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Review Article

Biotechnology for Prospective Medical Science

Second Generation Biofuel – An Alternate Source of Energy

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Abstract: The use of fossil fuels in the current situation has been increased. These fossil fuels do not come under the category of sustainable sources due to its depleting nature. Fossil fuels also pollutes the environment by increased emission of Greenhouse Gases (GHG). In order to produce an alternate renewable energy and to reduce the percentage of environmental pollution caused, many technologies like solar energy, tidal energy, wind energy etc. came into existence. But all these forms of energy can be produced in terms of electricity. To meet the increasing fuel demand, renewable biofuels came into the field where biomasses are utilized to produce biofuels. Second generation renewable biofuels are produced from conventional plant sources with higher oil content whose efficiency is determined by the amount of Green House Gases (GHG) emitted and the life cycle cost whether positive, neutral or negative. High yielding energy crops such as sugarcane, corn, switchgrass, wheat etc. are subjected to various processes / fermentation to yield biofuels like bioethanol, biobutanol, biohydrogen, biodiesel etc. The US and Brazil are replacing the demand for gasoline with around 15% of bioethanol and it has a higher octane rating, increased engine's compression ratio, increased thermal efficiency and ultimately reduces atmospheric pollution emissions. In future biofuels will be recognized as alternative fuel in transportation, energy generation, heat producer, charging electronics, environmental friendly cleaners, cooking, lubricants, adhesives etc. This review provides a brief outline about the second generation biofuels which would serve as an alternative energy in the near future.

Keywords: Biofuel, saccharification, liquefaction, lignocelluloses, cellulose, lignin, fermentation.

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1. INTRODUCTION

Biofuel production research in the current scenario has dramatically increased due intensification of civilization, transport, demand for fossil fuels etc.[1]. The global demand has been reported as 32.4 billion gallons in 2013 and it is predicted to increase to 51.1 billion gallons in 2022[2]. It has been depicted that about 13 fold energy consumption increased in the 20th century which is greater than the rate of population growth[3-5]. Predicted analysis between 2006 and 2013 revealed that 80% of fuel consumption was against transport and it has diminished the supply of fossil fuels and stimulated the use and production of biofuels as alternative source[6-10]. Evidence of biofuel consumption in EU27 countries from 2006 and 2008 was 5625 Kilotons of oil to 10064 Kilotons where the biofuel production increased from 5639 million tons to 8165 million tons[11]. Anticipated rising energy demand has developed to an alternate method of usage of biomass for energy production[12,13]. Biomass waste (maize, sugarcane, sugar beets, rice husk) were worldwide used for production of biofuels in most developing countries[14]. The energy from biofuel is considered to be a promising sustainable alternative source for its eco friendly, economic nature for future[15]. Nowadays sources for production of biofuel are well studied and reviewed that the availability of bioenergy from feedstock had been recommended for its special and abundant availability of cellulosic and lignocellulosic nature[16,17]. India, is known for its agricultural resources and enormous supply of waste is wasted (1/3rd as waste) every year[18,19]. Developing countries which depend on natural resources grow energy crops (eg) Brazil which is located mainly between Equator and Tropic of Capricorn is growing crops for about 851 million hectares[20]. With 30 years of experience, Brazil has developed bioethanol industries from sugarcane (since the climatic conditions suit the growth and high yield)[21]. A study from NIPE/ UNICAMP stated that Brazilian Government encourages the increase of sugarcane production to meet the demand of gasoline in 2025 and this can be overcome by substituting ethanol (5% 102 billion litres)[22]. Agro industrial traders are acting as cross border in biofuel production in US and European countries followed by Canada and Mexico [23,24]. Use of maize has shifted towards use of jatropha and palm oil as alternative feed stocks in Central America and Soy in neighboring Uruguay, Paraguay and Bolivia[25]. The present review article focuses on the importance and strategic plans adopted in various countries for production of biofuels from biomass (wealth from waste).

1.1 CHARACTERISTICS OF BIOFUEL

It has been predicted that by 2050, quarter of the world will depend on biofuel for transport fuel because of low carbon, non petroleum fuel, minimal changes to vehicle stock[26] and this will support the economic development for the country[27]. Biofuel usage has substantially increased from 2012 (25%) of anhydrous and hydrated ethanol (19.1 Mm^3) and biodiesel (2.2 Mm^3)[28]. Research on Greenhouse Gas Emission (GMG threshold) reduction by Renewable Fuel Standard (RFS) the biofuel should have 50% lower emission and it will classified as "Advanced Biofuel" and 60% as "Cellulosic Biofuel" and other biomass registered 44% (Corn ethanol) and 68% (sugar cane ethanol)[29,30]. Experimental analysis of biobutanol with gasoline in various compositions (100% Butanol, 50% gasoline and Butanol and 95% gasoline) were listed in the city of Sao Paulo for 120 km. It was found that the emission of biogenic CO_2 was 231.5 by 100% butanol and 104.2 in the second combination and 9.53 in the third case of 95% gasoline and CO_2 was nil in 100% butanol and 127.3 and 221.09 in other cases[31]. Elementary analysis of biocrude oil revealed carbon (75.2%), hydrogen (8.2%), nitrogen (0.5%), sulphur (0.3%), oxygen (15.8%), ash content (0.48%), bound water (3.8%)[32,33]. The cellulosic biocrude through Hydrothermal Liquefaction consisted of 4.4 mg/g aldehyde which was present as 5 – hydro-xymethyl-furfural (5-HMF) and 3.9 mg/g of ketone as 3-methyl-1,2 cyclopentanedione, 2,5-hexanedione and 1-(2-furanyl)ethane etc. and phenolic compounds such as guaiacol and creosol[34]. The leading bioethanol producers are Brazil with 19,000 million litres (ML) which is equivalent to 10.44 million litres of oil (MTOE) followed by China with 1,840 ML with 1.01 MTOE which is followed by Canada of 1,000 ML with 0.55 MTOE and USA ranked first with 26,500 ML and 14.55 MTOE[35]. Bioethanol comprises of oxygen (35%) which influence complete combustion of fuel and imparts reduced particulate emission [36,37] and even 10% ethanol blends GHG emission to 12.19% when compared to fossil fuel source [38] and 85% ethanol as fuel reduces nitrogen oxide to 10%, particulate to 20% and sulphate emission to 80%, respectively compared to conventional gasoline. The out/input ratio of various biomass is tabulated in Table 1.

Table 1 : Out/input ratio of various biomasses

Crop Biomass	Output / input ratio	References
Corn	6.9 - 9.5	39
Alfalfa corn	2.9-3.1	40
Switchgrass	10.8-11.3	40
Sugar beet	1.8	41
Winter cereals	2.5-2.8	42

1.2 BIOFUEL AS ALTERNATIVE ENERGY SOURCE

Around 16 distilleries in Pakistan are functioning effectively and produce 506.33 Million Litres (ML) of alcohol from 1.687 Million Tonnes of molasses[43]. It is also reported that Pakistan is exporting ethanol to European Union around 141.3 ML in 2004, 212.16 in 2006 etc[44,45]. In Brazil 25% of sugarcane production is utilized for 25% butanol and 50% of ethanol production[47]. The second generation biobutanol has more advantages that the photochemical oxidation was reduced from 30% to 20%[48] and this has proved that bioethanol has been considered eco friendly fuel in Brazil[28]. More recently, Brazil started concentrating on corn fermentation with yeast cells and proved that the process was faster (34-36h) when compared to the traditional process (45-60h)[49,50]. Dry weight of sugarcane bagasse contain 60% of lignocellulosic and cellulose sources which are accountable for ethanol production by acid or enzymatic hydrolysis[51]. Researchers still have technological challenges to overcome the pretreatment process, use of suitable enzymes, improve the efficiency of bagasse hydrolysis etc. for production of highly efficient second generation ethanol and butanol[52,53]. Hemicellulosic has been classified as micro and cellulose as macro fibrils[54] and lignin provides the structural role of the matrix[55] and these are demonstrated to be the major constituents for biofuel production by enzymatic hydrolysis[56]. First generation ethanol was produced basically from sugarcane and corn worldwide and production was 25 billion gallons where Brazil and the US contributed 85% globally[57]. In the second generation of biofuels low valued lignocellulosic materials from forestry, agriculture were used for production wherein improved pretreatment, fermentation processes were subjected to meet the challenges in 20th century[58]. The application of thermostable enzymes was used to achieve ethanol production of 40-50%[59] assisting liquefaction process. Characterization of bioethanol production were performed enzymatically using many acids of different concentrations (eg. Sulphuric, hydrochloric, hydrofluoric, formic phosphoric and nitric acids)[60] and with common catalyst[61] and varied temperature [62]. Three best fermentation process for ethanol production from lignocellulosic sources are Separate Hydrolysis and Fermentation (SHF), Saccharification and Fermentation (SF) and Simultaneous Saccharification and fermentation (SSCF) etc[63,64]and the microbial strains plays an important role for efficient production[65] and it is proved *Saccharomyces cereviae* as ultimate choice of strain[66] and *Scheffersomycesshehatae* (*Candida shehatae*) is also proved to be promising in ethanol production[66].

1.3 ADVANTAGES OF SECOND GENERATION BIOFUELS

The biofuels obtained from sugar and vegetables oils are classified under first generation biofuels. Whereas, the second generation biofuels are obtained from cellulose, hemicelluloses or lignin sources. The advantages of second generation biofuels are

- Less greenhouse gas emission (upto 86%)
- Fully biodegradable
- Environmentally friendly
- It can be directly supported or blended with petrol in some proportion (ethanol)
- Lower energy density
- Enhance and safeguard energy security[67]

The second generation technology includes processes like thermochemical, gasification, pyrolysis, torrefaction, hydrothermal liquefraction[68,69](Table 2).

Table 2: Second generation technologies

Process	Temperature & Pressure	Products
Thermochemical routes	150-3740C	Hydrogen, carbon monoxide, carbon dioxide, methane
Gasification	>700^{0}C	Diesel, biomethanol, gasoline
Pyrolysis	430^{0}C under pressure	Bio oil
Terrefaction	200-320^{0}C	Bio oil
Hydrothermal Liquefaction	400^{0}C and higher than atmospheric pressure	Bio oil, bio crude

1.4 CURRENT STRATEGIC PRODUCTION OF BIOFUEL

Biofuels obtain energy by the process of biological carbon fixation. United States leading the top most producer is biofuels (1,557 petajoules/year) where, they have gradually increased from 187 thousand barrels per day in 2000 to 1.8 million barrels per day in 2019. A statistic representation of leading countries was published by Ian Tiseo, September 2, 2020 is represented in the Table 3.[70]

Table 3: Biofuel production in developed countries

Countries	Biofuel production (Petajoules)
United States	1557.1
Brazil	992.2
Indonesia	275.5
Germany	143.4
France	113
China	111.3
Argentina	102.8
Thailand	95.6
Netherlands	79.2
Spain	66.7

The largest consumers of biofuels in US are National Army (Vehicles are fueled using 10% ethanol). US mainly uses corn (5.55 billion bushels in 2018) and soybeans for biodiesel production and according to Energy Information Administration (EIA) US produced 16.061 billion gallons of ethanol.Brazil which ranks seconduses sugarcane and soybeans and the production 30.755 billion litres of ethanol in 2018 which was 9% more than 2017. Germany produced 75.8 thousand barrels/day in 2018 (2.9% of global biofuel production in 2018). ADM Olmuhle Hamburg (American group) is one of the major producer of biodiesel in Germany. Argentina leads the fourth global production (70.6 thousand barrels / day in 2018) which accunts for 2017% of world's production. There are 19 bioethanol plants of 1.4 billion litres/year production and sugarcane is the raw material in all these plants. Gela bio refinery in Europe which Was launched in August 2019 utizies second generation raw materials and produced 7,50,000 tonnes annually of biofuel (70% of SO_2, CO_2, dust are reduced).

1.5 ALCOHOLIC FUELS

Aliphatic alcohols (CnH2n+1 OH)such as methanol, ethanol, proponal and butanol are used as fuel for internal combustion engines. The advantage of alcoholic fuels recorded is that they all have High Octane Rating which tends to increase their fuel efficiency. These alcoholic fuels are derived from fossil fuel and biomass and their Research Octane Number (RON) and Motor Octane Number (MON) and their energy density are tabulated in Table 4[71].

Table: 4 Alcoholic fuel characteristics

Fuel	Energy density (megajoules / Liter)	Research Octane Number (RON)	Motor Octane Number (MON)
Butanol	-30	96	78
Ethanol	-20	109	90
Proponal	-24	108	118
Methanol	-16	109	89

Alcoholic fuels have the potential to reduce NOx (25-32%), CO (12-24%) and MC (20-22%) due to lower carbon to hydrogen ratio and improved engine efficiency. Propane and butanol are less toxic and less volatile than methanol. The cellulosic fermentation with *Clostridium acetobulicum* processes produces proponal and butanol with extremely unpleasant smell. Swiss company (Butaico GmbH) adopts modified yeast in the production of butanol and on combustion they give out CO_2, water and heat. Ethanol has been used as rocket fuel and even in lightweight rocket powered racing aircraft and the energy content of ethanol is compared with other fuels is tabulated in Table 5[72] and schematic representation of biofuel production in Fig.1[73]

Table 5: Comparison efficiency of ethanol

Fuel	Energy content (MJ/L)	Research Octane Number (RON)
Methanol	17.9	109
Ethanol	21.2	109
E85(85% ethanol + Gasoline 15%)	25.2	105
LPG	25.3	
Aviation gasoline	33.5	100
Gasohol	33.7	93
Regular gasoline	34.8	91
Diesel	38.5	25

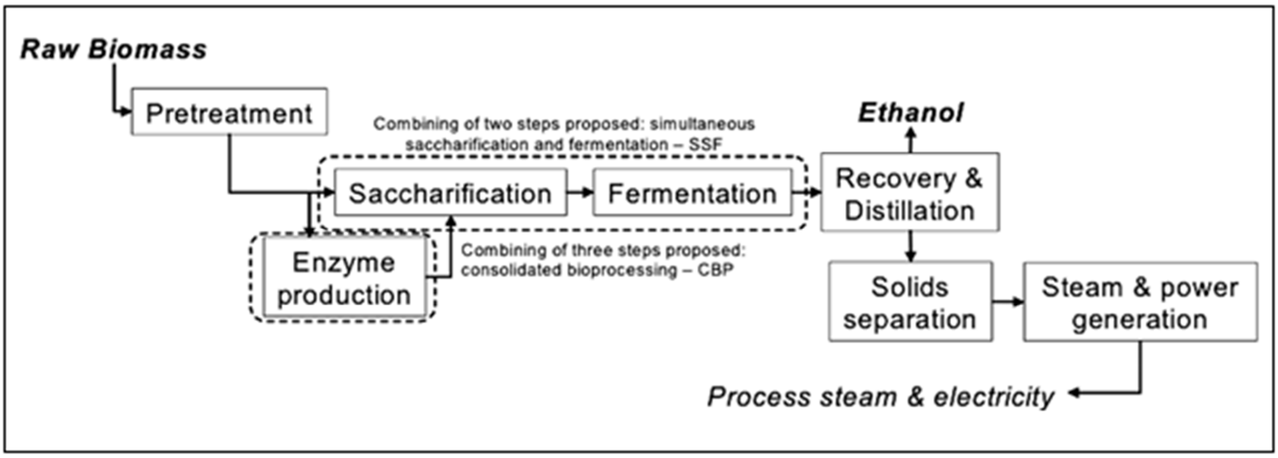

Fig 1: Schematic representation of ethanol production

1.6 ADVANCED BIOFUEL PRODUCTION THROUGH METABOLIC ENGINEERING

The demand in biofuel usage has raised the diversity of microorganisms in strain optimization in production of biofuel at industrial sectors in large scale. The engineered microorganisms (*E.coli, S. cerevisae and Yarrowia lipolytica)* are more effective in the production of advanced biofuel from lignocellulosic biomass[74]. In recent years isobutanol and n butanol has become more popular because of its low water solubility, anti knock properties and energy densities[75]. Clostridium strains are proven to produce butanol through acetone-butanol-ethanol (ABE) fermentation[76,77]. Engineerded *E.coli* has greater commercial implementation in which isobutanol pathway is altered to anaerobic conditions and secondly the cofactor preference for NADH over NADPH in ilvc and adhA[78,79]. E coli in another engineered method proved to increase the yield of isobutanol by eccretion of ammonia and deleting both gdhA and glnA gene (used in nitrogen assimilation)in Erlich-like pyruvate pathway. Six fold improvement of n butanol production (120 mg/L) was observed in heterologous expression of cytosolic pdh gene (inhibits ethanol and glycerol formation)using *S.cerevisiae*[80] and 242.8 mg/L of n butanol titers was reported in keto acid pathway by double deletion of ilv2 and adh1 by directing cytosoli c pyruvate to mitochondrial threnine metabolism[82]. Two distinct pathways such as isopentenyl diphosphate (IPP) and dimethyl allyl diphosphate (DMAPP). And proved to be the precursors of isoprenoid fuels serving more effectively in jet engines[83]. E.coli strains proved to efficient in the isoprenoid derived branched C_5 alcohol production by overexpression of mudF gene and alleviating the rate limiting step of dophosphate hydrolysis[84]. The purity of the product was improved whrn the pathway overexpressed the absence of idi gene in IPP to DMAPP[85]. Another proven evidence is that in optimization of MVA pathway, when multiple – omicsdata analysis, coupled with ribosome binding site (optimization for onudB) increased the production to 2.2g/L with 1% glucose substratum[86.87]. Convential biodiesels were produced from plant oils and animal fats through fatty acid ethyl esters (FAEE) and fatty acid methyl esters (FAME) via trans estification of lipids. Fatty acid biosynthesis are more advantangeous for its purities, improving performance etc. *E.coli, S.cerevisiae and Yarrowia lipolytica* (Oleaginous yeast) proved to be very efficient in fatty acid production of convential biodiesel and petroleum based diesel[88]. High yield of production was achieved by altering DNA binding affinity (response to acyl CoA) or by implementation of malonyl CoA (FapR) or supply of acetyl CoA and consumption of malonyl-CoA/ACP[89] or by linking upregulated FadfR or bypass ATP-dependent activation of acetyl CoA (C_2)[90] or optimized lipoylation pathway[91] .

2. *CONCLUSION*

Fossil fuels are limited and there may be a demand for coal, oil and natural gas and therefore the biofuels can work as an alternative form of fuel and reduce the pollution rate in the future. Biofuels are produced locally and therefore many countries have reduced their dependence on fossil fuels. Around more than 84% of the world's petroleum is utilized in the US and demand for transportation fuel is dramatically increasing day by day. The current scenario in the improvement in pretreatment, efficacy of enzymatic digestion, fermentation processes using efficient new strains will definitely increase the production of biofuel and strategic performance of eco friendly environment. Collective efforts in strain development, optimization process, novel biological pathways production rate and yield etc. will now be capable of developing economically feasible high yielding and efficient biofuels in industrial sectors. Apparent direction of recent advancement in metabolic engineering titers of these biofuel production requires significant improvement before commercial scale production.

3. AUTHORS CONTRIBUTION STATEMENT

Dr.R.Devika- Writing and compilation, M Subathra- Typing of manuscript, Dr. Madusudhan and others- Collection of articles.

4. CONFLICT OF INTEREST

Conflict of interest declared none.

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