical pre-treatment
3.2.1 Steam explosion or autohydrolysis
A promising pre-treatment technique that increases biomass's accessibility to cellulase assault is steam explosion [39]. Levulinic acid, xylitol, and alcohols can be produced from the biomass fractionates using this potential pre-treatment technique without the need for a catalyst [21]. This process involves heating the biomass for a few minutes using high-pressure steam (20-50 bar, 160–290 °C); the reaction is then terminated by abruptly decompressing to air pressure [26, 39]. The individual fibers are separated when steam is permitted to expand within the lignocellulosic matrix [21]. The economic appeal of steam-explosion pre-treatment stems from the high recovery of xylose (45–65%) [39, 40].

3.2.2 Liquid hot water method
The hemicellulose is hydrolyzed by compressed hot liquid water (at pressure above saturation point) in the liquid hot water method [39]. Using a hydrothermal pre-treatment technique, a significant proportion of hemicellulosic sugars are released as oligomers. Typically, the treatment lasts for 20 minutes at pressures more than 5 MPa and temperatures between 170 and 230 °C. Nevertheless, it also plays a role in the synthesis of trace levels of undesirable degrading substances such furfural and carboxylic acid, which are highly harmful to the fermentation of ethanol because they prevent microbial development [29, 41]. The method of xylose recovery is appealing from an environmental and economic standpoint, as it doesn't require any chemical or acid, and its recovery rate is relatively high (88-98%) [39].

3.2.3 Ammonia fiber explosion
Liquid ammonia and steam explosion are used in the ammonia fiber explosion (AFEX) pre-treatment [21]. Using a high temperature and pressure treatment followed by a quick pressure release, AFEX is an alkaline thermal pre-treatment that exposes lignocellulosic materials. Small particle size is not necessary for the effectiveness of biomass with higher lignin contents (such as softwood newspaper) or for causing the solubilization of only a very small fraction of solid material, primarily hemicellulose [28, 29]. This method also does not produce inhibitors of the downstream processes. The low processing time and ease of use are its benefits. When applied to substrates that have less lignin than sugarcane, it works better.

3.2.4 CO_2 explosion
The way that CO_2 explodes is comparable to how steam and ammonia explode do. But unlike ammonia explosions, which result in the creation of inhibitors, CO_2 explosions are less expensive [32, 40]. Yields from conversion are greater than those from the steam explosion method [40].

3.3 Chemical pre-treatment
Using diluted acid, alkali, ammonia, organic solvent, SO_2, CO_2, or other chemicals are examples of chemical pre-treatment techniques. These techniques are simple to use and produce good conversion yields quickly.

3.3.1 Acid pre-treatment
One of the most crucial methods is acid pre-treatment, which attempts to produce large yields of sugars from lignocellulosic materials. Acids that are concentrated or diluted (often 0.2% to 2.5% w/w) and heated between 130 and 210 °C are typically used. Among other types of acids, including phosphoric acid, nitric acid, and hydrochloric acid, sulfuric acid is frequently employed for acid pre-treatment [46]. To increase cellulose hydrolysis, acid pre-treatment might use concentrated or diluted acids [21]. Polysaccharides are attacked by the acidic medium, particularly hemicelluloses, which hydrolyze more readily than cellulose [46].

3.3.2 Alkaline pretreatment
The lignin matrix is broken down by an alkaline pre-treatment of lignocellulosics, releasing cellulose and hemicellulose for enzymatic breakdown [48]. When lignocellulose is treated with alkali, hemicelluloses, lignin, and silica dissolve, uronic and acetic esters hydrolyze, and cellulose swells, all of which contribute to the disruption of the cell wall. Swelling causes cellulose to lose some of its crystallinity. Paper or cellulose derivatives can be made from the end residue, which is mostly cellulose [46]. This procedure makes use of sodium, potassium, calcium, and ammonium hydroxides. Compared to other pre-treatment technologies, alkaline pre-treatment techniques use lower temperatures and pressures [27].

3.3.3 Wet oxidation
Wet oxidation involves treating the feedstock material with water and oxygen or air at temperatures higher than 120 °C [52]. One liter of water is added to every six grams of biomass. This method facilitates the movement of hemicelluloses from the solid to the liquid phases. It doesn't hydrolyze the hemicellulose molecules that are freed. Sugar oligomers are the byproducts of hemicellulose hydrolysis during moist oxidation [46]. Wet oxidation has been the subject of numerous investigations employing various substrates as a pretreatment technique [52-54].

3.3.4 Organosolv pretreatment
Another way to Delignify lignocellulosic materials is by organic solvent or Organosolv pulping operations. By

distilling the organic solvent, the lignins can be isolated and burning the liquor is not necessary when using mixes of organic solvent and water. Some of these pre-treatments are as follows: 90% formic acid and 50% carbon dioxide and 50% alcohol/water mixture combined are examples of pressurized carbon dioxide [46]. Other diverse organic solvents that can be employed in delignification processes include acetic acid, performic acid, ethanol, methanol, acetone, etc. [56]. Rice straw pre-treated with ionic solutions and ammonia produced a 97% conversion of cellulose to glucose [88].

3.4 Biological pre-treatment

Microorganisms like as brown rot, white rot, and soft rot fungi can aid in the degradation of the lignocellulosic complex, releasing cellulose. Lignin and hemicellulose can be degraded by biological pre-treatment [28, 29, 32], with white rot fungi appearing to be the most efficient microbe. White and soft rots target lignin as well as cellulose, whereas brown rot targets cellulose [32]. A cellulase-less mutant was created to selectively break down lignin and stop cellulose from being lost, although most biological pre-treatments have relatively slow rates of hydrolysis. Because there is less mechanical support, this method is safer and uses less energy [28, 29]. Its application is hampered by low yields and low hydrolysis rates, although it requires no chemicals [21, 40]. White rot fungus have been used to biologically pretreat bamboo culms at a low temperature of 25 °C [88]. Better delignification was shown in the case of *Phlebia* sp. MG-60, a marine microbe, when the substrate was supplemented with a nutrient medium like Kirk's Medium as opposed to sterilized water [46]. Long times are typically required for bio-delignification. It was found that the raw material's cellulose and lignin concentrations could be significantly decreased in a biological pretreatment study that used a variety of microorganisms to extract the sugars from the lignocellulosic matrix of sugarcane waste. *Aspergillus terreus* was observed to reduce the cellulose content by around 55.2%, whilst delignification was shown to occur at a rate of almost 92% [57].

4. Enzymatic hydrolysis

The crucial process of saccharification, which transforms complex carbs into simple monomers, is required to produce bioethanol. Enzymatic hydrolysis uses less energy and a more benign environment than acid hydrolysis [58]. It has been observed that pH 4-5 and a temperature between 40-50 ℃ are ideal for cellulase [39]. It has also been found that 50 ℃ temperature and pH 4-5 are ideal for xylanase assay conditions [88]. Consequently, compared to acid or alkaline hydrolysis, enzymatic hydrolysis has advantages due to its low toxicity, low utility cost, and low corrosion [28, 59]. Furthermore, enzymatic hydrolysis does not produce any inhibitory by products [58]. On the other hand, highly substrate-specific cellulase enzymes perform enzymatic hydrolysis.

Here, the enzymes cellulase and hemicellulase, respectively, cleave the cellulose and hemicellulose linkages. Glucan is found in cellulose, while several sugar units including mannan, xylan, glucan, galactan, and arabinan are found in hemicellulose. B-glucosidases, endo and exoglucanases, and other cellulase enzymes are involved. Exoglucanase (1,4-b-D glucan cellobiohydrolase or E.C. 3.2.1.91) eliminates the cellobiase units from the free chain ends, endoglucanase (endo 1,4-D glucanhydrolase or E.C. 3.2.1.4) targets the low crystallinity regions of the cellulose fiber, and b-glucosidase (E.C. 3.2.1.21) hydrolyzes the cellobiose units to glucose [23, 59]. A combination of at least eight different enzymes, including a-L-arabinofuranosidases, endo-1,4-b-D mannanases, b-mannosidases, acetyl xylan esterases, a-glucoronidases, and a-galactosidases, are known as hemicellulolytic enzymes, which are more complex [60].

While hemicellulose yields a variety of pentoses and hexoses, cellulose is hydrolyzed to produce glucose. The enzyme cellulase is produced by a number of species of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Bacillus*, *Bacteroides*, *Ruminococcus*, *Acetivibrio*, *Microbispora*, and *Streptomyces*. Numerous fungi have also been identified to produce cellulase, including *Trichoderma*, *Penicillium*, *Fusarium*, *Phanerochaete*, *Humicola*, and *Schizophyllum* sp. [28, 61]. Trichoderma is a well-researched fungal strain that produces cellulase and hemicellulase among the different cellulolytic microbial strains [62]. Trichoderma can manufacture three endoxylanases, five endoglucanases, and at least two cellobiohydrolases [62, 63]. Nevertheless, b-glucosidase activity, which is crucial for polymer conversion, is absent from *Trichoderma* [59, 64]. *Aspergillus*, on the other hand, produces b-glucosidase with remarkable efficiency [59]. Numerous studies have been conducted on *Trichoderma* cellulase treated with additional b-glucosidase [65-67]. When *Aspergillus niger* ZU-07 cellobiase and *Trichoderma reesei* ZU-02 cellulase were combined, the hydrolysis yield increased to 81.2% and the cellobiase activity was increased to 10 CBU/g of substrate [68].

The yields of monomer sugars from lignocellulose are influenced by several factors. The primary determinants of the enzymatic hydrolysis of lignocellulosic material are temperature, pH, and mixing rate [59, 69]. Substrate concentration, cellulase enzyme loading, and surfactant addition are other parameters that impact yield [28, 70, 71]. Substrate inhibition may result from high substrate concentration. The primary expense of the lignocellulosic ethanol process is attributed to cellulase [23]. In order to reduce both the amount of time needed for hydrolysis and the amount of cellulase loading, an effective pre-treatment that reduces cellulose crystallinity and removes lignin to the greatest extent possible must be chosen [72]. By adsorbing lignin onto themselves, surfactants alter the surface of cellulose. This stops the enzyme from attaching to lignin in an ineffective manner and reduces enzyme loading [73].

Numerous investigations on the enzymatic hydrolysis of cellulosic biomass to sugars have been published. The enzymatic hydrolysis of maize stalk hemicellulose at 30 ℃ and pH 5 was investigated by Belkacemi and Hamoudi [74]. After ten hours, sugar was released with 90% saccharification. Cellulase from *T. reesei* ZU-02 and Cellobiase from A. niger ZU-07 were used by Chen *et al*. [68] to investigate the enzymatic hydrolysis of maize straw. Tween 80 at a concentration of 5 g/L increased the hydrolysis yield by 7.5%. According to Borjesson *et al*. [71], the addition of PEG enhanced the soft lignocellulose's enzymatic conversion from 42% to 78% at 16 hours, with 50 ℃ being the ideal hydrolysis temperature. According to Xu *et al*. [62], *T. reesei* broke down 68.21% of the rice straw that had been prepared with alkali, whereas alkali-assisted photocatalysis, which followed enzymatic hydrolysis, produced a 73.96% conversion rate. After enzymatic hydrolysis, wheat straw prepared with alkaline peroxide demonstrated a 96.75% yield, while wet wheat straw Pretreated with atmospheric autocatalytic Organosolv yielded a yield exceeding 75% [75].

5. Fermentation

Several microbes use the saccharified material for fermentation. However, the absence of suitable microorganisms that can effectively ferment both pentose and hexose sugars prevents the industrial use of lignocelluloses for the generation of bioethanol [29]. An ideal microorganism should be able to separate hydrolysis and fermentation, have a high ethanol yield and productivity, and be able to utilize a wide variety of substrates in order for ethanol production to be commercially feasible (SHF). Although the SSF process does not require separate reactors and can boost ethanol yields by removing end product inhibition, it is still preferable to the standard SHF process for manufacturing ethanol. Due to the various optimal temperature ranges for the enzymes employed in fermentation and hydrolysis, there are some disadvantages even though it is similarly fairly priced [20, 39, 40]. More xylose to xylitol conversion under SSF conditions would account for some of the greater ethanol yield coefficient from SSF [78]. Research has indicated that SSF is a superior substitute for SHF [20, 21]. The presence of toxic chemicals that impede the growth and fermentation activity of the microbe may be the cause of the delayed xylose consumption during SHF fermentation [78]. The use of thermotolerant microorganisms, such as *Kluyveromyces marxianus*, which has been engineered to survive the higher temperatures required for enzymatic hydrolysis, can eliminate the disadvantage of SSF [20].

Consolidated bioprocessing (CBP) and simultaneous saccharification and co-fermentation (SSCF) are two further options to SSF or SHF [46]. Cellulase synthesis, biomass hydrolysis, and ethanol fermentation are all done in one reactor in CBP [20]. Another name for the procedure is direct microbial conversion (DMC). Direct cellulose-to-ethanol fermentation is often achieved by the use of mono- or co-cultures of microorganisms. Purchasing enzyme or producing it does not require capital investment when using CBP [40, 79]. This kind of action has been demonstrated by several fungi, including *Paecilomyces* sp., *Fusarium oxysporum*, and *Neurospora crassa*, as well as by bacteria like *Clostridium thermocellum*. However, because to its lengthy fermentation times (3–12 days) and low ethanol yields, CBP is not an effective technique [80]. The co-fermenting microorganisms in SSCF must be able to function at the same pH and temperature [39]. It was reported that *Saccharomyces cerevisiae* and *Candida shehatae* worked well together for the SSCF procedure [39]. For improved sugar utilization, sequential fermentation has also been used, employing two different microorganisms at different stages of the fermentation process: *S. cerevisiae* for hexose utilization in the first phase and *C. shehatae* for pentose utilization in the second phase. However, the ethanol yields obtained are not very high [26].

S. cerevisiae, Escherichia coli, Zymomonas mobilis, Pachysolen tannophilus, C. shehatae, Pichia stipitis, Candida brassicae, Mucor indicus, and other native or wild type microorganisms are some of the ones used in the fermentation [20, 21, 26, 29, 76, 77, 81, 82]. The finest ones should all be able to tolerate high ethanol concentrations, high temperatures, inhibitors found in the hydrolysate, and cellulolytic activity. For optimal production benefits and full utilization of the sugars in the hydrolysate, genetically modified or engineered microorganisms are employed.

Genetic engineering has been used to advance many elements of fermentation, including improved and broad substrate use, larger yields, and faster recovery rates. Numerous genetically engineered microbes have been created, including recombinant *E. coli* KO11 [83], *P. stipitis* BCC15191 [78], *P. stipitis* NRRLY-7124 [81, 82], *C. shehatae* NCL-3501 [84], and *S. cerevisiae* ATCC 26603 [81]. *Thermoanaerobacter* sp. and *Clostridium* sp. are two examples of strict anaerobic hemophilic bacteria that have been suggested [26, 29] to investigate the advantages of fermentation at high temperatures. Other developed thermotolerant microbes include *Z. mobilis*, *Candida lusitaniae*, and *K. Marxianus* [20].

6. Conclusion

It has been predicted that lignocellulosic biomass will be a key component of the economically viable bioethanol production process. Even though lignocellulose has lower theoretical ethanol yields (g ethanol/g substrate) than sugar and starch, these conventional sources are not enough to meet the world's needs for bioethanol production. Agricultural wastes are abundant in nature, less expensive, and renewable in that sense. There is no need for separate land, water, or energy requirements for agricultural wastes. The four main challenges for feedstock are price, availability, harvesting, and handling. To produce fermentable monomers with high concentrations in the hydrolysis process, the issue is to devise an effective method for depolymerizing cellulose and hemicellulose. In this regard, the most effective substitute method for saccharification of complicated polymers may be enzymatic hydrolysis. To maximize the efficiency of the enzymatic hydrolysis process, numerous attempts have been made to lower the cost of the cellulase enzyme. Lastly, the employment of recombinant microbial strains and the co-fermentation of xylose and glucose provide obstacles in the context of fermentation design. In summary, new research and effective technology should be used to overcome the conversion process's technological constraints in order to successfully develop and optimize bioethanol production from agricultural wastes in the near future.

7. References

1. Uihlein A, Schbek L. Environmental impacts of a lignocellulosic feedstock biorefinery system: An assessment. Biomass and Bioenergy 2009;33:793-802.
2. Ballesteros I, Negro MJ, Oliva JM, Cabanas A, Manzanares P, Ballesteros M. Ethanol production from steam-explosion pre-treated wheat straw. Applied Biochemistry and Biotechnology. 2006;130:496-508.
3. Campbell CH, Laherrere JH. The end of cheap oil. Scientific American; c1998. p. 78e83.
4. Lynd LR, Wang MQ. A product e nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. Journal of Industrial Ecology 2003;7:17-32.
5. Dhussa A. Biogas in India. Methane to markets partnership. Meeting of agriculture subcommittee; c2004 Apr 22.
6. Lime M, Ekstrandh A, Englesson R, Persson E, Bjornsson L, Lantz M. The Swedish biogas potential from domestic raw materials. Swedish, Malmo: Avfall Sverige Utveckling. Report No. 2008;02:2008.
7. Demirbas A. Bioethanol from cellulosic materials: A renewable motor fuel from biomass. Energy Sources 2005;27:327-33.
8. Lewis SM. Fermentation alcohol. In: Godfrey T, West S, editors. Industrial enzymology. 2nd ed. New York:

Stockton Press; c1996. p. 12-48.
9. Taherzadeh MJ, Karimi K. Acid based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bio resources. 2007;2(3):472-99.
10. Cadoche L, Lopez GD. Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. Biology of Wastes. 1989;30:1-7.
11. Binod P, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S, *et al*. Bioethanol production from rice straw: An overview. Bioresource Technology 2010;101:67-74.
12. Duff SJB, Murray WD. Bioconversion of forest products industry waste Cellulosics to fuel ethanol: A review. Bio-resource Technology. 1996;55:1-33.
13. Karimi K, Kheradmandinia S, Taherzadeh MJ. Conversion of rice straw to sugars by dilute acid hydrolysis. Biomass and Bioenergy. 2006;30:47-53.
14. Wati L, Kumari S, Kundu BS. Paddy straw as substrate for ethanol production. Indian Journal of Microbiology. 2007;47:6-29.
15. Vlasenko EY, Ding H, Labavitch JM, Shoemaker SP. Enzymatic hydrolysis of pretreated rice straw. Bioresource Technology. 1997;59:10-19.
16. Chen Y, Sharma-Shivappa RR, Chen C. Ensiling agricultural residues for bioethanol production. Applied Biochemistry and Biotechnology. 2007;143:80-92.
17. Ogawa M, Yoshida N. Nitrous oxide emission from the burning of agricultural residue. Atmospheric Environment. 2005;39(19):1-9.
18. Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy. 2004;26:61-75.
19. Berg C, World bioethanol production, the distillery and bioethanol network. Available from: http://www.distill.com/worldethanolproduction.htm; 2001.
20. Bjerre AB, Olesen AB, Fernqvist T. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnology and Bioengineering. 1996;49:68-77.
21. Balat M, Balat H, Oz C. Progress in bioethanol processing. Progress in Energy and Combustion Science. 2008;34:51-73.
22. Glassner D, Hettenhaus J, Schechinger T. Corn stover potential: recasting the corn Sweetner industry. In: Janick J, editor. Perspectives on new crops and new uses. VA: ASHS Press, Alexandria; c1999. p. 74-82.
23. Banerjee S, Mudliar S, Sen R, Giri B, Satpute D, Chakrabarti T, *et al*. Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. Biofuels, Bio products and Bio refining 2010;4:77-93.
24. Peiji G, Yinbo Q, Xin Z, Mingtian Z, Yongcheng D. Screening microbial strain for improving the nutritional value of wheat and corn straws as animal feed. Enzyme and Microbial Technology. 1997;20:1-4.
25. Lee J. Biological conversion of lignocellulosic biomass to ethanol. Journal of Biotechnology. 1997;56:1-24.
26. Sanchez ÓJ, Cardona CA. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresource Technology. 2008;99:70-95.
27. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtazapple M, *et al*. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology. 2005;96:73-86.
28. Sun Y, Cheng J. Hydrolysis of lignocellulosic material for ethanol production: A review. Bioresource Technology. 2002;96:73-86.
29. Talebnia F, Karakashev D, Angelidaki I. Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. Bioresource Technology. 2010;101(13):44-53.
30. Pandey A. Handbook of plant-based biofuels. New York: CRC Press; c2009.
31. da Silva ASA, Inoue H, Endo T, Yano S, Bon EPS. Milling pre-treatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. Bio-resource Technology. 2010;101:02-9.
32. Prasad S, Singh A, Joshi HC. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resources, Conservation and Recycling. 2007;50:1-39.
33. Mtui GYS. Recent advances in pre-treatment of lignocellulosic wastes and production of value added products. African Journal of Biotechnology. 2009;8(8):398-415.
34. Fan LT, Gharpuray MM, Lee YH. Cellulose hydrolysis biotechnology monographs. Berlin: Springer; c1987. p. 57.
35. Hu Z, Wen Z. Enhancing enzymatic digestibility of switch grass by microwave assisted alkali pre-treatment. Biochemical Engineering Journal. 2008;38:69-78.
36. Sridar V. Microwave radiation as a catalyst for chemical reactions. Current Science. 1998;74(5):46-50.
37. Ooshima H, Aso K, Harano Y, Yamamoto T. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. Biotechnology Letters. 1984;6(5):89-94.
38. Kitchaiya P, Intanakul P, Krairiksh M. Enhancement of enzymatic hydrolysis of lignocellulosic wastes by microwave pretreatment under atmospheric pressure. Journal of Wood Chemistry and Technology. 2003;23(2):17-25.
39. Neves MA, Kimura T, Shimizu N, Nakajima M. State of the art and future trends of bioethanol production, dynamic biochemistry, process biotechnology and molecular biology. Global Science Books; c2007. p. 1-13.
40. Hamelinck CN, Hooijdonk GV, Faaij APC. Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass and Bioenergy. 2005;28:384-410.
41. Taherzadeh MJ, Karimi K. Pre-treatment of lignocellulosic wastes to improve ethanol and biogas production: A review. International Journal of Molecular Sciences. 2008;9:21-51. DOI:10.3390/ijms9091621.
42. Yu Q, Zhuang X, Yuan Z, Wang Q, Qi W, Wanga W, *et al*. Two-step liquid hot water pre-treatment of Eucalyptus grandis to enhance sugar recovery and enzymatic digestibility of cellulose. Bio-resource Technology. 2010;101:1-9.
43. Wana C, Zhou Y, Li Y. Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility. Bioresource Technology. 2011;102:1-9.
44. Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ. Pre-treatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bio-resource Technology. 2010;101:51-61.
45. Wyman CE, Dale BE, Elander RT, Holtzapple M,

Ladisch MR, Lee YY. Comparative sugar recovery data from laboratory scale application of leading pre-treatment technologies to corn stover. Bio-resource Technol 2005;96:26-32.
46. Cardona CA, Quintero JA, Paz IC. Production of bioethanol from sugarcane bagasse: status and perspectives. Bioresource Technology. 2009;101(13):54-66.
47. Mosier N, Hendrickson R, Ho N, Sedlak M, Ladisch MR. Optimization of pH controlled liquid hot water pre-treatment of corn stover. Bio-resource Technology 2005;96:86-93.
48. Pandey A, Soccol CR, Nigam P, Soccol VT. Biotechnological potential of agro-industrial residues. I: sugarcane Baggase. Bio-resource Technology 2000;74:69-80.
49. Sun RC, Lawther JM, Banks WB. Influence of alkaline pretreatments on the cell-wall components of wheat-straw. Industrial Crops and Products. 1995;4(2):27-45.
50. Kumar R, Wyman CE. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pre-treatment of poplar by leading technologies. Biotechnology Progress. 2009;25:02-14.
51. Saha BC, Iten LB, Cotta MA, Wu YV. Dilute acid pre-treatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochemistry. 2005;40:693-700.
52. Martín C, Klinke HB, Thomsen AB. Wet oxidation as a pre-treatment method for enhancing the enzymatic convertibility of sugarcane bagasse. Enzyme and Microbial Technology. 2007;40:26-32.
53. Banerjee S, Sen R, Pandey RA, Chakrabarti T, Satpute D, Giri BS, *et al*. Evaluation of wet air oxidation as a pretreatment strategy for bioethanol production from rice husk and process optimization. Biomass and Bioenergy. 2009;33:1-6.
54. Ahring BK, Licht D, Schmidt AS, Sommer P, Thomsen AB. Production of ethanol from wet oxidised wheat straw by Thermoanaerobacter mathranii. Bioresource Technology. 1999;68:3-9.
55. Pedersen M, Meyer AS. Influence of substrate particle size and wet oxidation on physical surface structures and enzymatic hydrolysis of wheat straw. Biotechnology Progress. 2009;25(2):399-408.
56. Zhao X, Cheng K, Liu D. Organosolv pre-treatment of lignocellulosic biomass for enzymatic hydrolysis. Applied Microbiology and Biotechnology. 2009;82:15-27.
57. Singh P, Suman A, Tiwari P, Arya N, Gaur A, Shrivastava AK. Biological pre-treatment of sugarcane trash for its conversion to fermentable sugars. World Journal of Microbiology and Biotechnology. 2008;24:67-73.
58. Ferreira S, Durate AP, Ribeiro MHL, Queiroz JA, Domingues FC. Response surface optimization of enzymatic hydrolysis of Cistus ladanifer and Cytisus striatus for bioethanol production. Biochemical Engineering Journal. 2009;45:192-200.
59. Taherzadeh MJ, Karimi K. Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bio-resources. 2007;2(4):7-38.
60. Jorgensen H, Kutter JP, Olsson L. Separation and quantification of cellulases and hemicellulases by capillary electrophoresis. Analytical Biochemistry 2003;317(1):85-93.
61. Rabinovich ML, Melnik MS, Boloboba AV. Microbial cellulases (review). Applied Biochemistry and Microbiology. 2002;38(4):05-21.
62. Xu J, Takakuwa N, Nogawa M, Okada H, Morikawa Y. A third xylanase from Trichoderma reesei PC-3-7. Applied Microbiology and Biotechnology. 1998;49: 18-24.
63. Sandgren M, Shaw A, Ropp TH, Wu S, Bott R, Cameron AD, *et al*. The X-ray crystal structure of the Trichoderma reesei family12 endoglucanase 3, Cel12A, at 1.9 Å resolution. Journal of Molecular Biology. 2001;308(2):295-310.
64. Kovács K, Szakacs G, Zacchi G. Comperative enzymatic hydrolysis of pre-treated spruce by supernatants, whole fermentation broths and washed mycelia of Trichoderma reesei and Trichoderma atroviride. Bio-resource Technology. 2009;100:1-7.
65. Krishna SH, Reddy TJ, Chowdary GV. Simultaneous saccharification and fermentation of lignocellulosic wastes to ethanol using a thermotolerant yeast. Bio-resource Technology 2001;77(2):1-6.
66. Itoh H, Wada M, Honda Y, Kuwahara M, Watanabe T. Bioorganosolv pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. Journal of Biotechnology. 2003;103:73-80.
67. Ortega N, Busto MD, Perez-Mateos M. Kinetics of cellulose saccharification by Trichoderma reesei cellulases. International Bio deterioration Biodegradation 2001;47(1):7-14.
68. Chen M, Zhao J, Xia L. Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. Carbohydrate Polymers. 2008;71:1-5.
69. Olsson L, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial Technology. 1996;18(5):12-31.
70. Alkasrawi M, Eriksson T, Börjesson J, Wingren A, Galbe M, Tjerneld F, *et al*. The effect of Tween-20 on simultaneous saccharification and fermentation of softwood to ethanol. Enzyme and Microbial Technology. 2003;33(1):1-8.
71. Börjesson J, Peterson R, Tjerneld F. Enhanced enzymatic conversion of softwood lignocellulose by poly (ethylene glycol) addition. Enzyme and Microbial Technology. 2007;40:54-62.
72. Eggman T, Elander RT. Process and economic analysis of pre-treatment technologies. Bio-resource Technology. 2005;96:19-25.
73. Eriksson T, Börjesson J, Tjerneld F. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. Enzyme and Microbial Technology. 2002;31:53-64.
74. Belkacemi K, Hamoudi S. Enzymatic hydrolysis of dissolved corn stalk hemicelluloses: reaction kinetics and modeling. Journal of Chemical Technology and Biotechnology. 2003;78:02-8.
75. Saha BC, Cotta MA. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. Biotechnology Progress. 2006;22:49-53.
76. Sukumaran RK, Surender VJ, Sindhu R, Binod P, Janu KU, Sajna KV, *et al*. Lignocellulosic ethanol in India: prospects, challenges and feedstock availability. Bioresource Technology. 2010;101:26-33.

77. Girio FM, Fonseca C, Carvalheiro F, Duarte CL, Marques S, Bogel-qukasik R. Hemicelluloses for fuel ethanol: A review. Bioresource Technology. 2010;101:775-800.
78. Buaban B, Inoue H, Yano S, Tanapongpipat S, Ruanglek V, Champreda V, *et al*. Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting Pichia Stipitis. Journal of Bioscience and Bioengineering. 2010;110(1):18-25.
79. Lynd LR, van Zyl WH, Mcbride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: An update. Current Opinion in Biotechnology. 2005;16(5):77-83.
80. Szczodrak J, Fiedurek J. Technology for conversion of lignocellulosic biomass to ethanol. Biomass Bioenergy. 1996;10:67-75.
81. Moniruzzaman M. Alcohol fermentation of enzymatic hydrolysate of exploded rice straw by Pichia Stipitis. World Journal of Microbiology and Biotechnology 1995;11:46-57.
82. Nigam JN. Ethanol production from wheat straw hemicellulose hydrolysate by Pichia Stipitis. Journal of Biotechnology. 2001;87:17-27.
83. Takahashi CM, Lima KGC, Takahashi DF, Alterthum F. Fermentation of sugar cane bagasse hemicellulosic hydrolysate and sugar mixtures to ethanol by recombinant Escherichia coli KO11. World Journal of Microbiology and Biotechnology. 2000;16:29-34.
84. Abbi M, Kuhad RC, Singh A. Fermentation of xylose and rice straw hydrolysate to ethanol by Candida shehatae NCL-3501. Journal of Industrial Microbiology. 1996;17:3-20.
85. Georgieva TI, Mikkelsen MJ, Ahring BK. Ethanol production from wet exploded wheat straw hydrolysate by thermophillic anaerobic bacterium Thermoanaerobacter BG1L1 in a continuous immobilized reactor. Applied Biochemistry and Biotechnology. 2008;145:99-110.
86. Erdei B, Barta Z, Sipos B, Réczey K, Galbe M, Zacchi G. Ethanol production from mixtures of wheat straw and wheat meal. Biotechnology for Biofuels. 2010;3:7-16.
87. Das P, Ganesha A, Wangikar P. Influence of pre-treatment for deashing of sugarcane bagasse on pyrolysis products. Biomass and Bioenergy. 2004;27:45-57.
88. Alizadeh H, Teymouri F, Gilbert TI, Dale BE. Pretreatment of switch grass by ammonia fiber explosion (AFEX). Applied Biochemistry and Biotechnology. 2005;121e124:33-41.