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# Spirulina and Its Potential in Bioproduct Production: a Review

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## Abstract

The use of the microalgal biomass in biorefineries can produce different products, such as biofuel, food, feed, and bioactive compounds. In addition, the microalgae used in waste treatment can have its biomass exploited, such as *Spirulina* sp., which shows satisfactory results in the studies performed. Thus, the present study aimed to describe the production of *Spirulina* bioproducts in biorefineries, demonstrating its benefits and its potential for sustainable development. The study utilized an integrative literature review methodology, and the research question was "What bioproducts can be developed from a *Spirulina* biorefinery and how can they contribute to sustainability?". The articles were initially screened based on their title and abstract, and only those that fit the research question were selected for full reading. The reviewed studies suggest that the processing of microalgae within biorefineries has significant potential for synergy, sustainable processes can be developed that maximize the use of microalgae biomass and minimize waste and emissions. *Spirulina* can be used to treat wastewater from food processing plants, where microalgae could absorb nutrients and organic matter while producing biomass that could be harvested and converted into other bioproducts. However, challenges such as high operating costs, variability in biomass and effluent composition, and the need for scalable technologies must be addressed to fully realize the benefits of microalgae in biorefineries. Further research and investment in this area are necessary to develop cost-effective, scalable, and sustainable microalgae biorefineries, involving interdisciplinary collaborations between different fields and partnerships between academia, industry, and government.

**Keywords:** Sustainability, Wastewater, Biomass, Microalgae

## 1. Introduction

Microalgal biomass has gained significant attention as a valuable resource in biorefineries, offering a wide range of products including biofuel, food, feed, and bioactive compounds such as carbohydrates, pigments, oils, and proteins (Costa et al., 2020). However, the widespread utilization of microalgal biomass is still limited by various challenges, such as high production costs, low biomass yields, inconsistent quality and composition, and technical and regulatory barriers (Brasil et al., 2017; Olguín et al., 2022).

One key advantage of microalgae is their remarkable ability to biologically fix $CO_2$, making them vital contributors to the global carbon cycle. These photosynthetic organisms have the capacity to sequester atmospheric $CO_2$ as well as absorb and convert nutrients from aquatic sources and combustion/exhaust gases ($NOx$, $CO$, $CO_2$, and $SOx$) (Patel et al., 2017; Mohan et al., 2020). Consequently, microalgae can play a crucial role in the treatment of industrial waste, which often requires remediation prior to discharge into water bodies (Schmitz; Magro; Colla, 2012).

By harnessing the potential of microalgae for waste treatment, it becomes possible to exploit their biomass and establish circular processes that promote resource reuse, waste management, and sustainable production (Costa et al., 2019a; Esteves et al., 2021). Among the various microalgae species evaluated for this purpose, *Spirulina* sp. has demonstrated promising results, exhibiting high efficiency in nutrient removal and biomass production (Li et al., 2019). Moreover, its integration into circular economy models presents an opportunity to optimize resource utilization and minimize environmental impact.

The circular economy paradigm emphasizes the intelligent and efficient use of resources, aiming to reduce resource extraction, waste generation, and emissions through closed-loop systems (Suárez-Eiroa et al., 2019). By adopting a circular economy approach in biorefineries, the recirculation of resource flows can be maximized, resulting in cost-effective biosystems with enhanced performance and productivity (Costa et al., 2019a; Velenturf; Purnell, 2021). This approach focuses on waste minimization and seeks to generate economic and environmental benefits.

Thus, the objective of this study is to explore the production of bioproducts from *Spirulina* in biorefineries, highlighting its advantages and potential for sustainable development. By elucidating the benefits of *Spirulina* cultivation and the integration of circular economy principles, this research aims to contribute to the advancement of sustainable practices in biorefinery systems.

## 2. Methods and Materials

An integrative literature review methodology was adopted for this study. The review involved analyzing and synthesizing relevant studies from the literature to draw conclusions and develop a hypothesis (Broome, 2000). The methodology followed the steps proposed by Whittemore and Knafl (2005) and was guided by the question: "What bioproducts can be developed from a *Spirulina* biorefinery and how can they contribute to sustainability?"

To conduct the literature search, several databases were utilized, including Web of Science, Google Scholar, and CAPES Periodicals. The searches were not limited by the initial period or document type, and articles written in English, Portuguese, and Spanish were considered. The selection process began with a preliminary screening of titles and abstracts based on the guiding question. Subsequently, the selected articles were thoroughly read, and studies that did not align with the guiding question were excluded from the review.

### *2.1 Microalgae Cultivation for Sustainable Biomass Production and $CO_2$ Biofixation*

Microalgae are photosynthetic microorganisms that require different cultivation conditions and can grow rapidly, even under unfavorable conditions (such as variations in light and temperature), compared to plant sources (Esteves et al., 2021). Through photosynthesis, these microorganisms convert water, carbon dioxide, and light into oxygen and biomass, and different species of microalgae have been identified with unique biochemical or physiological characteristics (Rizwan et al., 2018).

To achieve high biomass productivity, the supplementation of a carbon source, such as $CO_2$, is often necessary for cultivation. The cost of this nutrient can represent a significant portion, approximately 60%, of the total nutrition costs (Costa; Morais, 2011). Therefore, increasing the concentration of dissolved $CO_2$ in the culture media is crucial to enhance biofixation rates (Vargas-Estrada, 2020). The utilization of alternative sources such as $CO_2$ derived from industrial waste can help mitigate the problems associated with $CO_2$ emissions, reduce nutrient costs, and potentially generate carbon credits (Costa; Morais, 2011).

The *Spirulina* genus is classified as a cyanobacterium, which can adapt to different environmental conditions (Vonshak, 1997). Its biomass is often employed in the production of different compounds due to its high protein content (~60 %), but this genus has also been studied for its $CO_2$ biofixation capacity and impact on greenhouse gas reduction (Costa et al., 2019a). In addition, these cyanobacteria have been getting added to cultivation in effluents because they produce a high amount of biomass and have easy cultivation (Nogueira et al., 2018), considering this, among the algal strains considered suitable for large-scale biorefinery production is *Spirulina* (García et al. 2017).

## 3. Result

### *3.1 Microalgae Cultivation and Efficiency*

The stages of cultivation, disruption, and separation of microalgae have production limitations as well as high cost involved. For cultivation, not only carbon sources are needed, in order to occur autotrophic growth, about 30 elements must be present, where macronutrients can be provided in concentrations of grams per liter and micronutrients in milligrams per liter in culture media (Grobbelaar, 2013; Chowdury; Nahar; Deb, 2020).

Various formulations exist for synthetic culture media specific to each microalgae species, while special formulations can also be developed using commercial fertilizers, natural waters, or residual coproducts from other processes (Grobbelaar, 2013; Rosa, 2018). Therefore, achieving an efficient and optimal process necessitates finding a balanced condition among specific operating parameters for each microalgae species, including $CO_2$, pH, salinity, temperature, light intensity and quality, in addition to other essential growth nutrients (Rizwan et al., 2018; Chowdury; Nahar; Deb, 2020).

For the cultivation of *Spirulina* sp. for example, Zarrouk medium is used, composed of: $NaNO_3$, $K_2HPO_4$-$3H_2O$, $MgSO_4$-$7H_2O$, $CaCl_2$-2 $H_2O$, $FeSO_4$-$7H_2O$, 2NaMg, $NaHCO_3$, NaCl, $K_2SO_4$, H3BO$_4$, $MnCl_2$-$4H_2O$, $ZnSO_4$-$7H_2O$, $CuSO_4$-$5H_2O$ and $MoO_3$ (Grobbelaar, 2013). The optimal temperature is in the range of 35-38 ℃, and the minimum temperature for growth is approximately 15-20 ℃. Maintaining pH control above 9.5 is crucial and can be achieved by adding $CO_2$, which not only regulates pH but also serves as an essential inorganic carbon source for photosynthesis and high productivity (Borowitzka, 2018).

*Spirulina* cultivation can be carried out in various types of closed photobioreactors, such as tubular and plate reactors made of glass or plastic, with different configurations including horizontal, vertical, conical, and inclined, and mixing achieved through air pump systems. Closed photobioreactors offer advantages such as large illumination surface, higher productivity, improved mass transfer, and better contamination control (Ugwu et al., 2008).

After the cultivation of the microalgae, their collection is performed, being separated from the culture medium, so that they can be reused in the process and the product proceeds to the next production steps. To perform the treatment of *Spirulina* biomass, prior to its extraction process, its drying is performed by drum-drying, freeze-drying or spray-drying (Rizwan et al., 2018). The rupture step, on the other hand, is necessary to ensure the efficiency of the extraction step, and is performed so that the cellular material of the microalgae becomes available by breaking the membrane and that all components remain intact, using equipment with high energy expense. After the rupture, the separation must be performed, a step responsible for extracting the different components of the microalgae (Vanthoor-Koopmans et al, 2013).

### *3.2 Biomolecule Extraction from Spirulina: Methods and Advancements*

*Spirulina* biomass contains a wide range of biomolecules that possess numerous properties and applications, making them highly valuable for extraction purposes (Vernes et al., 2019). However, during the extraction process, it is crucial to carefully select methods that are fully biocompatible to preserve the bioactivity of the extracted molecules. The versatile microalgal biomass offers the potential to obtain various products, including biofuels that utilize $CO_2$-neutral energy, as well as molecules such as carbohydrates, pigments (chlorophyll, carotenoids, and astaxanthin), oils, and proteins (Costa; Morais, 2011; Vanthoor-Koopmans et al., 2013).

However, microalgal biomass production presents many commercialization challenges, such as the amount of energy used in the process, high cost with nutrients for growth, and harvesting techniques (Vanthoor-Koopmans, et al., 2013; Elrayies, 2018). Recently, new extraction techniques are being developed, which do not use toxic solvents, have shorter extraction time and better performance (Imbimbo et al, 2020).

Solid-liquid extraction of water-soluble bioactive compounds, such as proteins and pigments, is usually performed by maceration in aqueous solvents (Vernes et al, 2019). Lipids and carotenoids, on the other hand, are usually extracted by organic solvents, such as hexane, chloroform, acetone, methanol, and diethyl ether (Saini; Keum, 2018). Conventional extractions usually require organic solvents, long extraction times, and usually need the dried biomass (Imbimbo et al, 2020).

Regarding protein extraction, Käferböck et al. (2020) evaluated the potential of applying pulsed electric fields in enhancing cell disintegration and subsequent extraction of *Spirulina* compounds was investigated. The method enabled a 90% increase in C-phycocyanin extraction, in addition to the extraction of 95.5% of total protein, showing better results compared to the sphere milling method. The fractions obtained also presented higher purity and lower environmental impact than conventional methods.

Similarly, Ayekpam et al. (2021) evaluated the applicability of an eco-friendly method for extraction of phycocyanins from dry biomass of *Spirulina platensis*, employing nanoparticles, polyethylene glycol, and salts. The silver nanoparticle-based extraction resulted in the highest yield and purity, which were higher than conventional extraction. Thus, this method is advantageous over conventional approaches because it is environmentally friendly, safe, and not too expensive.

Larrosa et al. (2018) demonstrated an improved extraction yield of biocompounds from *Spirulina* for biofilm production. The samples exhibited a phycocyanin content ranging from 75.0 to 85.4 $\text{mg.g}^{-1}$ and total phenolic compounds ranging from 41.6 to 41.9 $\text{mg.g}^{-1}$. The biofilm with the most desirable characteristics displayed a tensile strength of 3.69 MPa, water vapor permeability of $1.67\text{x}10^{-11}$ $\text{g.m.s}^{-1}.\text{m}^{-2}.\text{Pa}^{-1}$, and exhibited thermal stability.

Pohndorf et al. (2016) investigated various lipid extraction processes for *Spirulina*, involving different methods of biomass drying (tray and spray bed), cell disruption (microwave, autoclave, and grinding), and solvent extraction (hot and cold). The cold extraction method yielded an average lipid content of 5.8%, while the hot extraction method yielded an average of 1.7%.

For comparison of different polysaccharide extraction methods, Wang et al. (2018) evaluated the hot water, alkali, ultrasound-assisted, and freeze-thaw extraction methods. As a result, the alkaline extraction method showed the best results, with the final extract consisting of 71.65% polysaccharide and 8.54% protein. A brief description of further optimization studies for extraction of biocompounds from *Spirulina* are shown in Table 1 and an illustration of the extraction process is shown in Figure 1.

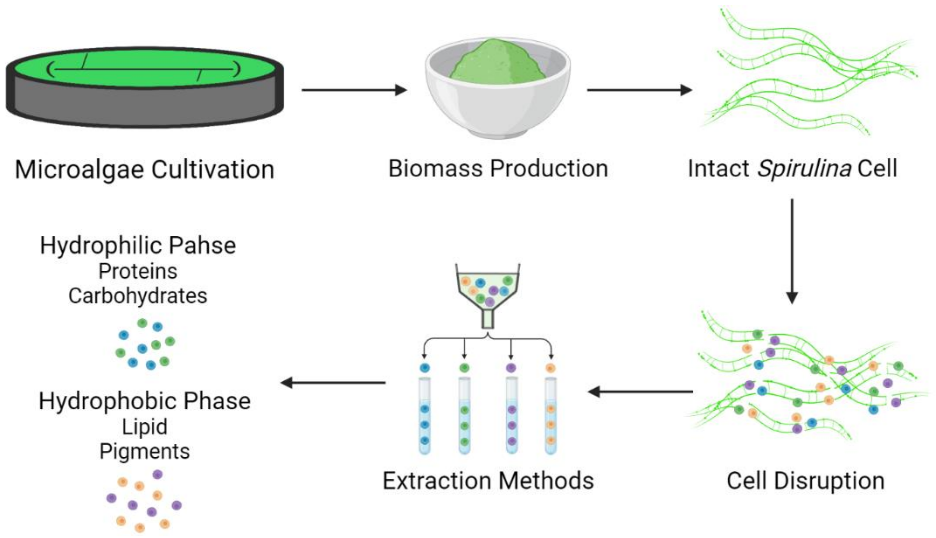

Figure 1. Extraction process of *Spirulina* biomolecules

Table 1. Extraction studies of *Spirulina* biocompounds

| **Compound** | **Method** | **Result** | **Ref** |
|---|---|---|---|
| Lipid | By hot and cold solvent | The cold extraction method showed better results than the hot extraction | Pohndorf et al. (2016) |
| | Solvent-based, ultrasound assisted and mechanically stirred | The integrated method was effective, with the final sample presenting a lipid content of 8.7% | Neag et al. (2022) |
| Protein | By solvent, optimized by ultrasound with heat and pressure | Ultrasound technology allowed higher protein extraction compared to the conventional method | Vernes et al. (2019) |
| | By solvent, with optimization of ultrasound extraction | *Spirulina* protein concentrates had higher desired functional properties than other samples | Yücetepe et al. (2019) |
| Phycocyanin | By utrasound with biphasic liquid flotation | The integrated method increases the efficiency of extraction and recovery of the compound | Chia et al. (2020) |
| | Different solvents as extraction medium, in conventional, ultrasonic and microwave method | The highest phycocyanin content was extracted using $CaCl_2$, one of the samples resulted in a more vivid blue, more phycocyanin and antioxidant activity | Ilter et al., (2018) |
| Carbohydrates | By hot water, by alkali, ultrasound-assisted, and by freeze/thaw | The alkaline extraction method showed the best results | Wang et al. (2018) |
| | Optimize solid/liquid ratio, temperature, and extraction time | Yield of 8.3% polysaccharides, composed of 53% rhamnose and phenolic content of 45 mg GAE.g$^{-1}$ | Chaiklahan et al. (2013) |
| | Optimize alkali extraction with ultrasound and mechanical agitation | The extraction was effective and resulted in 75.76% protein and 41.52% carbohydrate | Lupatini et al. (2017) |
| | Compare polymer aqueous biphasic system (ABS) method with ionic liquid-based ABS | The ionic liquid-based ABS method was more efficient, with the extraction of 83.26% phycocyanin and recovery of 73.89% carbohydrate | Choi et al. (2021) |

## 4. Microalgae Biorefineries Towards Sustainable Production

Biorefineries encompass the production of biofuels and value-added products from biomass, integrating bioprocesses and chemical technologies to minimize environmental impact (Rizwan et al., 2018). In the realm of microalgae biorefineries, the focus is on reducing production costs through integrated processes, waste reduction, and efficient conversion of biomass into energy, chemicals, polymers, feed additives, and more (Costa et al., 2019b).

Although microalgae hold great promise as a feedstock for biofuels, biofertilizers, food, and feed, the high cost associated with manufacturing compounds derived from algal biomass remains a challenge for economic competitiveness. The cultivation of microalgae necessitates artificial lighting and precise temperature control, which introduces expenses that impede the widespread utilization of microalgal biomass (Funk et al., 2021).

Microalgal biorefineries leverage process integration and equipment to extract high-value chemicals from microalgal biomass (Siddiki et al., 2022). Focusing solely on the production of a single product from microalgal biomass is less financially rewarding and constrains economic viability (Kim et al., 2019). Therefore, incorporating recovery processes into bioproduct manufacturing adds value to the value chain and aligns with the goals of the circular economy.

To extract high-value chemicals from microalgal biomass, microalgal biorefineries leverage process integration and specialized equipment (Siddiki et al., 2022). Focusing on producing a single product from microalgal biomass yields limited financial returns and hampers economic viability (Kim et al., 2019). Thus, incorporating recovery processes into bioproduct manufacturing adds value to the value chain and aligns with the principles of the circular economy.

By subjecting microalgal biomass to multiple processing stages, it becomes possible to convert it into primary and secondary commercial products, leading to both environmental and economic benefits (Kim et al., 2019). Consequently, all stages of microalgae exploitation in biorefineries can be developed with a long-term sustainability perspective, aiming to achieve environmental advantages (Mohan et al., 2020).

The utilization of microalgae for the treatment of biorefinery wastewater represents an economically viable and environmentally friendly approach for the production of microalgae-based bioproducts (Clarens et al., 2010). Employing wastewater as a nutrient source for microalgae cultivation offers an efficient pathway for wastewater treatment (Kashyap et al., 2021). Algae efficiently consume nutrients present in wastewater, contributing to bioremediation efforts and reducing costs (Clarens et al., 2010). Cultivating microalgae in wastewater holds promise as a sustainable biorefinery strategy, where the utilization of wastewater as a nutrient source presents an alternative to utilizing microalgae solely for bioproduct production (Kashyap et al., 2021).

### *4.1 Wastewater Treatment and Bioproduct Production from Spirulina*

The methodologies commonly used for the treatment of effluents have several disadvantages, such as overhead costs and energy consumption, which are responsible for about 25 to 40% of the energy costs of industrial plants, plus the use of chemicals during the process, low treatment efficiency and potential waste of resources (Lee et al., 2019; Song et al., 2020; Esteves et al., 2021).

Currently, the use of microalgae for effluent treatment is regarded as one of the most promising technologies for nutrient removal, particularly for phosphorus and nitrogen, as these microorganisms possess a high capacity to recover nutrients from the medium (Li et al., 2019; Song et al., 2020). Recent studies have showcased the bioremediation potential of microalgae in wastewater, demonstrating their effectiveness in removing nutrients, heavy metals, and even carbon capture (Chai et al., 2021).

Among their advantages, microalgae offer the possibility of reusing the biomass generated during operations, eliminating the need to treat it as process sludge. Additionally, microalgae have the potential to contribute to the reduction of $CO_2$ emissions through their biofixation capacity (Ruiz et al., 2013; Esteves et al., 2021). The ability of microalgae to eliminate emerging contaminants is also noteworthy, with different microalgae species exhibiting specific capabilities to eradicate various types of contaminants from the environment (Chai et al., 2021). This process is illustrated in Figure 2.

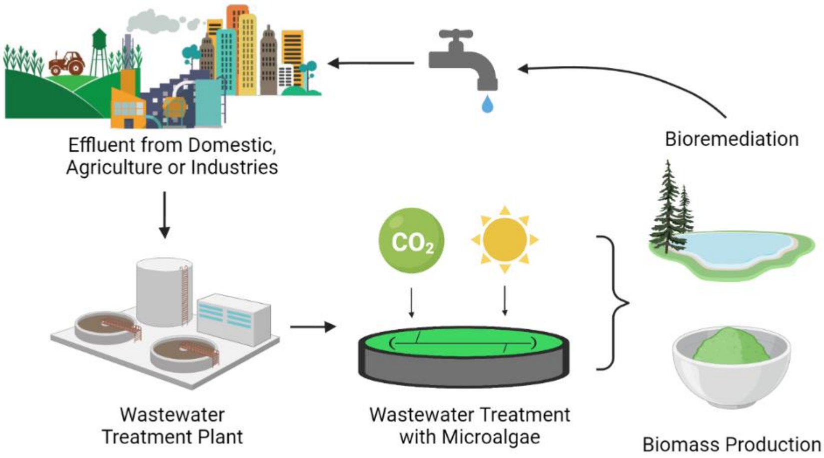

Figure 2. Bioremediation process with microalgae

Almomani and Bhosale (2021) investigated the biosorption efficiency of toxic metals from wastewater using *Spirulina platensis*. The results demonstrated satisfactory removal rates of 95%, 87%, and 63% for Aluminum, Nickel, and Copper, respectively. In another study, Nogueira et al. (2018) examined the cultivation of *Spirulina platensis* in fish farming effluent to reduce inorganic nutrient levels for effluent discharge. The findings indicated that the maximum cell density of S. *platensis* resulted in the production of 0.22 g.L-1 of dry biomass and a maximum productivity of 0.03 g.L-1 day-1, with the nutrient levels in the treated effluent complying with Brazilian environmental regulations for effluent disposal.

Han et al. (2021) conducted a study utilizing *Spirulina* for the treatment of soy sauce wastewater and the production of value-added biomass. The research revealed that microalgae exhibited robust growth when cultivated in 100% raw soy sauce wastewater, with a maximum biomass yield of 1.984 g.L-1. The removal efficiencies for total nitrogen and chemical oxygen demand were 81.76% and 84.08%, respectively.

Furthermore, the maximum protein, chlorophyll a, and carotenoid content were measured as 65.57%, 7.57, and 2.78 mg.g-1, respectively. These studies exemplify the significant benefits of microalgae cultivation in wastewater treatment, as microalgae assimilate various pollutants, and their biomass can be utilized for the production of value-added compounds.

### *4.2 Nutritional and Functional Potential of Spirulina Biomass in Bioproducts*

*Spirulina platensis* is one of the richest sources of protein of microbial origin (Lupatini et al., 2016), and can exhibit beneficial health properties as well as being used as a protein supplement or food ingredient due to its functional properties. The production of microalgae for food and feed is constantly increasing, being consumed in approximately 80 countries and produced in more than 22 countries (Costa et al., 2019b).

The most promising market for microalgae is composed of its use in human nutrition, as a nutritional supplement, for example, and also as a relevant substitute for raw materials commonly used in animal feed (Costa et al., 2019b). There are already commercial products that use *Spirulina* in their formulation, such as M&M's® chocolates, which use the microalgae as a natural colorant, and in cereal drinks from brands such as Nature's Own®, AIK CHEONG and Super NutreMill, to increase the nutritional value of these products (Tang et al., 2020).

The protein content of this microalgae ranges from 50% to 70% of its dry weight (Falquet; Hurni, 1997), these proteins can also be hydrolyzed into bioactive peptides, which have received much attention due to their health benefits and biological activities (Vo et al., 2013). In addition, it is an excellent source of phycocyanin, as its protein fraction contains about 200 $g.kg^{-1}$ of phycocyanin (Su et al., 2014), a pigment commonly used in the food and cosmetic industries as a natural dye due to its blue color, which can also show anti-inflammatory, antioxidant, and anticancer properties (Vonshak, 1997).

Figueira et al. (2011), evaluated the enrichment of gluten-free breads with *Spirulina* biomass and attested an increase of more than 15% in protein content, in addition to an enhancement in the concentration of different amino acids in the enriched bread. In a study by Ilona et al. (2018), the authors evaluated the effectiveness of *Spirulina* in the diet of calves. The content of erythrocytes, hemoglobin, total protein, albumin fraction, and glucose in the blood of calves from experimental groups was higher than the level of the control group. Also, compared to the control group, the level of profitability was higher.

In general, carbohydrates can constitute 15-25% of the dry weight of this microalgae, composed in different concentrations of glucose, fructose, sucrose, glycerol, mannitol and sorbitol (Falquet; Hurni, 1997). Wuang et al. (2016) evaluated the feasibility of cultivating these microalgae with aquaculture effluent for fertilizer production. The remediation potential of was found to be good for ammonium and nitrate removal and *Spirulina*-based fertilizers improved the growth of *Eruca sativa*, *Ameranthus gangeticus*, *Brassica rapa* ssp. *Chinensis* and *Brassica oleracea alboglabra*.

Regarding lipids, *Spirulina* biomass can have a lipid content ranging from 5.6% to 7% of its dry weight. These lipids can be divided into a saponifiable fraction (83%) and an unsaponifiable fraction (17%), which mainly consists of paraffin, pigments, terpene alcohol, and sterols (Falquet; Hurni, 1997). Ananthi et al. (2018) explored the potential of *Spirulina* sp. for biodiesel production and synthesis of silver nanoparticles in a single fermentation process, offering a sustainable manufacturing alternative. The microalgae exhibited favorable physicochemical properties for biodiesel production and demonstrated efficient renewable energy generation. Furthermore, satisfactory synthesis of nanoparticles with antimicrobial properties against pathogens such as *Bacillus* sp., *E.* coli, *Klebsiella* sp., *Proteus* sp., and *S. aureus* was achieved.

*Spirulina* biomass also has other compounds of interest, such carotenoids, mainly composed of beta-carotene (700-1700 mg.kg$^{-1}$, comprising up to 80% of the total carotenoids), the remainder being composed of fucoxanthin and cryptoxanthin. In addition, its biomass is rich in different vitamins, containing, for example, 50-190 mg.kg$^{-1}$ of Vitamin E, 34-50 mg.kg$^{-1}$ of Vitamin B2 and 30-46 mg.kg$^{-1}$ of Vitamin B6. The most relevant minerals in *Spirulina* biomass are iron (580-1800 mg.kg$^{-1}$), calcium (1300-14000 mg.kg$^{-1}$), phosphorus (6700-9000 mg.kg$^{-1}$) and potassium (6400-15400 mg.kg$^{-1}$) (Falquet; Hurni, 1997).

Gad et al. (2011) analyzed the protein extract of *Spirulina* with respect to antioxidant activity, radical scavenging and hepatoprotective effects against $CCl_4$ in vivo, to attest to its use as a functional food for people with liver diseases. The study reaffirmed the antioxidant capacity of *Spirulina*, as well as the possibility of the extract to prevent $CCl_4$-induced liver damage, attesting to free radical scavenging properties and antioxidant activity. According to the authors, the positive effect of its extract is due to its content of proteins, lipids, and carbohydrates, in addition to elements such as zinc, magnesium, manganese, selenium, and vitamins. A compilation of these studies and more is shown in Table 2.

Table 2. Different applications of *Spirulina* compounds for bioproduct production

| **Purpose** | **Result** | **Ref** |
|---|---|---|
| Pigment | The phycocyanin extract showed good properties and good pH stability, the coloring factors were sufficiently low in all samples and the drinks remained stable in cold | García et al. (2021) |
| | The addition of phycocyanin to gelatin referred to a higher moisture content, water activity, but showed a bright blue coloration as well as stability in the production of gelatin candies | Dewi et al. (2018) |
| | The addition of phycocyanin to yogurts decreased Streptococcus thermophilus and *Lactobacillus bulgaricus* counts, allowed for increased firmness, color stability, and no pathogen growth | Mohammadi-Gouraji (2019) |
| Food | *Spirulina* can be used as a functional food for people with liver diseases | Ge et al. (2011) |
| | Increased protein content and amino acid concentration in enriched gluten-free bread | Figueira et al. (2011) |
| | Increased protein, ash, lipid, and carotenoid content in enriched snacks | Lucas et al. (2018) |
| Biofuel | *Spirulina* sp. may have physicochemical properties suitable for biodiesel production | Ananthi et al. (2018) |
| | The biomass had 79.5% conversion, 7.1% biodiesel yield and 290 mL/g for biogas | Sumprasit et al. (2017) |
| | *Spirulina* biomass increased with higher amount of nitrate, but there was reduction in lipids, the density, viscosity, and flash point of biodiesel were 0.78, 4.67, and 107 ℃, respectively | Can et al. (2017) |
| | The direct conversion of *Spirulina* biomass to biomethane had an energy potential of 16 770 kJ.kg$^{-1}$, while bioethanol production from hydrolyzed biomass showed 4 664 kJ.kg$^{-1}$ | Rempel et al. (2019) |
| Animal Feed | The supplementation of feed in diets of red hens can influence the production of more nutritious eggs, obtaining a proper color and no modification of yolk quality | Rey et al. (2021) |
| | *Spirulina* has been shown to be effective in the diet of calves, increasing the content of erythrocytes, hemoglobin, total protein, albumin fraction and glucose in the blood, and increasing the profitability | Ilona et al. (2018) |
| Supplement | *Spirulina* enrichment can produce shakes with increased 4.98% protein and 10.8% ash, contributing to the nutritional supplementation of the elderly | Santos et al. (2016) |
| | The enrichment of supplements for athletes contributed to the increase of minerals (electrolyte repositories), proteins and carbohydrates (enhancers and muscle recovery supplements) | Carvalho et al. (2018) |
| | *Spirulina* supplementation can be used to balance and reduce blood lipids, as well as modify carbohydrates and lower blood sugar levels | Huang et al. (2018) |
| Biofertilizer | There was a significant increase in zinc levels of all *Spirulina* biofortified samples, which were *Amaranthus gangeticus*, *Phaseolus aureus* and Tomato | Anitha et al. (2016) |
| | Potential remediation of ammonia and nitrate from aquaculture effluent, and ability to enhance growth of *E. sativa*, *A. gangeticus*, *B. rapa* ssp. *Chinensis*, *B. rapa* ssp. *Chinensis* and *B. oleracea alboglabra* | Wuang et al. (2016) |
| Bioactive Compounds | Antioxidant capacity and shows potential to prevent $CCl_4$-induced liver damage | Gad et al. (2011) |
| | Antioxidant capacity against DPPH and ABTS radicals and β-Carotene/Linoleic acid system | Machado et al. (2017) |
| | Antimicrobial activity of *Spirulina plantentis* extract could be observed against C. *albicans* and *E. coli* | Pratita et al. (2019) |

## 5. Circular Economy

The circular economy model aims to make the best use of resources by restoring and regenerating the environment (Velenturf; Purnell, 2021). Studies show that, in addition to contributing to sustainable development through achieving several UN Sustainable Development Goals (Schroeder et al., 2019), the circular economy can also function as a tool for sustainable development. In 2019, Suárez-Eiroa et al. proposed a new definition for the circular economy, adapting it for application in a new conceptual model, which presents elements that enable the evaluation of resource use and implementation of process improvements.

The principles of circular economy application were described by Webster (2015), which outlines the minimums that must be guaranteed for the circular economy to be happening, which define that:

1. Every natural capital should be preserved by controlling resource use, prioritizing the use of renewable resources in nature;
2. There should be optimization of resource use, to have a great life cycle, with better performance and subsequent regeneration;
3. The development of products that can be remanufactured, refurbished, and recycled should be encouraged, allowing a circular supply chain.

Studies indicate that using the circular economy can promote economic growth by creating new businesses and jobs, reducing material costs, increasing resource supply security, and reducing the impact on the environment (Kalmykova et al., 2018). Companies already using the circular economy show success stories in reducing waste, providing solutions for new products, reformulating products, and creating digital business models for existing technologies (Velenturf; Purnell, 2021).

### *5.1 Exploiting Spirulina for Circular Economy: Enhancing Yield and Reducing Waste*

Research shows that there are different ways to exploit *Spirulina* to increase its yield and reduce process waste. These studies highlight the potential of integrating microalgae into the circular economy, as discussed in chapters 3.2, 4.1, and 4.2. They investigate the application of *Spirulina* in bioremediation processes, optimization of extraction techniques, cost reduction, and utilization of different compounds present in *Spirulina* to establish a holistic and circular approach within biorefineries.

In addition to these possibilities, scientists have been actively developing sustainable methods that prioritize the conservation of natural resources and minimize pollution associated with the production process. Sequential extractions have been optimized to maximize the utilization of the same biomass, thereby reducing waste generated during the process. For instance, Solis et al. (2021) conducted a study focused on optimizing microalgae biorefineries while considering sustainable economics. Their approach aimed to maximize profitability and minimize environmental impact by emphasizing resource recovery and recirculation. The study employed a life cycle assessment methodology, encompassing microalgae cultivation, utilization, and disposal stages. Notably, the research explored the production of biodiesel, glycerol, biochar, and fertilizer as potential end-products.

From the study, it appears that the production with the greatest environmental impact is with cultures in photobioreactors and with centrifugation, since they consume more energy. Land use also contributes significantly to the overall environmental impact, primarily due to nutrient utilization during cultivation. Among the different process routes investigated, cultivation in open lagoons with flocculation harvesting and solvent extraction emerged as the approach with the least environmental impact. This highlights the importance of considering cultivation and extraction techniques in achieving a balance between profitability and environmental sustainability within the biorefinery system.

In another study conducted by Chaiklahan et al. (2018), an economic feasibility analysis was conducted for the extraction of phycocyanin, lipids, and polysaccharides from *Spirulina*. The study revealed that the yield of phycocyanin was 8.66% of the biomass dry weight, while the yields of lipids and polysaccharides were 3.55% and 0.72%, respectively. Importantly, the economic analysis indicated that the production of phycocyanin alone was already economically viable, with a production cost of $249.70 per kilogram. Furthermore, it was suggested that the phycocyanin content should not fall below 15% of the dry weight to ensure a positive return on investment.

In a study conducted by Ferreira-Santos et al. (2021), the focus was on optimizing the sequential extraction of various compounds from *Spirulina*, including phycobiliproteins, phenolics, chlorophyll, carotenoids, lipids, and ashes. Three different methodologies were employed to disintegrate and rupture the microalgae cells: conventional aqueous extraction, ohmic heating, and enzymatic treatment. The aim was to intensify the extraction process and facilitate the use of sustainable technologies, ultimately leading to the recovery and isolation of different microalgae fractions. This approach aligns with the principles of waste reduction and the application of circular economy strategies.

In a study by Hultberg et al. (2017), the authors tested the integration of the biogas effluent remediation process as a source of nutrients during *Spirulina* cultivation compared to standard microalgae cultivation. There was a higher concentration of the pigment phycocyanin in the effluent-based medium, there were no statistical differences in the protein and amino acid concentration of the media tested, and there was a slight increase in the total amount of lipids in the biomass produced in the effluent-based medium. The authors conclude that the effluent from the biogas process supported good growth of *Spirulina*, producing biomass of sufficient quality for biogas production.

In the same vein, Bose et al. (2022) proposed a circular methodology to produce biofuel (biomethane), biofertilizer (digester) and food (*Spirulina* powder) using agricultural feedstock (grass silage and cattle manure slurry). After optimization, the authors defined a methodology that enables $CO_2$ emission reduction with good productivity. Furthermore, the authors conclude that *Spirulina* outperforms most protein food alternatives in terms of $CO_2$ emissions, terrestrial and aquatic footprints. Alobwede et al. (2019) evaluated the possibility of applying the circular economy to the recycling of biomass for use on agricultural land for enhancing soil quality and nutrition of cultivars. The authors attested that *Spirulina* enabled the increase of total nitrogen, phosphorus, and nitrate in soil.

Aiming to find an alternative to the use of freshwater, Bezerra et al. (2020) evaluated the cultivation of *Spirulina* in seawater without added nutrients, which showed growth ($X_{max}$= 2.17 $g.L^{-1}$), an increase in carbohydrate content and productivity of 203% and 52%. This demonstrates that seawater is an alternative growth medium, with additional advantages for biomass and carbohydrate production.

## 6. Challenges

Microalgae offer a sustainable and promising alternative for the production of bioproducts, and ongoing research aims to explore their full potential within biorefineries. Integrating microalgae cultivation with effluent treatment in biorefineries presents an opportunity to harness their benefits in a more sustainable manner. However, to fully realize the potential of microalgae production, further studies are needed to evaluate scalability. Currently, limitations such as high production costs, low biomass yields, inconsistent quality and composition, and technical and regulatory barriers hinder large-scale production (Brasil et al., 2017; Olgu ń et al., 2022).

The economic viability of microalgae production processes remains challenging to define precisely. Establishing economic parameters for *Spirulina* and other microalgae cultivation requires comprehensive consideration of all biomass processing steps and specifications for their utilization (Costa et al., 2019b). Moreover, the high cost of microalgae cultivation is attributed to the complex cultivation process, nutrient demands, and energy-intensive harvesting and extraction methods (Gifuni et al., 2018).

Besides, the possibility of contamination, the nutrient removal mechanism, and impurities in the biomass after processing are just some of the challenges that need to be overcome (Javed et al., 2019). The use of wastewater for microalgae cultivation can result in the accumulation of contaminants in the algal biomass, making it unsuitable for use in animal feed or food supplements. As wastewater can contain a range of pollutants, these contaminants can accumulate in the microalgae biomass and may pose a risk to human and animal health if consumed, requiring further studies to enable contaminant removal mechanisms for biomasses intended for human and animal consumption (Brasil et al., 2017).

Contamination of biomass can occur due to adsorption of contaminants on the surface or due to intracellular accumulation. It is important to identify strategies and develop methods to reduce the concentration of contaminants in the growth medium or to manipulate culture conditions to inhibit contaminant uptake by microalgae, thereby mitigating biomass contamination (Markou et al., 2018).

Another challenge is that effluent characteristics are determinants of the efficiency of microalgae-based systems for waste treatment. These characteristics include the concentration of nutrients and pollutants, turbidity, color, and pH, which must be characterized after cultivation to determine the optimal microalgae cultivation conditions (Molinuevo-Salces et al., 2019). In addition to the variability in wastewater composition, there is also with respect to microalgae, which can affect the quality and quantity of biocompounds that can be produced, making it challenging to develop standardized production processes that can be economically viable and scalable (Gifuni et al., 2018).

In addition, there is a need for policy creation and regulatory frameworks to support the development and adoption of microalgae-based biofuels (Gifuni et al., 2018), as well as more incentives for biofuel production, which will boost the development of microalgae-based biofuel industries (Brasil et al., 2017).

In order to be able to realize biomass integration in large-scale industries, it is necessary to overcome these challenges in microalgae cultivation, such as the possibility of integrating wastewater treatment industries with biomass production technologies.

## 7. Conclusion

The reviewed studies suggest that there is significant potential for synergy in the processing of microalgae within biorefineries. By adopting circular economy principles such as waste valorization, closed-loop systems, and renewable energy integration, sustainable processes can be developed that maximize the use of microalgae biomass and minimize waste and emissions. For example, using *Spirulina* to treat wastewater from food processing plants, where microalgae could absorb nutrients and organic matter while producing biomass that could be harvested and converted into other bioproducts.

The production of a wide range of bioproducts from microalgae can also be supported by advances in biotechnology, enabling increased productivity, quality, and diversity of microalgae-derived products such as biofuels, bioplastics, nutraceuticals, and pharmaceuticals. However, some challenges need to be addressed if the benefits of microalgae in biorefineries are to be fully realized, such as high operating costs, variability in biomass and effluent composition, the need for robust and scalable technologies, and the possible risks associated with large-scale microalgae production when used in effluent treatment.

To achieve cost-effective, scalable, and sustainable microalgae biorefineries that contribute to the transition towards a circular and bio-based economy, interdisciplinary collaborations between biologists, engineers, chemists, and economists are essential. Partnerships between academia, industry, and government entities are also crucial for fostering innovation, knowledge exchange, and the implementation of supportive policies and regulations.

In conclusion, the future of microalgae biorefineries holds great promise for the efficient utilization of biomass, waste reduction, and the production of valuable bioproducts. Continued research, technological advancements, and collaborative efforts are necessary to overcome challenges, optimize processes, and pave the way for a sustainable and economically viable microalgae-based bioeconomy.

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## Table of Abbreviations

ABS: Aqueous Biphasic System

ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

COD: Chemical oxygen demand

DNA: Deoxyribonucleic acid

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

E. coli: Escherichia coli

GAE: Gallic Acid Equivalent

NOx: Nitrogen oxides

OD: Optical density

PBR: Photobioreactor

Ref: Reference

RNA: Ribonucleic acid

S. *platensis*: *Spirulina platensis*

sp.: Species

UN: United Nations

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