



## Review

## Biodiesels from microbial oils: Opportunity and challenges

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## ABSTRACT

Although biodiesel has been extensively explored as an important renewable energy source, the raw materials-associated cost poses a serious challenge on its large-scale commercial production. The first and second generations of biodiesel are mainly produced from usable raw materials, e.g. edible oils, crops etc. Such a situation inevitably imposes higher demands on land and water usage, which in turn compromise future food and water supply. Obviously, there is an urgent need to explore alternative feedstock, e.g. microbial oils which can be produced by many types of microorganisms including microalgae, fungi and bacteria with the advantages of small footprint, high lipid content and efficient uptake of carbon dioxide. Therefore, this review offers a comprehensive picture of microbial oil-based technology for biodiesel production. The perspectives and directions forward are also outlined for future biodiesel production and commercialization.

## 1. Introduction

The extensive usage of non-renewable fossil fuel has negatively impacted on global climate change (Rakopoulos et al., 2014). Different from fossil fuel, biodiesel has been considered as an environmental-friendly alternative energy source, which is sulphur-free, nontoxic and biodegradable (Lund et al., 2014). In general, biodiesel is produced via the transesterification of oils (e.g. fatty acids) and short-chain alcohols with appropriate catalysts. The first-generation biodiesel is primarily produced from edible vegetable oils, such as soybean oil, rapeseed oil, sunflower oil etc. It should be noted that shifting agricultural land from food production to energy crops is highly debatable, while against the original intention of biodiesel production. However, the impact of biodiesel production on food production, in some situation, also depends on circumstances. For example, soybean oil, a by-product from animal feed production and animal fat, have been used as feedstock for biodiesel production in Brazil (De Oliveira and Coelho, 2017). In most countries, biodiesel production from crops will continue worsen global food shortage and inevitably compromise future food supply.

The quality of the first-generation biodiesel is generally incomparable with the petroleum diesel in terms of viscosity, density, heating value, cetane number and congealing point (Table 1). It should also be realized that the raw materials used for producing first-generation biodiesel generally account for 70–95% of the total production cost,

which is an obvious hurdle for large-scale biodiesel production (Ling et al., 2014).

So far, extensive effort has been dedicated to exploring non-food oils for production of the second-generation biodiesel, such as oils derived from *Jathropa curcas*, *Brassica carinata* etc. (Pinzi et al., 2009). However, a large area of land for plantation of these materials is still needed, which turns out to be challenging in highly urbanized countries. Hence, there is an urgent need for alternative feedstock, more importantly, production of which should not compete with agro-land for crops. In this regard, microbial oils produced by various kinds of microorganisms (e.g. bacteria, yeast, fungus and microalgae) have attracted increasing attention as a feedstock for biodiesel production due to their unique characteristics of short growth cycle, small footprint, high lipid content, and no requirement on agricultural land (Subramaniam et al., 2010). However, the situation is still very challenging towards large-scale commercialization of microbial oil-based biodiesel, e.g. some technical obstacles (e.g. harsh microbial culture condition, complicated and costly microbial harvesting and oil extraction etc.) will need to be overcome in the future.

Apart from raw materials, high-efficiency and cost-effective catalysts are highly desirable for transesterification. Currently, homogeneous catalysts (e.g. sodium hydroxide, sulphuric acid etc.) have been developed for commercial biodiesel production due to its high efficiency and low cost (Baskar and Aiswarya, 2016). However, the

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**Table 1**

Properties comparison of the soybean-biodiesel and petroleum-diesel. (Ma et al., 2017; Pinzi et al., 2009; Efe et al., 2018).

Properties	Unit	Diesel	Soybean	ASTM D-6751
Viscosity at 40 °C	Mm <sup>2</sup> /s	2.95	4.25–4.50	1.9–6.0
Density at 20 °C	g/mL	0.82	0.87	0.82–0.9
Heating Value	MJ/kg	45.95	33.5–39.8	–
Cetane number	–	51	47.1	≥ 49
Congeaing point	°C	–6	–5	–

ASTM D-6751: American Society for Testing and Materials D-6751.

recovery and reuse of these soluble catalysts from the end products of transesterification appeared highly difficult. To tackle such challenges, some heterogeneous catalysts have been employed for biodiesel production, which can be easily recovered from the crude biodiesel products and recycled back to the production process (Dai et al., 2015, 2016). However, it should be pointed out that there are many challenges associated with the heterogeneous catalysts, such as complexity in preparation, harsh reaction condition, and unstable activity.

Although there are several reviews on microalgae-based biodiesel production technologies with the focus on evaluating the feasibility of using microalgae-based biodiesel to petroleum diesel (Sivaramakrishnan and Incharoensakdi, 2017; Mohan et al., 2015; Chen et al., 2018; Faried et al., 2017; Zhu et al., 2017), this review covered a broad spectrum of oleaginous microorganisms for biodiesel production instead of microalgae, and offered a comprehensive picture of the state of art of biodiesel production from microbial oils, including the advanced cultivation and harvesting methods of holophytic and heterotrophic microorganisms, the latest developments of enhanced lipid production and advanced biodiesel conversion methods based on novel catalysts and the new discovery of biodiesel-synthetic pathways. Meanwhile, the potential benefits, limitations and challenges associated with large scale microbial oil-based biodiesel production were also outline, together with the perspectives and directions forward.

## 2. Microbial oils for biodiesel production

Biodiesel can be produced from various environmentally friendly feedstocks including microalgae, *Jatropha Curcas*, vegetable oil, waste cooking oil etc. (De Oliveira and Coelho, 2017). According to Web of Science, the number of publications on biodiesel derived from microalgae showed an increasing trend in the period of 1991 to 2015 (Zhang et al., 2018). This clearly indicates a growing interest in biodiesel produced from microalgae-based oils.

Microbial oil, known as single cell oil, can be produced by

oleaginous microbes (e.g. yeast, fungus, microalgae etc.) with carbohydrate, hydrocarbon and grease as carbon sources (Subramaniam et al., 2010). Triglyceride made up of polyunsaturated fatty acid (PUFA) has been known as the main component of microbial oil, while the fatty acids with high unsaturation degree (e.g. C16 and C18) are chemically similar as vegetable oils (e.g. rapeseed oil, palm oil, soybean oil etc.) (Wang et al., 2015). These indicate that the quality of biodiesel produced from microbial oil should be generally acceptable. Microbiologically, the oleaginous microorganisms producing lipid can be classified into three different family groups, i.e. microalgae, fungi (moulds and yeast) and bacteria (Ratledge and Wynn, 2002), among which bacteria are less capable of producing lipid as they only can synthesize specific lipid and PUFA. Therefore, microalgae and fungi have been considered as the key lipid producers.

Holophytic microalgae can synthesize lipid in a similar way to the green plants, i.e. they can convert carbon dioxide into autologous biomass through photosynthetic fixation of inorganic carbon, followed by transformation into lipids through a series of induced reactions. This approach has the advantages of high lipid content, short growth cycle and less land demand compared to the processes using the conventional energy crops as feedstock (Chisti, 2007). In addition, heterotrophic microorganisms (e.g. fungi, yeasts, bacteria and heterotrophic microalgae) can all grow on various carbon sources, including organic waste or low-grade biomass, for large-scale lipid production (Ling et al., 2014). To improve lipid production, some advanced microbiological methods, such as cell fusion, mutation breeding, genetic modification, have been explored as well. Fig. 1 showed the processes involved in biodiesel production from the oleaginous microbe including microbial cultivation, harvesting, lipid extraction and biodiesel conversion.

## 3. Holophytic microalgae for lipid production

Holophytic microalgae are single-cell, photosynthetic microorganisms which can utilize sunlight and carbon dioxide to grow and accumulate lipid up to about 70% under the favorable conditions. Generally, the lipid produced by holophytic microalgae is one to two-magnitudes higher than that from the oil-producing crops (Chisti, 2007). The biodiesel production by holophytic microalgae is a multiple-step process involving (i) conversion of carbon dioxide to autologous biomass through photosynthesis, (ii) transformation of carbon into lipids via a series of induced reactions and (iii) transferring intracellular lipid to extracellular lipid. Ultimately, biodiesel can be obtained through catalysis and transesterification of extracted lipid and short chain alcohol (Fig. 1). It should be realized that the uptake of carbon dioxide by microalgae may largely offset the emission of carbon dioxide generated from the combustion of biodiesel, suggesting an achievable carbon-

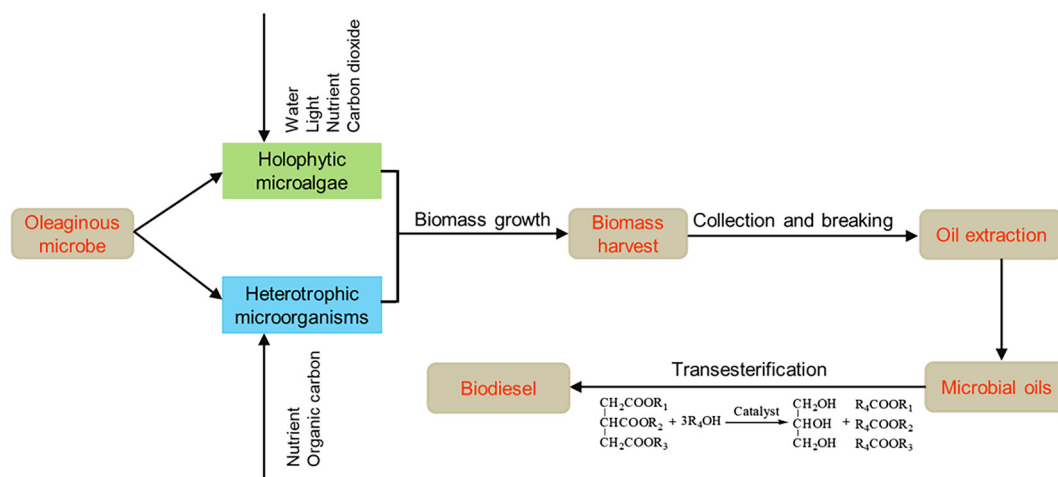


Fig. 1. Processes involved in biodiesel production from oleaginous microbe.

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