

BIOFUELS- A NEED FOR BETTER FUTURE

Sandeep Kaur¹, Rajeev Sharma^{2,3}, Shubham Bansal^{2,3}

¹*Department of Biotechnology, Shri Guru Granth Sahib World University, Fatehgarh Sahib (India)*

²*Department of Chemistry, Multani Mal Modi College, Patiala (India)*

³*Department of NSS, Multani Mal Modi College, Patiala (India)*

ABSTRACT

An ever increasing global energy demand and depleting fossil fuels have led to energy crisis in the world and global leaders are looking for alternative fuel sources. Bioethanol or biofuel has emerged to be a promising candidate in this field. As a petroleum blender, it provides a cheap, renewable alternative to fossil fuels. It also has added advantages like increase in its octane number (108) for better engine performance and supply of oxygen for better combustion leading to reduction in the harmful emissions. The sources for biofuel production are damaged cereals and lignocellulosic wastes, which are readily available in an agricultural country like India. Indian energy demand increase by 5 percent every year, with road transportation being the highest utilize of gasoline and diesel. The use of bioethanol as an energy source will not only help meet the needs of increasing vehicle population of India but also stabilize Indian economics, which is burdened by import of fossil fuels from foreign nations. Being cheap and eco-friendly, biofuels are emerging as fuels of the future.

I INTRODUCTION

Ethanol or bioethanol as it may be called is the largest volume fermentatively produced organic solvent of immense utility as chemical feedstock, fuel supplement and gasoline extender. The ever rising cost of fossil fuel internationally has forced major world economics to examine renewable and cheaper alternatives to fossil fuel to meet their energy demands. Bioethanol has emerged as the most suitable renewable alternatives to fossil fuel as their quality constituents match diesel and petrol, respectively. In addition, it is less polluting than their fossil fuel counterparts. Environmental concerns and the desire to be less dependent on imported fossil fuels have intensified worldwide efforts for production of ethanol from starch and sugar producing crops.

Ethanol derived from agricultural sources, as distinct from petrochemical source is referred to as bioethanol. It is clear, colourless liquid, which boils at 78 °C. The bioethanol is widely used in industries, medicine and motor fuels. It is also used in antifreeze compounds and rocket fuels, pharmaceutical, printing, cosmetics *etc.* The common viable method adopted for ethanol production is through microbial fermentation of cane juice. The other substrates are sweet sorghum, corn, sugarbeet, sweet potato *etc.* which are rich in sugar. Apart from these, the cereal crops can also serve as alternate source.

Ethanol has emerged as a major motor fuel and fuel additive recently due to depleting fossil fuels and fluctuating crude oil prices. While US, Canada and Australia permit upto 10% blending of ethanol with

gasoline, its concentration goes till 22% in Brazil [1]. The mixture of gasoline and ethanol is termed as gasohol. Use of gasohol not only reduces the use of gasoline but also has additional advantages like increase in its octane number (108) for better engine performance and supply of oxygen for better combustion leading to reduction in the harmful emissions [2]. Such important properties of ethanol have led to increase in its production in the world from 195 million gallons in 1980 to 13.17 billion in 2006 [3]. Brazil and the United States are the world's largest producers of fuel ethanol. While Brazil uses sugarcane ethanol, the United States produces it from corn [4]. Ethanol gives efficient combustion as compared to gasoline, although it gives just two-thirds of energy and heat value of petrol [5]. In unmodified diesel engine, it greatly reduces visible smoke, when used up to 15% ethanol [6].

Biodiesel is produced through transesterification of vegetable and residual oils and fats and it can work as full substitute with minor engine modifications [7]. Bioethanol which substitutes for gasoline in flexi-fuel vehicles, also serves as feedstock for ethyl tertiary butyl ether (ETBE) which blends more easily with gasoline. Biogas or biomethane can be used in gasoline vehicles with slight adaptations, is produced through anaerobic digestion of liquid manure and other digestible feedstock [7].

II NEED FOR FUEL ETHANOL

The global temperature has increased by half a degree Celsius since the beginning of 20th century. The period between 1990 and 2006 have recorded an average of 10 of the warmest years since 1850, showing that this trend has speeded up [8-10]. The carbon dioxide emissions have doubled over last three decades from being less than 15000 M ton in 1971 to nearly 25000 M ton per year in 2003. The global energy demand has increased tremendously and it is assumed that if the trend continues, there will be 60% increase in CO₂ emissions by 2030 [11]. This alarming increase in green house gases led the countries of the world to adopt remedial measures and Kyoto protocol aimed at reduction in overall greenhouse gas emissions was signed by 163 countries. This protocol implemented the objective of the UNFCCC to fight global warming by reducing greenhouse gas concentrations in the atmosphere to 'a level that would prevent dangerous anthropogenic interference with the climate system. The Protocol's first commitment period started in 2008 and ended in 2012. A second commitment period was proposed in 2012, will continue up to 2020. The transport sector accounts for more than half of world's total oil consumption and hence contributes substantially to greenhouse gases emissions in the world [11]. The small oil dependent units (cars, buses) make the structure of transport sector difficult and expensive to change. Hence, biofuels shows the potential as an alternative fuel, which will not only decrease the overall CO₂ emission but also improve energy security.

III ENERGY DEMANDS IN INDIA

India has the highest energy demand in transport sector, with 60% of total goods and 80-85% of total passenger traffic using road infrastructure [12]. India had 142 million registered motor vehicles in year 2010-11 and there is 8-10% annual growth in vehicle population. It was estimated that total registered vehicles by the end of 2013 will be 165 million [12].

Currently, 73% of fuel demand is met by diesel followed by gasoline at 20% and it is expected that there combined demand will grow by more than 5 percent over the coming years. It is also estimated that by the end of this decade, the average demand for transport fuels will rise to 167 billion litres and will grow further to 195 billion litres by 2023 [12], (Table 1).

Table 1: Fuel Use projection in India

Calendar Year	2015	2016	2017	2018	2019	2020	2021	2022	2023
Gasoline Total	28	30	32	35	37	40	43	47	50
Diesel Total	94	97	97	101	110	115	119	124	129
On-road	56	58	58	61	66	69	72	74	78
Agriculture	11	12	12	12	13	14	14	15	16
Construction/mining	4	4	4	4	4	5	5	5	5
Shipping/Rail	5	5	5	5	5	6	6	6	6
Industry	10	11	11	11	12	13	13	14	14
Power generation	7	8	8	8	9	9	10	10	10
Jet Fuel total	8	9	9	10	11	12	13	14	15
Total Fuel Markets	130	136	136	143	159	167	176	185	195

Source: Industry and Trade sources [12].

India is fourth largest global contributor to carbon emissions. Government of India (GOI) transport policy has targeted EURO-III and IV vehicle emissions norms [13] for vehicles, which in turn require adoption of clean and green fuel [12].

IV RAW MATERIALS

Ethanol is produced by fermentation of sugary and starchy materials. The raw materials for ethanol fermentation are classified into three types: sugars, starches and cellulose materials. Sugars (from sugar cane, sugar beets, molasses and fruits) can be converted to ethanol directly. Starches (from corn, cassava, potatoes and root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. Cellulose (from wood, agricultural residues, waste sulfite liquor from pulp and paper mills) must likewise be converted into sugars, generally by the action of mineral acids. Once simple sugars are formed, enzymes from microorganisms can readily ferment them to ethanol. Molasses, which contains 50:50 of sugars: organic and inorganic compounds, including water is most widely used material for ethanol fermentation. It is thick, dark-colored syrup produced during refinement of sugar.

Limited availability of molasses and its alternative uses led to search for other alternatives and agricultural biomass esp. damaged food grains can act as a potential substrate. These substrates include corn (maize), wheat, oats, rice, potato and cassava. On a dry basis, corn, wheat, sorghums (milo) and other grains contain around 60

to 75% (w/w) of starch, hydrolysable to hexose with a significant weight increase (stoichiometrically the starch to hexose ratio is 9:10), and these offer a good resource in many fermentation processes [14]. India is second largest producer of rice in the world having produced about 136 millions tones of rice in 2007 [15]. Food Corporation of India (FCI) categorizes the damaged grains in the five classes on the extent of damage. Corn has emerged as primary raw material for biofuel production in the United States [16]. The United States emerged as master producer of bioethanol in 2009, producing 55% of worlds total biofuel with 10.9 billion gallons [1].

Agriculture residues, forestry residues and municipal solid waste are the three sources of cellulosic materials. Besides these there are materials grown specifically for fuel production such as woody or herbaceous high productivity energy crops or trees produced by conventional forestry [17]. Sugarbeet and sweet sorghum can be utilized for bioethanol production besides sugarcane [18,19] examined potential of fodder beets juice for fermentation to ethanol.

High starch content of cereals makes them good source for conversion to biofuel and biobased products. Fermentation of such grains is somewhat more complex than fermentation of sugars, because starch must first be converted into sugar and further into ethanol. Starchy materials cannot be directly metabolized by *Saccharomyces cerevisiae* and need to be converted to hexoses or pentoses sugars by amylolytic enzymes: α -amylase and glucoamylase. The other method of converting starch to sugars is cooking at high temperature (140-180 °C). Then resulting sugar is converted into ethanol by use of yeast, producing carbon dioxide as co-product.

Before microbial enzymes were readily available in the market, grain based ethanol was produced using malt or Koji as enzyme source. The use of microbial enzymes for alcohol production from starch was first reviewed by Aschengreen [19] and various enzyme-based cooking processes were described in 1981. A review of the production of ethanol from whole grain was made by Lyons in 1983 [20] and later by Lewis [21] in 1996. Fuel ethanol is recovered by distillation after anaerobic fermentation.

Table 2: Overview of starch content, gelatinization temperature and expected yield of alcohol from various raw materials used for alcohol production.

Raw material	Typical starch content in %	Gelatinisation temperature, °C	Alcohol yield (litres per 100 kg)	Protein content in %
Barley	54 – 65	53° - 63°	34 - 41	9.0-14.0
Maize	60 – 63	68° - 74°	38 - 40	9.0-10.0
Manioc/Tapioca (Meal)	65 – 80	51° - 65°	40 - 50	0.5-2.0
Rye	55 – 62	55° - 70°	35 - 37	8.0-16.0
Sorghum	55 – 65	70° - 78°	36 - 42	8.0-10.0
Triticale	63 – 69	55° - 70°	40 - 44	13.0-16.0

Wheat	58 – 62	58° - 65°	36 - 39	10.0-14.0
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Source: www.biokemi.org [22]

V BIOFUELS AND INDIA

India is an agricultural country. It had production of 93.9 million tons (Mt) of wheat, 104.6 Mt of rice, 21.6 Mt of maize, 20.7 Mt of millets, 357.7 Mt of sugarcane, 8.1 Mt of fibre crops (jute, mesta, cotton), 17.2 Mt of pulses and 30.0 Mt of oilseeds crops, in the year 2011-12 [23]. A considerable amount of food grains are damaged each year due to improper storage and rodent infestation. These damaged food grains which are unfit for human consumption can be used for production of fuel ethanol. [24] have reported that 50 million tones of damaged rice was lying unutilized in the FCI warehouses. An estimated amount of 1.3 mt of food grains (mostly rice, wheat and maize) were wasted in Food Corporation of India (FCI) godowns between 1997 and 2007 [25]. Indian government spent about Rs. 259 crore to clean away rotten food grains. Every year food grains worth Rs. 58,000 crore are lost due to wastage [25]. This utilization of damaged grains for an industrial application will not only alleviate the pollution problem in the country, but also help in management of such a huge biomass.

The availability of biomass in India is estimated at about 500 million tons per year covering residues from agriculture, processing industries, and forestry. Between 120 and 150 million tons of surplus agro industrial and agricultural residues per year could be made available for power generation. This estimate is based on a survey by the Indian Ministry of New and Renewable Energy, which indicated that 15 to 20 percent of total crop residues could be used for power generation, without altering the primary crops' present uses.

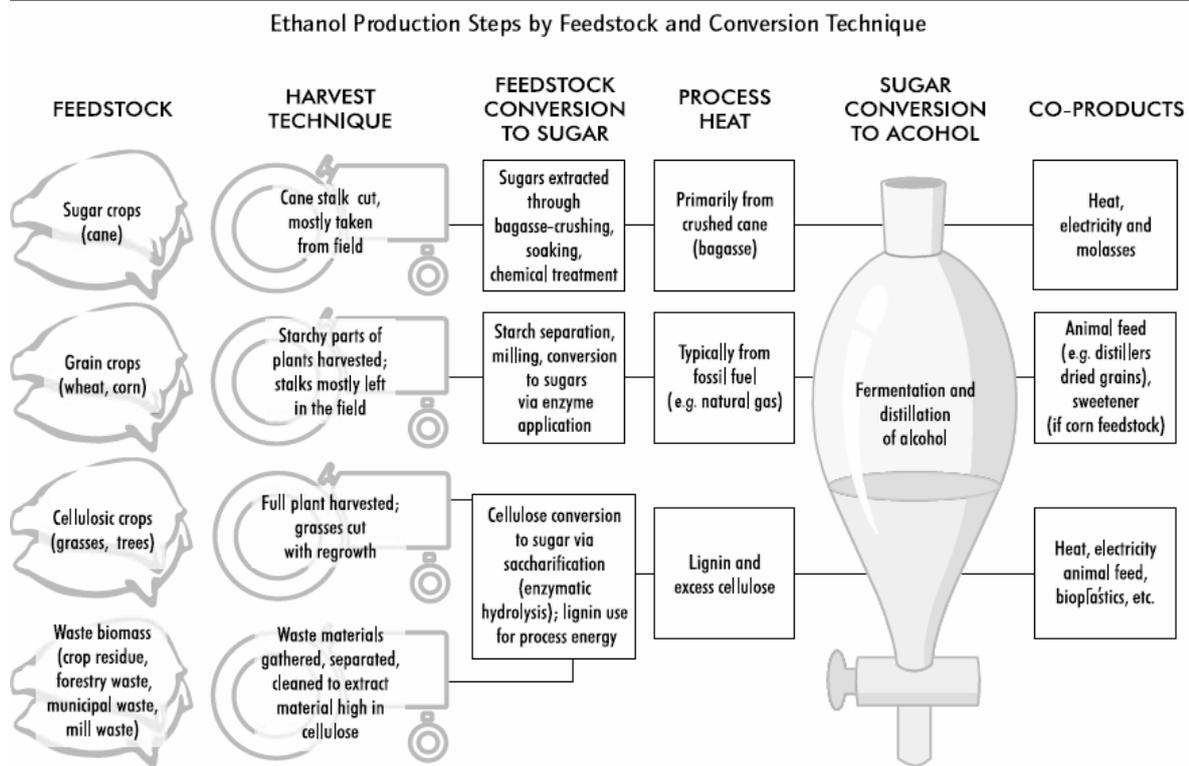
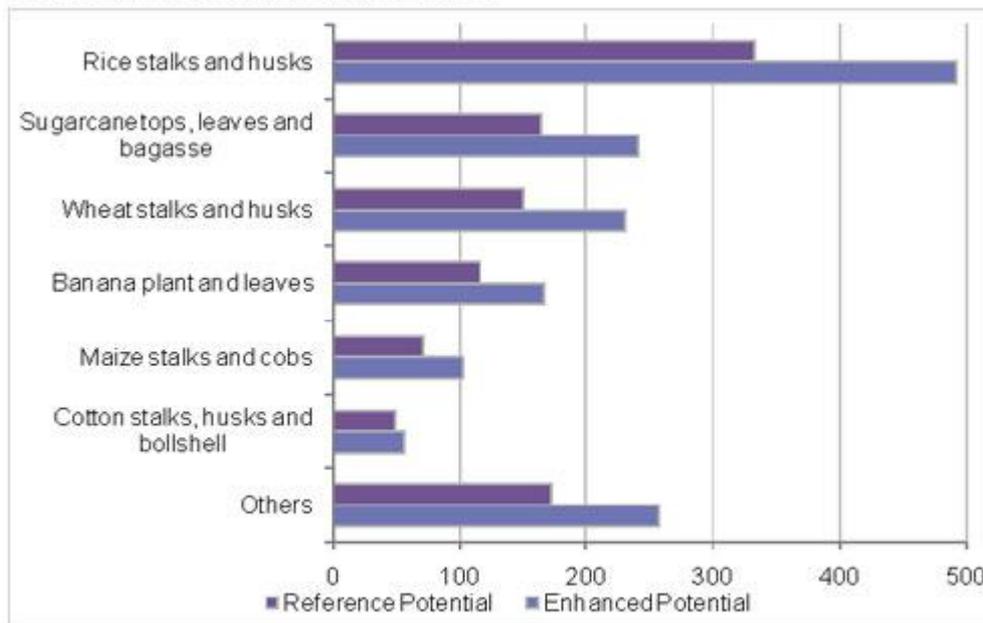


Figure1: Illustration of ethanol production (Source IEA, 2004)

Additionally, biomass has been playing important role as fuel for sugar mills, textiles, paper mills, small and medium enterprises (SME) and has significant potential in breweries, textile mills, fertilizer plants, paper and pulp industry, solvent extraction units, rice mills, and petrochemical plants. The total estimated biomass power potential in India is estimated at 31,000 MW of which the surplus power generation through bagasse cogeneration is estimated at 10,000 MW. [12]

Crop residue potential in 2020 (m tonnes)



Source: Bloomberg New Energy Finance, UN Food and Agriculture Organization (FAO)

India has 330 distilleries which can produce over 4 billion litres of rectified spirit (alcohol) per year in addition to 1.5 billion liters of fuel ethanol. Of this total, about 143 distilleries have the capacity to distill around 2 billion liters (GOI, 2013) of conventional ethanol per year and could meet the demand for 5-percent blending with gasoline. India produces conventional bioethanol from sugar molasses. Production of advanced bioethanol is in its research and development.

VI BIOETHANOL PRODUCTION PROCESS

Ethanol is produced by process of fermentation. Fermentation is a metabolic process that converts sugar to acids, gases and/ alcohol. It is carried out by yeast and bacteria. In alcoholic or ethanol fermentation glucose, fructose and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products in yeasts. As this conversion takes place in absence of oxygen, alcoholic fermentation is considered an anaerobic process. One glucose molecule is broken down into two pyruvates, which then break down into two acetaldehydes which are then converted to two ethanol molecules. *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia kudriavzeii* are the yeasts which bring about the alcoholic fermentation.

Bioethanol from molasses: The process consists of following steps. [27]

1. **Crushing and extraction:** After chopping the sugar cane, it is washed in counter current flowing warm water. The bagasse containing less than 0.5% sugars are squeeze dried to remove maximum

sugars. The extracted sugars are concentrated in evaporates and crystalline sugars are separated in centrifuges.

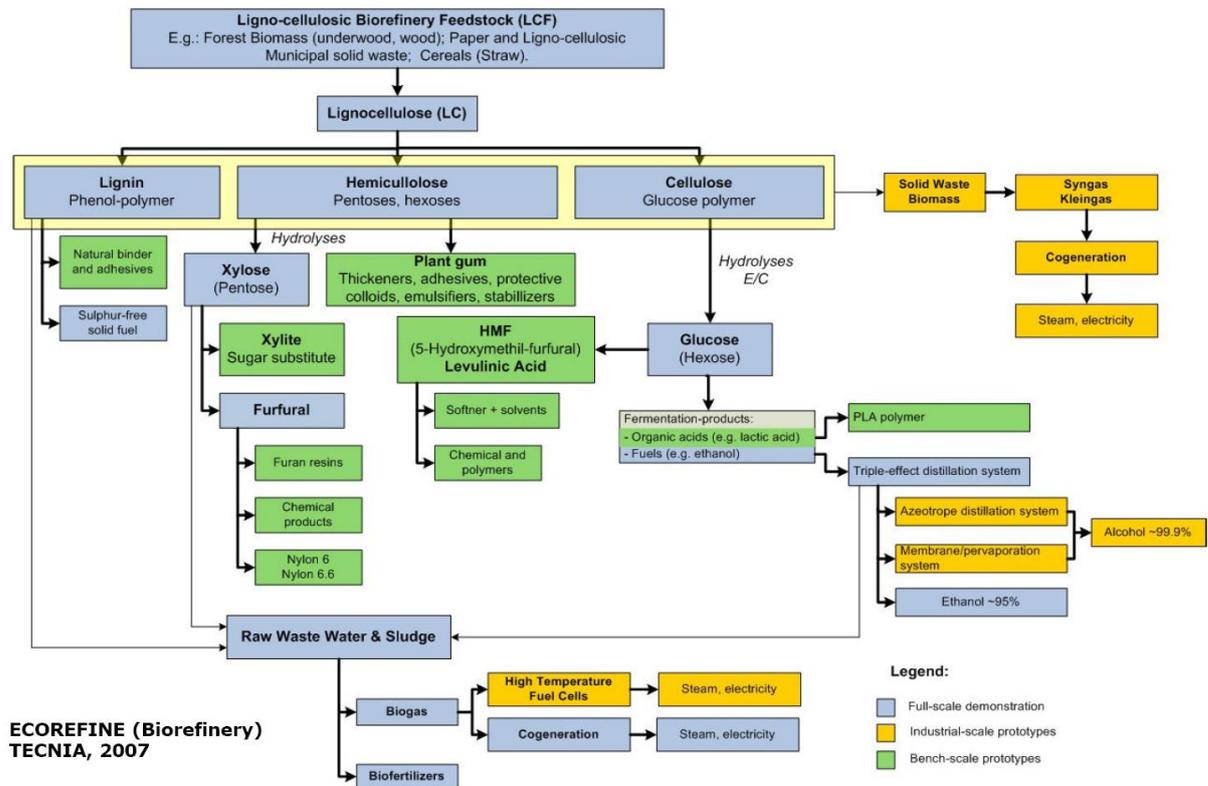
2. **Molasses fermentation:** The liquid residue (molasses) left behind contains approximately sugar: mineral matter in 50:50 ratio. This mixture is mixed with yeast and minerals and allowed to ferment.
3. **Distillation:** The fermented mash on completion of fermentation contains 10% alcohol. The mash containing alcohol, non-fermentable solids and yeast cells is feeded into a multicolumn distillation system, which separates alcohol from solids and water.
4. **Denaturation:** Ethanol is then denatured with 0-5% gasoline at the time of transport to make it unfit for human consumption [28].

Bioethanol from Starchy sources: The starchy sources include grains and feedstock and the process consists of following steps. [27]

1. **Milling:** The feedstock is first milled and grounded into flour, which is then sieved to extract pure starch. The alternative method is to mix the feedstock with water, ground it, sieve it to extract pure starch and then dry it in the sun (wet milling).
2. **Liquefaction:** The pure starch is mixed with water and alpha-amylase in a sterile container and heated at 120-150 °C to liquefy the starch. The high temperature also reduces the bacterial count in the mash [28].
3. **Saccharification:** The secondary enzyme glucoamylase is added to the mash to convert the liquefied starch to fermentable sugars. The process of liquefaction and saccharification is carried on simultaneously and usually glucoamylase is added almost 6-12 h after alpha-amylase.
4. **Fermentation:** After saccharification the mash is cooled down to the room temperature and yeast is added for fermentation, so that the sugars can be converted to ethanol [29].
5. **Distillation:** The fermented mash on completion of fermentation contains 10% alcohol. The mash containing alcohol, non-fermentable solids and yeast cells is feeded into a multicolumn distillation system, which separates alcohol from solids and water.
6. **Denaturation:** Ethanol is then denatured with 0-5% gasoline at the time of transport to make it unfit for human consumption [28].

Bioethanol from lignocellulosic sources:

The second generation of biofuels is being produced from lignocellulosic sources. Cellulose and lignin are structural components of plant cell and can be derived from trees, grasses, cereals etc. They comprise almost 60-80% of plant material. But cellulose and lignin are complex polymers and hard to break down as compared to starch [30]. The hydrolysis stage is more complex and expensive. It needs pre-treatment like steam explosion, acid and alkali hydrolysis. After hydrolysis the by-products need to be further treated and separated for fuel production. An outline of the process is given below



First and second generation of biofuels

The first generation biofuels were produced from cereal crops and molasses. They are characterized by their ability to be blended with petroleum-based fuels and can be combusted with internal combustion engine [31]. On commercial basis about 50 billion litres of first generation biofuels are produced annually. The problem with first generation biofuels started with concerns about environmental impacts and carbon balances [31]. Their major disadvantage is on food-vs- fuel debate, as there has been increase in food prices due to increase in fuel production using cereal crops [32].

Plants produce many industrially important primary and secondary metabolites. The primary metabolites are carbohydrates which include simple sugars like starch and glucose and complex materials like cellulose and lignin. The secondary metabolites include gums, resins, rubbers, waxes, terpenes, steroids, alkaloids etc [33]. While the primary metabolites have potential for biofuels production, the secondary metabolites are utilized for high value chemicals using integrated processing techniques [31]. Though biomass shows potential for biofuels, but it needs tremendous efforts to develop systems for production, conversion and utilization of feedstocks [34,35] reviewed current and future technologies related to separation methods for lignocellulosic biorefineries for production of ethanol and other products. Clark *et al.* [36] reported the use of green chemical technologies to transform low value waste biomass to waxes and ethanol etc. The role of different type of catalytic processes for production of biofuels was reported by Chew *et al.* (2008). [37]

The lignocellulosic biomass is cheap and abundant non-food material available from plants [31]. But as discussed they are not cost effective and there are a number of technical barriers needed to be overcome before full potential of lignocellulosic feedstocks can be realized [38]. Plant biomass is the most abundant biological resource available and hence it is seen as the promising raw material for fuels [31]. As there is a constant increase in demand for biofuels, cereal crops can only satisfy a proportion of the increasing demand [39]. Cellulosic ethanol and Fischer-Tropsch fuels are examples of second generation biofuels. Though non-commercial at this time it is anticipated that they could significantly reduce CO₂ production and do not compete with food crops [31].

Table 3: Chemical composition of common lignocellulosic biomass

Constituents	Hardwood (%)	Softwood (%)
Cellulose	40-5	40-5
Hemicellulose	25-35	25-35
Lignin	20-25	20-25
Pectin	1-2	1-2
Starch	Trace	Trace

Source: Miller (1999) [40].

Currently, most abundant cellulosic feedstocks derived from plant residues in the U.S, south America, Asia and Europe are from corn stover, sugarcane bagasse, rice, wheat straws respectively [42,43,1].

A co-culture of *S.diaeticus* and *S. cerevisiae* 21 produced 24.8 g l⁻¹ ethanol in a single step fermentation using raw unhydrolyzed starch, which was 48% higher as compared to yield obtained with monoculture of *S. diaeticus* [5]. In monoculture batch fermentations maximum ethanol was produced in 48 h at 30 °C using 6 g l⁻¹ starch [4]. Increase in grain sorghum concentration from 8 to 28% had no effect on final yield but led to decreased rate of fermentation because of higher slurry concentration which took eight days to ferment [45]. Badger (2002) [46] studied that 9.4- 10.91 (2.5-2.9 gallons) of pure ethanol can be produced from 25.3 kg bushel of maize grain with 15% moisture.

D-xylose is the second most important sugar forming in the hemicellulose portion of the plant cell wall and constitutes one-third of sugars in lignocellulosic feedstocks after enzymatic or acidic decomposition [47]. But *Saccharomyces cerevisiae*, primary industrial yeast used for ethanol production, converts only hexose sugars such as glucose and is not able to co-ferment glucose and xylose [48]; [1]. But advances in genetic and enzymatic technologies have led to expansion in *S. cerevisiae* capabilities to ferment different sugars simultaneously [49]. There is a wide range of fungal and recombinant bacteria available which is capable of fermenting xylose sugar, but they are unable to adapt to fermentation process conditions and produce only low ethanol yields [50,51]. Besides this, there are microbial contaminants present in cellulosic material, which compete with fermenting yeast for nutrients and produce toxic end products, which leads to considerable loss in ethanol yields [52,1]. Toxic components, primarily acetic acid along furfural, hydroxymethyl furfural and

phenolic components are also formed as a result of pretreatment processes [53,54]. Another problem to be dealt with is the lignin side effects on enzymatic hydrolysis [60,55]. For xylose utilization, after it is taken through cell membrane, xylose is isomerized to xylulose which is then converted to glyceraldehydes-3-phosphate and fructose-6-phosphate in pentose phosphate pathway [56]. In *S.cerevisiae* xylose uptake is inefficient as compared to glucose [5]. The xylose transport K_m values have been reported between 130 mM- 1.5 M [57], which are almost 5-200 fold higher than glucose [5]. *S.cerevisiae* can catabolise xylulose [58], but due to absence of an active isomerization system, it cannot isomerize xylose to xylulose [41,5]. To overcome this, attempts have been made to introduce bacterial xylose isomerase in *S. cerevisiae* [59]. The pathway in yeast requires reduction of D-xylose to xylitol by D-xylose reductase using NADH or NADPH. Xylitol is then oxidized to D-xylulose with NAD^+ by XDH [5]. The complexities arise due to cofactor specificities as it leads to cofactor imbalance, if cell is unable to compensate it in metabolism. The pentose phosphate pathway is a non-oxidative pathway with many reversible steps, operating near equilibrium. Phosphorylation of xylose to xylulose-5-phosphate is an irreversible step with large differences in Gibbs free energy, which move the reaction forward [41]. Although recombinant *S. cerevisiae* strains have been designed having successful expression of xylose reductase, xylulose dehydrogenase and xylulose kinase, the rate of xylose utilization is very low and conversion yields are poor due to xylitol accumulation [5]. The reason for poor conversion may be the limitations in different steps as cited above.

VII CONCLUSION

The increasing demand of fossil fuels is affecting economy of nations of the world. Bioethanol can prove to be a useful resource to meet these energy demands. The first generation biofuels were prepared from cereal grains which have given way to second generation of lignocellulosic feedstocks. Lignocellulosic feedstocks have the advantage of being in abundance and renewable. Also, there is no food vs fuel controversy for these second generation biofuels which was faced by the first generation. Being in abundance they hold a tremendous potential as biofuels. The improvements in pre-treatment process, enzymatic hydrolysis and development of genetically modified organisms for better utilization of lignocellulosic sugars and efficient recovery technologies will decrease the capital cost their full potential.

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