



ADVANCEMENTS AND CHALLENGES IN BIOFUELS PRODUCTION: A COMPREHENSIVE REVIEW

Author: #Ramendra Singh Verma, Student, Department of Biotechnology, RR Institute of Modern Technology (AKTU), Lucknow, Uttar Pradesh, India,

#Aashutosh Sharma, Student, Department of Biotechnology, RR Institute of Modern Technology (AKTU), Lucknow, Uttar Pradesh, India,

#Govind, Student, Department of Biotechnology, RR Institute of Modern Technology (AKTU), Lucknow, Uttar Pradesh, India,

***Sumit Pandey, Assistant Professor, Department of Biotechnology, RR Institute of Modern Technology (AKTU), Lucknow, Uttar Pradesh, India,**

***Dr. Dheerendra Kumar, Assistant Professor, Department of Biotechnology, RR Institute of Modern Technology (AKTU), Lucknow, Uttar Pradesh, India,**

#All Authors contributed equally

Abstract:

It is increasingly clear that biofuels can be a viable source of renewable energy in contrast to the finite nature, geopolitical instability, and deleterious global effects of fossil fuel energy. Collectively, biofuels include any energy-enriched chemicals generated directly through the biological processes or derived from the chemical conversion from biomass of prior living organisms. Predominantly, biofuels are produced from photosynthetic organisms such as photosynthetic bacteria, micro- and macro-algae and vascular land plants. The primary products of biofuel may be in a gas, liquid, or solid form. These products can be further converted by biochemical, physical, and thermochemical methods. Biofuels can be classified into two categories: primary and secondary biofuels. The primary biofuels are directly produced from burning woody or cellulosic plant material and dry animal waste. The secondary biofuels can be classified into three generations that are each indirectly generated from plant and animal material. The first generation of biofuels is ethanol derived from food crops rich in starch or biodiesel taken from waste animal fats such as cooking grease. The second generation is bioethanol derived from non-food cellulosic biomass and biodiesel taken from oil-rich plant seed such as soybean or jatropha. The third generation is the biofuels generated from cyanobacterial, microalgae and other microbes, which is the most promising approach to meet the global energy demands. In this review, we present the recent progresses including challenges and opportunities in microbial biofuels production as well as the potential applications of microalgae as a platform of biomass production. Future research endeavors in biofuel production should be placed on the search of novel biofuel production species, optimization and improvement of culture conditions, genetic engineering of biofuel-producing species, complete understanding of the biofuel production mechanisms, and effective techniques for mass cultivation of microorganisms. The cost-effective production of biofuels from renewable materials will begin to address energy security and climate change concerns. Ethanol, naturally produced by microorganisms, is currently the major biofuel in the transportation sector. However, its low energy content and incompatibility with existing fuel distribution and storage infrastructure limits its economic use in the future. Advanced biofuels, such as long chain alcohols and isoprenoid- and fatty acid-based biofuels, have physical properties that more closely resemble petroleum-derived fuels, and as such are an attractive alternative for the future supplementation or replacement of petroleum-derived fuels. Here, we review recent developments in the engineering of metabolic pathways for the production of known and potential advanced biofuels by microorganisms. We concentrate on the metabolic engineering of genetically tractable organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* for the production of these advanced biofuel.

Keywords: *Biofuel, Bioenergy, Alternative Energy Source, Biomass, Biochemical Process.*

I. INTRODUCTION OF BIOFUEL

Presently, three major issues are in front of human beings: hunger, the lack of energy and the deterioration of the environment (Popp et al. 2014). It is obligatory to fight with all the three vehemence simultaneously, because any one of these is capable to extinct our civilization (Escobar et al. 2009). The ease of access to energy is the basic driving force behind the socio-economic progress and the

vital element to sustain human's current elevated standard of living (Walker et al. 2016; Arshad and Ahmed 2016). Globally consumption of energy has been almost doubled up in recent times (Bentley 2016) and fossil fuels share more than 80% (Pfenninger and Keirstead 2015). When we talk about energy, it is clear to each and every person that its saving is the best attitude to be privileged by minimizing irrational use and enhancing the utilization efficiency (Abdmouleh et al. 2015) as fossil fuel reservoirs are depleting fast (Hook et al. 2014). Up till now human's energy requirements has been met by the fossil fuels (coal; oil; gas) since many decades[1]. Alternative cheap and environment friendly energy is the hot issue in today's world. Fossil fuels account for over 80.3% of the primary energy consumed in the world, and 57.7% of that amount is used in the transport sector (Escobar et al. 2009). Burning of conventional fuels results in the harmful emissions of greenhouse gases such as carbon dioxide (CO₂), nitrogen oxide (NO_x), volatile organic compounds (VOC) and hydrocarbons (HC); incremental for the climate changes (Chavez-Baeza and Sheinbaum-Pardo 2014; Friedlingstein et al. 2014; Reuter et al. 2014). Although such fuels give the best cost/benefit ratio; but at the same damage the environment. Fossil based diesel is an essential fuel for running vehicles, power plants and motor engines in the transportation, agricultural and industrial sectors (Emanuel and Gomes 2014; Orsi et al. 2016) and remained the most merchandising commodity among primary products trade in 2010 (Janaun and Ellis 2010). Transportation sector spent more than 30% of the energy supply globally, in which above 80% is by the road transport (Holmberg and Erdemir 2015). Worldwide almost 60% oil supply is consumed by this sector (Bilgen 2014), practically operating on gasoline, diesel oil almost 97.6%, with a small amount from liquid natural gas (Ramadhas et al. 2004; Murphy et al. 2013)[2].

Now the world has been challenged by global warming problem (IPCC 2014). The release of Carbon dioxide from the combustion of fossil fuels, the key contributor to the process has generated the interest in promoting biofuel as one of the leading renewable energy sources (Kumar et al. 2013). Table 1.1 shows the major benefits of the biofuel[5]. The sustainable production of biofuel is a valuable tool in stemming climate change (Creutzig et al. 2015), boosting local economies, particularly in lesser-developed parts of the world (van Eijck et al. 2014a; van Eijck et al. 2014b), and enhancing energy security for all (Jatrofuels 2012; Hughes et al. 2014)[8]. Advancement in renewable biofuel sources; cling to solution key of the dual difficulties, running down the fossil fuel reservoirs and environmental pollution (Smith 2013). Therefore, exploration of novel, renewable, environment friendly, clean, reliable and economically feasible energy resources is serious requisite of the day (Dale et al. 2014). The discharge of greenhouse gases through the burning of fossil fuels in transport sector alters the natural equilibrium of environment[7]. The world has now started to realize the problem and syndromes created by conventional fuels (Karwat et al. 2014). To minimize the fossil fuels role, the exploration of renewable substitutes, the biofuel like bioethanol, biogas and biodiesel are on rise (Ho et al. 2014). Biofuel, biodiesel, biogas and bioethanol are currently available in the market, already being used for various types of engines (Prasad et al. 2007; Demirbas 2008; Janaun and Ellis 2010; Shahid and Jamal 2011; Geczi et al. 2015; Malakhova et al. 2015; Choudhary et al. 2017)[10].

II.IMPORTANCE OF BIOFUELS IN SUSTAINABILITY ENERGY

Worldwide transportation is responsible for 25% of energy demand and nearly 62% of oil demand; generation of electricity involves single largest consumption of fuel in the world. More than 60% of power generated comes from fossil fuels. Fossil fuels were generated after millions of years after the anaerobic decomposition of prehistoric organic matter buried under the ground[12]. Stocks of fossil fuels are limited, due to the fast consumption of the fuel and due to industrialization, stocks of fossil fuels are depleting very fast. Limited availability has become the reason for its high cost in due course of time. Environmental consequences of fossil fuels and concerns about exhausting petroleum supplies, high oil prices and the need to mitigate the greenhouse effect have spurred the search for renewable transportation biofuels. Biofuels have emerged as an important alternative fuel on world stage. It belongs to renewable category of fuels[10]. The biofuels have environmental benefits, are economically competitive, and can be produced in large quantities. Biofuels are defined as any hydrocarbon fuel which are obtained from organic matter in a short span of time such as days, weeks or even months from contemporary biological processes like agriculture aerobic digestion, unlike fossil fuels which are obtained by geological process after millions of years. Biofuels may exist in solid, liquid, or gas state. Wood, charcoal, and waste bagasse are solid; ethanol, methanol, propanol, butanol and plants oil are liquid; methane gas and syngas are examples of gaseous biofuels. The production of biofuels requires energy to grow crops and convert them to biofuels. Subsequently, there is increasing interest in the use and production of fuels obtained from plants or organic waste. Biofuels are biomass materials that are directly used as solid fuel or transformed into gaseous or liquid fuels which can be easily stored, so that the harnessed energy can be released through combustion when needed. Biofuels may simply be perceived as liquid or gas transportation fuels derived from biomass. Various biomass raw materials such as energy crops, agricultural residues or forest products can be used to produce biofuels. There is thus a boom in biofuel industries in many developing countries. As the demand for biofuels increases, they have enormous biomass resources which are becoming more valuable. Bioenergy is obtained from these fuels due to the carbon present in them which are generated by carbon fixation process. In this process, atmospheric carbon dioxide is converted into the organic compounds which are found in living organism. Different compounds are produced through this process such as protein, fat and sugar. Any of these may act as source of energy in biofuels. Biofuels are obtained from different sources such as starch feedstock, cellulose feedstock like food crops, wood, sawdust, grasses, waste from domestic and industrial processes, organic part of municipal waste, animal fat and vegetable oil of rapeseed, mustard, soybean, sunflower, palm, jatropha plant, dried manure, waste from farming, animal husbandry, forestry, algae, fungi and different microbes. Increasing interest has been dedicated to bioconversion of biomass into fuel ethanol, which is considered as an alternative to fossil fuels and the cleanest liquid fuel (Li et al. 2009). Biofuel, on the other hand, is derived from biomass, which can be produced eventually year after year via sustainable farming practices. This indicates biomass and biofuel are renewable. Since last three decades, there has been acceleration in the research on improving production of biofuels for both ecological and economical reasons [18], [19], [22]. The recuperation is primarily for its use as an alternative to petroleum-based fuels. Despite the fact that biofuel can be synthesized by chemical processes such as reacting ethylene with steam (Anuj et al. 2007), biofuel is a renewable energy source produced mostly by the sugar fermentation process (Oyeleke and Jibrin 2009). Biofuels may be considered as hope of energy security

that stands as an alternative fuel. They are responsible for providing 2.7% of world fuels which is used for road transport. In 2010, biofuel production was 105 billion litres which was approximately 17% more in comparison to 2009 production level. International energy agency has set the target of 25% of fuels used in transport to be obtained from biofuels by the year 2050 to minimize the dependence on petroleum and coal. In 2011, there was provision of blending of biofuel in 31 countries and 29 states. Bioethanol and biodiesel have maximum consumption in the world [34], [27]. In 2010, the overall world production of bioethanol was 86 billion litres with USA and Brazil as the topmost bioethanol producing countries. Both of these countries generate 90% of bioethanol of the world. Brazil tops the list when we compare the consumption of biofuels in the world; it has a provision of 95% blending of ethanol with gasoline. In other countries, blending with ethanol are found between 10 and 15%, usually blending has to be decided by the performance of engine and exhaust emission with respect to engine load and speed. USA and Australia have provision of 10% ethanol blending in gasoline. India has approved the 5% blending of biofuels in petrol. Ethanol fuel blends are extensively sold in USA. The most common blend comprises of 10% ethanol and 90% petrol (E10). To run on E10 the vehicle engine require no modification and vehicle warranties are also not affected. Only flexible fuel vehicles can run on E85 which has up to 85% ethanol and 15% petrol blends (Tanaka 2006). European Union is the largest producer of biodiesel which accounts for nearly 53% of world production in the year 2010. In France, 8% biodiesel blending can be used in all vehicles using diesel as fuel. Similarly Russia holds 22% of world forest and it is the world leader in the supply of biomass. According to the latest estimate of Grand View Research, it is expected that global market of enzyme may reach \$17.5 billion by the year 2024 by the exponential demands of bioethanol (enzymes are used in synthesis) emerging from many developing countries. Extensive research is also being carried out in India on biofuel. Some experts are of the view that vegetable oil blending of linseed, neem and mahua can be tried in diesel engine in different proportion. The rise in the use of biofuels is inevitable. However, biofuel production is questioned from a number of angles. The concern arises on the implications of its emissions, and increased prices of corn that is used for biofuel production, with a particular implication on food security (Wikipedia 2008). Biofuels may in days to come offer a viable substitutes, but “the implications of the use of biofuels for global security as well as for economic, environmental, and public health need to be further evaluated” (EEA 2006)[10].

III. FEEDSTOCK SELECTION AND AVAILABILITY

Biochars are carbon (C) rich materials typically produced via biomass pyrolysis at relatively low temperatures (300–700 °C) under limited oxygen conditions (Lehmann and Joseph 2009). Biomass, the feedstock for biochar creation, is typically derived from agricultural and forestry waste products, municipal waste, green and food waste. The creation of biochar from these products places C into a recalcitrant form which could last hundreds to over thousands of years (Spokas 2010; Kuzyakov et al. 2014; Wang et al. 2016), suggesting that biochar could aid in climate change mitigation (Tripathi et al. 2016) as one of the few negative greenhouse gas emission technologies with sustainable development co-benefits (Smith et al. 2019). Over shorter time scales (e.g., one to several years), biochars have been proven to improve environmental quality by sorbing heavy metals and organic contaminants (e.g., Sigua et al. 2019; Cui et al. 2019; Novak et al. 2019a), positively affect soil water relations (e.g., Lentz et al. 2019; Kammann et al. [19] 2011), reduce greenhouse gas emissions (e.g., Fuertes-Mendizábal et al. 2019; Borchard et al. 2018; Jeffery et al. 2016), and improve crop growth (e.g., Laird et al. 2017; Novak et al. 2016; Liu et al. [13] 2013). Although creation of biochars for the above purposes may seem simply based on feedstock selection, biochar production for environmental improvements is complex. Feedstock selection, pyrolysis temperatures, and pyrolysis types can all greatly influence the final biochar product (Cao et al. 2017; Cha et al. 2016). Thus, increasing the understanding of the interaction between feedstock, pyrolysis temperature, and production technique (i.e., either fast or slow pyrolysis) would help biochar stakeholders make more well-informed choices for its use. In terms of feedstock, understanding how initial feedstock properties influence final biochar characteristics is important. Feedstocks have been shown to play a major role in creating biochars with distinctly different chemical properties (Funke and Ziegler 2010; Novak et al. 2019b). In relative terms, wood-based biochars contain more C and lower plant-available nutrients, manure-based biochars show opposite trends, and grass-based biochars typically fall somewhere in between woody and manure biochars (Ippolito et al. 2015). However, these properties may be altered by pyrolysis temperature and pyrolysis technique used for biochar creation. Pyrolysis temperature and production technique play key roles in creating biochars with various chemical and structural properties [19]. For example, nutrient availability changes drastically as pyrolysis temperature is increased (Clough et al. 2013; Nguyen et al. 2017). Specifically, with increasing pyrolysis temperature one typically observes increasing biochar C, P, K, Ca, ash content, pH, specific surface area (SSA), and decreasing N, H, and O content (e.g., Weber and Quicker 2018; Ippolito et al. 2015). These biochar characteristics are driven by forcing C into more condensed, recalcitrant forms, the creation of oxide/carbonate mineral phases (e.g., P, K, Ca) leading to greater ash content and higher pH, and the loss of N, H, and O via volatilization. Volatilization losses further concentrate other remaining elements (Kim et al. 2012; Kinney et al. 2012). When choosing pyrolysis type, in general slow pyrolysis tends to produce biochars with greater N, S, available P, Ca, Mg, surface area, and cation exchange capacity (CEC) as compared to fast pyrolysis. The above aspects of biochar creation have led individual researchers to pay attention to biochar end-product properties. Current literature contains an untapped wealth of information regarding feedstock, pyrolysis type and temperature choices. However, there is some uncertainty in the literature with a vague description of how biochar characteristics are influenced by feedstock choice, pyrolysis type, and temperature employed. Thus, we chose to provide in this review a clearer picture by synthesizing existing literature to create a true comprehensive review of biochar properties based on feedstock choice, pyrolysis temperature, and pyrolysis type. Presenting this type of data is paramount for improving our understanding of the factors used to create biochar and characteristics within the final product. Establishing these comparisons should aid biochar practitioners and stakeholders in making well-informed decisions for biochar use as amendments in soils and for environmental mitigation purposes in mine spoils or metal contaminated soils. The objective of this work was focused on comprehensively reviewing how different feedstocks, pyrolysis temperatures, and production techniques affect biochar physicochemical properties, total and available nutrient content, other characteristics, and what these properties indicate when biochar is used for agricultural benefits [10] [28] [32] [40] [33].

IV. RECENT DEVELOPMENTS ON INTEGRATED BIOCHEMICAL PROCESS

In recent years, the dairy industry has seen tremendous growth with the development of innovative technologies to increase the productivity of milk and milk-based products. The development of the dairy industry has led to the generation of a huge amount of waste. As compared to 2017, a total of 2.2% increase in global milk production accounting for 843 million tonnes and a 2.9% increment in dairy products in 2018 accounting for 75 million tonnes was reported (FAO, 2019). It has been estimated that 6–10 L of wastewater is generated for the processing of one liter of dairy milk (Gramegna et al., 2020). The dairy wastewaters should be managed properly for the safety of the environment and human beings. The pollution control board assigns discharge standards for effluent treatment in the industries (Sivaprakasam and Balaji, 2021). Therefore, industries should follow the norms before their discharge to the water bodies. Although, processes such as physical and chemical are available for the treatment of wastewater originating from the dairy effluents (Vialkova et al., 2019, Qin et al., 2021a). In dairy wastewater, various colloidal particles are present. These colloidal particles can be removed using several traditional methods, such as ion exchange, flocculation, flotation, coagulation, membrane filtration, and solvent extraction[38]. Of these methods, flocculation and coagulation are considered as the most suitable methods, which involve the addition of synthetic coagulants including fava bean, chitosan, common bean and *Moringa oleifera* (Tripathi et al., 2021). Membrane separation can be used for the recycling of wastewater (Pasotti et al., 2020). But in membrane separation, the proteinaceous materials stick to the membranes producing a foul smell[33]. A solution to this problem could be the use of absorbents and coagulants before membrane separation. Recent technologies such as reverse osmosis and nanofiltration could be effective in recovering protein, lactose, and water reusability. As compared to conventional technologies, these new technologies are stable, flexible, clean, and energy-saving (Roufou et al., 2021a, Roufou et al., 2021b) [26]. Dairy sludge is also a major component of dairy wastes. In the treatment of sludge, anaerobic digestion is considered an effective method for the treatment. Anaerobic digestion takes place in four steps such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Adghim et al., 2020, Liu et al., 2021a). Sludge stabilization is performed by sludge disintegration in anaerobic digestion (Zhang et al., 2021). In addition to the anaerobic digestion, various integration technologies have emerged for the treatment of dairy wastes to produce value-added products[27]. A new approach known as bio-electrochemical systems combines electrochemical and biological strategies to produce useful products such as hydrogen (Adesra et al., 2021). These integrated technologies can be classified as microbial fuel cells, enzymatic biofuel cells, microbial desalination, microbial electrosynthesis, and microbial solar cells. Dairy waste can be used to produce various value-added products. It can be used to produce bioplastics (Tripathi et al., 2021), biodiesel (Kavitha et al., 2019), bioenergy (Asunis et al., 2020a), biochemical (Asunis et al., 2020b), ethanol (Zheng et al., 2022), biofuel (Choudhary et al., 2021), functional beverages (Lawton et al., 2021), biohydrogen (Ayil-Gutiérrez et al., 2021), biocatalyst (Bhusari et al., 2021), organic fertilizer (Alharbi et al., 2021), single cell protein (Gaur et al., 2017), microbial fuel cells (Ahmad et al., 2019), and organic acids (Marcus et al., 2021, Qin et al., 2021b)[15][18][19]. Of the various useful products, polyhydroxyalkanoates (PHAs) are very popular and can be produced utilizing dairy waste such as milk whey (Colombo et al., 2019). Milk whey contains proteins, fats, and lactose. Therefore, it can be fermented to produce PHAs (Tripathi et al., 2021). Moreover, the added advantage is that milk whey does not require any pretreatment steps like enzymatic or chemical hydrolysis. In this context, a study used bacterium *Bacillus megaterium* strain Ti3 for PHAs production utilizing dairy whey (Israni et al., 2020). The study resulted in high yield and productivity. Another study worked on the cheese whey and used anaerobic fermentation to produce carboxylic acid followed by PHAs production (Domingos et al., 2018). Dairy waste can also be used for the production of biodiesel (Kavitha et al., 2019). For the production, dairy waste scum is obtained from dairy industries. The process involved the use of CaO obtained from eggshells for transesterification of dairy scum. In a study, cheese whey powder was used to produce D-lactic acid by using the *Lactobacillus bulgaricus* CGMCC 1.6970 (Liu et al., 2018). Concentrated whey can be used for the production of chalcone 3-hydroxylase and flavanone 3-hydroxylase (Hausjell et al., 2019, Awasthi et al., 2021a) [23], [26], [19].

V. CURRENT SITUATION OF BIOFUEL PRODUCTION AND ITS ENERGY

There is a huge demand of fuel due to its consumption in transportation, energy generation and industrial purposes. Recently, the demand for petroleum-based fuel has resulted in a number of economic and environmental concerns and attentive efforts are needed to encourage the emerging alternative fuels (Gowen and Fong, 2011). Biofuels, produced from biomass, render an environmentally clement and cost-efficient solution for fossil fuel impoverishment. These substitutive and inexhaustible sources of fuel as biodiesel and bioethanol have captivated increasing attention from industry, decision-makers, and scientists because of their valuable advantages (Xing et al., 2012). The narrative about the biofuels as a prospective alternative to petroleum-based transportation fuels have been growing during the past few years (Gowen and Fong, 2011; Stephanopoulos, 2007). Specifically, the production of ethanol and butanol are mainly based on sugar or starch feedstock fermentation, whereas the biodiesel is being yielded by trans-esterification of lipids obtained from soya beans, canola seeds and other crops (Munasinghe and Khanal, 2010). The cost-effective and inexhaustible raw materials such as lignocellulosic feedstock derived from agricultural wastes (such as sugar cane bagass, sugar beet or corn stalks) and energy crops (such as poplar or switchgrass) are used to manufacture biofuels adding an advantage of not severely affecting food supplies (Bajpai, 2017; Kovačić et al., 2017). However, various investigative and technology related obstacles are involved in the deployment of lignocellulosic biomass as a raw material for the biofuel industry. Many microbial strains are well-known for fermentation potential yielding biofuels. Among yeasts, *Saccharomyces cerevisiae* is one of the most widely utilized microbes for fermentation of monomeric sugars for ethanol production on industrial scale. The bacterial species employed for fermentation purpose include *Zymomonas mobilis*, *Clostridium thermosaccharolyticum*, *C. thermohydrosulfuricum*, *Thermoanaerobacter mathranii*, *T. Brockii* and *T. ethanolicus*[29][32][34][37]. The site-specific genome editing is a leading domain of genomics that seems to be helpful in the improvement of microbial strains for biofuel production. The genetic engineering is a widely used method for site-specific modifications in the genome for the manipulation of a particular feature in the indigenous microorganisms including knocking-down, knocking-out, and knocking-in the genes. This contradicts the conventional genetic engineering where the gene to be manipulated is first singled out and then manipulated in vitro and added back to the host, or a heterologous gene is inserted by genetic transformation

methods to change a particular feature of the organism (Garneau et al., 2010; Wiedenheft et al., 2012). The RNA-guided endonuclease-mediated (REM) and modified endonuclease-mediated (MEM) site-specific genome editing methods have been recently employed for strain improvement. CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-associated nuclease 9), a natural bacterial defense system, is a characteristic example of REM based genetic engineering method and multifaceted tool for genetic engineering that employ a guide RNA (gRNA) to point Cas9 to a particular sequence. This facile RNA-guided genome-engineering technology has been regarded as a groundbreaking tool in biology and has multiple inventive uses in biofuel manufacturing (Xu et al., 2016). The CRISPR/Cas9 technology has proven to be a novel and smart tool in industrial research and have been employed to engineer the genomes of various microorganisms such as bacteria, yeast, filamentous fungi, and algae (DiCarlo et al., 2013; Fang et al., 2017; Jiang et al., 2013; Wang et al., 2016). The instigators of CRISPR/Cas9 have modified this tool into an adaptable and robust approach for genetic engineering. This review summarizes generations of biofuels, processes and microbes involved in biofuel production with special emphasis on discoveries of CRISPR/Cas9-mediated genome editing in microorganisms in order to link these discoveries for improvement of biofuels production. Biofuel sources and its generations. Biofuels are liquefied fuels that are generated from the different biological materials such as animal matter and waste plants (Azad et al., 2015; Rodionova et al., 2017) [36]. These are mainly categorized into two types like primary and secondary biofuels. The primary biofuels generally used in their unrefined form for cooking, heating, and production of electricity. Some of the examples of primary biofuels are fuel-wood, pellets, wood chips, crop remains, and landfill gas (Rodionova et al., 2017). Secondary biofuels are refined form of primary biofuels, which are generated either in the form of solids (e.g. charcoal), liquids (e.g. biodiesel, bioethanol, and bio-oil), or gases (e.g. biogas, and hydrogen). The secondary biofuels that are used in vehicles and numerous industrial processes include biodiesel, bioethanol, and biogas. These are manufactured by the biological processing of biomass (Doshi et al., 2016).

VI. GENERATION OF BIOFUELS

Based on the raw materials and biological processes that are employed for their manufacturing, secondary biofuels are further classified into four generations as shown below:

i. First generation biofuels:

Starch (from potato, barley, wheat and corn) or sugars (from sugar-beet and sugarcane) are usually fermented to manufacture first-generation biofuels such as bioethanol and butanol. Where, bioethanol is regarded as the most eminent biofuel of the first generation which is generated by fermenting carbohydrates distilled from crop plants (Naik et al., 2010). Crop plants with a high concentration of carbohydrates are fermented with the help of enzymes synthesized by *S. cerevisiae* to get bioethanol. The *S. cerevisiae* work on six-carbon sugars (usually glucose) to produce bioethanol (Nigam and Singh, 2011). Another highly efficient first generation biofuel is biodiesel which is manufactured by trans-esterification or cracking of straight vegetable oils of sebacious plants (e.g. sunflower, palm, rapeseed, soybeans and coconut etc.), commonly used for cooking purposes (Rodionova et al., 2017). They offer multiple advantages especially to underdeveloped countries by considering the fact that lower production costs of biofuels enable low-paid societies to have access to inexhaustible forms of transport fuels (Demirbas, 2011). Substantial economic and environmental constraints are also associated with the first-generation biofuel production systems apart from their various advantages. For example, as the production efficiency expands, their competition with agriculture for cultivable land also increases. This is the most frequent problem associated with the production and use of first-generation biofuels in the last few years (de Vries et al., 2010) [1].

ii. Second generation biofuels:

Novel starch, sugar and fatty crops like jatropha, cassava or miscanthus are used to manufacture second-generation bioethanol and biodiesel with the help of traditional technologies. Biobutanol and syndiesel® are some other popular second generation biofuels which are manufactured from lignocellulosic materials (e.g. straw, wood, and grass) (Rodionova et al., 2017). The use of total above-ground biomass for the manufacture of second-generation biofuel provides a better land use efficiency in comparison to first-generation ones. Moreover, reduced cost of raw material, as well as utilization of inedible lignocellulosic biomass (the woody part of plants), which does not compete with food, lend an advantage to second-generation biofuels (Havlik et al., 2011; Schenk et al., 2008). Non-edible components of corn or sugarcane, forest harvesting residues, agricultural residues, as well as wood processing waste such as leaves, straw or wood chips are used as sources of lignocellulosic material. Nevertheless, the technology which is used to transform lignocellulosic materials into sugars is costly and entails the use of special enzymes. This simply means that manufacturing second-generation biofuels on a commercial scale are not feasible currently (Brennan and Owende, 2010) [5].

iii. Third generation biofuels:

Microalgal biomass is used to generate third generation biofuels. Microalgae are autotrophic life forms of aquatic environment e.g. cyanobacteria (Brennan and Owende, 2010; Lee and Lavoie, 2013). The growth yield of the microalgal biomass in comparison to traditional lignocellulosic biomass is very peculiar (Scott et al., 2010). In contrast to conventional crops, micro-algal biomass gives 15–300 times more oil for biodiesel production. The high energy index, minor cost, eco-friendly and completely inexhaustible feedstock are few of the prodigious benefits that make microalgae an exceptional source of biomass. It mainly reduces the stress on already decreasing water and land resources because of its ability to grow on undeveloped land and water which is not appropriate for food production. The most promising feature which enables algae to be used as third generation biofuel is their excessive oil content. Among all the species of green algae, *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Dunaliella salina* are the most utilized species for obtaining biofuels owing to their high oil content (around 60–70%) (Azad et al., 2014). With multiple advantages, it also has its pitfalls as the third generation biofuel technology is in its infancy. The major drawback is its high estimated cost and reliance on fossil fuels in production steps elevating the environmental concerns (Liang et al., 2009; Scott et al., 2010) [8].

iv. Fourth generation biofuels:

The fourth-generation biofuels are manufactured by making use of modern techniques such as geo-synthesis or low pressure, advanced biochemistry, petroleum-hydro-processing and low-temperature electrochemical processes. These techniques use the captured carbon from the environment to produce fourth generation biofuels (Azad et al., 2015). The different authors have defined the fourth-generation biofuels in diverse ways. For example, Lü et al. (2011) has utilized metabolically engineered forms of microalgae to produce fourth generation biofuel. This concept has been applied to manufacture inexhaustible fourth generation biofuels by using chemical processes (Cheng et al., 2011). While Demirbas (2009) defines fourth generation biofuel as the changeover of biodiesel and vegetable oil into biogasoline by utilizing the advanced technologies. Unlike fourth generation biofuels, the second and third generation biomass feedstocks take up CO₂ while cultivating and transforming it to fuel. Two processes have been cited in the literature for the manufacturing of fourth generation biofuels. In the first method, the carbon that is available by industry emitted CO₂, is captured into the water with the electrochemical process and is used to manufacture liquid methanol. Electricity and heat energy can be manufactured by using the inexhaustible energy sources like wind, solar, geothermal, hydro etc. (Azad et al., 2015). A sequence of electrolytic cracking and catalytic production is performed in these processes which guides to a low pressure and temperature electrochemical manufacturing process. Liquid inexhaustible methanol is a cost-efficient, eco-friendly fuel which can be mixed with biodiesel and aviation spirit. In the second method, old oil and gas fields or saline aquifers are used to geosequester the captured CO₂. This carbon captured in fourth generation biofuel manufacturing locks more carbon than it releases making it carbon negative rather than neutral. It has been reported that this method also decreases CO₂ emissions by replacing fossil fuels by capturing the CO₂ emissions from the environment [7], [4].

VII. TECHNO-ECONOMIC ANALYSIS OF BIOFUEL PRODUCTION

As a renewable substitute for petroleum fuels, biofuels have attracted increasing attention for economic, environmental, and energy security considerations. First-generation biofuels could be relatively easily converted to transportation fuels but lead to food versus fuel dilemma. Cellulosic biofuel feedstock such as corn stover, switchgrass, and woody biomass does not compete with food supply but highly recalcitrant (Carriquiry et al., 2011) [39]. US Environmental Protection Agency (EPA) revised the Renewable Fuel Standard in 2007, which aims to accelerate the domestic biofuel production and consumption. The Revised Renewable Fuel Standard (RFS2) mandates that by the year 2022, at least 16 billion gallons per year of cellulosic biofuels will be produced and consumed in the US (Schnepf, 2011). However, cellulosic biofuel production has been significantly below the blending targets established by the RFS2 due to technical immaturity and feedstock availability issues (Brown, 2015) [33]. Lignocellulosic biomass could be converted into bio-oil via pyrolysis, and the biomass pyrolysis can be followed by bio-oil cracking, gasification, or hydroprocessing to produce transportation fuels (Wang et al., 2013a). The mechanism research shows that fast pyrolysis of cellulose biomass yields to products such as pyrans, furans, and linear small molecular compounds (Wang et al., 2012). The pyrolysis behaviors and structural features are significantly affected by the process conditions (Wang et al., 2015). Researchers also use thermogravimetric analysis coupled to Fourier transform infrared spectroscopy to analysis the evolution of typical pyrolysis products (Wang et al., 2013b). The major challenge faced by the cellulosic biofuel industry is that investors are not willing to take the risk to construct commercial scale facilities, and lack of real facility cost information for the production systems prohibit the improvement of production system to reduce costs and uncertainty (Brown, 2015). Techno-economic analysis (TEA) has been widely adopted to overcome this dilemma. Process models are developed to simulate the production systems at a commercial scale [26]. Materials and energy balances are developed. Cost analysis is then employed to evaluate the economic feasibility of the production system at commercial scale (Zhang et al., 2013a). TEA, as a simulation approach, is highly dependent on the model assumptions, which could lead to significant inaccuracy and even errors. Another major barrier for commercialization of cellulosic biofuels is transporting bulky solid biomass over a long distance. This is mainly caused by the low energy density of lignocellulose biomass and a large collection radius due to the limitation of biomass availability. In general, logistics cost for transport biomass from farmland to biorefinery can make up 50–75% of the feedstock cost (Harland, 1996) and more than 35% of the total production cost of advanced biofuel is feedstock cost (Wooley et al., 1999). TEA studies typically focus on the technical and economic performance for a single facility and neglect the upstream biomass collection and transportation as well as the downstream biofuel transportation and distribution. However, with the importance of supply chain configurations in the economic feasibility evaluation of cellulosic biofuels, TEA should incorporate the supply chain configurations explicitly rather than the simplify assumption of a flat feedstock cost and biofuel price at the facility gate. Recently, researchers have worked on incorporating pre-determined simple supply chain configurations to estimate biomass feedstock cost for integrated pathways (Manganaro and Lawal, 2012, Zhang and Wright, 2014). However, in reality, feedstock availability, logistic cost, and biofuel demands will all affect evaluation of economic feasibility (Li and Hu, 2014). This serves as the major motivation for this proposed approach to incorporate logistic settings into the techno-economic analysis. There has been an increasing body of literature on supply chain network design for the biofuel industry (Li and Hu, 2014, Li et al., 2014, Zhang and Hu, 2013). Design and management of logistic flow includes the raw materials, work-in-process, and finished products from source of raw materials to the point of consumption (Rogers and Brammer, 2009). In order to incorporate supply chain design into TEA study, logistic information such as biomass availability, transportation cost, and demands distributions is necessary. A decision method and optimization model is necessary to determine the optimal facility locations and capacities as well as the logistic flow decisions for biomass supply and biofuel distribution [19], [18], [14].

VIII. ENVIRONMENTAL IMPACT AND SUSTAINABILITY

Currently, the transportation sector accounts for approximately 50% of the global oil consumption and produces approximately 25% of the global energy-related CO₂ emissions [1]. Therefore, improving energy security and decreasing vehicle contributions to greenhouse gas (GHG) emissions and air pollution become primary goals compelling governments to seek alternatives to the petroleum fuels currently dominating transportation. Over the past few decades, several fuel candidates have emerged, such as liquefied petroleum

gas (LPG) [2], [3], [4], [5], compressed natural gas (CNG) [6], [7], [8], [9] and electricity for electric vehicles (EVs) [10], [11], [12], [13], [14], [15]. The above fuel alternatives have some benefits over petroleum, but they also show a number of drawbacks that reduce their ability to capture the market share. For example, they all require significant vehicle modifications and a new fueling infrastructure. As a result, except in a few places, both automakers and fuel suppliers are disinclined to make substantial investments in such an uncertain market. In contrast, biofuels have the potential to overcome the traditional barriers mentioned above. They have been regarded as sustainable options for reducing petroleum-dependence and GHG emissions in the transportation sector. In addition, biofuels could share the existing distribution infrastructure with little modification. In fact, many countries are implementing the use of biofuels. Low-percentage bioethanol blends, such as 10% bioethanol in conventional gasoline (known as E10), are already dispensed in many refueling stations worldwide [16], with a high level of compatibility with materials and equipment. In addition, biodiesel is also currently blended with conventional diesel in many countries, ranging from 5% (BD5) in France to 20% (BD20) in the USA, and is used as a neat fuel (100% biodiesel) by some trucks in Germany [17]. Expanding the use of biofuels would support several major policy objectives:

Energy security: Biofuels can readily replace petroleum fuels and, in many countries, can provide a domestic rather than an imported source of transport fuel. Even if imported, ethanol or biodiesel will likely come from regions other than OPEC (Organization of Petroleum Export Countries), creating a broader global diversification of supply sources of transport fuels. Environment: With lower GHG emissions over the whole fuel chain, biofuels are generally more climate-friendly than petroleum fuels. Either in their neat form or as blends with conventional petroleum fuels, vehicles running on biofuels emit less of some pollutants that exacerbate air quality problems, particularly in urban areas. Reductions in some air pollutants are also achieved by blending biofuels, though some other types of emissions (e.g., NO_x) might be increased this way[38].

Fuel quality: Refiners and automakers have become interested in the benefits of ethanol to boost fuel octane, especially where other potential octane enhancers, such as MTBE, are discouraged or prohibited.

The objective of the present paper is to study the lifecycle performance of biofuel vehicles using simulation tools. A biomass-to-wheels (BTW) analysis is performed to assess the potential benefits in energy savings and GHG-emission reductions for applying biofuels to the transportation sectors[16]. three types of biofuel vehicles, i.e., flexible fuel vehicles (FFVs), diesel vehicles (DVs), and fuel cell vehicles (FCV), are studied to investigate the effects of replacing conventional gasoline vehicles (GVs) on the GHG emission and the total energy consumption. First, the fuel economy is determined by using the simulation tools in MATLAB/Simulink [18]. Then, a comprehensive analysis of fuel-cycle energy consumption and GHG emissions for various biofuels is conducted using the GREET (Greenhouse gases, Regulated Emissions, and Energy use in Transportation) code .three types of biomass-feedstock are studied in the present work, i.e., corn, switchgrass, and soybean. The analysis comprises four fuel pathways, corn-to-ethanol, switchgrass-to-ethanol, soybean-to-biodiesel, and corn-based hydrogen. The pathway of petroleum-to-gasoline is also discussed for comparison. In conjunction with the vehicle fuel economy and the fuel-pathway results, the lifecycle performance in energy consumption and GHG emission of the biofuel-based transportation system are determined. The results obtained in the present work could enrich the information available for the public, industry and government to make biomass-informed decisions at this important policy-making time[22], [23].

IX. THE SOCIAL AND ENVIRONMENTAL IMPACTS OF BIOFUELS

The production of biofuels presents a new economic opportunity for Asia, as well as a possible mechanism for countries to reduce their greenhouse gas (GHG) emissions and enhance energy security. Their societal value depends on the extent to which they can address those needs, while at the same time minimising social and environmental costs [1], [2]. This paper examines the costs and benefits of biofuel production in an Asian context, focusing primarily on liquid biofuels. The opportunities, choices and risks relating to biofuel production vary considerably between different Asian countries. (the) People's Republic of China is the most populous country on earth, and can ill-afford to turn fertile cropland over to biofuel production. India, with a more decentralised government, is searching for ways to improve the lives of the rural poor, many of whom have been left behind by rapid economic growth. Indonesia, also decentralized, is struggling to protect its tropical forests, among the most diverse in the world. Bangladesh is one of the poorest and most densely populated countries in the world, with limited capacity to formulate and implement sound policies. In contrast, Japan has a highly developed economy and will feature as an importer rather than a producer of biofuels. Other Asian countries also vary greatly in terms of population size, state of development, average income, existing patterns of land-use, and quality of governance. To some extent, the level of demand for more socially and environmentally responsible biofuels will come from consumers. Social equity and environmental protection are important issues for people in Asia, as they are for people in other parts of the world who consume Asian exports [3]. At present, these considerations appear to play a relatively minor role in consumer decisions in most Asian countries, but they already have considerable effect on export markets. Consumer concerns about the role of biofuels in tropical deforestation, for example, have had a large influence on the acceptability of crop-based feedstocks for use as biofuel in Europe. In the medium to long term, biofuel development founded on social and environmental responsibility is more likely to be publicly acceptable and to contribute to economic development. The aim of this paper is to provide an overview of the social and environmental impacts of biofuel production in Asia. The major impacts are considered under three headings: those that are largely a product of land-use change, those that are associated with particular feedstocks, and those associated with technology and scale. This is a useful division, although it is somewhat artificial, as all of these factors interact. The paper ends with a discussion of some of the most promising ways to improve social and environmental responsibility, including current initiatives.

X. IMPACTS OF LAND-USE CHANGE ON BIODIVERSITY: A COMPREHENSIVE ANALYSIS

Land-use change is an important form of global pressure affecting biodiversity (e.g. Sala et al., 2000, UNEP, 2002, UNEP-RIVM, 2003, Zebisch et al., 2004). The most important type of land use in Europe is agriculture, with 34% of the European terrestrial area used for crop production and 14% for grassland (Verburg et al., 2006). Higher-scale studies on the effects of land-use change on biodiversity have focused mainly on 'major land-use types' with little attention paid to the intensity of land use (e.g. Sala et al., 2000). Agricultural landscapes are considered to be homogenous matrices. In practice, there is a large heterogeneity in farming systems and management practices. Low-intensity farming systems are critical to nature conservation and protection of the rural environment (Bignal and McCracken, 1996), while large-scale input-intensive systems can cause major environmental problems in agricultural and surrounding non-agricultural ecosystems (Donald et al., 2001, Benton et al., 2002). The biodiversity in agricultural landscapes depends largely on the intensity of land use, so an assessment of changes in agricultural biodiversity at the European scale needs spatially explicit information on land-use intensity. We can distinguish between input intensity, which is measured by input variables, e.g., chemical fertilizer, pesticides, and output intensity, measured as production per unit land area and time (Turner and Doolittle, 1978)[23], [32], [37]. Farming systems differ regionally in intensity and have, in the past, shown large changes. Post-war agricultural policies in the EU focused mainly on increasing agricultural productivity by promoting technical innovations and by ensuring the rational development of agricultural production (as laid down in article 33 of the EC Treaty). These policies can be considered successful in as far as they have resulted in increased yields and enhanced capacity for self-sufficiency. However, increased agricultural intensity has also resulted in an increasing pressure on biodiversity, and this is likely to continue (Tilman et al., 2001). Petit et al. (2001) indicated that agricultural intensification would be the most important form of pressure on biodiversity in the coming decades. In response to biodiversity loss, environmental objectives and landscape preservation in recent years have become prominent issues in the EU Common Agricultural Policy (CAP) and related environmental policies. The EU has committed itself to halt biodiversity loss by 2010 (EU, 2002). This study reported on here is incorporated into the EURURALIS project (Klijn et al., 2005), a scenario study aiming to stimulate discussion on the future of Europe's rural areas (Westhoek et al., 2006). In this paper we aim to: (1) assess the land-use intensity and relating biodiversity in agricultural landscapes in the EU25, for the current situation (2000) and to (2) analyze the impact of (agricultural) land-use change on biodiversity for the four EURURALIS scenarios for 2010, 2020 and 2030. The database of the EU Farm Accountancy Data Network (FADN) and other farming statistics were used to classify farm types according to land-use intensity. Attribution of ecosystem quality values to farm types was based on a literature review carried out for the GLOBIO3 modelling development. Ecosystem quality values for other land-use types, using the same methodology, were attributed, so that biodiversity tradeoffs between agricultural intensification and expansion of extensively managed agricultural land can be analyzed. The EURURALIS scenario storylines and outcomes of the GTAP (Global Trade Analysis Project) model (Hertel, 1997, Van Meijl et al., 2006), IMAGE (Integrated Model to Assess the Global Environment) (IMAGE Team, 2001, Eickhout et al., in press) and CLUE (Conversion of Land Use and its Effects) (Veldkamp and Fresco, 1996, Verburg et al., 2006) models used in EURURALIS were used to model future changes in land-use intensity and biodiversity[40], [36].

XI. MICROALGAL BIOFUEL REVISITED: AN INFORMATICS-BASED

The extensive use of fossil fuels has led to global climate change, environmental pollution, health problems, and an energy crisis associated with the irreversible depletion of traditional sources of fossil fuels [1]. Many countries are thus turning their attention to the development of new, clean, and sustainable energy sources [2], [3]. Among the various potential sources of renewable energy, biofuel, the fuels obtained from biomass (i.e., organic matter derived from plants, animals, and microorganisms), are of great interest and are expected to play a crucial role in the global energy infrastructure in the future. In contrast to other forms of renewable energy (e.g., wind, tidal, and solar energy), energy is chemically stored in biofuels and biofuels can be used in existing engines after blending to various degrees with petroleum diesel, resulting in lower carbon monoxide (CO) and sulfur oxide (SO_x) emission levels. Microalgae are the fastest growing photosynthesizing organisms and, in addition to consuming carbon dioxide (CO₂) and nitrogen (N)-based compounds such as ammonium, microalgae are one of the most important producers of oxygen on earth [6], [7]. Microalgal biomass contains approximately 50% carbon by dry weight [8]. During the production of 100 tons of microalgal biomass using natural or artificial light, approximately 180 tons of CO₂ can be fixed [9]. Although all CO₂ absorbed by an alga to produce fuel oil is released back into the environment as soon as the oil is burnt and the residual biomass degrades [10], no more extra CO₂ is produced in the production and consumption of microalgal biofuel, which maintains carbon balance in environment and may relax global warming associated with the consumption of fossil fuels and emission of CO₂. Given their rapid growth and ability to convert solar energy into chemical energy via CO₂ fixation, microalgae have been considered one of the most promising sources of oil for the production of biodiesel [11], [12]. In addition, microalgae are used as feedstock for a wide variety of practical and potential metabolic products, such as food supplements, pharmacological substances, lipids, enzymes, biomass, polymers, toxins, and pigments [13], [14]. Microalgal cultivation is a costly process due to the large amounts of water, inorganic nutrients (mainly N and phosphate (P)), and CO₂ needed [15], [16]. Compared to the microalgal cultivation by using freshwater, N and P fertilizer, and purchased CO₂, biodiesel production from microalgae may be more environmentally sustainable, cost-effective, and profitable if combined with processes such as wastewater and flue gas treatments, in which freshwater is replaced with wastewater and flue gases are used as a source of carbon and inorganic nutrients for microalgal culture [17], [18]. However, the availability of sufficient concentrated CO₂ from burning coal or other fossil fuel is still a major impediment to production of algal fuel oils at a meaningful scale [10], and other CO₂ source (e.g., oil refinery and biomass power plant, etc.) should be further taken into account. Although the entire chain of microalgal biofuel production, including culture selection [1], [19], [20], [21], [22], [23], [24], [25], cultivation and pest control [12], [17], [26], [27], [28], [29], [30], [31], [32], [33], harvest [18], [31], [34], [35], and lipid production and processing [18], [21], [36], [37], [38], [39], [40], has been extensively studied and reviewed during the past few years, the production cost is too high for industrialization, and the price of algae-based biodiesel (\$2.76/kg) remains strikingly higher than that of normal diesel (\$0.95/kg) [41]. To obtain a comprehensive and

systematic overview of trends in microalgal research, particularly in microalgal biofuel research, we retrieved and extensively analyzed manuscripts and patents related to this topic and published between 1900 and May 2015. This informatics study provides useful guidance for future research in the field of microalgae, particularly microalgal biofuel. Moreover, the challenges/problems of microalgae biofuel production and recent advances of their solution were reviewed, and the perspective view of the future R&D needs and trends are proposed.

XII. BIOFUELS (ALCOHOLS AND BIODIESEL) APPLICATIONS

The world is presently confronted with the twin crises of fossil fuel depletion and environmental degradation. Indiscriminate extraction and lavish consumption of fossil fuels have led to reduction in underground-based carbon resources. The search for alternative fuels, which promise a harmonious correlation with sustainable development, energy conservation, efficiency and environmental preservation, has become highly pronounced in the present context. The fuels of bio-origin can provide a feasible solution to this worldwide petroleum crisis. Gasoline and diesel-driven automobiles are the major sources of greenhouse gases (GHG) emission [1], [2], [3]. Scientists around the world have explored several alternative energy resources, which have the potential to quench the ever-increasing energy thirst of today's population. Various biofuel energy resources explored include biomass, biogas [4], primary alcohols, vegetable oils, biodiesel, etc. These alternative energy resources are largely environment-friendly but they need to be evaluated on case-to-case basis for their advantages, disadvantages and specific applications. Some of these fuels can be used directly while others need to be formulated to bring the relevant properties closer to conventional fuels. Due to the recent widespread use of petroleum fuels in various sectors, this study concentrates on assessing the viability of using alternative fuels in the existing internal combustion engines. The present energy scenario has stimulated active research interest in non-petroleum, renewable, and non-polluting fuels. The world reserves of primary energy and raw materials are, obviously, limited. According to an estimate, the reserves will last for 218 years for coal, 41 years for oil, and 63 years for natural gas, under a business-as-usual scenario [1], [5], [6]. The enormous growth of world population, increased technical development, and standard of living in the industrial nations has led to this intricate situation in the field of energy supply and demand. The prices of crude oil keep rising and fluctuating on a daily basis. The crude oil prices are at near record levels and are stabilizing at about US\$65 per barrel now. This necessitates developing and commercializing fossil-fuel alternatives from bio-origin. This may well be the main reason behind the growing awareness and interest for unconventional bio energy sources and fuels in various developing countries, which are striving hard to offset the oil monopoly. Environmental concerns have increased significantly in the world over the past decade, particularly after the Earth Summit '92. Excessive use of fossil fuels has led to global environmental degradation effects such as greenhouse effect, acid rain, ozone depletion, climate change, etc. There is a growing realization worldwide that something constructive has to be done soon to reduce the GHG emissions. In the Kyoto conference on global climate change, nations world over have committed to reduce GHG emissions significantly.

Use of various fossil fuels such as petroleum products and coal lead to several environmental problems such as reduction in underground-based carbon energy sources, serious modifications in earth's surface layer, subsidence of ground surface after extraction of fuels and minerals etc. Usage of these fossil fuels has led to increase in CO₂ levels in atmosphere from 280 PPM in pre-industrial era to 350 PPM now. These CO₂ levels are still climbing as a function of fuel burnt leading to greenhouse effect, acid rains, smog and change of climate world-over. These environmental implications are being felt in day-to-day life in the form of changing weather patterns, more severe winters and summer globally, foggy conditions in several parts of the world for a prolonged period during winter months. The combustion of fossil fuel has an adverse affect on human health through increased air pollution in cities, acid rains, build up of carbon dioxide, changing heat balance of the earth, etc. In fact, projections for the 30-year period from 1990 to 2020 [8], [9], [10] indicate that vehicle travel, and consequently fossil-fuel demand, will almost triple and the resulting emissions will pose a serious problem. The main reason for increased pollution levels, in spite of the stringent emission norms that have been enforced, is the increased demand for energy in all sectors and, most significantly the increased use of automobiles. The global population of motor vehicles on the roads today is half a billion, which is more than 10 times higher than what was in 1950. Combustion of various fossil fuels leads to emission of several pollutants, which are categorized as regulated and unregulated pollutants. Regulated pollutants are the ones, whose limits have been prescribed by environmental legislations (such as USEPA, EURO and Bharat norms) whereas there are some pollutants for which no legislative limits have been prescribed. These are categorized as unregulated pollutants. Regulated pollutants include NO_x, CO, HC, particulate matter (PM) and unregulated pollutants include formaldehyde, benzene, toluene, xylene (BTX), aldehydes, SO₂, CO₂, methane etc. [11], [12], [13].

These regulated as well as unregulated pollutants contribute to several harmful effects on human health, which are further categorized as short- and long-term health effects. The short-term health effects are caused by CO, nitrogen oxides, PM, (primarily regulated pollutants) formaldehyde etc., while long-term health effects are caused mainly by poly-aromatic hydrocarbons (PAH's), BTX, formaldehyde, (primarily unregulated pollutants) etc. CO is fatal in large dosage, aggravates heart disorders, affects central nervous system, and impairs oxygen-carrying capacity of blood by forming carboxy-hemoglobin. Nitrogen oxides cause irritation in respiratory tract. HC's cause drowsiness, eye irritation, and coughing [14], [15], [16]. These pollutants also contribute towards several regional and global environmental effects. Regional environmental effects such as summer smog are because of aldehydes, carbon monoxides, nitrogen oxides etc. Winter smog is because of particulate. Acidification is caused by nitrogen oxides, sulfuric oxides etc. Several global effects like ozone layer depletion, global warming etc. are caused by CO₂, CO, methane, non-methane hydrocarbons, nitrogen oxides etc. [17], [18]. Transportation and agricultural sector is one of the major consumers of fossil fuels and biggest contributor to environmental pollution, which can be reduced by replacing mineral-based fuels by bio-origin renewable fuels. There are a variety of biofuels potentially available, but the main biofuels being considered globally are biodiesel and bio-ethanol. Bio-ethanol can be produced from a number of crops including sugarcane, corn (maize), wheat and sugar beet. The last two are currently the main sources of ethanol in Europe [19]. Biodiesel is the fuel that can be produced from straight vegetable oils and edible.

CONCLUSION

Microalgae can be an answer to the primary concern of imbalance in food, fuel, bio-crude, and energy as sustainable alternative crop, but the microalgae research is still in its infancy. Most of the potential commercialization models suggest biorefinery approaches to make microalgal biofuel production economical, where the microalgal biomass is used for extraction of bio-crude, and valorization of co-product(s) can maximize the chances of commercialization. Jet biofuels based on *Euglena* wax esters have great potential as an alternative to cope with GHG emissions in transportation sector. However, the current upstream and downstream processes for *Euglena*-based jet biofuel production have not been sufficiently optimized. To achieve industrial production, further aggressive research on strain development and up-scaling cultivation processes are required. Fungi-assisted bio-flocculation of microalgae provides a new potential for wastewater treatment. The co-culture of microalgae and fungi not only save the harvesting cost, making the wastewater treatment economically feasible and bringing more significant pollutant removal efficiency than that of mono-culture. The stable system formed by the combination of fungi and microalgae is more resistant to the harsh environment. Of the several methods available for producing biodiesel, transesterification of natural oils and fats is currently the method of choice. The purpose of the process is to lower the viscosity of the oil or fat. Although blending of oils and other solvents and microemulsions of vegetable oils lowers the viscosity, engine performance problems, such as carbon deposit and lubricating oil contamination, still exist. Pyrolysis produces more biogasoline than biodiesel fuel.

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