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Botryococcus braunii for biodiesel production

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ABSTRACT

This paper presents a review on microalga *Botryococcus braunii* (*B. braunii*) with an emphasis on lipid production for possible industrial commercialization in biodiesel production. Summarizing the nature of this alga is fundamental to establish the detail environment conditions for optimal biomass growth and the production of lipids, as well as upstream processing technologies for biodiesel production. This study focuses the attention on identifying possible avenues for further investigation in the design of cultivation systems and the integration of process technologies at industrial scales. The advantages and disadvantages of biodiesel production processes based on these algal lipids are also analyzed. A particularly promising process involving *B. braunii* combines flue gas and wastewaters to produce lipids, converting them to biodiesel by supercritical (SC) direct trans/esterification (esterification followed by transesterification). We conclude that such processes using *B. braunii* have considerable potential for the production of cleaner and more efficient biodiesel than current methods, reducing the environmental impact of fossil fuel use.

Contents

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1. Introduction

The high carbon dioxide (CO_2) concentration in the atmosphere, mainly the result of fossil fuel combustion, is thought to be one of the primary causes of global warming [1]. Biodiesel, being a renewable fuel, presents a promising alternative solution over fossil diesel. Biodiesel is typically generated by chemical reactions (commonly known as trans/esterification) between lipids (*e.g.*, triacylglycerols, abbreviated TAGs), free fatty acids (FFAs), and alcohol (commonly methanol or ethanol), which generate fatty acid alkyl esters. A variety of lipid-rich feedstocks can be used to produce biodiesel. These include: naturally derived crops; refined, used or waste vegetable oils; animal fats; and algae lipids or sewage sludge. Algae are a particularly promising lipid source due to their high lipid content and possibility to fix atmospheric CO_2 through photosynthetic capture. They can also be cultivated on non-agricultural land or in aquatic environments, using wastewater as a nutrient source and without competing with food production or impacting land use.

Algal biodiesel industrial production is still far from being commercially viable, but it appears to be potentially suitable [2,3]. Current problems include: (i) the considerable usage of non-agricultural land, fertilizers and water; (ii) high energy demands; (iii) the relatively high up-front capital costs and the high price of biodiesel; and (iv) the poor transportation, storage, combustion, and drivability performance of biodiesel. Several studies have pointed out that fatty acid methyl esters (FAME) present a variety of problems when applied to automobile fuels [4]. However, the efficiency and quality of biodiesel production are heavily influenced by the lipid extraction and lipid conversion methods used [5–7], and so the choice of feedstock is vital. The development of energy efficient and profitable technologies through process integration is, therefore, a high priority for algal biodiesel production.

The lipid content, the fatty acids (FAs) composition of TAGs and quality (for example, iodine value (IV), cetane number (CN), FFAs content) are the most important factors for selecting the right species of microalgae. Polar and neutral lipids are preferred for the biodiesel production. *Phaeodactylum tricornutum*, *Chlorella vulgaris*, *Chlorella protothecoides*, *B. braunii*, and *Nannochloropsis salina* are identified to produce such lipids [8]. Among all mentioned species, *B. braunii* emerges as a promising lipid source because of its widespread occurrence in aquatic environments and high lipid content. According to an Aquatic Species Program (ASP) report [9], *B. braunii* would not function well as a feedstock for the lipid-based fuel production due to its slow growth (doubling time of 72 h). The ASP also found this alga lipid to be less suitable for transesterification (*i.e.* creating biodiesel), having most of its lipids as C29 to C34 aliphatic hydrocarbons, and less abundance of TAGs and C18 FAs. The FA profiling of *B. braunii* (IBL-C117) is not appropriate for producing a pure American Society of Testing and Materials (ASTM) nor the Brazilian Agency for Fuels and Biofuels (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis, ANP) grade biodiesel [10]. However, newer research shows that the doubling time could be reduced to 48 h and that both algal lipid composition and FA profiling are strongly affected by its environment growth conditions.

The aim of this paper is to obtain a comprehensive picture of *B. braunii* biodiesel production to identify technologies with improved performance indicators. First of all, *B. braunii* is considered as a biodiesel feedstock referring to its nature, cultivation (including contamination problems and nutritional requirements), harvesting, lipid extraction, and treatments to obtain FAMEs. Then, biodiesel production processes based on these algal lipids are analyzed, highlighting both their advantages and disadvantages. It is worth to emphasize that while relevant and readable reviews have been published on the biodiesel production from various algal species [11–14], none has included *B. braunii*.

2. *B. braunii* as a biodiesel feedstock

B. braunii is a green, pyriform shaped planktonic microalga, with colonies growing in the cluster and that can be found in temperate or tropical oligotrophic lakes and estuaries, widespread in freshwater and brackish lakes, reservoirs, ponds, or even ephemeral lakes. *B. braunii* strains can be found in all climate zones except Antarctica [8]. Cell size (length $\times$ width) is in the range of (8–9 $\times$ 5) μm to (13 $\times$ 7–9) μm [15]. Among various micro and macro algae species, *B. braunii* has been identified as the most promising for biofuel production, because of its excellent lipid production capability (content up to 65%, see Table 1). Although this microalga is known for high amounts of hydrocarbons and ether lipids [16,17], its high content of saturated and monounsaturated FAs as well as TAGs [18,19] makes it suitable for biodiesel production. The percentages of saturated, monounsaturated and polyunsaturated FAs in *B. braunii* dry biomass revolve around 9.85%, 79.61% and 10.54%, respectively [10]. Most of the FAs are synthesized during the steady-state phase of algae growth, with the highest content of palmitic and oleic acids located intracellularly, but also in small amounts in the extracellular medium.

Lipid secretion in the extracellular medium is an unusual phenomenon of *B. braunii* because all known species of microalgae have cytoplasmic lipids. For example, the FAs lipids biosynthesis by *B. braunii* begins in the plastid, elongates within the endoplasmic reticulum, transfers to the plasma membrane of the endoplasmic reticulum or *via* Golgi bodies and then secretes to the cell surface [46]. Lipid droplets are seen on the surface of the cell apex [46]. The lipid yield and composition vary with the strain, growth conditions and cell aging [47–49].

3. Toxic blooms and competition

The colonial *B. braunii* is a bloom-forming alga. The cause of the blooms and their subsequent damage to the populations of other organisms has been widely studied [50,51]. Blooms are characterized with a high lipid content, about 40% of the dry biomass, compared with 18% for the culture collection strain [18]. Furthermore, the lipid composition of bloom strains is represented mostly by saturated and monoenoic FAs. The dominant FAs are palmitic, oleic, and linolenic acids. These FAs allow *B. braunii* to dominate in natural environments due to their toxic effects on fish and zooplankton. Nonetheless, *B. braunii*'s blooming nature is advantageous for biodiesel production due to quantity and quality of the