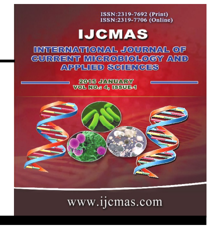

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Review Article

Biofuel from agricultural waste: A review

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A B S T R A C T

Ethanol an important biotechnology product in terms of volume and market values is intensely researched and out of many findings, one good finding suggests that lignocelluloses may prove to be one of the most useful alternatives to renewable energy sources. Sugar substances like molasses, sugarcane juice and starch based materials like corn, rice and wheat have proved to be promising raw materials for the ethanol production. Lignocelluloses (cellulose, hemicelluloses, lignin, etc) from plant materials are thus upcoming source of bio-ethanol production. Various agricultural wastes are rich sources of sugars that can be efficiently fermented to ethanol with the help of appropriate fermenting organisms. We at our lab focus on enhancement in production of bioethanol upon cohesive saccharification and fermentation process. Our target is built up an efficient protocol for simultaneous saccharification and fermentation targeting utilization of agricultural wastes. This review is a survey of various aspects of bioethanol production, focussing India. This review also gives a clear picture of various generations of biofuels, their benefits and also their limitations.

Keywords

Bioethanol,
Agriculture,
Ethanol,
Biofuel,
Saccharification,
Fermentation

Introduction

Rapid increase in demand of limited oil reserves has received tremendous attention for development of new renewable fuel in order to meet the global energy requirements (David et al. 2008). The biodegradable, renewable fuels (biodiesel, bioethanol) may be a promising alternative energy sources with minimal pollutants like sulphur, nitrous oxides, carbon dioxide) (Blanco et al. 2007). In a specific definition biodiesel is described as renewable and non toxic fuel that consist of mono-alkyl esters of long chain fatty acids derived from animal fats or vegetable oils (Vicente et al. 2007). Derivatives of methyl esters and glycerol from vegetable oils (Bourna et al. 2005) have been used since ages for biofuel production. Research has reached a reasonable commercialization stage in various European countries where since more than ten years biofuel is used as commercial fuel and it also adds value to the existing counterpart such as helping in restoring lubricity (Wilson 2002; Vermeersch 2002; Bournay et al. 2005). Brazil and United states lead world in production of valuable bio-ethanol (USDA

2006). Use of microalgae, yeast and bacteria are vigorously tracked as potential renewable alternate and researchers have investigated that microalgae serve as potential producers and can be cultivated with marginal land with water unsuitable for irrigation purpose (Wahlen et al. 2012; Chisti et al. 2007; Scott et al. 2010; Pittman et al. 2011; Wahlen et al. 2011) where as an alternate actually rare selected species of oleaginous yeast and bacteria accumulate at high concentration (Waltermann et al. 2000; Ageitos et al. 2011; Wynn et al.2005; Wahlen et al. 2012). Yeast and bacteria needs an abridged source of carbon to meet their energy needs. Most of these strains metabolize a varied range of carbohydrates, such as residual sugars from food production (e.g., molasses or lactose) or biomass hydrosylate (Voss et al. 2001; Ykema et al. 1998; Yu et al. 2011). Also, many studies have established the functional properties of biodiesel derived from numerous sources to determine the potential to replace petroleum diesel (Cecrle et al. 2011; Bugos et al. 2011; Kocak et al. 2007; Gumus et al. 2007; Bradley et al. 2012).

Environmental concerns over fossil fuel usage and alternate energy sources

India is nearing to 8% share in global CO_2 emissions **(**World Energy Insight 2013). The increasing fossil energy usage, the uncertain supply of energy resources and recent politico-economic transitions has led to focus of world leaders on the so called "energy trilemma" which refers to energy security, energy equity and environmental sustainability. Energy security is an issue concerning the supply of energy resources facilitating the availibility to masses and energy equity in turn refers to the affordability of a given energy resource. Ultimately, concerns are about environmental sustainability for ensuring low carbon emmision and also reducing other green house gases emmision to scale down global warming (World Energy Issues Monitor 2014). Renewable energy sources are being sought after as solution to the finitude of fossil fuels; the solution to the question of sustainability. The prime renewable energy sectors are hydropower generation, geothermal energy, solar power, wind energy, and the biofuels comprising mainly of biodiesel and bioethanol production as energy sources.

Annual capital expenditure on fossil fuels (oil, gas and coal) extraction, its transportation and on the process of oil refining has crossed double the values since the year 2000 to 2013 (World Energy Outlook 2014). Economists, policy makers and governments around the world are facing several stark issues related to their populations. The key issues includes persistent concern to ensure energy security, economic development with sub, persistence of agriculture and employment and climate change mitigation policies and challenges to reduce GHG emissions.

Biofuels

Biofuel are chracterized as vegetable oil, Biodiesel, solid biofuels, bioethers, bioalcohols, biogas etc. They are broadly divided as first generation, second generation, third generation and fourth generation biofuels. A steady rise in biofuel consumption is projected from 1.3 mboe/d in 2011 to 2.1 mboe/d in 2020 and whereas the same in 2035 at 4.1 mboe/d. By 2035, biofuels are projected to meet 8 % of total road transport fuel requirement from the current share of 3%. Ethanol hold the prime share as biofuel , making upto

75% of the total biofuel use till now. The heaviest demand regions for biofuels include US, India, China, Brazil and the EU accounting for nearly 90% of global biofuel demand until 2035 (world enrgy outlook 2010).

First generation biofuels comprise of biofuels derived from starch, sugar, vegetable oil or even animal fats processed by conventional methodologies. Vegetable oils can be used as biofuels after proper atomisation and reduction in viscosity. Biodiesel is a non- petroleum based fuel which contains short aliphatic chain, mostly methyl or alkyl, esters. Biodieselis synthesized by transesterification of animal fat or vegetable oil.Bioethers (Fuel ethers, Fuel oxygenates)serve the purpose of octane enhancers. They reduce pollutant emmissions and thus improve fuel, qualitatively. Biogas on the other hand is the result of fermentation of organic matter by anaerobic microbes. Main component of biogas is methane. Syngas is obtained by subjecting the biomass to pyrolysis followed by combustion and gasification (Mohammad 2012). Solid biofuel refers to the agricultural waste, wood, domestic waste, fodder waste and dried manure such as animal dung. Mainly energy is harvested by direct combustion in domestic use and small scale enterprises (Rahman et al. 2008). Pollution is the main setback with such an energy source. Bioalcohols are derived by fermentation of carbohydtrates, either simple sugars like sucrose or xylose, or complex ones like starch and cellulose (Josserand et al. 2008). Cellulosic bioalcohols are difficult to obtain.Various materials like wheat, corn, sugarcane or sugar beets can serve as raw materials. Conceptually, it is the enzymatic digestion of complex sugars, then fermentation of released sugars followed by distillation and drying (Bianca et al. 2009).

Second generation biofuel are obtained by processing a range of non-food crops and biowastes. Waste biomass, the stalks of agriculture crops such as wheat and paddy, corn stover and wood can serve as raw material. The aim is to harvest the abundant carbohydrate reserves in form of cellulose in the biomass or the complex lignocellulose content in woody materials as substrate for ethanol production (Braun 2008).

Third generation biofuel includes those where algae serves as feedstock for biofuel production and these fuel are referred as 'oilgae' . Algae are characteristically low input biomass with high yield potential. There is much scope to work on this generation biofuel (Byenlee et al. 2008).
Fourth generation biofuel includes those innovative approaches that are being worked out for example developing technology which can convert vegetable oil into gasoline. Certain firms are engaged in making genetically engineered microorganism which can produce fuel directly from CO_2 . Crops can also be genetically engineered which enhances CO_2 consumption by plants to a level more than what is released on combustion, bringing out carbon negative cycle (Picket et al. 2008; Bianca 2009).

Bioethanol: Production, Biomass and Microbes

Ethanol has been graded as excellent as a fuel candidate for modern combustion engines in motors. It has an octane number 98 which is better than gasoline with an octane number of 80 (Coelho et al. 2004) and incurs lower evaporative loss compared to gasoline attributed to lower vapor pressure than the latter. Ethanol is less flammable than its counterpart, gasoline, in air, making it a safer candidate as for fuel choice (Moreira et al. 1999). Ethanol can be used with a compression ratio of 12:1 in motor combustion engines (than that of gasoline at 8:1), which takes it ahead as a fuel choice, yet again. Due to higher compression ratio, ethanol is 15 % more efficient which in turn compensate the lower energy density (Goldemberg 2008).

Bioethanol is obtained as product of fermentation of simple sugars in the biomass with help of microorganisms performing enzymatic digestion. Ethanol has been produced in industries by synthetic catalytic processing of petroleum products such as ethylene. For industrial application, synthetic ethanol has the maximum share as it is cheaper to produce as compared to biomass derived ethanol (Biewinga et al. 1996). The leading bioethanol producers as well as consumers are Brazil and United States, which is a result of bioethanol upscaling policies, post 1973 oil crisis, to combat fuel insecurity (Gnansouna et al. 2005).

The consumption and production of biofuel globally has increased 7% in 2013 to a level of 116.6 billion litres, after a slight decline observed in 2012. Volume of fuel ethanol increased 5% going up to a level of 87.2 billion litres (IEA, World Energy Outlook. 2013). India's Biofuel Policy had regulations concerning ethanol blending with gasoline under the Power Alcohol Act, 1948. The Ethanol Blended Petrol Program was launched by government of India in January, 2003 in 9 states and 4 Union Territories for promoting the mandate of blending ethanol with gasoline upto 5% level (Acharya et al. 2004). A blending level of 20% for both biodiesel and bioethanol is projected to be achieved by 2017 (Basavaraj et al. 2010).

Substrate for bioethanol production via fermentation are the carbohydrates generally classified as monosaccharides, disaccharides and polysaccharides, all of which can be used for ethanolic fermentation with varying degree of yields. They can be used either directly for fermentation like the monosaccharides, or first subjected to digestion or chemical degradation and subsequent fermentation as in case of polysaccharides (Biewinga et al. 1996). The prominent natural monosaccharides are pentose as 5-C carbohydrates and glucose as 6-C carbohydrate. Plants contain disaccharides sucrose, predominantly found in crops like sugarcane, maltose and lactose.

Biomass for ethanol production can be harvested from crops meant for food industry, either in form of cereal grains i.e. corn, wheat or as crop residues such as wheat straw or corn stover etc. Other sources include crops which give the byproduct as substitute for bioethanol such as molasses and sugarcane bagasse from the sugarcane industry (Rasul et al. 2004). The use of whole grain described as first generation and use of residues as second generation biofuel respectivelyas discussed earlier. Leading bioethanol producers like Brazil and United states diverted the surplus harvest in agricultural crops for example that of corn in US and sugarcane in Brazil towards bioethanol industrial production.

The correlation between agriculture especially food crops and bioethanol then became a prominent factor contributing to vlatility in prices of ethanol as fuel (Wyman et al. 2004). These factors very well describes our need for second generation and third generation biofuel.

Feedstock harvested/collected are delivered to ethanol plant and stored therefo following which conditioning of the same is done in order to reduce unwanted fermentation and contamination. Pretreatment enhances quantity of fermentable carbohydrate. Various fermentation methodology particularly batch, fed batch or continuous fermentation (Raven et al. 1999) may be employed. Yeast immobilization and recyling of substrates are done to increase efficiency of the system which enhances productivity (Gnansouna et al. 2005).

Biomass saw an escalating demand in generation of heat energy, power generation and transport sectors. The reported total primary energy usage of biomass touched approximately 57 EJ in 2013. Global share of final energy consumption for the year 2012, is 78.8% fossil fuels, 2.6% nuclear power and 19% renewable sources (includes, geothermal, solar, hydropower, wind, biofuel etc.). Biomass are categorized as traditional or modern where, traditional biomass refers to the solid biomass combusted and is commonly used in the rural communities of developing countries. Modern biomass refers to source of energy which is "derived efficiently from solid, liquid and gaseous biomass fuels for modern appllications "(Renewables Energy Policy, Global Status Report 2014). Various feedstocks has been tried out on labscale trials and others which are used in commercial production of bioethanol. Global production of bioethanol has seen corn and sugarcane as predominant feedstocks at commercial level (Mohan et al. 2008). Other commercial feedstocks include sugarbeet and wheat. Basedon type of carbohydrate feedstocks are characterized as Sucrose feed stocks, starch feedstock and lignocellulosic biomass.

Sucrose is primarily obtained from crops such as sugarcane, sugarbeet, sweet sorghum etc. Sugarcane, sugarbeet, sweet sorghum etc served as first generation abiofuel and very studies on these feed stocks have been done (Balat et al. 2009; Badger et al. 2002; Charles et al. 2014; Kumar et al. 2006; Laban et al. 2013; Fortmeier et al. 1995; Montross et al. 2009). Sugarcane was a prefered one because of it being cheaper, sweet sorghur require dless fertilizer and so on. Still utilization of feedstock for fuel production was a major limitation as it could hamper worls economy.

Starch feedstock is chemically a homopolymer of glucose units, with two different types of linkages among the chain, one showing the α (1,4) linkages forming the amylose which is made up of nearly 1000 glucose residues and the other type which is a highly branched chain structure , with side branching formed by α (1,6) linkage., forming the Amylopectin which may contain 10 thousand to 1 lac glucose residues (Gnansouna et al. 2005).

Enzymatic hydrolysis of starch is done by glucoamylase enzyme which converts starch into D- glucose. Now, glucose as monosaccharide can be proceeded for fermentation.

Corn comprises of 60 % starch and is leading feedstock for bio-ethanol production (Kumar et al.2014). Cassava is another starch based feedstock used for bioethanol production and contains 50-70 % starch (Peláez et al. 2013).

The major constraints in the starch fermentation with *Saccharomyces cerevisae* lies in inability of this yeast to ferment starch which requires addition of α-amylase and glucoamylase enzyme addition to break α (1,4) and α(1,6) linkages respectively. Secondly, starch requires cooking of starch substrate at higher temperature (Mabee et al. 2010).

Hydrolysis of corncob material for producing reducing sugar with *Streptomyces* sp. cellulase and ethanol fermentation of cellulosic hydrolysate was carried out. *Streptomyces* sp. T3-1culture enhanced the yield of reducing sugars with the production of CMCase, Avicelase and β-glucosidase activity of 3.8, 3.9 and 3.8 IU/ml, respectively (Farrell et al. 2006; Ohgren et al. 2005). The conversion of cellulose to glucose was favoured by CMCase, Avicelase, and β-glucosidase.(Wingren et al. 2003) It was observed that the synergistic interaction of endoglucanase, exoglucanase and β-glucosidase revealed efficient hydrolysis of cellulosic substrate (Sun,et al. 2002). The overall reducing sugar yield reached 53.1 g/100g dried substrate after 5 days of incubation. Further fermentation of cellulosic hydrolysate containing 40.5 g/l glucose was performed using *Saccharomyces* which have important effects and future applications concerning to fabrication of ethanol from agricultural cellulosic waste (Chuan et al. 2011).

The physical properties of pure ethanol limits its use in the same infrastructure as used to deliver retail gasoline and also in standard automobile engines at high blend ratios. Also, ethanol proves to be corrosive on rubber and plastic in the car engine (Randy et al. 2013).

Since ages presences of the microbes in the soil acts as a deciding factor to judge the benefits of a particular land for planting. Additionally the soil microorganism plays an important role in putrefaction, fermentation and synthesis. The microorganism under fermentative growth is subcategories into useful fermentation and harmful fermentation, also known as fermentation and putrefaction respectively (Bradley et al. 2012). In order to overcome Industrial constraint organisms growing at a higher temperature were preferred to work with. Thermophilic bacteria like *Bacillus Aerophilous* are known for the efficiency in fermenting sugars (Lynd,et al. (2008). Cellulolytic bacteria either in isolation or combination belonging to the genera *Thermoanaerobacter* and *Thermoanaerobacterium* (Lynd et al. 2005) have proven to ferment all the sugar in the biomass. Patel et al. reported that lactic acid can be produced from cellulose at very low cellulase concentration, using a thermophilic *Bacillus* species (Patel et al. 2005). *T. saccharolyticum* is known for hemicellulolytic organism (Shaw et al. 2008). Both aerobic and anaerobic bacteria can produces hydrolytic enzyme to carry out hydrolysis of a wide range of material, such as lignocellulosic material. cellulase production with high specific activity has been reported from bacteria belonging to *clostridium, streptomyces, bacillus , thermomonospora, ervinia and cellulomonas* . sinces the growth rate of anaerobic bacteria is very low and requires aerobic growth conditions the production of cellulase from has been gaining tremendous attention . Tricoderma among the fungal genera tricoderma is the most exploited genera for the production of cellulase (Mohanram et al. 2013).

Lignocellulosic biomass is a promising alternative energy source that can be utilized to produce ethanol. The conversion processes involves two steps: Hydrolysis of cellulose in the lignocellulosic biomass for production of reducing sugars, and fermentation of the sugars to ethanol. The cost of production of ethanol from lignocellulosic materials is comparatively high on the basis of current technologies. The main challenges are the low yield and high cost of the hydrolysis process. However, many efforts are being made in order to improve the hydrolysis of lignocellulosic materials by pre-treating the of lignocellulosic materials for removal of lignin and hemicellulose and by optimizing the cellulase enzymes and loading of enzymes. Furthermore, simultaneous saccharification and fermentation efficiently removes g an inhibitor to cellulase activity, i.e. glucose, thus enhancing the yield and rate of cellulose hydrolysis (Ye Sun et al. 2001). Fermentation is carried out by yeast both aerobically or anaerobically. The measurement of gas pressure present inside the fermentation vessel is used to determine the metabolic activity of the yeast (Afschar et al. 1996; Akoh et al. 2009). Yeast are great sugar fomenters namely *Saccharomyces cerevisiae*, *Pichia pastoris*, *Pichia stipitis* (Lee et al. 2003) etc. Some ferment only hexose, some only pentoses while few of them effectively ferment both pentose and hexose.

Conclusion

We could understand that though production of bioethanol is highly researched and even various evidences of its being commercialized are known, still many challenges lies ahead in making ita sustainable source. These include sufficient supply of molasses at a given site to reduce cost of transportation etc., to develop an economically effective production system. There are also various physical and chemical factors (Sanchez et al. 2008) limiting production of liquid fuels such as ethanol and biodiesel. These factors include obtaining less factor of sunlight by plants in some countries; obtaining ethanol from the produced site requires use of large amount of fossil fuel, low yield of oil crops and that extraction of oil from oil crop is energy intensive process (Sanchez et al. 2008).

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