



Role of Oleaginous Microorganisms in the Field of Renewable Energy

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The world increasingly requires biodegradable and renewable products in all production fields, with the vast volume of emissions generated by the fuel sector presenting a difficult issue that needs to be addressed. Biodiesel is a sustainable energy source generated by lipids obtained from the biomass of plants, animals, or microbes. The production of biodiesel has undergone multiple stages of development in order to identify the most sustainable method for its generation and utilization. The search for the optimum sources and procedures has been put to the test, and the solution is slowly but steadily approaching. Plants such as palm trees, sugar beets, and corn were originally intended to be utilized as raw materials for biofuel production. Plant cultivation requires arable land and a certain period to grow, which lowers the productivity in terms of g/L/day. Moreover, owing to the global food shortage, this approach was not sustainable, as it required a great deal of fresh water for plant growth. However, microorganisms can produce lipids in a variety of conditions and do not require fresh water to do so [1].

Following this revelation, the use of oleaginous microorganisms as a source of lipids became popular. These microorganisms have sparked the interest of scientists around the world in recent decades as a potential feedstock for renewable bioenergy as well as a variety of other highly valuable ingredients such as omega-3 fatty acids (DHA, EPA), pigments (astaxanthin, lutein, and squalene), vitamins, and proteins for the pharmaceutical, nutraceutical, food, and cosmetic industries. Microorganisms are known to be natural lipid producers, some of which accumulate more than 20% w/w of lipids and are thus considered oleaginous microorganisms. These microbes can synthesize the vast majority of fatty acids from short hydrocarbonated chains (C6) to long hydrocarbonated chains (C36), which may be saturated, monounsaturated, or polyunsaturated fatty acids. The fatty acid composition determines whether the lipids produced by oleaginous microorganisms are used as feedstock for biodiesel production or as nutraceuticals. Thraustochytrids, fungi, and certain microalgae are widely recognized for producing very long-chain PUFA, whereas microalgae, bacteria, and yeasts are mostly involved in the production of lipids suitable for biodiesel.

When it comes to the production of lipids, oleaginous yeasts and microalgae are favorable microorganisms, among others. The genus *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces* contain species of oleaginous yeasts, some of which may accumulate lipids up to 80% w/w of their dry cell weight. Additionally, the lipid metabolism of these oleaginous yeasts is well-known. In an attempt to enhance the economic viability of microbial lipid production, oleaginous yeast strains have been grown on several waste materials and non-edible lignocellulosic biomasses without competing with food sources [2]. For example, *Rhodosporidium toruloides*-1588 was cultivated on an undetoxified hydrolysate of hardwood and softwood sawdust for the production of lipids as feedstock for biodiesel production [3]. In another case, *Candida tropicalis* synthesized lipids when cultivated in olive mill wastewater [1]. This oleaginous yeast not only reduced



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). the organic load and phenols from the olive mill wastewater but also synthesized lipids along with lipase (203 U·L⁻¹) and protease (1105 U·L⁻¹) [1].

The cultivation of microalgae is readily scalable in fermenters or bioreactors, unlike plant cultivation. Microalgae can adopt various modes of nutrition to grow, which can be classified as autotrophic, heterotrophic, mixotrophic, and photoheterotrophic. In autotrophic cultivation, microalgae rely on an inorganic carbon source and energy form photosynthesis to synthesize biomass and valuable metabolites, while in the mixotrophic condition the inorganic carbon source is replaced by organic carbons. Instead of using an inorganic carbon source and light, some microalgae can grow under heterotrophic conditions by using an organic carbon source in dark conditions. Various species of *Chlorella* can easily be cultivated in the waste sources obtained from industries or non-edible lignocellulosic biomass. In autotrophic cultivation, microalgae can transform 9 to 10% of solar energy into biomass with a theoretical yield of approximately 77 $g/m^2/day$, corresponding to 280 ton/h/year, which can be slightly lower with large-scale outdoor or indoor cultivation. In photobioreactors, the actual yield seems to be lowered due to the inaccessibility of the active radiation to each cell. For an equal distribution of light energy to reach all the cells at the same strength and convert the maximal amount of light energy to biomass, the proper shaking and mixing of the culture in the bioreactor is required. As such, a photobioreactor's design is critical; it might vary between different species of microalgae according to their demands for light intensity, temperature, gas mixtures, and so on. Large-scale cultivation can be performed in an open raceway pond, but it is highly affected by contamination [4]. To circumvent this problem, large-scale cultivation has shifted into closed tubular photobioreactors [5]. This type of bioreactor is mainly composed of transparent glass or plastic, where the culture is circulated with a certain velocity. Tubular systems come in a variety of configurations, including single planes of horizontal tubes and several planes of vertically stacked horizontal tubes (fence-like systems). The open raceway pond is primarily used for the generation of microalgal biomasses, which are then processed into biofuel, but the biomass cannot be used for food, whereas tubular bioreactors may be used for both purposes. Due to the combined benefit of water treatment and biomass generation, replacing a commercial cultivation medium with wastewater containing vital nutrients is becoming more popular. For instance, Porphyridium cruentum was cultivated in ultra-filtered swine wastewater to remediate it and simultaneously produce lipids with a fatty acid profile suitable for biodiesel production [6].

Ocean cultivation systems have lately garnered interest for commercial-scale microalgae production owing to benefits such as the mixing of the culture by ocean waves, the use of soluble nutrients, the vast availability of the area, and so on, all of which result in lower cultivation and maintenance costs. Although microalgal cultivation can be scaled-up, it has some drawbacks associated with the photobioreactor's design, the maintenance of axenic cultures, and the cost of the transparent material used in photobioreactors that hinders its application. The heterotrophic cultivation of microalgae results in higher biomass yields—reaching as high as 100 g/L of dry biomass—than those obtained from autotrophic cultivation, as well as higher productivities. Heterotrophic cultures do not require any special type of bioreactor to grow and require 12 times less area to grow than autotrophic cultivation, and can be easily scaled up to 100,000 L. Of course, heterotrophic cultures also have drawbacks in terms of the availability of microalgal species that can grow in the dark, the cost of organic carbon sources, contamination, and the inability to create light-induced metabolites.

Despite the pros and cons associated with the different modes of nutrition for microalgae, they can shift to multiproduct biorefinery, where some products can only be obtained in high quantities under a certain mode of nutrition. For example, protein, carbohydrate, and carotenoids are usually synthesized in high quantities during the autotrophic mode of nutrition, whereas lipids are generally synthesized under the heterotrophic mode. The production of biolubricants, which promote their usage as renewable and biodegradable substitutes for conventional mineral oil-based lubricants, is another sustainable application for microbial lipids [7].

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