



## Third generation biofuels: A nutritional perspective in enhancing microbial lipid production

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### ABSTRACT

The third generation biofuels derived from oleaginous microorganisms have gained traction recently as the potential feedstock in generating fuel for energy production, reducing the direct dependence on fossil fuels. However, commercialization of microbial technology for biofuel production remains intricate and questionable due to many factors concerning the life cycle assessment and techno-economic feasibility of microorganisms-based biofuels. This review initially focuses on the nutritional aspects in enhancing the biomass and lipid yields from various microorganism feedstock, serving as impetuses for the production of third generation biofuels. Nutrient optimizations in terms of nutrient starvation, supplementation and balancing technique are discussed in relation to their respective effects on the microbial biomass and lipid productions. More importantly, the economic perspectives of oleaginous microorganism cultivations are also reviewed and strategized using alternative and non-conventional nutrient sources for possible technology scale-ups and commercialization.

### 1. Introduction

Energy production remains high in demand stemming from the continuous rise in human population and world globalization. The increasing trend of energy consumptions has resulted in the overreliance on non-renewable energy sources preponderantly fossil fuels (coal, petroleum, and gas) which would soon exhaust the current global supplies. Thus, not being able to satisfy the future demands for perpetual energy production and consumption. Besides, the usage of fossil fuels has brought about many environmental concerns such as global warming due to the greenhouse gases emission during the combustion of fossil fuels in producing energy. Thereby, the alternative energy sources which are readily available, facilely accessible and greener in nature are very much needed. Of having little sustainable alternatives, the world is turning to biofuels for answers to global energy woes. The well-known biofuel, i.e., biodiesel, is conventionally recognized to have combustible potential comparable with fossil fuels in addition to its renewability for incessant applications. In fact, the research progress in renewable energy realms inclusive of biofuels has ratcheted up tremendously in comparison with a decade ago (Fig. 1).

Biofuels are derived from renewable fuel sources, e.g., various organic matters which can be categorized into two main categories,

namely, primary and secondary biofuel sources. The primary sources use unprocessed organic materials directly as a fuel, whereas secondary sources are the resulting fuels (e.g., ethanol, biodiesel, etc.) produced from processing of various biomasses and used to power vehicles or for industrial applications [1]. Biodiesel in general is a synthetic diesel-like fuel composed of a mixture of fatty acid methyl ester (FAME) with quality commonly satiating the requirements decreed by either the European (EN 14214) Standard or American Society for Testing and Materials (ASTM D-6751) Standard. Biodiesel production has been gaining prominence as an alternative liquid fuel source recently and this is evident by the surge of biodiesel production throughout recent years. The biodiesel production in United States (U.S.) alone had increased about 23% from 0.082 million barrels per day in 2015 to 0.101 million barrels per day by 2016 and this figure was also estimated to further increase to 0.105 million barrels per day by 2017 and 0.109 million barrels per day by 2018 (Fig. 2) [2]. At present, oil products account for about 93% of energy consumption in the transport sector of which biofuels (bio-ethanol and biodiesel) contribute to 4% of the global transport fuel [3]. As biodiesel is a synthetic diesel-like fuel, it can be directly used as a fuel or blended with petroleum diesel and used in diesel engines with little or no modification [4,5]. In fact, the mechanisms and infrastructures needed for biodiesel applications have

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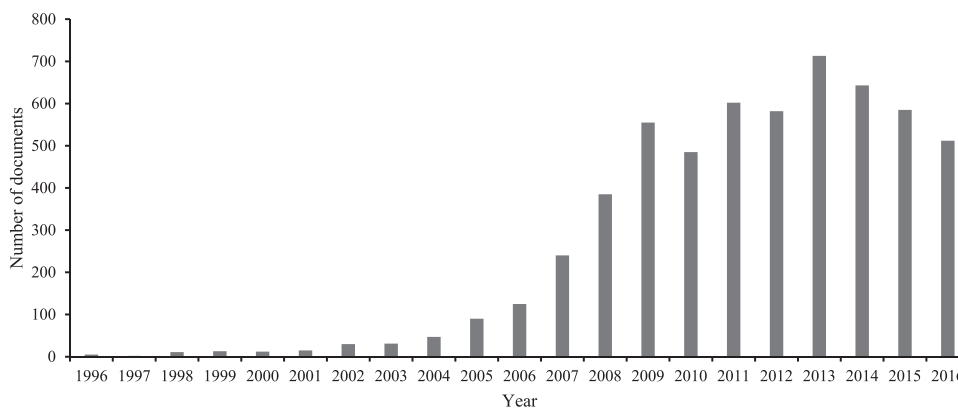


Fig. 1. Number of documents published under renewable energy topics from year 1996 to 2016. Data extracted from Scopus (2017).

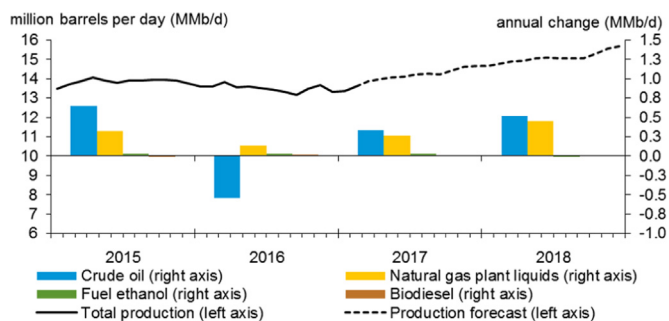


Fig. 2. U.S. crude oil and liquid fuels production (million barrels per day) [2].

already existed and continuously developed, besides being simulated in existing diesel engines [6,7]. The attractiveness of biodiesel in comparison with the conventional petroleum diesel fuel is that its applications are much safer and cleaner for the environment. While having virtually similar technical properties as diesel fuel, biodiesel is more advantageous due to its portability, ready availability, renewability, higher combustion efficiency and lower sulphur and aromatic contents [4]. The biodiesel also renders greater reductions in particulates and carbon monoxide upon the combustion as opposed to diesel fuel. Studies into evaluating the performances and emissions of pure biodiesel and blend mixtures with diesel oil in the non-modified and modified compression ignition engines have been implemented and reported in literatures [8–11].

Biofuels from secondary sources are further subcategorized into first, second and third generation biofuels which are different in terms of raw material sourcing and technological processes done during the conversion into biofuels. Starting with the first generation of biofuels, the sources are derived from edible oil bearing crop plants such as palm oil, corn, soybean, sunflower, etc [12]. However, there are serious concerns over food-versus-fuel debates when it comes to the first generation biofuels [1,12,13]. Issues over feedstock sourcing, impact on biodiversity, land availability for growing agricultural crops, and global food crisis are among the firm criticisms lambasted by environmentalists and non-government organizations [14,15]. With that, lignocellulosic feedstock from plant biomasses came into the development of second generation biofuels [1]. Indeed, the second generation biofuels cover a wider range of feedstock in the sense that they are mainly derived from non-edible feedstock such as lignocellulosic plant biomasses, agriculture residues (e.g., bagasses, straws, etc.), and waste products such as waste cooking oil [13]. These feedstocks are advantageous because it can counter the food-versus-fuel issues present in the first generation biofuels. Also, the examples of plants bearing oil-seeds that have been categorized under the second generation biofuels as well are *Jatropha curcas*, *Croton megalocarpus*, *Cerbera manghas*, etc. These plants were found to be an attractive alternative feedstock for

biofuel production due to its similarities with the edible oils and capabilities to grow under non-arable lands, making way for effective land utilization [12,13]. Nevertheless, growing these plants for second generation biofuels would entail regular irrigation and nutrient replenishment with good management practices in ensuring consistent oil yields [12,16]. As a matter of sustainability, these feedstocks are not economically and practically viable for stable energy supply due to their low conversion rates and lack of sourcing materials [5,11]. To date, the exploitations of various oleaginous microorganisms (microalgae, bacteria, yeast, and fungi) have resulted in the rise of third generation biofuels. The use of microalgae biomass feedstock had advantages over the other microbial species since it possessed higher growth tendencies and biomass productivities, in addition to its ability to accumulate large cell lipid content (20–77%) [1,12,17]. Besides, the residual microalgae biomass also could be further converted into other value added products such as bio-methane, bio-oil, bio-ethanol, bio-hydrogen, etc. through series of biorefinery processes [18]. The status summaries of the first, second, and third generation biofuels are shown in Fig. 3.

As of late, the most promising renewable feedstock for biodiesel production is from microalgae biomass. The annual productivity and oil content of microalgae is far superior than that of any oil-seed crop. For instance, a high-yielding oil crop i.e. oil palm would need 24% total cropping area to meet 50% of the U.S. transport fuel demands whereas microalgae would only need 1–3% total cropping area in meeting the exact same demand for transport fuel, thus, cultivating microalgae in the same acreage devoted to oil crops would have easily sated the demands for petroleum diesel fuel usage, even with the modest microalgae productivity [19,20]. Tilman et al. [21] termed 'biofuels done right' to be feedstock with low greenhouse gas emissions while having little to no food competition. In this case, microalgae is proven to be favorable as it made way for an effective land utilization through degraded or abandoned land usage. This can minimize the direct and indirect land-clearing associated to biofuel expansion which would potentially lead to the creation of long-term carbon debt and biodiversity loss. Concisely, these benefits are impossible to be materialized with first and second generation biofuel feedstock [21,22]. With the increasing consumption of petroleum liquid fuels especially in the transportation sector [23] (Fig. 4), there would be limitations of continuous dependence on such non-renewable energy source and the rise of biodiesel usage in replacing these liquid fuels would be inevitable. Thus, taking sustainability and renewability into account, microalgae cultivation would be a more suitable and realistic approach towards future biodiesel production. While there are many papers and reviews reporting on the microbial production technologies with emphasis on microalgae, there are still lacking of documents associating to the nutritional modes in cultivating microalgae feedstock, along with the nutritional supplementation techniques applied in altering the biochemical compositions in microbial cells.

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