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Mini-Review

Research of Sustainable Jet Fuel Production Using Microbes

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Abstract

Global climate change, coupled with rapidly increasing oil prices and energy demand around the world, has paved a way for intense research in the biofuel sector. Stakeholders in the aviation industry have started to focus on bio-jet fuel. Bio-jet fuel is regarded as a sustainable solution to greenhouse gas emissions and energy demand. This paper provides a brief review of the biofuel production technologies, the role of bacteria in producing hydrocarbons and the recent advancements in microbial engineering to enhance the biofuel production. Finally, this paper concludes by highlighting the challenges and future research implications in bio-jet fuel production.

Introduction

Atmospheric greenhouse gases (GHG) such as carbon-di-oxide (CO_2), methane, nitrous oxide, and fluorinated gases contribute to global climate change. Due to anthropogenic activities, GHS emission levels are increasing tremendously during the past few decades. The Environmental Protection Agency (EPA) report stated that in 2016 of all U.S. greenhouse gas emissions from human activities, the CO_2 emission accounted for about 81.6% while methane contributed about ten percent (10%), and nitrous oxide accounted for six percent (6%) [1].

Pacala and Socolow (2004) [2] reported that the CO_2 emission from fossil fuels at the global level was seven Gt (gigatonnes) of carbon per year. In the United States alone, between 1990 and 2016 the carbon dioxide emissions increased by about four percent (4%) [1]. This increase in emissions is attributed to the rapid increase in economy, population, and demand for air transport. The NASA climate change department reported that the CO_2 emission in March 2018 was 408 ppm, which is significantly high [3]. Among the different industries that contributed to these GHG emissions in the U.S, the fossil fuel combustion in the transportation sector alone accounted for 34% of total CO_2 emission and 27% of total GHG emissions [1]. Within the transportation sector, the demand for air travel is increasing rapidly. Based on compound average growth rate (CAGR) of 3.7%, the International Air Transport Association has forecasted that the demand for air travel will double by 2035 [4].

Airlines are spending approximately 28% of their operating cost to fuel their fleets [5]. This high operating cost and the volatility in fuel prices are the driving force for the aviation industry to develop biofuel. Climate change has also necessitated the reduction of the carbon footprint at the global level, and as the aviation industry emits more CO_2, IATA has developed guidelines to reduce CO_2 emissions by improving fuel efficiency by one and a half (1.5%) which will aid in achieving carbon neutrality by 2020 and reduce the emissions by 50% in 2050 [6]. Sustainable bio-jet fuel offers low carbon emissions. The life cycle assessment (LCA) of biofuels from different renewable processes decreased the CO_2 emissions by approximately 80% based on the production technology [7]. The feedstocks used for biofuel production are biological in origin, namely jatropha, algae, camelina, halophytes, agricultural and forest residues, etc., and microbes play a significant role in biofuel production from these feedstocks. This paper will discuss specifically the biofuel production technologies, role of microbes in producing hydrocarbons (alkanes), and conclude by briefly reviewing the recent advancements in microbial engineering to enhance the biofuel production.

Alternative Aviation Fuels and Their Production Technologies

Aviation industry uses two types of jet fuel namely, Jet A and Jet A1 (kerosene-based fuel, which are mixtures of hydrocarbons, primarily alkanes) and Jet B (gasoline-based fuel). Among these two fuels types, kerosene-based fuels are mixtures of hydrocarbons, predominantly alkanes. Kallio *et al.*, (2014) [8] reported that alkanes significantly reduce both the freezing and boiling points of the aviation fuel, and so it is a vital product to develop bio-jet fuels. Hence, most of the recent research works are targeting alkanes as an essential product for bio-jet fuel synthesis [9]. There are mainly two methodologies for producing alkanes; the first method is by producing triglycerides and fatty acids by utilizing oleaginous crops and then chemically converting that to alkanes; the second method is based on pyrolysis of biomass to produce syngas and then chemically converting that to alkanes [9]. Microorganisms have the exceptional ability to produce fatty acids and some microbial species are able to convert the fatty acids into alkanes/alkenes. The innovations and development of biotechnology and bioengineering have paved the way for more research in the field of alkane production through microbes.

The alternative aviation fuels that are gaining popularity are sustainable in nature as they are predominantly produced from renewable resources, and the related GHG emissions are also comparatively lesser than in the use of fossil fuels. Moreover, these alternative fuels are compatible with the conventional fuels. The different types of alternative aviation fuels that are being developed are Hydroprocessed Renewable Jet fuels (HRJs) or Hydroprocessed Esters and Fatty Acids (HEFA) [10], Fischer Tropsch fuels (FT fuels) [11], liquid bio-hydrogen and bio-methane [12], biodiesel [13], and bio-alcohols [14].

The technologies used for producing renewable jet fuels are classified broadly into two types based on the type of processes utilized for its production viz. thermochemical process and biochemical process [15]. Thermochemical processes include Biomass to liquid process (BTL process), Fischer Tropsch process (FT process), and hydroprocessing. While the biochemical process consists of direct sugar to hydrocarbon process (DHSC) and alcohol to jet (ATJ) process [9]. Wang and Tao (2016) [16] classified the conversion pathways into four types based on raw materials (alcohol, oil, gas, and sugars) used for fuel production namely alcohol to jet (ATJ), oil to jet (OTJ), gas to jet (GTJ), and sugar to jet (STJ). These pathways are certified by American Society for Testing and Materials (ASTM) [17].

Alcohol to jet (ATJ) pathway is used to convert butanol and ethanol to jet fuels using processes like alcohol dehydration, oligomerization, and hydrogenation. HEFA/HRJ fuels are produced through oil to jet (OTJ) synthesis, which utilizes different process such as catalytic thermolysis or hydrothermal liquefaction and the hydro-treated depolymerized cellulosic jet process [8,16]. Gas to jet (GTJ) involves two pathways namely Fisher Tropsch Biomass to Liquid (Fischer-Tropsch Synthesis Paraffin Kerosene [FT-SPK]) and gas fermentation process. In the FT process, the biomass is first converted to syngas, then converted to liquid hydrocarbons through F-T synthesis while in gas fermentation pathway the biomass is transformed into syngas which is fermented to either ethanol or butanol and finally upgraded to jet fuel through the ATJ process. Sugar to jet (STJ) pathway includes the biological and catalytic conversion of sugars to hydrocarbons [17].

Role of Microorganisms, and Current Status of Microbial Biofuel Production

Among these technologies described, the utilization of lignocellulosic material to produce bioethanol contributes significantly towards the commercial production of sugars from renewable resources. This is a promising approach towards the sustainable bio-jet fuel production. This conversion is brought about either by the catalytic upgrading of sugars to hydrocarbons pathway [18] or by biological fermentation [16]. There are two types of fermentation, namely direct fermentation, which refers to the conversion of plant materials to sugar and then sugar to alcohol or indirect fermentation referring to the pyrolysis of plant materials followed by the conversion of syngas to ethanol by using acetogenic bacteria [19]. Indirect fermentation is rarely used.

When compared with the catalytic upgrading of sugars to hydrocarbon pathway, the biological fermentation method has more advantages. It does not require chemical catalysts, high energy/temperature reactions, and the conversion can be carried out in a single fermentation tank. Due to these reasons, STJ technology is regarded as a significantly promising pathway for the production of bio-jet fuels. STJ technology with special reference to biological conversion and the role of microbes in biofuel production will be further discussed briefly.

Biofuel production involves different process such as identifying the potential plant source, isolation of the microbial strains (fungal, and/or bacterial species), and designing the conversion pathway for efficient conversion of plant materials to sugars, and then converting that to ethanol using microbes either utilizing native strains or genetically engineered strains. The most important step in biofuel production is the conversion of the raw material to sugar.

Different plant materials such as agricultural crops, grasses, weeds, algae are used for biofuel production and their composition (cellulose, hemicellulose, lignin etc.) varies widely. Based on this composition of the plant material, different kinds of microbes, optimum conditions for their incubation, and other requirements for biofuel production, will also differ considerably [20]. The ideal microbe will possess a high tolerance to inhibitors, high metabolic fluxes, optimum utilization of substrates, and have deregulated pathways for sugar transport and produce a single fermentation product.

Fermentation (Ethanol Production)

Many research works are carried out to identify the potential microorganisms that are able to bring about these transformations and to develop a technologically and economically viable method based on this conversion. Irrespective of the plant material used, the starch, cellulose or hemicellulose were first degraded to pentose, and hexoses and later it is fermented into ethanol. There are different fermentation pathways (Example: hetereolactic acid fermentation by lactic acid bacteria such as *Leuconostoc* species and mixed acid fermentation by enteric bacteria) that produce ethanol as one of the end product. Nonetheless, ethanol has to be the primary product for the commercial production of biofuel. Especially, the yeast Saccharomyces cerevisiae and gram-negative bacteria *Zymomonas mobilis* produce two moles of ethanol per mole of hexose. Both these microbes convert pyruvate to alcohol through pyruvate decarboxylase/alcohol dehydrogenase enzymes. However, the conversion of hexoses to ethanol using *S. Cerevisiae* is widely adopted at an industrial level production [21]. Since, plant materials consist of both cellulose and hemicellulose (precursor of xylose, a pentose sugar), microbes that can efficiently and effectively metabolize these two sugars are required for the industrial production of alcohol. Several microorganisms such as the fungi *Penicillium capsulatum* and *Talaromyces emersonii* [22], the thermophilic *actinomycete Thermomonospor fusca* [23], the hyperthermophile *Caldicellulosiruptor saccharolyticus* [24] completely depolymerize hemicellulases to xylose. Usually, xylose is metabolized to pyruvate through the pentose phosphate pathway. Different microbes such as anaerobic fungi and different groups of mesophilic and thermophilic anaerobic bacteria are able to metabolize xylose [25].

Consolidated Bioprocessing (CBP)

CBP is another significant strategy adopted at the industrial level to produce biofuel in an economically efficient way. CBP refers to the microbial conversion of plant materials to biofuels without enzymatic treatments [26]. This conversion is brought about by either a single microbe or a combination of microbes. The anaerobic, thermophilic bacterium *Clostridium thermocellum* is one of the significant candidates used in CBP. Another potential candidate from the same genus is *C. Bescii* and it was actually engineered to produce ethanol [27].

Similarly, the mesophilic and filamentous fungus *Trichoderma reesei* has also been engineered to produce ethanol [28]. Yamada, Taniguchi, Tanaka, Ogino, Fukuda, and Kondo (2010) [29] suggested that an alternative efficient way is to engineer an ethanol producing microbe to digest cellulose. Microbes such as *S. Cerevisiae, E. Coli* and *Z. Mobilis* were engineered to digest cellulose through the expression of heterologous cellulolytic enzymes. Lynd, Currie, Ciazza, Herring, and Orem (2008) [30] utilized thermophilic gram-positive *Firmicutes* that belongs to the orders *Clostridiales* and *Thermoanaerobiales* and their research proved that *Clostridium thermocellum* has the highest cellulose degradation ability among the order *Clostridiales. Clostridium phytofermentans* is a gram-positive, anaerobic bacteria that ferments a wide variety of polysaccharides, oligosaccharides, and monomeric sugars to produce mainly ethanol [31]. *Clostridium Acetobutylicum* is another interesting species which predominantly produces butanol, along with acetone, and ethanol [32]. In general, several clostridia species are able to produce sugar by degrading the cellulose and then convert sugar to ethanol. Though promising, the amount of ethanol produced (on a w/v scale) rarely exceeds 5% and CBP using thermophilic anaerobes have not yet been commercialized. *Geobacillus thermoglucosidans* has the ability to convert lignocellulosic biomass to bioethanol [33].

Microbes Producing Alkane/Alkene (Fermentation of Sugar to Alkane/Alkene)

Fu, Chi, Ma, Zhou, Liu, Lee, and Chi (2015) [34] stated that most of the eubacteria synthesize low amount of alkanes. As it is synthesized intracellularly, its separation and purification from the cells are quite difficult. On the other hand, few prokaryotes are able to produce alkanes both intracellularly and extracellularly. Among the prokaryotes, anaerobic bacteria especially the species belonging to the genus Clostridium, and Desulfovibrio have exhibited significant results in producing alkanes mainly C_{25} to C_{35} [35]. In cyanobacteria, alkanes are found in lipid droplets and are actually packed with quite a lot of hydrophobic energy-dense compounds [36].

Han, McCarthy, Calvin, and Benn (1968) [37] reported that several species of cyanobacteria are able to produce a hydrocarbon that is within the appropriate range for jet fuel (C_{15} to C_{19}). But the biosynthesis pathway of alkane in cyanobacteria was not well known for a long time. Only in 2010, Schirmer, Rude, Li, Popova, and Cardayre, [38] explained the alkane biosynthesis pathway in cyanobacteria. In this cyanobacterial pathway, fatty acyl-ACP is converted to alkane/alkene (C_{15} to C_{19} especially C_{17}). Beller, Goh, and Keasling, (2010) [39] studied another pathway in which alkene is produced by *Micrococcus luteus,* a gram-positive bacteria. They reported that in this pathway alkane/alkene is produced as long chain hydrocarbon (C_{23} to C_{33}) with at least one double bond. This is referred to as head-to-head condensation of fatty acids. It begins with the formation of fatty acyl-CoA (or ACP) which is converted to β-ketoacyl-CoA by acyl-CoA dehydrogenase, an enoyl-CoA hydratase, and a 3-hydroxy acyl-CoA dehydrogenase in a sequential way and ultimately it leads to the alkene biosynthesis. Park, Tanabe, Hirata, and Miyamoto (2001) [40] reported that *Vibrio furnissii* synthesizes alkanes between C_{15} and C_{24}. This pathway starts with fatty acid which is then converted into alkane and is referred to as hypothetical vibrio pathway.

Engineering Microbes for Alkane Production

Although a lot of microorganisms are able to produce alkanes naturally, the production level is not so high. Especially, being photosynthetic in nature cyanobacteria caught the attention of the biofuel industry as it is able to synthesize alkanes from sunlight and carbon dioxide. Several research works are being carried out with different methodologies by altering the growth and environmental conditions, and microbes are genetically engineered to increase the alkane production level to a commercial level. Wang, Liu, and Lu (2013) [41] were able to increase the alkane production in *Synechocystis* sp. PCC 6803 by 8.3 times higher than the wild strain. This high production rate was achieved by genetically modifying *Synechocystis* strain by deleting the β ketolyase phaA, and overexpressing the native FAR (Fatty acyl-ACP reductase) and ADO (Aldehyde-deformylating oxygenase) enzymes. Kageyama, Waditee-Sirisattha, Sirisattha, Tanaka, Mahakhant, and Takabe (2015) [42] were able to double the alkane production in the nitrogen-fixing cyanobacterium *Anabaena sp*. PCC 7120 by exposing it either to nitrogen deficiency or salt stress. While Peramuna, Morton, and Summers (2015) [43] genetically modified the *Nostoc punctiforme* cells and exposed them to high light conditions in order to overexpress the genes that encoded FAR, ADO, and a lipase to increase the production of alkanes. They were able to increase the production from 1.12% to 12.9% of CDW (cell dry weight). Despite the developments attained, the cyanobacterial alkane production amount is still low and the biofuel industry shifted its attention to identify new microbes.

Due to the limitations related to the alkane production using cyanobacterial species, biofuel industry is attempting to develop new microbial platforms to produce alkanes. The industry specifically prefers certain microbes such as *Escherichia coli, Bacillus subtilis, Aspergillus nidulans,* and the yeast, *Saccharomyces cerevisae* either due to their production abilities or suitability for industrial production. The first attempt to engineer the bacterial platform to express the cyanobacteria pathway in *E. Coli* was made by Schirmer *et al.* (2010) [38]. By expressing FAR and ADO from *S. Elongates* PCC7942 the engineered *E. Coli* MG 1655 were able to produce 25mg/l of pentadecane and heptadecene. When the *S. Elongates* ADO was replaced with ADO from *N. Punctiforme* PCC 73102 the alkane/alkene production went up to 80mg/l. When a modified medium was utilized the alkane/alkene (tridecane, pentadecene, pentadecane and heptadecene) production increased up to 300mg/l and this methodology was patented by the biofuel company LS9 [44]. Harger, Zheng, Moon, Ager, An, Choe, *et al.* (2013) [45] engineered *E. Coli* to produce different alkane/alkene ranging from C_{13} to C_{17}. By overexpressing the FAR and ADO enzymes from *S. Elongates,* and fabH2 from *B. Subtilis,* the engineered *E. Coli* BL 21 was able to produce alkane at a level of 98.3mg/l [45]. Choi and Lee (2013) [46] developed a new *E. Coli* strain by deleting fadE, fadR and overexpressing fadD. This new strain was able to produce alkanes such as C_9, C_{12}, C_{13}, and C_{14}. Choi and Lee (2013) [46] reported a production level of 580.8mg/l. Alkanes (C_{13} to C_{17}) were produced directly from FFA (free fatty acids) by overexpressing the reductase complex from *Photorhabdus luminiscens* and ADO from *N. Punctiforme* in *E. Coli* [47]. This method produced up to 8mg/l of alkanes and the Shell Company patented this method [47]. Song, Yu, and Zhu (2016) [48] engineered *E. Coli* to overexpress FAR and ADO from *S. Elongatus* PCC 7942, the transcription factor fadR and mutated the gene that encodes aldehyde reductase, yqhD. This engineered *E. Coli* BL 21 predominantly produced alkanes/alkene between C_{15} and C_{17} at a rate of 255.6mg/l. Thus several *E. Coli* strains have been engineered to produce advanced biofuel such as isopropanol, n-butanol, n-propanol, isobutanol, 2-methyl-1-butanol and 3- methyl-1-butanol [49].

The first recombinant yeast *S. Cerevisiae* that produced alkanes was reported by Bernard, Domergue, Pascal, Jetter, Renne, Faure, *et al.* (2012) [50]. Buijs, Zhou, Siewers, and Nielsen (2015) [51] tested the cyanobacterial pathway of alkane production in the yeast *S. Cerevisiae*. They engineered *S. Cerevisiae* by overexpressing FAR and ADO from *S. Elongatus* PCC 7942, overexpression of the *E. Coli* ferredoxin and the ferredoxin NADP+ reductase (Fdx and FNR) and deleting hfd1. The engineered strain produced alkanes such as C_{13}, C_{15}, and C_{17} and the production level was 22μg/l CDW. Foo, Susanto, Keasling, Leong, and Chang (2016) [52] used FFA instead of glucose to produce alkanes. The alkane production was about 70 μg/l. This methodology was further improved by Zhou, Buijs, Zhu, Qin, Siewers, and Nielsen (2016) [53] by substituting glucose as a substrate instead of FFA and they achieved a production of 10.4g/l of FFA.

Beopoulos, Cescut, Haddouche, Uribelarrea, Molina-Jouve, and Nicaud (2009) [54] reported that the oleaginous yeast *Yarrowia lipolytica* is capable of storing up to 36% of CDW as lipids and it is considered as a potential strain for the industrial biofuel production. Liu, Pan, Spofford, Zhou, and Alper (2015) [55] reported that the engineered strain of *Yarrowia lipolytica* is able to accumulate 39.1g/l of lipid. The black yeast *Aerobasidium pullulans var melanogenunm* is able to accumulate up to 66.3% of CDW as lipids (26.7% palmitic acid, 44.5% oleic acid and 21% linoleic acids) [56]. Kohn and Kim (2015) [57] isolated approximately 46 microbial isolates that are able to produce C_3 to C_8 hydrocarbons. They identified species of *Actinomyces sp., Enterococcus Faecium, E. Hirae, E. Coli, Clostridium Glycolicum, Proteus sp.* and *Tissirella sp.* Oleaginous yeast is a promising candidate but requires more research to be completed with regard to its metabolic pathway, and genetics to commercialize its utilization in the alkane production. The current demand for sustainable fuels can be fulfilled only when these alternative microbes which have a high potential to produce alkane is characterized fully.

Conclusion

As the global consumption of sustainable biofuel increases and the aviation sector relies on the utilization of bio-jet fuel to achieve its target in reducing the GHG emission, it is essential to discover new methodologies to produce renewable jet fuel. There are still a lot of challenges associated with the commercialization of sustainable bio-jet fuels. ICAO and the regional aviation authorities should develop policies to bridge the price gap between bio-jet and conventional jet fuel and should provide incentives to encourage the biofuel production. The entire supply chain from feedstock production to the distribution of bio jet fuel will have to be well developed.

Among the production pathways discussed earlier, the majority of the biofuel produced today is through HEFA pathway. Out of all the commercial operation, only AltAir facility in California produces chiefly bio jet fuel using Ecofining technology. The others produce mainly biodiesel and a small quantity of bio jet fuel. The operational capacity of all the HEFA facilities accounts only for 4.3 bln L/y. In HEFA pathway Ecofining and NEXBTL are the two main production technologies that are commercialized so far. NEXBTL technology is used by Neste Company in Finland, Singapore, and the Netherlands while Ecofining technology is used by AltAir, Emerald Biofuels, Diamond Green Diesel, Renewable Energy Grouo (US), and ENI (Italy) [58].

Other methods that utilize biomass, lignocellulosic and algal sources, to produce bio-jet fuel are still in demonstration stage with promising results. This conversion uses FT, ATJ, STJ pathways. This paper discussed the microbial production systems that converted the sugars to alkane based jet fuel. Though these engineered microbial systems are not yielding the productivity at a commercial level, in the future as the supply of fossil fuel decreases, and the applied bioengineering, biotechnological, and microbiological research improves, this will definitely provide a competitive alternative for the shift towards sustainable bio jet fuel.

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