Bioethanol Production from Lignocellulosic Materials - An Overview


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Abstract

Energy consumption has increased steadily as the world population has grown and more countries have become industrialized. The fossil fuels, such as Crude oil, Coal and natural gas have been the major resources to meet the increased energy demand. However, they are gradually being depleted to extinction because they are not renewable. Moreover, serious environmental and ecological problems have been aroused during their exploitation and use. Therefore, there is great interest in exploring alternative energy source to maintain the sustainable growth of society. Ethanol, a clean and renewable energy source, which can be produced through fermentation from renewable biomass, has drawn much attention from the government and researchers. Apart from an alternative to traditional energy sources, ethanol has been widely used as a solvent or feed stock in pharmaceutical and chemical industries. However, fermentative production of ethanol has been limited using current maize starch based technology because of raw material shortage and high cost. A potential method for low cost fermentative production of ethanol is to utilize lignocellulosic materials such as agricultural wastes. We would now discuss and cite out the rapid progress in the field of bioethanol from lignocellulosic materials over the past few decades.

Keywords: bioethanol, alternative energy, lignocelluloses, renewable biomass, fossil fuels.

Introduction

Global demand for energy continues to grow due to the expanding human population and industries. The major energy demand is still supplied from conventional fossil fuels such as oil, coal and natural gas. Major petroleum-based fuels can be replaced by renewable biomass fuels such as bioethanol, bio-diesel and biohydrogen. Ethanol has been used by humans as intoxicating ingredient in alcoholic beverages. The isolation of a relatively pure alcoholic compound was first obtained by the Islamic alchemist who developed the art of distillation. Ethanol has long been considered as a suitable alternative to fossil fuels either as a sole fuel in cars with dedicated engines or as an additive in fuel blends with no engine modification requirement when mixed up to 30%. Henry ford designed a car model called the model T to run on ethanol and quoted ethanol as "the fuel of the future". Ford and others continued to promote the use of ethanol. By 1938, an alcohol plant in Atchison, Kansas was developed with the capacity of producing 18 million gallons of ethanol a year. Ethanol production in the United States grew from 175 million gallons to 1.4 billion gallons in a span of 18 years. Worldwide production capacity of ethanol in 2005-06 was about 45-49 billion liters per year, and is expected to reach 115 billion liters in 2015. The use of fuels blended with ethanol gave two mandatory types namely (E10) and (E85) which contain 10% and 85% of ethanol respectively. Most E85 using vehicles fall under the US government vehicles. The transportation of this ethanol has also become of great concern. When blended ethanol is run through pipelines, the ethanol gathers the moisture content from the pipelines. Hence the ethanol and gasoline need to be brought through different tankers and are to be blended at another terminal before loading into trucks (Licht,F.O., 2006). Research has been carried out for the production of ethanol from different sources: cellulosic bio-mass (Licht,F.O., 2006), cassava (L. Davis, et al., 2005), sago (Z.S. Kadar, et al., 2004), sorghum (Y. Sun, et al., 2002), blackstrap molasses (Z. Yu, et al., 2003), and maize (A. Demirbas, et al., 2003). In particular, ethanol produced from biomass by fermentation is expected to find use as fuel and in the production of various chemicals (H. Shigechi, et al., 2002). Among the products obtained from such sources, our review will highlight the production of bioethanol from lignocellulosic wastes.

Three main reasons for the production of bioethanol from cellulosic biomass are: i) it is renewable, (ii) doesn't emit harsh gases like CO₂, SO₂, NOₓ into the environment and (iii) it holds the key factor to the economy. The only low cost fermentation substrate that can meet the demands of oil of the future is lignocellulosic biomass. The cellulose biomass consists of mainly waste of inedible cellulose fibers that form the stems and branches of most plants. Grain crops, switch grass, crop residues like corn stalks, wheat straw, rice straw, grasses and wood residues are the many forms of cellulosic biomass. Cellulose has been deemed to be comprised of half of all the organic carbon on the planet. An analysis by NRDC (National Research Defense Council) has found that cellulosic ethanol can provide a solution for the US transportation fuel needs by 2050 with out decreasing the production of food and animal feed. Cellulosic biomass has been a challenge for scientists to convert it into ethanol. They have tried using harsh acids like sulphuric acid and high temperatures to try and breakdown the matrix of cellulose into sugars. An economical process has never been developed using traditional chemistry.

The scientists are thriving to find a cost effective process for the production of ethanol (Z. Yu, et al., 2003). Numerous biotech firms are in the process of perfecting enzymes for cellulosic ethanol. Assorted bio refineries are being constructed to the intake of the cellulosic biomass.

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and to convert them to quality sugars for fermentation of ethanol. There have been great dramatic breakthroughs in the production of cellulosic ethanol. In 2001, the cost of cellulase enzyme was the greatest barrier for the production of the cellulosic ethanol at a cost of $5 per gallon. It was then highly reduced to a mere amount of 10–18 cents per gallon. This was an enormous 30 fold decrease. Numerous companies like Diversa Corporation, Dyadic international invested large in the research and development field and came out successfully. America is the largest continental producer of bioethanol constituting 22.3 billion liter / year. Asia stands second with 5.7 billion/year. Europe, Africa and Oceanic countries follow the list. The first commercial production of cellulosic ethanol was produced by the Iogen Corporation in 2004. Abengoa bioenergy was the first to construct cellulosic ethanol production plant in commercial scale. The plant could process 70 tonnes of agricultural waste to produce 1 million gallon of ethanol in an annual basis. The DOE (Department of energy) of the USA has granted $10 million to develop a next generation dry mill corn ethanol plant. These plants will be able to produce ethanol from corn and residual fibers, which require processing of the cellulase enzymes. 80% of the world’s biomass consists of forest plants. Hence, the raw material cellulose is the highly preferred choice for the production of ethanol. Lignocellulosic biomass comprises mainly of compounds cellulose, hemicellulose and the adjoining molecule lignin (A. Demirbaş et al., 2004). Cellulose is the 6-carbon sugar, glucose arranged in bundles. Hemicellulose primarily consists of 5-carbon sugars and Xylose. Five distinct steps are involved in converting the lignocellulosic biomass to bioethanol: (1) biomass delignification to release cellulose and hemicellulose from the lignin-cellulose-hemicellulose complex, (2) depolymerization of the cellulose and hemicellulose carbohydrates to produce their respective free sugars (pentoses and hexoses), (3) fermentation of the free sugars to produce ethanol, (4) ethanol recovery and (5) effluent treatment (C.A.C. Alzate, et al., 2006)

Raw materials of lignocelluloses obtained from chemical pulps consist of large percent of cellulose content of 60-80. These cellulosic materials being away from the human food chain add up to its positives. 42% cellulose and 21% hemicellulose in wood, the maximum theoretical yield of ethanol can be calculated to be 0.32 grams of ethanol per gram of wood. This is based on complete conversion of cellulose and hemicellulose to sugars, and conversion of sugars to bioethanol at theoretical yield of 0.51 g/g. Lignocellulosic materials may be hydrolyzed with the emission of radiation by gamma ray or microwave irradiation or electron beam irradiation. However, these processes are commercially unimportant.

Considering the feedstocks used, we have waste form the fields, which cannot be used for edible purposes. There is a large amount of these waste present in abundance. These wastes can be taken in and used as lignocellulosic substrates for the production of bioethanol (J.D. Murphy, et al., 2005).

Most frequently used microbial strains for the production of ethanol were the fungi and bacteria. Researchers showed improved bioethanol production from lignocellulosic waste materials by Saccharomyces sp. (K.Saravanakumar, et al., 2013). It converts the reducible sugar glucose by fermentation into ethanol. The fermentation conditions depend on the microbes used. Pure culture techniques are used to culture the selective species of microbe and grown in culture medium. Then the strains are extracted and cultured in the substrate medium for the process of fermentation. A bulk fraction of the money invested for the production of bioethanol is used for the pre-treatment purpose. Pretreatment is breaking of lignin and extracting cellulose, glucose and other mono sugars which increase the yield of bioethanol (C. Munoz, et al., 2011).

Another major processing step involved is the saccharification of cellulose. Pentose and hexoses are obtained from hemicellulose and cellulose by means of breaking down of the H₂ bond by means of hydrolysis conc. Sulphuric acid or HCl are used for the hydrolysis of lignocellulosic materials (M. Lewandowska, et al., 2007). Through these acids may reduce or degrade certain amount of these sugars, the use of dilute acids will take increased time comparatively. The important two factors needed to make the process economically viable are to optimize sugar recovery and recovery of acid for recycling. The advantage of using conc. acid is the potential for high sugar recovery.
The acid and sugar are separated by ion exchange and the concentration of the acid is revived using multiple effect evaporates. There are also other means of pretreatment by means of steam explosion, ammonia fiber explosion, CO2 explosion, ozendysis, alkaline hydrolysis, oxidative delignification, organosolvent process and other biological treatment (K.Karimis et al., 2006). The consolidated resources for the production of bioethanol are given in table 1.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Author/Year</th>
<th>Strain/Media formulation</th>
<th>Raw material/Substrate</th>
<th>Pre-treatment method</th>
<th>Fermentation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Davis et al., (2005)</td>
<td><em>Zymomonas mobilis.</em> (25g xylulose, 5g yeast extract 10 mg/ml tetracycline culture - 70°C frozen culture)</td>
<td>Wheat – acid hydrolysate supplemented with glucose in 3 seed tank</td>
<td>Dilute acid pre-treatment for hemicellulose recovery. Sulphuric acid -0.25 – 2% heated for specific temperature 180°C, 100°C, 120°C) time 0.5 – 5.5 hours</td>
<td>Fermentation media pH controlled 3M NaOH temp. Temperature-30°C, agitation – 200 rpm.</td>
</tr>
<tr>
<td>2</td>
<td>S. Zhu et al., (2005)</td>
<td><em>Trichoderma reesi</em> Media – MGYP medium at 33°C for 12 hour in orbital shaker. Inoculum conc. 1.5x10^8 yeast cell/ml</td>
<td>Raw wheat straw</td>
<td>Conventional alkaline pretreatment. 20 gm 160ml of 1% Sodium Hydroxide and boiled in 500 ml beaker for 60 minutes. Dried at 65°C 2 days. (MW assisted alkali pretreatment) MW microwave (700W in microwave oven for 25 minutes)</td>
<td>Fermentation media in the wheat (Pre-treated), Temperature- 35°C - 45°C pH – 4.8 – 5.8 160 rpm/min HRT – 72 hours fermentation 96 hours conventional.</td>
</tr>
<tr>
<td>3</td>
<td>Tucker et al., 2004</td>
<td><em>Saccharomyces cerevisiae</em> (ATCC. 200062) Pitchia stipitis (ATCC PTA 3717)</td>
<td>Wet distillers grains (Wet – DG)</td>
<td>Acid pretreatment Time: 5.40 minutes. Temp – 140 - 185°C (depends on substrate) H2SO4 -1.1% - 3.27%</td>
<td>Fermentation media with wet distillers grain (Wet DG) temperature - 32°C, pH – 5, rpm – 150, HRT – 24 hours preferred</td>
</tr>
<tr>
<td>4</td>
<td>E. Gnansounou et al., 2003</td>
<td><em>Saccharomyces cerevisiae.</em> (normal standard media for culture strength improvisation)</td>
<td>Sorghum bicolor (Sweet sorghum of china)</td>
<td>Crushing and extraction of juice for fermentation</td>
<td>Fermentation media with extract from sweet sorghum. Temperature-33°C - 35°C pH – 5 rpm – 160</td>
</tr>
<tr>
<td>5</td>
<td>S. Zafar et al., 2006</td>
<td><em>Kluyveromyces spp.</em> (Crude whey, 3.4% lactose (MTCC 1288) Culture strength 24 hours</td>
<td>Crude whey</td>
<td>---</td>
<td>Fermentation media with crude whey temp. 34°C, pH-4.5 (maintained by 6N NaOH)</td>
</tr>
<tr>
<td>6</td>
<td>ZS Kadar et al., 2002</td>
<td><em>Kluyveromyces marxianus</em> (Y01070)media – glucose 50.0 g/l, yeast extract 2.5g/l, peptone 5.0 g/LKH2PO4, MgSO4, NH4Cl(0.2g/l) Temp -30°C.</td>
<td>Old conjugated card board (OCC) and paper sludge, paper waste</td>
<td>Enzyme pretreatment cullulase and logen cellulose</td>
<td>Fermentation media with OCC and paper waste separately. Temp – 40°C pH –4.5 – 5.3 (maintained by 10% NaOH or H2SO4) HRT – 96 hours.</td>
</tr>
<tr>
<td>7</td>
<td>L. Mojoric et al., 2006</td>
<td><em>Saccharomyces cerevisiae</em> (media with glucose) 32 Deg C/48 hours Culture strength</td>
<td>Corn meal (Dry milling process)</td>
<td>Enzyme pretreatment alpha amylose from beta licheniforms and gucoamylase from A. Niger</td>
<td>Fermentation media with corn stover pretreated by physicochemical and enzymatic method. Temperature –30°C pH – 5 HRT – 72 hours Aerobic fermentation (batch and fed batch)</td>
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<tr>
<td></td>
<td>Authors</td>
<td>Yeast Type</td>
<td>Media Description</td>
<td>Pretreatment Method</td>
<td>Fermentation Method</td>
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<tr>
<td>8</td>
<td>Karimis et al., 2006</td>
<td>Baker yeast (Saccharomyces Cerevisiae)</td>
<td>standard yeast media 30°C / 24 hours, culture pH – 5, culture strength</td>
<td>Physico chemical pretreatment. SO2 treatment by impregnation with steam. Enzymatic hydrolysis by cellulase enzyme.</td>
<td>Fermentation media with corn stover pretreatment by physicochemical and enzymatic method. Temperature 30°C pH – 5 HRT – 72 hours Aerobic fermentation (batch and fed batch)</td>
</tr>
<tr>
<td>9</td>
<td>K. Kurini et al., 2005</td>
<td>Muror indiens Pichia Stipitis glucose</td>
<td>peptone agar media – as agar slants. Temp - 30°C pH – 5.5 spore suspension used for strength improvement</td>
<td>Mechanical disruption using pulveriser. Acid pretreatment (H2SO4 – 0.5%) 18 hours, mixture is steamed in to a high pressure reactor. Then cooled. pH – adjusted to 5</td>
<td>Fermentation media with pretreated rice straw. Aerobic or anaerobic 50ml inoculation in 100 ml of mixture. Temperature -30°C pH – 5 + 0.1 HRT – 72-96 hours</td>
</tr>
<tr>
<td>10</td>
<td>K. Karini et al., 2005</td>
<td>Pitchia Stipitis (glucose yeast malt extract)</td>
<td>Agar slants 30 – 0.5°C</td>
<td>Mechanical disruption using pulveriser. Acid pretreatment (H2SO4 – 0.5%) 18 hours, mixture is steamed in to a high pressure reactor. Then cooled. pH – adjusted to 5</td>
<td>Fermentation media with pretreated rice straw. Aerobic or anaerobic 50ml inoculation in 100 ml of mixture. Temp -30°C pH – 5 + 0.1</td>
</tr>
<tr>
<td>11</td>
<td>Panagioton et al., 2004</td>
<td>Fusarium oryssporum potato dextrose agar.</td>
<td>pH – 6 strength – 3-4 days</td>
<td>Glucose basal media</td>
<td>Aerobic potato dextrose agar. Temp - 30°C 200 rpm pH – 6 HRT – 2 days O2 concentration– 30%min</td>
</tr>
<tr>
<td>12</td>
<td>Yu et al., 2003</td>
<td>Saccharomyces cervisiae Zymomonas mobilis.</td>
<td>Pitchia spp. (Yeast extract, urea, KH2PO4, MgSO4.7H2O, hydrate)</td>
<td>Cellulose pyrolysate</td>
<td>Yeast – 30°C/140 hours/ 150 rpm Same even for Pittichia spp.</td>
</tr>
<tr>
<td>13</td>
<td>M. Lewando wsk et al., 2005</td>
<td>Saccharomyces cervisiae ph-5, (maintained with MHCL), immobilized in calcium alginate has enzyme immobilized. (β-galactosidase with glutaraldehyde) 30pC</td>
<td>20% lactose pasteurization was carried at 80°C for 20 min</td>
<td>Neutralization method followed by dilution with distillation H2O</td>
<td>Semi continuous ethanol fermentation Temperature-30°C Ethanol separation by per-vaporization</td>
</tr>
<tr>
<td>14</td>
<td>Persassner et al., 2005</td>
<td>Bakers yeast pH – 5.5 30°C 24 hours culture</td>
<td>Wood chip (salix)</td>
<td>Steam pretreated treated with dilute H2SO4 for excess for 90 min.</td>
<td>Media with wood chip Temperature-30°C pH-5 (auto maintained by 10% NaOH) 350 rpm, 24 hours.</td>
</tr>
<tr>
<td>15</td>
<td>L. V. A. Reddy et al., 2005</td>
<td>Saccharomyces cerevisiae (CFTR1101)</td>
<td>Malt extract peptone yeast extract MPYD (liquid medium broth)</td>
<td>Pulverization (Physical pretreatment method)</td>
<td>MPYD media (broth) + finger millet (germinated) Temperature-30°C 100 rpm pH – 5 HRT – 48 hours</td>
</tr>
</tbody>
</table>
Strains used

Microorganism plays a vital role in the fermentation. They are the harvesting units of the substrate to product (biofuel). Most commonly, fungi and bacterial cultures are widely used for the fermentation procedure. However fungi play an alarming role in the fermentation. As it is known that, the entire fermentation setup started with fungi *Saccharomyces cerevesiae* traditionally. In the recent days, *Kluveromyces spp*, the thermo tolerant yeast gives high yield of bioethanol. Most of the fermentation procedure involves the yeast as the biological agent converting the monosaccharide to ethanol in an anaerobic condition. Also certain bacteria are included in the biofuel production. Moreover, the genetically engineered microorganisms are also being used for the entire process of conversion. *Escherichia coli* and *Saccharomyces spp* are genetically modified for the production of biofuel. However the efficiency of conversion mainly depends on the substrate used and the pretreatment method employed along with the fermentation process.

**Substrate used for fermentation to produce bioethanol**

Various kinds of substrates are at present being used for biofuel production. But our review is mainly concerned about the major lignocellulosic materials that are being widely used as the substrate for the fermentation in the process of producing biofuel. Lignocellulosic materials generally include all the kind of crop residues, agriculturally waste products and the biomass constituents. Each and every are of the above that can be feasible used are discussed in the following article.

<table>
<thead>
<tr>
<th>No.</th>
<th>Authors</th>
<th>Substrate Used</th>
<th>Treatment Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>X.M.Ge et al., 2005</td>
<td>Schizosaccharomyces pombe</td>
<td>Reducing sugar containing biomass</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>General pretreatment methods</td>
</tr>
<tr>
<td>17</td>
<td>R. Millati et al., 2004</td>
<td>Rhizopus spp, Mucor spp, Rhizomucor spp, (totally 12 stains) Used pH-5.6 Temp - 50°C (PDA slant potato dextrose agar)</td>
<td>Spruce tree wood hydrolysate</td>
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<td></td>
<td></td>
<td></td>
<td>Fermentation media with spruce the wood hydrolysate.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(Pretreated) Temperature-37°C pH – 5.5 HRT – 7 days</td>
</tr>
<tr>
<td>18</td>
<td>Qian Xiang et al., 2004</td>
<td>Liriodendron totipifera</td>
<td>Saw dust</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>General fermentation condition previously</td>
</tr>
<tr>
<td>19</td>
<td>M.B.Allesteros et al., 2002</td>
<td>*Kluyveromyces. Marxianus, 42°C 150 rpm 16 hours culture</td>
<td>Plant biomass</td>
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<td>Fermentation media with plant biomass (pre treated)</td>
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<td></td>
<td></td>
<td></td>
<td>Temperature-42°C HRT-160 hours culture</td>
</tr>
<tr>
<td>20</td>
<td>Y. Fujita et al., 2002</td>
<td>Genetically modified yeast strains</td>
<td>Cellulosic materials</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>General pretreatment methods</td>
</tr>
<tr>
<td>21</td>
<td>H. Golias et al., 2002</td>
<td>*Klebsiella oxytoca, Zymomonas mobilis, (variety of species)</td>
<td>Sources of reducing sugars</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maintained at a pH of 5.0 and Temperature-35°C – 37°C.</td>
</tr>
<tr>
<td>22</td>
<td>N. Fuji et al., 1998</td>
<td><em>Saccharomyces pastorianeus</em></td>
<td>Porous cellulose carriers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fermented for 10 days at 30°C with 50 rpm</td>
</tr>
<tr>
<td>23</td>
<td>SudhaRani et al., 1998</td>
<td><em>Clostrium thermocellum</em> In CMS medium</td>
<td>Paddy straw sorghum sewer, de shelled corn cabs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fermentation conditions maintained for the specific substrates.</td>
</tr>
<tr>
<td>24</td>
<td>L.O.Ingram et al., 1995</td>
<td><em>Kluyveromyces oxytoca</em></td>
<td>Assorted raw materials</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maintained at a pH of 4.8 at 50°C</td>
</tr>
</tbody>
</table>
Once after selection of a particular substrate it is highly regarded to find which sort of the pretreatment method may be adapted for the efficient hydrolysis of the substrate and moreover since this biomass is regarded as an efficient renewable source of energy, process is carried out so that this biomass can be converted into biofuel by the action of microorganisms. Some of the biomass that are widely being used are – crop residues, corn and corn wastes like corn stover, barley and barley waste material, oats, oat grain, rice, rice waste, wheat, wheat hydrolysate, sorghum, sugar cane bagasse etc. Recently, for the past couple of decades, woods, salix, millet, lactose waste, lactose, whey are also being widely used as the raw material for the fermentation to produce bioethanol. Wood, millet and other crop residues require a different sort of pretreatment like acid hydrolysis, alkaline hydrolysis and also enzyme based hydrolysis (S. Zhu, et al., 2006).

Corn

Corn is the major food crop of North America, Africa (26%), Europe (12%) and South America (9%). North America is regarded as the highest producing area of corn. Most of the corn is used as animal feed. About 64% is secondly used as human food. Nearly 5% of the corn is wasted as logistic waste. Hence, if this wasted part is being used as the substrate for bioethanol production, then it has been calculated statistically that 9.3 gallons of bioethanol can be produced. Some of the waste products in corn milling are dry distiller's grain, non edible waste, etc. Lignin is rich in corn stover. Based on all these statistics available, corn can be used as the efficient substrate for the biofuel production.

Barley

Barley is also an important crop with annual production of 62% in Europe and Asia (15%). Apart from food to animals and humans, 4% of barley is wasted. 4% of barley can be effectively converted into 1.5 gallons of bioethanol which can effectively replace 1.1 gallons of gasoline. Barley also produces DDGS (Distillers Dry Grain and Solubles) which can be effectively used as the substrate for bioethanol production. There is not much information available right now regarding the DDGS. However, this DDGS and wasted barley grain can be effectively used for the biofuel production.

Oat

Oat is also an important crop variety aimed to feed the animal stock and to less present humans. Highest producer is the Europe (64%) followed by North America (21%), Oceania (5%). The lost oat is very less of the order 2%. If this wasted oat is being converted to biofuel it can replace 161 million litres of gasoline and there by producing 225 million litres of bioethanol. Oat straw is globally available which could produce bioethanol. Oat straw is a waste crop residue with major percent of cellulose and lignin with certain other polysaccharides. On the whole, oat and oat straw can together produce about 3.16 gallons of bioethanol. Hence, bioethanol and fuel production using oat is gaining importance.

Rice

Rice is yet another important food crop, mainly concerned for human needs than animals. Asia is the largest producer of rice with more than 90%. Wastage in rice is around 4.8%. Hence, research works are being carried out in most countries to effectively convert this substrate in to biofuel. Already 12.3 gallons of bio alcohol have been produced by the western countries and other parts of Asia. Tribals of eastern part of India and Tibetan regions are known to produce alcohol for drinking purpose under the name ‘sake’. Even the rice straw just like other waste agricultural byproducts can be used as the effective means to produce alcohol as a fuel.

Wheat

Wheat and wheat straw with wheat grains that are being wasted can be potentially counted in to the effective means of biofuel through fermentation. Asia (43%), Europe (32%) are the primary producers of the wheat. Second largest producer is India while first being china. The use of substrate as wheat is limited and at the same time, the production of ethanol as biofuel from wheat is also less. Wasted wheat grain, wheat dry milling together constitutes the substrate for biofuel production. Wheat straw is potentially being used as substrate for the biofuel production. Europe is currently producing bioethanol in large extent.

Sorghum

Sorghum is another primary crop in Africa (33%) and North America (23%). Sorghum is mainly used as animal waste and almost equally for humans. 6% of the sorghum produced is being wasted annually. Sorghum grain waste, sorghum dry milling, sorghum straw are collectively used for producing biofuel. The sorghum usage for bioethanol production is carried out only in North America and not in Africa, because of low yield of the straw and grain. Nearly 4.9 gallons of bioethanol is being produced.

Sugar cane bagasse

Sugarcane bagasse is the straw like substance that is left over as waste after extracting juice. This sugarcane bagasse is extensively being used as the substrate for the ethanol production. However, in recent times, bagasse is directly being used for the production of the biogas or gobar gas. The above mentioned sources are the most common sources for producing bioethanol. Apart from these substrates wood, salix, millets, are also intensively being used in many cases. The overall view of the biofuel and bioethanol producing industries are - any substrate which has polysaccharide material with lignin in it can be potentially utilized and adapted for the bioethanol production. However for the effective and enhanced production of the bioethanol, foremost thing to be considered is the pretreatment method. This is because of the amount or percent of ethanol conversion to substrate is directly related to the hydrolysis of the polysaccharide substrate that has taken place. Hence, based on this, criterion and the pretreatment method that can be possibly adapted for biofuel production in a proficient way.

Pretreatment methods

A potential and efficient way to produce ethanol is through the utilization of lignocellulosic material like crop residues, grasses, saw dust, wood chips and solid animal waste. Research works has almost been so concrete in the preparation methods for producing ethanol using lignocellulosic materials. However, it is high time to increase the percent of ethanol being produced by this substrate usage. Among the methods, for increasing the percent, the steps needed to be improvised in the pretreatment methods. Hence, now we can deal a bit with the various pretreatment methods being applied in this regard (A. Demirbas, et al., 2003).

Hence, various methods being applied are broadly classified into four categories.

- Physical pretreatment
Physiochemical pretreatment

The streams adapted in these techniques are Steam explosion (auto-hydrolysis), Ammonia fiber explosion (AFEX) and CO2 explosion.

Steam Explosion

Chipped biomass is treated with high pressure steam and then the pressure is swiftly reduced due to which the cellulose material under goes explosive decompression. The temperature being used is 1600°C – 2600°C; it is continued for few seconds to several minutes before it is introduced into atmospheric pressure. Hemicellulose degradation and lignin transformation is obtained at high temperature and hence cellulose hydrolysis potential is increased by treatment with dilute Sulphuric acid. Under conditions of steam explosion for optimal treatment of cellulose it uses two concepts. High temperature and low residence time or low temperature and high residence time (S.J.B. Duff, 1996). However, lower temperature and longer residence time can be employed beneficially and has high percentage substrate pretreatment, Advantages of steam explosion are it is low energy requiring process compared to physical methods of pretreatment. It includes also no environmental costs. Steam explosion is too cost effective for hard woods, agricultural wastes and other high cellulose rich residues. Limitation is some of the new residues formed become highly inhibitory product to the fermentation procedures involved. However, when using this technique it needs careful selection of the substrate and also the temperature and residence time adapted.

Ammonia fiber explosion AFEX

When instead of steam, if liquid ammonia at high pressure is being used as the gaseous medium for a particular period of time it is called as ammonia fiber explosion (AFEX). AFEX concept is similar to steam explosion. This pretreatment method can be employed satisfactory for grass straw, herbaceous crop residues and several lignocellulosic materials like alfalfa, wheat straw, wheat chaff, barley straw, corn stover, rice straw, municipal solid waste, softwood, newspaper and bagasse. AFEX is found not to solubilize hemicellulose compared to acid pretreatment and steam hydrolysis (E.Y. Vlasenko, et al., 1997). Various percentage of hydrolysis has been obtained for various substrates mentioned above. However, some of major disadvantage include reducing the cost and protecting the environment, ammonia need to be recycled after pretreatment. It is done using a process of superheated ammonia at 200 0C is passed through the AFEX treated substrate which vaporizes the rest of ammonia in the treated substrate. The ammonia is collected if a pressure controller is used for the recovery. The main assert of this technique is it does not produce any inhibitory products as in the previous techniques being employed. Also, small particle size is not compulsory for this sort of pre-treatment technique.

CO2 explosion

This is again similar to steam and ammonia pretreatment methods. CO2 explosion is used for pretreatment of lignocellulosic materials. In this, it is found that during pretreatment, carbonic acid is formed from CO2 which increases the hydrolysis rate. However it is found that, this method yields relatively low percentage conversion to glucose compared to steam or ammonia explosion (Y.Z. Zheng, et al., 1998) compared CO2 as efficient and cost effective way of pretreatment compared to steam and AFEX with no inhibitory product being obtained finally. However, this treatment method can be efficiently modified by using other gases for treatment in such a way that it produces no toxic products.
products at the end, and has high percentage of substrate treatment. It should also be noted that new method employed in physico chemical method be cost effective and efficient.

**Chemical pretreatment**

Chemical pretreatment involves wide range of choice of selection. Five methods are available in this technique.

**Ozonolysis**

Ozone can be used to degrade lignin and hemicellulose in many lignocellulosic materials. Ozone can act on substrate like wheat straw, bagasse, green hay, peanut, pine (W.C. Neely, 1984) cotton straw (D. Ben-Ghedalia, et al., 1983) and saw dust (P.F. Vidal, 1988). Lignin is not effectively treated by means of ozone. Ozonolysis has following treatment advantages: i) It produces no toxic residues. ii). It removes lignin to a good extent. iii). It is cheap processing wise as it requires no much increases or decrease in temperature pressure. Disadvantage mainly includes the large amount of ozone required which makes the process expensive.

**Acid hydrolysis**

Concentrated acids like Sulphuric acid, hydrochloric acid are used in acid hydrolysis. They are toxic, corrosive and hazardous in the reactors being used. These are highly efficient of pretreatment methods. But care to be taken so that the reactor paves favorable way for the process to be carried. In case, if acid of high concentration being used then it should be made a note that, after treatment of substrate, the acid needs to be removed for economically feasible process information. At present, acid with lower concentration that is dilute acid hydrolysis is carried out most successfully. Dilute sulphuric can be used for pretreatment of cellullosic materials. At moderate temperature it has low conversion rate of substrate to sugar molecules. However, in the case of high temperature dilute acid treatment the cellulose hydrolysis rate is satisfactory. Two processes are involved in acid hydrolysis. They are i) High temperature continuous flow process – this process is for low solid loading temperature of the order -16000C is used. The solid concentration is around 10% weight of substrate/weight of reaction mixture (A.O. Converse, et al., 1988). ii) Batch process – low temperature – temperature maintained is less than 16000C. The solid concentration is less than 10% – 40% is high solid concentration (A. Esteghlalian, 1997). For ethanol, the production is most feasible at pH greater than 5 to 8. Hence in this case, the acid used need to be neutralized. Hence for this reason this method is highly cost effective than the physico chemical process employed.

**Alkaline hydrolysis**

Similar to acids, some bases can also be used for pretreatment method and of lignocellulosic materials. Its effect depends on the lignin content of the materials (J.D. McMillan, et al., 1994). The porosity of the lignocellulosic material increases when the cross linking xylan and hemicellulose bonds are being removed. This is primarily done by the saponification process by the alkaline materials. Generally NaOH of various strengths are being employed in the pretreatment process. Ammonia which is an alkaline chemical is also used in this regard. Hence apart from acids, alkali solutions can also be used to a great extend in the pretreatment processes.

**Oxidative delignification**

In Oxidative delignification process hydrogen peroxide is used. It is a strong corrosive agent. Lignin in the substrate is greatly degraded by peroxidases enzyme with presence of H2O2 (A.M. Azzam, 1989). This method of pretreatment is not satisfactory and not widely applied.

**Organosolvent process**

Organic or aqueous organic solvent are used in this process. The various organic acids used in this method are the methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetra hydrofurfuryl alcohol. In this process inorganic acid catalyses are also incorporated for the organosolvent process. But when the organosolvent process is carried at high temperature, inorganic acid catalyst addition is necessary. In this process after hydrolysis of the organosolvent, it is highly essential to remove the solvent as it may prove to be inhibitory for the process of fermentation and might be toxic to the microorganism used in most cases. Thus, of the various methods employed for pretreatment in chemical methods most efficient are the acid hydrolysis and alkaline hydrolysis which are of great importance even today.

**Biological pretreatment**

Biological pretreatment method uses the usage of microorganisms that has the capability to degrade lignin and hemicellulose in the substrate. Generally various fungal strains are used exhaustively as they can break the lignin and hemicellulose effectively (T. Lebeau et al., 1996).

**Various fungal strains and other microorganisms used for the production of bioethanol**

Brown, white and soft rot fungi. Some of sub spp. used are – Pleurotus ostreatus, Phanoschaete sordida, Pyenoporous cinnabarius 115, Sporotrichum pulmententum, Ceriporiopsis subvenispora, Cyathus stercoreus, Penicillium chysosporium etc. White rot fungus produces lignin peroxidases and manganese dependent peroxidase during secondary metabolism for the degradation of lignin. Enzymes like polyphenol oxidases, laccases, H2O2 producing enzymes and quinone reducing enzymes can also degrade the lignin and hemicellulose effectively (T. Lebeau et al., 1996).

**Enzymatic hydrolysis of cellulose**

It involves usage of enzyme cellulase for the pretreatment of cellulose. The cellulase enzyme degrades the cellulose into the reduced sugar.
These are the various pretreatment methods employed for enhancing hydrolysis of the substrate. The most widely applied are depending on the nature of the substrate, the hydrolysis rate, time of reaction, cost effectiveness and above all the environmental issues related to the methods. Thus, based on the substrate and its nature, pretreatment methods are selected.

Thus, on the whole, various researchers in this field have produced varying results of ethanol produced using variety of substrate and also diversified strains of microorganisms. The various conditions like temperature, aeration, agitation, HRT and the microorganisms used are given as an account for the past five years from 2001 – 2006 for better reference.

**Analytical methods**

Different kinds of analytical techniques have been used for various analysis purposes. Glucose to Bioethanol is analysed using basic techniques like DNS method to highly advanced techniques like the HPLC (High Performance Liquid Chromatography) and GC (Gas Chromatography). They install and use various kinds of chromatograms. The mostly used chromatograms are the Aminex HPX series. There are different models in the series like the Aminex HPX – 87H and Aminex HPX – 87Pb and more. The complete data is taken in and processed using computers employing softwares like Microsoft excel and its advantageous versions. Also assorted databases like the BORWIN data collection are used for references. Using these data's and results, numerous graphical representations are plotted and their progress is studied carefully.

**Conclusion**

The world's crude oil is high on its decreasing phase and the need for an alternative is in its exponential phase. Though it has been cued out that ethanol can be the apt alternative, the commercialization of it in a successful phase may take a little time. If the cost of the cellulase enzyme is reduced even more, there is a great scope for the commercialization of the industry. Experiments are going on to insert a cellulase coding gene is reduced even more, there is a great scope for the commercialization of the industry. Experiments are going on to insert a cellulase coding gene and multiply them, thus a huge watch in the genetics sector.

The people all over the world are watching over for a quick breakthrough in the field of production of bioethanol to provide an economical and ecofriendly fuel and scientists all over the globe expect a model for enzyme adsorption and hydrolysis of microcrystalline cellulose with slow deactivation of the adsorbed enzyme. Biotechnol. Bioeng. 32 p.p. 38 – 45.


