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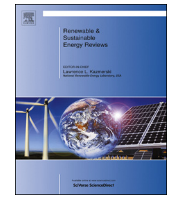

Concepts and studies on lipid and pigments of microalgae: A review

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ABSTRACT

This review describes compounds produced microalgae, such as biodiesel, lipids, fatty acids (FA), triacylglycerides (TAG), and pigments (phycobilins, chlorophylls, and carotenoids). We discuss the factors inducing the accumulation of these metabolites and their economic importance. We focused on cell wall breaking methods of microalgae used to produce biodiesel. A special approach was made to extremophile microalgae used in biodiesel production. The type of methodology used in the cultivation and the use of extremophiles microalgae can permit feasible biodiesel production.

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1. Introduction

The dwindling oil reserves, rising levels of carbon dioxide, and global warming increased governmental interest in renewable energy, such as biofuels. Plants rich in lipids or carbohydrates are used to produce, respectively, derivates of fatty acids and alcohols, such as oxi-compounds. Accordingly, several crops have been used for biofuel production, like sugarcane (*Saccharum officinarum* Linn.), sugar beet (*Beta vulgaris* Linn.), cassava (*Manihot esculenta* Crantz), soybean (*Glycine max* Linn (Merrill), canole (*Brassica napus* Linn var. *oleifera* Moench), and palm (*Elaeis guineensis* Jacq.) [1].

Biodiesel is the main biofuel derived from oils and vegetable or animal fats. Biodiesel is usually produced by transesterification in the presence of methanol or ethanol and alkaline catalysis, resulting in a mixture of alkyl esters from fatty acids [2,3]. The transesterification method was developed in 1937 by George Chavanne, who patented the "Procedure for the transformation of

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vegetable oils for their uses as fuels" [4]. Biodiesel production is mainly based on grease raw materials, such as palm oil, canola oil, animal tallow, and soybean oil [5]. However, the prices of these commodities are regulated internationally and affect biodiesel price [6]. For example, about 80% of biodiesel cost vary with the price of grease raw materials [7], suggesting that new greases sources are needed in order to reduce the biodiesel price.

As a result, there is growing interest in using microalgae for biodiesel production, especially because some produce about 150 times more oil per hectare than soybean [8]. Soybean is the main oilseed used for biodiesel production in Brazil. However, turning biodiesel production from microalgae into an economically viable activity is still a challenge [9].

Producing biofuel from microalgae has many advantages [10], such as: (1) high growth rate; (2) lower demand for water than commercial crops; (3) High efficiency in CO_2 mitigation; (4) smaller areas for cultivation. However, there are also disadvantages, such as: (1) dependence of light incidence and penetration into the aquatic environment to ensure high biomass production (2) difficulty of developing simple and inexpensive procedures to convert lipids into biodiesel.

Additionally, there are other drawbacks related to the selection of species with appropriate production time and yield of biomass, lipids, and pigments. Ideal species would also have biomass easily separable from the culture medium, adapted to low-cost cultivation conditions, and resistant to invasive organisms.

Thus, closed systems (photobioreactors) and open ponds (raceways) are used for microalgae cultivation on a large scale and both have advantages and disadvantages. The cost to build and operate a closed system is higher than ponds. Conversely, it requires less light and area, besides having volumetric productivity about 13 times higher than raceways [11].

Key aspects of lipid metabolism in microalgae have been analyzed to improve biodiesel production. For example: (1) stress may increase lipid content [12]; (2) manipulating the growing medium can increase biomass productivity [13]; (3) some microalgae can survive in extreme environments, which facilitates production in raceway and decreases contamination [14]; (4) the lipid metabolism is known at the molecular level ([15]; and 5) some microalgae produce valuable pigments, such as astaxanthin, lutein, and β-carotene, which can prevent and treat diseases [16].

Thus, the cultivation of microalgae for biodiesel production involves not only the selection and production of species, assessment of types and amount of lipids, but also the potential market for co-products.

2. Lipids of microalgae

Lipids can be classified into two groups: polar and neutral. They are insoluble in water but soluble in most organic solvents. Polar lipids include phospholipids and glycolipids, while neutral lipids include acylglicerids (tri, di- and monoglycerides) and free fatty acids. Microalgae use neutral lipids as energy source and polar lipids to form cell membranes. Nonetheless, microalgae also have fatty-acid free components that are not converted into biodiesel, such as steroids and pigments [17]. As a result, higher production of pigment implies lower production of fatty acids. Therefore, although some microalgae produce high lipid content [18,19], this not necessary means a high biodiesel production. Accordingly, the percentage of lipid content per dry weight of microalgae may range from 1.5% to 75% (Table 1), and a given microalga can produce different amounts of lipids depending on the culture medium and procedure. For example, the lipid content of *Chlorella vulgaris* ranged from 12% to 26% and *Botryococcus braunii* ranges from 14% to 75%.

Table 1
Lipid content in different cyanobacteria and microalgae.

Division	Lipid content (%)	Reference
Cyanophyta		
Lyngbya birgei	12 ± 2.8	[22]
Spirulina maxima	6 – 7	[10]
Spirulina platensis	8.5 ± 2	[22]
Spirulina platensis	4–9	[10]
Synechocystis pevalekii	9 ± 2	[22]
Chlorophyta		
Ankistrodesmus falcatus	1.58	[23]
Ankistrodesmus gracilis	7.9–20.5	[24]
Auxenochlorella protothecoides	39.3 ± 0.8	[25]
Auxenochlorella protothecoides	32.9 ± 0.6	[25]
Botryococcus braunii	14.0–28.6	[26]
Botryococcus braunii	25–75	[19]
Chlamydomonas rheinhardii	21	[10]
Chlorella pyrenoidosa	2	[10]
Chlorella sorokiniana	19	[27]
Chlorella spp.	10.5	[23]
Chlorella vulgaris	12.0 ± 0.56	[28]
Chlorella vulgaris	26 ± 0.5	[25]
Chlorella vulgaris	14 – 22	[10]
Chlorococcum infusionum	11.3 ± 1	[22]
Dunaliella bioculata	8	[10]
Dunaliella salina	6	[10]
Scenedesmus acuminatus	1.58	[23]
Scenedesmus dimorphus	16–40	[10]
Scenedesmus obliquus	12–14	[10]
Scenedesmus obliquus	6.18 ± 0.20	[29]
Scenedesmus quadricauda	1.9	[10]
Spirogyra sp.	11-21	[10]
Spirogyra orientalis	21 ± 2.5	[22]
Heterokontophyta		
Navicula minima	16.2 ± 0.6	[22]
Nitzschia spp	3.68	[23]
Phaeodactylum tricornutum	9.40 ± 1.77	[28]
Euglenophyta		
Euglena acus	5.78	[23]
Euglena gracilis	14–20	[10]
Rhodophyta		
Catenella repens	8 ± 1.5	[22]
Ceramium manorensis	8 ± 1.9	[22]
Geledium pusillum	9.7 ± 2.8	[22]
Dinophyta		
Gymnodinium sp.	29.6	[30]

The main Chlorophyta studied for biodiesel production are unicellular, such as *Auxenochlorella protothecoides*, *Chlorella vulgaris*, *Chlamydomonas rheinhardii*, and *Dunaliella salina*. Since growth rate of these species are faster, lipid productivity is higher than in other divisions. But the divisions Euglenophyta and Dinophyta have potential to be used for biodiesel production, since their lipid amount is greater than some Chlorophyta (Table 1). Cyanophyta are also evaluated for biodiesel production, due to their high cell growth rate. However, some produce toxic and bioaccumulative substances, such as microcystin, which is carcinogen [20].

It is also important to assess the types of fatty acids in microalgae, since they influence biodiesel quality, especially oxidative stability, cold filter plugging point, and contents of mono-, di- and triglycerides [21].

3. Fatty acids in microalgae

Several authors have evaluated fatty acid composition in microalgae [18,30–37]. Fatty acids are carboxylic acids with 4-36 carbons chains. Fatty acids without unsaturations are called saturated (SFA), those with only one unsaturated bond are called monounsaturated (MUFA), those with two are di-unsaturated

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