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Microalgal Biomass as a Source of Polyunsaturated Fatty Acids for Industrial Application: A Mini-Review

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Microalgal biomass has been considered a potential source of lipids, proteins, and carbohydrates for different industrial applications. Usually, molecules with a possibility of use in food, pharmaceutical, and cosmetic industry are added-value products that can make microalgae large-scale production economically viable. Examples of molecules present in microalgae biomass are triglycerides rich in Polyunsaturated Fatty Acids (PUFAs) widely studied due to the benefits to human health, including prevention of cardiovascular diseases and diabetes. Some marine microalgae such as *Isochrysis* sp., *Nannochloropsis* sp. and diatom *Phaeodactylum tricornutum* are known to exhibit high quantities of long-chain PUFA such as Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). In addition, some species show a high content of Monounsaturated Fatty Acids (MUFAs) on lipids, as *Botryococcus braunii*, which usually contains more than 50% of oleic acid. Other high-value molecules from microalgae biomass are the anti-oxidants and vitamin precursors named carotenoids, such as β-carotene, fucoxanthin, and astaxanthin. Besides lipid material, algal proteins can be used in nutrition depending on amino acid profile, as well as a source of high-value peptides. Also, polysaccharides are used as food additives as they exhibit anti-oxidant properties. This short review focused on the screening of different microalgae species to produce high-value lipids, aiming long-chain fatty acids production of industrial interest. Some aspects are considered in this work, such as biomass productivity and composition together with information on cultivation conditions. Therefore, it may help researchers and process engineers to select the adequate microalgae strain for application in large-scale plants in the food and pharmaceutical industry.

* 1. Introduction

Microalgae are promising sustainable feedstock for biofuels and other bioproducts once they exhibit high growth rates, are efficient CO2 sequesters, and do not require large areas of arable land to be cultivated. Furthermore, microalgae cells lack hemicellulose and lignin, thus making this biomass conversion advantageous over conventional lignin-cellulosic biomass once it does not require pre-treatment to remove these components.

Despite biofuels from this biomass being vastly studied, the process is usually not economic feasible due to high capital and operation costs involved. Hence, other high-value products obtained by fractioning biomass have been approached to improve the economics of a microalgae biorefinery (Chew et al., 2017; Dibenedetto et al., 2016; Frias et al., 2014; Suganya et al., 2016). Furthermore, environmental applications such as wastewater treatment combined with microalgae growth and harvesting could reduce capital investments. In order to develop this biorefinery, it is first necessary to study the selection of microalgae strains, the manipulation of stress conditions during cultivation to maximize the desired product and type of growth (Chen et al., 2017).

Microalgae are therefore a sustainable carbon source once it can capture CO2 during growth and convert it into different products: biofuels, bio-oil, sugars. Many species are also suitable as a source of high-value bioproducts for food and pharmaceutical industries, such as polyunsaturated fatty acids (PUFA) and carotenoids (Jeon et al., 2016). This work approaches the production of PUFA from microalgae integrated to a biorefinery concept to obtain different co-products thus developing a viable process for industrial applications.

* 1. Food additives: microalgal biomass composition

As discussed, microalgal biomass consists mainly of carbohydrates, proteins, and lipids, which can be applied in different food segments. More recently, they have been explored as a source of bioactive compounds to supplement nutritional and energy needs (Vaz et al., 2016), such as important polysaccharides, biopeptides, fatty acids, vitamins, among others.

Carbohydrates can be divided into mono-, di-, oligo-, and polysaccharides. And the most abundant sugar molecules are glucose, rhamnose, xylose, and mannose (Markou and Nerantzis, 2013). Some microalgae species have important carbohydrates for food consumption, apart from the possibility to produce biofuels by fermentation or another method. For example, brown and red algae may be a source for sulfated polysaccharides with antioxidant activities (Rocha de Souza et al., 2007). In green algae, carbohydrates are usually encountered as starch, which could be applied to produce biopolymers (Di Caprio et al., 2016).

Microalgae are also known as protein-rich biomass, thus are vastly used as animal feed and aquaculture as well as commercialized usually as powder or capsules for food supplementation (Hayes et al., 2017; van der Spiegel et al., 2013). They have an adequate amount of essential amino acids that are interesting for human nutrition, as well as lectins and phycobiliproteins, which are exploited for industrial applications. Furthermore, bioactive peptides with several health benefits, among those anti-hypertensive, anti-oxidative, and appetite suppression can be obtained. Widely commercialized species are *Spirulina* due to high protein content with several health benefits, and *Chlorella* that is rich in both proteins and polyunsaturated fatty acids (Bleakley and Hayes, 2017).

Finally, lipid droplets in microalgae cells are mostly formed by triacylglycerols (TAG) that can be applied as feedstock for several bioproducts, including edible oils. As mentioned, it is first necessary to select a microalgae strain with maximal TAG accumulation and optimal growth conditions (Klok et al., 2014).

Among lipid constituents for food supplementation, some fatty acids, which are building blocks for numerous lipids (de Carvalho and Caramujo, 2018), are known to provide health benefits. The eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are widely known as health beneficial and mostly present on marine microalgae species (Chauton et al., 2015), as from genre *Nannochloropsis*, *Pavlova*, *Isochrysis*, and *Schizochytrium* (Martins et al., 2013; Pinzon-Frias et al., 2015; Ryckebosch et al., 2014). Although it can also be found in high quantities freshwater species, for example, *Chlorella* species (Matos et al., 2016; Vidyashankar et al., 2015). Dietary habits, including PUFA and omega-3 consumption, were already associated with heart disease (Mente et al., 2009), stroke, and diabetes (Micha et al., 2017), embryonic development (Baéza et al., 2017), among others; thus, the importance of studying these sustainable sources.

Other important biomass constituent includes carotenoids, such as astaxanthin, β-carotene, and lutein that have benefits from colouring to antioxidants (Jeon et al., 2016).

* 1. Polyunsaturated fatty acids production from microalgae

Microalgae lipids can be source of PUFA that are mostly essential fatty acids from ω3 groups, such as α-linolenic (C18:3), EPA (C20:5), and DHA (C22:6), or from ω6 group, such as γ-linolenic (C18:3) and linoleic (C18:2) acids (Akoh, 2017). Besides these, other less common PUFA are produced by microalgae, such as C16:2, C16:3, and C16:4 (Yao et al., 2015) or longer-chain C24-C28 (Mansour et al., 2005).

Several microalgae, mainly marine species, are known to produce high quantities of PUFA. Table 1 shows the screening of potential microalgae species that were evaluated aiming these high-value fatty acids production. Seawater-grown microalgae are predominant, but freshwater species, for example *Chlorella vulgaris*, can also be evaluated as PUFA-sources. As observed on Table 1, *C. vulgaris* produced the highest PUFA concentration among the compared studies, and the second highest lipid content on biomass. The PUFA content in microalga lipids usually varies between species and can be found in both polar and neutral lipids (Feller et al., 2018). Besides the screening of microalgae with high PUFA production, growth conditions can improve lipid accumulation, as nitrogen starvation and inorganic carbon availability (Guihéneuf and Stengel, 2013).

Many long-chain PUFA are associated with several health benefits, and its consumption from natural sources as food supplement is indicated from young ages until adulthood. On the other hand, recent studies have focused on adding these essential fatty acids to commonly consumed food, such as dry pasta (De Marco et al. 2018) or producing monoacylglycerols (MAGs) rich in omega-3 PUFA (He et al., 2016; Solaesa et al., 2016). That way, other food ingredients in which these molecules are not naturally encountered could be enriched with these fatty acids. MAGs with PUFA can also exhibit anti-inflammatory and anti-proliferative effects, as indicates studies that produced MAG-EPA, MAG-DHA, and docosapentaenoic acid monoacylglycerol (MAG-DPA) (Khaddaj-Mallat et al., 2017). Consequently, these bioproducts would be interesting not only for food industries where MAGs are widely used as emulsifiers but could also be applied in pharmaceutical and medicinal areas.

Table 1: Brief literature review on PUFA production from microalgal biomass.

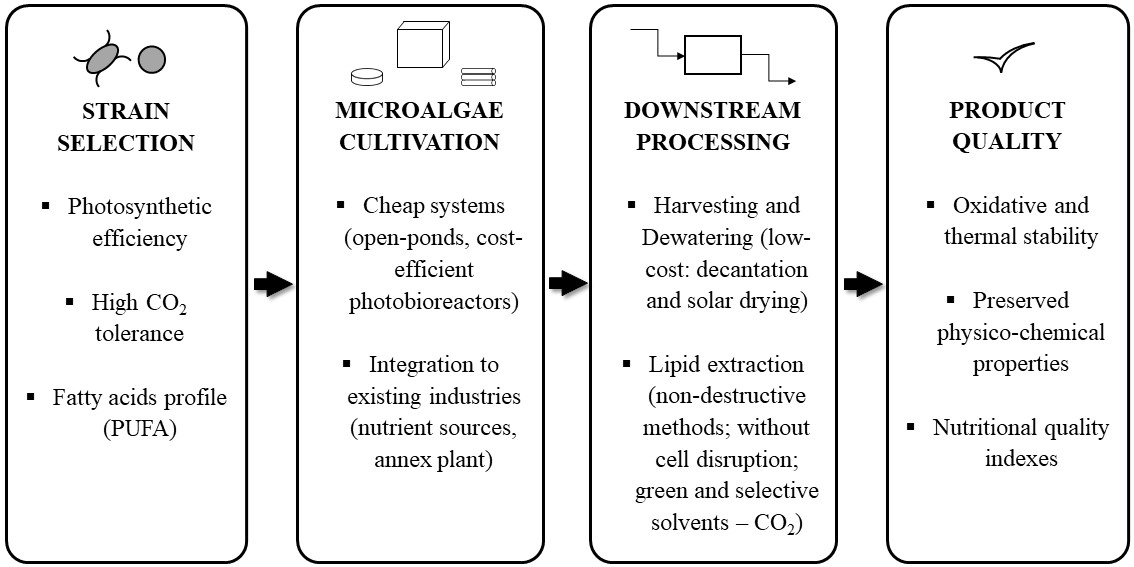
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| Microalgae | Growth conditions and  lipid extraction | Lipids /  wt% on biomass | PUFA /  wt% on lipids | Reference |
| *Chlorella vulgaris* | - | 12.8 | 59.4a | (Matos et al., 2016) |
| *Nannochloropsis gaditana* | - | 8.1 | 4.7a | (Matos et al., 2016) |
| *Nannochloropsis oculata* | 500 L open tank,  subcritical *n*-butane | 1.72 | 35.6 | (Feller et al., 2018) |
|  | - | 15.6 | 36.5a | (Matos et al., 2016) |
| *Nannochloropsis* sp. | - | 8.9 | 14.56 | (Rodríguez De Marco et al., 2018) |
| *Phaeodactylum tricornutum* | 500 L open tank,  subcritical *n*-butane | 1.28 | 25.8 | (Feller et al., 2018) |
|  | - | 14.9 | 42.5a | (Matos et al., 2016) |
|  | 1250 L raceway pond,  Bligh and Dyer | - | 38.4b | (Hamilton et al., 2015) |
| *Porphyridium cruentum* | 180 L cylinder,  subcritical *n*-butane | 2.4 | 38.5 | (Feller et al., 2018) |
|  | - | 5.3 | 59.1a | (Matos et al., 2016) |
| *Spirulina platensis* | - | 5.5 | 32.3a | (Matos et al., 2016) |

aPUFA/Σ(SFA+MUFA+PUFA)

bmol%

* 1. Economic viability for industrial application

Lipids from microalgal biomass is an important viable and vegan source for PUFA consumption. Therefore, it is important to evaluate the economics of microalgae production, which is dependent on biomass productivity and lipid yield (Chauton et al., 2015). For example, among the various growth parameters to be optimized, studies have shown that high oxygen supply is beneficial for cell growth and saturated fatty acids production other than PUFA (Sun et al., 2018). To summarize research findings and advances on process optimization, upstream and downstream steps on microalgae cultivation for lipid production are presented in Figure 1. Techno-economic analysis has pointed to a lower cost for EPA and DHA production using flat panel photobioreactors (FPBRs) with abundant sunlight availability (Chauton et al., 2015), compared to tubular systems and open pond raceway in two geographic locations studied. Moreover, FPBRs also represent environmental and economic advantageous systems regarding reduction of greenhouse gases emissions and of costs, mainly due to high energy demands, respectively (Posada et al., 2016).



*Figure 1: A guide for optimizing steps involved in lipid production from microalgal biomass.*

Besides choosing low-cost unit operations to cultivate microalgal biomass, harvest and extract lipids for further processing, associating microalgae growth to bioremediation, by profiting from waste streams that could be nutrient sources and provide water economy in existing industries, is a key aspect. However, it is essential to guarantee the quality of biomass and specific target products, as already pointed by recent studies (Koutra et al., 2018).

In this work, emphasis was given to a high-value lipid constituent, PUFA. But as mentioned, microalgae primary metabolites (carbohydrates, lipids, and proteins) and pigments can provide several bioproducts to be applied to a wide-range of fields, another step towards the viability of a bio-based refinery (Vuppaladadiyam et al., 2018, Ruiz et al., 2016). Overall, reducing capital and operating costs and high-value co-products obtained from microalgae are the main ideas towards a viable biorefinery.

* 1. Conclusions

Microalgae are a promising feedstock for many valuable bioproducts, among these, PUFA that are health beneficial and until now mostly obtained from animal sources. Several aspects must be addressed to make microalgae production in an industrial scale viable, including optimization of growth conditions and upstream processing to increase biomass and lipid productivities. As well as selecting between different downstream processing to reduce costs while maintaining product quality.

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