UNIVERSITY OF SANTA CRUZ DO SUL - UNISC
GRADUATE PROGRAM IN ENVIRONMENTAL TECHNOLOGY
DOCTORATE

Maiara Priscilla de Souza

EVALUATION OF THE BIOPRODUCTS OBTAINED FROM PERIPHYTIC BIOMASS HARVESTED IN AN ALGAL TURF SCRUBBER SYSTEM (ATS)

Santa Cruz do Sul
2021

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Doctoral Thesis presented to the Graduate Program in Environmental Technology, in the area of concentration on Environmental Technology Management, in the research line Microbiology Applied to Environmental Technology, of the University of Santa Cruz do Sul, as a partial requirement to obtain the title of Doctor in Environmental Technology.

Advisor: Dra. Rosana de Cassia de Souza Schneider
Co-advisor: Dra. Lisianne Brittes Benitez

Santa Cruz do Sul
2021

ACKNOWLEDGMENTS

GRATITUDE!! This is the first word I can express (now, and daily)! Today I finish another cycle, the Doctorate! Every day that I spend, I realize that this achievement is not only about the final result, but also about the entire path traveled until reaching this purpose.

At this end of this cycle, I would like to thank you for all the learning, not only in the academic/professional area, but in life! Some of my carefully planned goals were often not achieved, but they made me realize that it was exactly what I needed at that moment, to reach other goals that I didn't even consider.

I have learned in recent years, that no reward comes without effort and that the time wasted complaining about nonsense can be successfully converted into time to thank and get your hands dirty to achieve goals and make dreams come true. I realized that the great ally of this journey is inner peace, and that is priceless, so any spark that might disturb it must be erased. I realized that my squad of angels and beings of light to whom I pray so much, are not only on the spiritual plane as I imagined, but also living with me daily, through family, friends, colleagues and teachers.

Again, I summarize all this cycle in one word: GRATITUDE! For everything I've learned and for the people who make all the difference in my life ...

> "Tem gente que Deus coloca na nossa vida só pra nos dar paz. Que nos empurra para o melhor de nós, que nos guia para o caminho do bem. Gente que é sorriso em dia feio, e que é suporte quando parece faltar chão."
>
> (Autor desconhecido)

ABSTRACT

Effluent treatment is a worldwide trend, which is driven by economic, environmental and social gains. In this context, waste biomass valorization resulting from a bioremediation process is receiving attention since the microorganisms present in this process plays an important role in nutrients sequestration and can present a biomass with high-added value. Consequently, this could open up numerous possibilities for developing innovative products and sustainably strengthening the production of bioproducts. Considering these aspects, this research aimed to obtain bioproducts from periphytic biomass harvested in an ATS system, installed in the Lago Dourado Reservoir, Santa Cruz do Sul, RS, Brazil. For this, the thesis was subdivided into 5 manuscripts; each of them contributed to the strengthening of aspects necessary for the construction of this study. This division was made in the following items: (1) a review manuscript with bibliometric analysis about the main bioproducts that can be obtained from microalgae and the main areas that they can be applied; (2) a review manuscript about clean technologies associated with microalgae, to verify the best tools to have a cleaner process; (3) a research article that explores the identification, extraction and quantification of the main bioproducts that can be obtained from the periphytic biomass; (4) manuscript with a case study that associated the results found in manuscript 3 with data from obtained from industries to verify which of the bioproducts studied during the thesis, would have more chances of successfully entering in the market; (5) manuscript that analyzes the life cycle assessment of pigments production on a pilot scale, considering that this bioproduct was found in large quantities in the periphytic biomass. As general results, all the studies were fundamental for building knowledge on the main themes that would be worked on during the thesis. In addition, the possibility of already having publications of these studies shows that this research is of interest and that it can be used as a basis for building new knowledge for future studies. Besides, scientific and technological expertise can transform these bioproducts into services for the economic development of society, focusing on environmental issues and focusing on a cleaner production process.

Keywords: periphyton; biomass; waste valorization; bioproducts; clean technologies; Algal Turf Scrubber system.

RESUMO

O tratamento de efluentes é uma tendência mundial, a qual é impulsionada por ganhos econômicos, ambientais e sociais. Neste contexto, é importante a valorização de biomassa residual proveniente de um processo de biorremediação uma vez que os microrganismos presentes neste processo podem auxiliar no sequestro de nutrientes e apresentar uma biomassa com alto valor agregado. Como consequência, isso poderia abrir inúmeras possibilidades para o desenvolvimento de produtos inovadores e fortalecendo a produção de bioprodutos de forma sustentável. Considerando estes aspectos, este presente trabalho tem como objetivo principal valorizar uma biomassa perifítica remanescente de um processo de biorremediação, através de um sistema ATS, instalado em um lago de captação e abastecimento da população. Para isso, esta tese foi subdividida em 5 manuscritos, cada qual contribuído para o fortalecimento de aspectos necessários para a construção deste estudo. Essa divisão foi feita nos seguintes itens (1) manuscrito de revisão com análise bibliométrica sobre os principais bioprodutos que podem ser obtidos a partir de microalgas e quais os seus principais setores de aplicação; (2) manuscrito de revisão sobre as tecnologias limpas associadas as microalgas, de modo a verificar as melhores ferramentas para se ter um processo mais limpo; (3) artigo prático que explora a identificação, extração e quantificação dos principais bioprodutos que podem ser obtidos na biomassa perifítica alvo deste estudo; (4) artigo que realiza um estudo de caso associando os resultados encontrados no artigo 3, e com associações de dados de indústrias a fim de verificar quais dos bioprodutos estudados durante a tese, teriam mais chances de ingressar com sucesso no mercado; (5) manuscrito que realiza a análise do ciclo de vida dos pigmentos, em uma escala piloto, considerando que este bioproduto foi encontrado em grande quantidade na biomassa perifítica; Como resultados gerais, todos os estudos foram fundamentais para construção do conhecimento sobre os principais temas que seriam trabalhados durante a tese. Além disso, a possibilidade de já se ter publicações destes estudos, mostram que essa pesquisa é de interesse, e que pode servir como base para a construção de novos conhecimentos para estudos futuros. Além disso, conhecimento científico e tecnológico construídos, são uma possibilidade de transformar estes bioprodutos em serviços para o desenvolvimento econômico da sociedade, com um olhar pautado nas questões ambientais, focando em um processo de produção mais limpa.

Palavras-chave: perifíton; biomassa; valorização de resíduos; bioprodutos; tecnologias limpas; sistema Algal Turf Scrubber

SUMMARY

1 INTRODUCTION

The continued dependence on the energy sector leads to the search for alternatives that make it possible to minimize the emission of pollutants to the environment and, at the same time, make new energy sources available (Gielen et al. 2019). Due to the growing demand for these innovations, research in the environmental field is increasingly encouraged; however, developing new proposals is a very challenging task. Microalgae are considered a promising alternative in this field, as they can bioremediate while producing biomass that can be used for the production of compounds of commercial interest (Davis et al. 2011, Rawat et al. 2011, Tibbetts et al. 2015).

In recent years, many news reports from newspaper sources have reported a growing concern about the accumulation of algae and microalgae in various locations in Brazil (GLOBO, 2010; TRIBUNA (2010); GAZ, 2016) [1,2,3]. Although they are part of the ecosystem and are beneficial when in adequate concentrations, the proliferation of algae has become a problem, as it grows rapidly in rivers and lakes responsible for supplying water to the population. Therefore, water can have color, taste, and odor outside legal standards, which has adverse effects on human health. Recently, an algae proliferation problem has been reported in rivers in the Southeast and Midwest of Brazil, which was hampering the operation of hydroelectric turbines. To solve this problem, algae were transformed into raw material for bio-oil production [1].

The combined use of the Algal Turf Scrubber (ATS) system to remove nutrients from eutrophic waters and biomass production can reduce the costs involved in the process. The development of bioproducts from periphyton, obtained in the ATS system, is an adequate alternative since it is considered economically viable if compared to photobioreactors (Adey et al. 2011).

The biomass valorization for pharmaceutical, food, cosmetics and biofuels industries is becoming increasingly popular as it provides the possibility to reduce environmental impacts and diversify energy sources (Rawat et al. 2011). According to Song et al. (2021) the global bioenergy demand will increase significantly by almost 3-fold by 2060 and to follow this drastic increase, sustainable biomass resources such as

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[1] GLOBO. Usinas hidrelétricas transformam plantas aquáticas em biocombustível. <**https://g1.globo.com/jornal-nacional/noticia/2019/08/05/usinas-hidreletricas-transformam-plantas-aquaticas-em-biocombustivel.ghtml>, Acessado em 06/08/2019, 2019.**

algae, crops and wastes have to be used as efficiently as possible.

Water treatment through ATS, followed by biomass for the production of different bioproducts, can minimize the impacts related mainly to microalgae blooms due to the eutrophication of water bodies. This problem is reflected in the quality of the water consumed by the population. In this way, if the ATS is efficient, an adequate use of biomass can be done. Consequently, this combination will improve the economic and environmental aspects of water catchment in natural or artificial water reservoirs (Zhang et al. 2020).

Several studies demonstrate the variability in microalgae biomass exploitation such as vitamins from microalgae *Nannochloropsis* sp., *Pavlova pinguis, Stichococcus* sp., *Tetraselmis* sp. (Brown et al. 1999) and *Spirulina platensis* (Kumudha et al. 2010)*;* antioxidants using seaweed *Ecklonia radiatta;* pigments through microalgae *Phormidium autumnale* (Rodrigues et al. 2015); fatty acids and amino acids with *Chlorella* sp., *Chlorella saccharophila, Chlorella minutíssima* and *Chlorella vulgaris* (Hempel et al. 2012)*;* carbohydrates through microalgae *Kirchneriella* sp. (Frampton et al. 2013) and seaweed *Ecklonia radiatta* (Charoensiddhi et al. 2015); and bioproducts obtained simultaneously (nutrients, fatty acids, pigments, carbohydrates and proteins) with *Kirchneriella* sp. (Frampton et al. 2013).

Periphyton is composed of a variety of autotrophic and heterotrophic organisms that grow on surfaces in aquatic environments. It can be formed by benthic freshwater photoautotrophic algae and prokaryotes, by heterotrophic, chemoautotrophic organisms, fungi, protozoa, metazoans and viruses group (de Souza et al. 2020). This periphyton configuration ensures the elements fixation and assists in the biomass nutrients composition, demonstrating the possibility of remediation use. In this way, the water bioremediation using local biota is a useful alternative for contaminants removal of, since the existing technologies are usually not sufficient to carry out a complete treatment of these substances. These microorganisms, in addition to assimilating carbon, nitrogen and inorganic phosphorus for their development, also have properties to remove heavy metals and organic substances (Kumar et al., 2020; Lu et al., 2020).

Considering these aspects, the research developed in this doctorate thesis, is linked to line 4 (Microbiology applied to Environmental Technology) of the Postgraduate Program in Environmental Technology. This association constitute a tool to enable the ATS system as a treating method for eutrophic waters and with the possibility to obtain high-added value compounds of biotechnological interest. It is also noteworthy that this research presents innovation since, until now, there are few initiatives to effectively use the periphytic biomass from a bioremediation system, to obtain bioproducts.

Thus, the present thesis was divided into the introduction, main and specific objectives, methodology, manuscripts developed, final considerations and future perspectives. This information was organized in chapters that present the thesis in a general aspect and, chapters that correspond to each manuscript built to account for the proposed objectives.

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2 THESIS OBJECTIVES

2.1 General objective

To assess the potential for bioproducts obtention from periphytic biomass harvested in an ATS pilot system installed in the Lago Dourado Reservoir, Santa Cruz do Sul, RS, Brazil.

2.2 Specific objectives

- To conduct a bibliometric study of the main trends about bioproducts from microalgae and environmentally friendly methods associated with clean technologies;
- To evaluate the lipids, proteins, carbohydrates, antioxidants, pigments, and metals in periphytic biomass;
- To extract the major components of the ATS biomass considering environmentally friendly methods;
- To characterize bioproducts by liquid chromatography with diode array (HPLC-DAD), gas chromatography-mass spectrometry (GC-MS), ultraviolet spectrometry (UV-Vis), fourier transform infrared spectroscopy (FTIR) and inductively coupled plasma optical emission spectrometry (ICP-OES);
- To use multiple-criteria decision analysis (MCDA) to verify which of the target bioproducts are most likely to be placed on the market, on an industrial scale;
- To assess the possibility of adapting the optimized system to a pilot-scale through life cycle and economic assessment.

3 METHODOLOGY

3.1 Research outline

At first, the main concern was to reinforce the theoretical basis through articles that that could consolidate information about the subjects that would be the target of this study. For this, bibliometric mapping was used to obtain information about the relationship between microalgae and bioproducts, and microalgae with clean technologies. The periphyton is mainly formed by microalgae, then the searched from information were focuses on these microorganisms during the thesis has predominance of microalgae in its composition. After these initial researches, it was possible to develop two review articles (Manuscript 1 and Manuscript 2).

From the initial knowledge obtained, the periphytic biomass analysis formed during the bioremediation process in the ATS system (Manuscript 3) was performed. Antioxidants, carbohydrates, lipids, pigments, and proteins were the main bioproducts evaluated. In addition, ash and metals were also analyzed.

Through the data obtained in Manuscript 3 and with information from relevant data obtained in recent industries reports and scientific articles, the multicriteria decision analysis statistical tool was applied in order to verify which of the target bioproducts of this study could be more able to be commercialized on an industrial scale (Manuscript 4).

Based on the results of Manuscript 4, Life Cycle Analysis was performed for the production of pigments from the periphytic biomass formed in the ATS system (Manuscript 5). Thus, it was possible to obtain more data to produce this bioproduct on a pilot-scale.

The main process of the methodology can be seen in Figure 1.

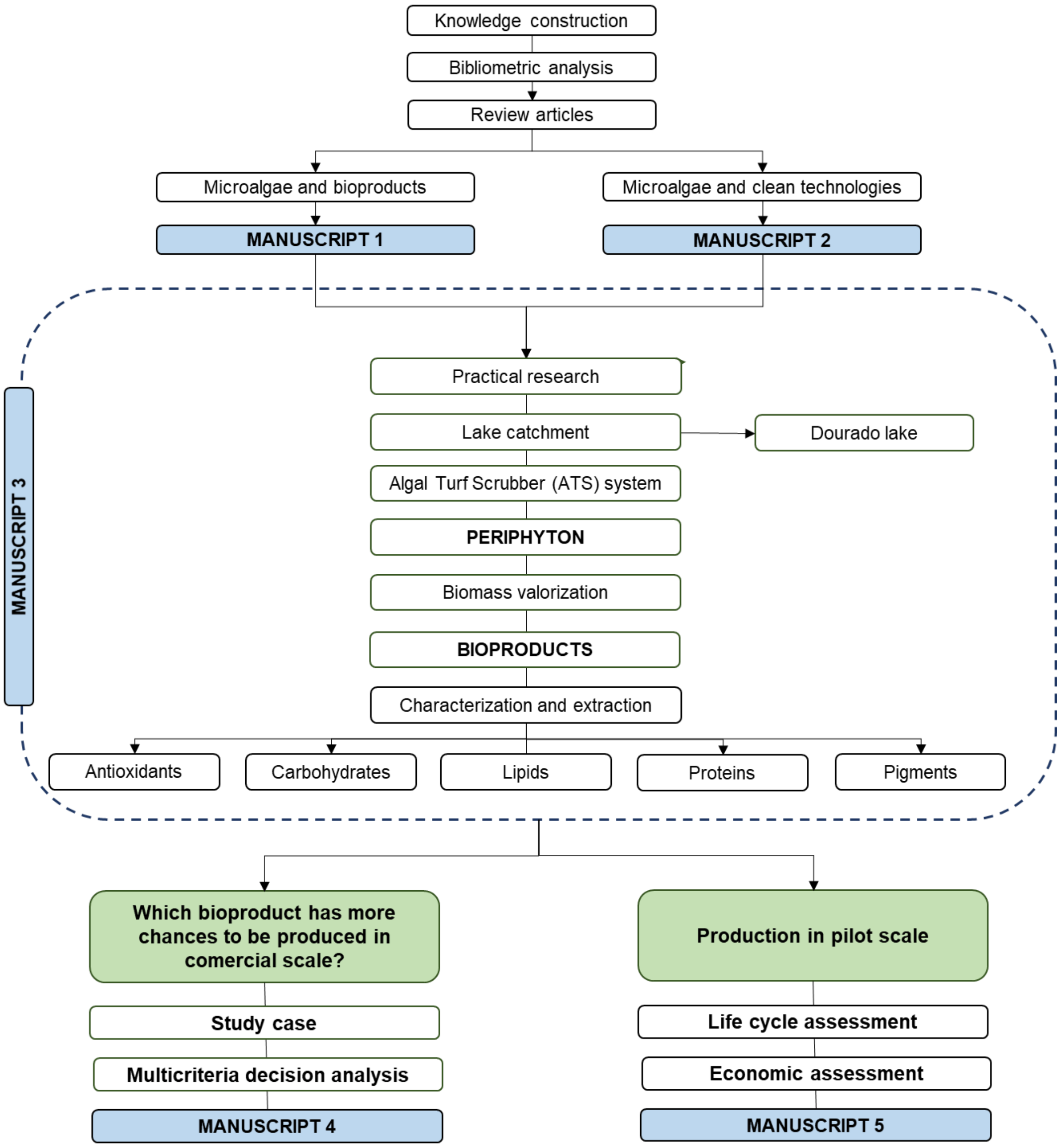

Figure 1 – General methodology of thesis development

3.2 Origin of the biomass used in the thesis development

The biomass used in this study was obtained from a previous study already published by our research group (Martini et al., 2019). This biomass was produced in an ATS system on a pilot scale (Fig. 1), installed in the Lago Dourado reservoir, Santa Cruz do Sul County, RS.

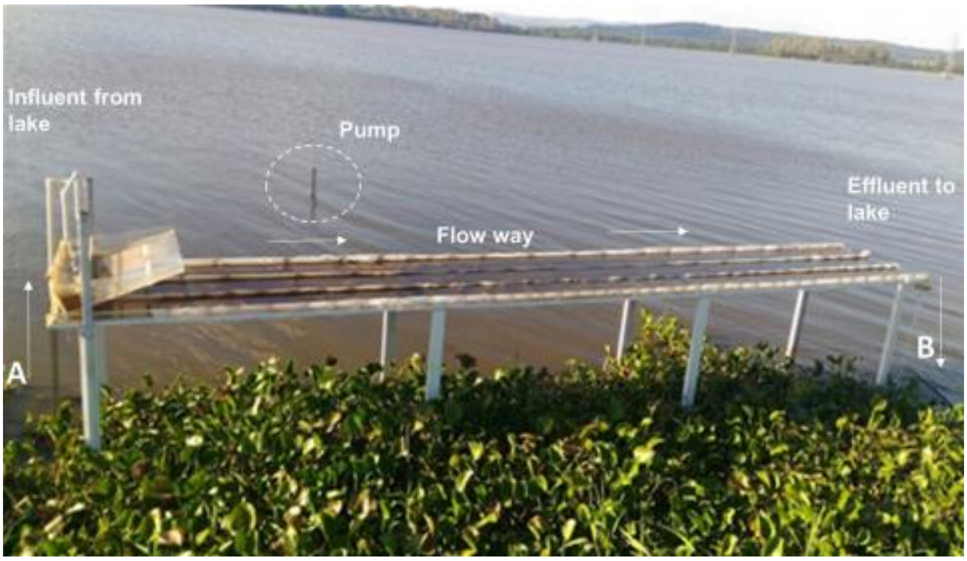

Figure 1 - ATS pilot system used in this thesis in Dourado Lake (Martini et al., 2019).

The dimensions of the ATS system were 5-m long and 1-m wide. The system was constructed by laying 3-mm thick sheets of polymethyl methacrylate (acrylic) on an iron structure with a 0.5% declining grade. The ramp was divided into three flow ways of 0.3 m each, for triplicate analyzes. A layer of a 0.27-mm nylon mesh screen was placed on the top of the acrylic to act as a periphyton attachment. The flow ways received water from Dourado Lake at a flow rate of approximately 2 L min^{-1} (Martini et al., 2019).

The biomass was harvested harvested manually at intervals of 1–3 weeks in hot season (March, August, and September) and cold season (April to July) in the southern hemisphere. In the hot season, the maximum temperature was 28.6 °C and the minimum temperature was 15.7 °C. The collected periphyton was maintained in a plastic bag. The biomass was spread out in trays and brought to a drying oven (model SL 101, Solab, Brazil) with air circulation and air exchange at 50 °C. After drying, the biomass was milled with a Willey-type cryogenic knife mill (Tecnal TE-680). The biomass was maintained in polypropylene (PP) tubes and stored in a freezer at <−20 °C until analysis (Martini et al., 2019).

4 MANUSCRIPTS

Based on these main objectives, the thesis was divided into 5 manuscripts, which were developed through the studies performed: 2 review articles, 2 research articles and 1 case study. A previous information will be explained about each manuscript situation, an after, each manuscript will be presented.

4.1 MANUSCRIPT 1 - Potential of Microalgal Bioproducts: General Perspectives and Main Challenges

Manuscript 1 is a review article about the main bioproducts that can be found in microalgae biomass. This article was published in 2018, in the periodic “Waste and biomass Valorization” with Qualis CAPES A2 (researched in 2021).

https://doi.org/10.1007/s12649-018-0253-6

REVIEW

Potential of Microalgal Bioproducts: General Perspectives and Main Challenges

Maiara P. de Souza[1] · **Michele Hoeltz**[1] · **Pablo D. Gressler**[1] · **Lisianne B. Benitez**[1] · **Rosana C. S. Schneider**[1]

Received: 20 November 2017 / Accepted: 26 February 2018

Abstract

Microalgae have received considerable attention due to several applications in which they can be used, such as bioremediation and the production of high-value products. Microalgal biomass is known for its richness and variety of bioproducts such as lipids, carbohydrates, proteins, pigments and antioxidants. They can be used for several purposes, for example, in the production of biofuels, food nutrition (human and animal), pharmacology and cosmetology. However, despite the importance of these applications, there are still certain difficulties to produce microalgae bioproducts in large-scale. The high general cost of microalgal production is one of the major disadvantages, since cultivation, extraction and biorefining steps are necessary. Unfortunately, these processes are frequently not compensated by the final product sales price. The identification of the target compounds present in biomass as well as the costs involved in these steps are necessary to promote microalgal development for agricultural, commercial and industrial applications. To focus on the main aspects related to the bioproducts present in biomass, this review aims to demonstrate the main concepts regarding bioproducts and microalgae, the relevant data about the sectors of application, and the main challenges and perspectives associated with these subjects by using bibliometric mapping.

Keywords Microalgae · Biomass · Bioproducts · Bibliometric mapping

Introduction

The current energy sector profile drives the search for alternatives to provide energy, minimize greenhouse gases emissions and for biomass valorization [1]. However, the development of new proposals is a challenging task. In this context, microalgae are a promising source to produce biomass due to several applications they can offer, such as bioremediation [2, 3], biofuel production [4–7] carotenoids, phycobiliproteins and polyunsaturated acids [8], among others [9].

Microalgal biomass has been widely studied and applied in many areas. The ability of these microorganisms to grow in inhospitable conditions, as well as the ability for metabolic adaptation under stress conditions, makes microalgal biomass an attractive raw material for large-scale cultivation and industrial applications [10]. Microalgae can also accumulate several types of metabolites in their structure and their susceptibility when they grow under stress conditions may be a factor to increase production. The nutritional value of these products can be influenced by the size, shape, digestibility and biochemical composition of microalgae. When the composition and concentration of the substances present in the biomass are modified, different properties and consequently different applications are obtained. The properties are mainly influenced by the conditions and the physiological state of the culture [11].

According to Grobbelaar [12], the interest in microalgal production to obtain biomass for different applications began essentially at Stanford University (USA) in a pilot plant. Subsequently, this activity also occurred in other countries, such as Japan, Germany, Israel, the Czech Republic and China. The first large-scale research of microalgal biomass production began in 1960 in Trebon, Czech Republic. At this time, it was also reported that the cyanobacterium *Spirulina platensis* (classified as *Arthrospira* sp.) grew naturally in lakes and was harvested and used as a food source by local people in Africa and China. In 1982, *Spirulina* sp. was for the first time described as polyhydroxyalkanoate (PHA) producer, however, nowadays is considered as one of the most

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* Michele Hoeltz
michelehoeltz@gmail.com

[1] Post Graduate Program in Environmental Technology, University of Santa Cruz do Sul, Av. Independência 2293, Bloco 53, Rio Grande do Sul CEP 96815-900, Brazil

prospective cyanobacterial for production of bioproducts like protein, phycocyanin or phycoerythrin [13, 14] and in recent years, biopolymers [15].

Initially, the main purpose of microalgal cultivation was producing functional foods; however, this branch of activity was not very well developed and did not instigate a great demand. This factor was considered to be one of the main limitations to the increase of the development of microalgal production on an industrial scale [16]. The development of new bioproducts is an innovative proposal in the field of microalgae. Considering the several applications obtained from microalgal bioproducts, it is possible to highlight their use in the pharmaceutical, food nutrition, cosmetic and biofuel industries [17, 18]. Effectively, microalgae show promising prospects for obtaining bioproducts due to the high value of their metabolites [19]. However, the costs required for their production should be considered, mainly in aspects related to efficient cultivation systems and bioproduct introductions on commercial and industrial scales.

There is a significant difference between the currently available technology for microalgal production and that needed to supply the global demand for some bioproducts. Technologies should be expanded by several orders of magnitude to be sufficient for commercial scale and to contribute significantly to economic development. Thus, in addition to the technological aspects, the production cost should also be taken into consideration. To achieve this economic progress, it is still necessary to solve some factors, which are usually related to biological, engineering and biomass separation [20–22].

In recent years, many review articles about microalgae and bioproducts were published, for example: biorefinery to obtain high value products from microalgae [22], bio- fuel production from microalgal biomass by using pyroly- sis method [23] or ultrasound treatment [24], biogas from microalgae emphasizing aspects related to cultivation, harvesting and pretreatment for anaerobic digestion [25], possibilities for conversion of microalgae oil into aviation fuel [26], recent developments on sustainable based biofuel and bioenergy production with microalgae [27], the use of marine microalgae for production of biofuels and chemicals [28] upstream and downstream processing of microalgae for biodiesel production [29] and advances in the field of liquid transportation biodiesel using these microorganisms [30]. All these publications are important, but most of these reviews present the current scenario and recent developments of biofuels, the main steps and methodologies to produce them and strategies to reinforce this sector. However, many of the bottlenecks in this area are justified by the difficult commercialization of these bioproducts. Even though the information related to the importance of bioproducts from microalgae is widespread, it is clear that several challenges are found and need to be considered in the production on an industrial scale. The difficulty of finding information in the literature to gain a current perspective of this scenario is another limitation in the economic analysis of the different compounds obtained from microalgae. Nowadays, there are relatively few industries that already work in this sec- tor and these data are difficult to find. Cultivation systems described in the literature are generally based on small-scale plants associated with short-term studies on production. In addition, the industrial plants already well-established do not reveal their production costs, which hinders obtaining a general view of the situation [31]. Thus, it is important to demonstrate some capital costs involved in industries that already operate in this area, to serve as support and to contribute for people who intend to start a business in this sector as well as to supply new information for future studies. Another aspect to be highlighted is that the reviews found in literature usually discuss the bioproducts from microalgae individually [32–35], which demonstrates the need to report studies that provide these information in a single review.

Considering the numerous applications that can be achieve by microalgae and due to the high value of biomass, the present review was developed in order to track and compile information about the production of microalgae and bioproducts on a commercial scale which were not identified until this moment in published reviews articles. The bibliometric mapping was used to evaluate the last 10 years of publications regarding bioproducts obtained from microalgae using VOSviewer software based on the Web of Science database. The systematic information, collection and critical analysis about the evolution, potential, obstacles to insert biomass in the market considering the already established industries worldwide correspond to an advance in this field. The estimation of future trends and perspectives about this subject through industry reports containing production data, costs and forecast in general can offer relevant and new information promoting a relevant impact in the area of this review.

Research Methodology

The research for this review focused on the main aspects involving microalgae bioproducts, general perspectives and main challenges in order to visualize the most relevant topics in this context, to bring new information about these points as well as to aggregate general concepts. First, a general search was conducted in different databases, such as Science Direct, Scopus and Web of Science. In this context, Fig. 1 was schematized to present the main bioproducts obtained from microalgal biomass and their applications. To facilitate access to information about microalgae and bioproducts Table 1 was created by searching the main studied microalgae, the target bioproducts, cultivation medium and system, extraction procedure, determinations and bioproduct content.

The bibliometric mapping was applied in order to verify the main topics discussed to date in the literature and investigate the relationships between the most cited words. The present review was organized via a search of studies described to date that could easily accessed with relevant information for consultation and the development of future studies.

For bibliometric analysis, VOSviewer version 1.6.6 was applied using the database of Web of Science from Thomson Reuters due to its comprehensiveness regarding the target subject, high-quality studies and availability of data analysis in VOSviewer. The articles were initially selected in Web of Science based on a combination of the words "microalgae" and "bioproducts" and by selecting data based on titles, abstracts and keywords of studies published between 2007 and 2017. As a result, 12,655 original publications were found and exported in Web of Science format for further analysis in VOSviewer. After this analysis, the topic "microalgae" AND "application" was searched using the same parameters as the previous search, and 1007 original publications were found. In the VOSviewer software, bibliometric maps were created based on text data using the previous exported database from Web of Science. The Web of Science fields from which terms were extracted were "title and abstract", and binary counting was used with a minimum number of 10 occurrences of a term. Then, the words presented in Figs. 2, 7 and 8 were selected.

Based on the results from bibliometric mapping, and during the development of the writing, the main bioproducts and sectors of application were described and discussed. Figures 3, 4, 5 and 6 were developed to complement important information and to be a didactic source for consultation. It was also verified that topics about the main challenges involving microalgae bioproducts for industrial applications had to be explored (Fig. 8).

Lastly, the critical opinion of the authors of this review was synthesized in order to facilitate the understanding of the subject in general, provide an outlook, critical analysis and prospects about microalgae, bioproducts and possibilities to commercialization.

Microalgae

Microalgae are living organisms that have photosynthetic ability and can be found in marine or freshwater environments. These microorganisms have simple requirements for their development, for example, water, light, CO_2 and other nutrients [36]. They can develop in several habitats, under extreme temperature, salinity and pH conditions [37, 38]. Microalgal growth occurs in general between 1 and 2 weeks and, under appropriate cultivation conditions, it is possible to achieve approximately 1–2 cell divisions per day [23].

During the photosynthetic process, microalgae can accumulate high-value compounds that are of commercial interest [39]. In addition, the high growth rate, the potential to

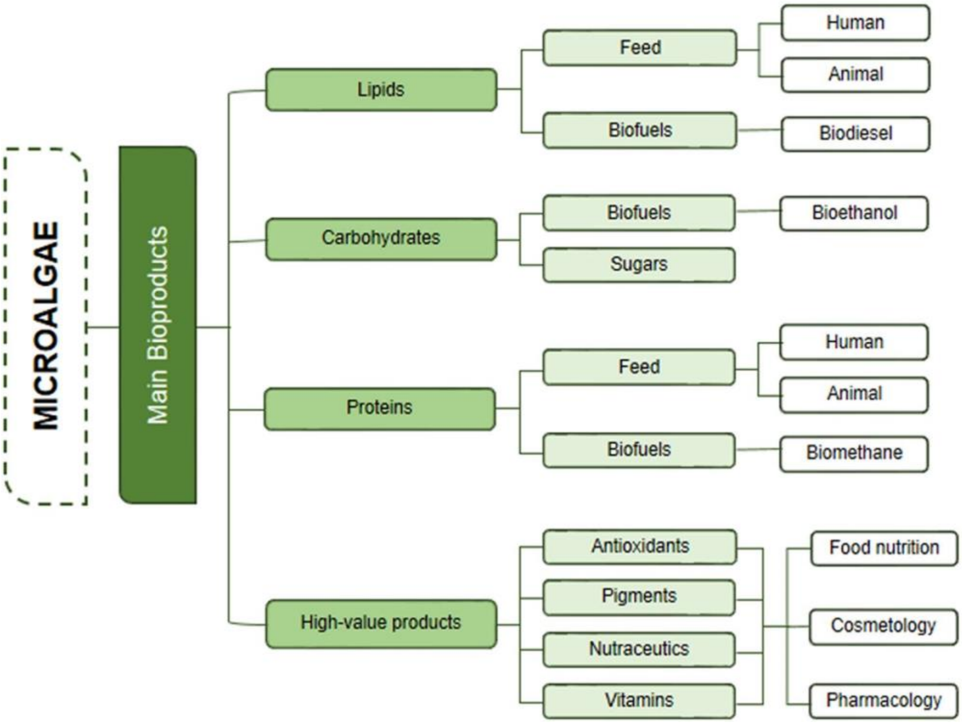

Fig. 1 Main bioproducts obtained from microalgal biomass and their applications

produce biofuels without competing with food resources and good development with CO_2 consumption, allowing great efficiency in the greenhouse gas mitigation are the main advantages of using microalgae as a feedstock [40].

Biomass characterization is necessary due to the high biological variability that can influence the composition and bioproducts [41, 42]. Microalgae may contain in their biomass several compounds, for example, vitamins from *Nannochloropsis* sp., *Pavlova pinguis, Stichococcus* sp., *Tetraselmis* sp. [11] and *Spirulina* sp. [43]; antioxi- dants from *Ecklonia radiata;* pigments from *Phormidium autumnale* [44]; fatty acids and proteins from *Chlorella* sp., *Chlorella saccharophila, Chlorella minutíssima* and *Chlorella vulgaris* [45]; carbohydrates from *Kirchneriella* sp. [46] and *Ecklonia radiata* [47]; and simultaneous bioproducts such as fatty acids, pigments, carbohydrates and proteins from *Kirchneriella* sp. [46]. All these microalgae provide possible commercial applications and can be used mainly in the production of biofuels, human and animal feed and high-value compounds for commercial applica- tions such as pharmaceutical, food and cosmetology pur- poses (Fig. 1).

Microalgal Bioproducts

Microalgal bioproducts can be obtained from different culture media and by different extraction techniques. To reveal more information about the studies involving bioproducts from microalgae, bibliometric mapping was used to evaluate the trends and the studies developed in this area in the last 10 years. The publications were evaluated with the VOSviewer software using the Web of Science database. Initially, the bibliometric search was made through a combination of the words microalgae and their main bioproducts (Fig. 2).

Several studies about microalgae and bioproducts have been reported. The keywords obtained by bibliometric mapping separated the bioproducts into main groups related specially to lipids, carbohydrates, proteins, antioxidants and pigments (Fig. 2a). A search for the combination of the most cited microalgae in the recent years and their relation to specific types of bioproducts was also carried out (Fig. 2b) and the main species related to these bioproducts were: *Chlorella* sp. and *Neochoris oleoabundan* for lipids, *Isochrysis galbana, Tetraselmis chuii* and *Skeletonema Costatum* for proteins, *Haematococcus pluvialis* and *Spirulina* sp. for pigments and antioxidants and *Chlorella* sp. for carbohydrates.

Reinforcing the results of the bibliometric mapping, Table 1 was developed. It is possible to visualize in Table 1 that microalgae were obtained in laboratory, pilot-scale, indoor or outdoor reactors. The compound analysis requires equipment such as spectrophotometers and chromatographs. Considering that the extraction methods for different compounds use solvents, the technology is still expensive, as is the production of microalgae. Depending on the type of bioproducts, it is possible to cultivate these microorganisms in wastewater, which has already been thoroughly investigated, especially for biofuels [48].

Each of the major bioproducts and the challenges involved in the production of biomass or the product itself will be discussed further. In addition, there is a possibility of using crude extracts for applications in anti-inflammatory, anti-cancer, anti-diabetes and antibacterial activities [56]. This potential brings several health benefits and may be an option to enable microalgae commercialization. The financial impact is directly linked to the production and separation due to the requirements for pharmaceutical use. Furthermore, by avoiding the extraction and biorefining steps, the products obtained from microalgae can be developed more quickly at larger scales, the benefits provided by these microorganisms can be exploited, and the costs reduced.

Lipids

Microalgae can contain a high amount of lipids, generally in the range of 15–77%, and the composition of this bioproduct can differ considerably in different species [57]. According to Chew et al. [22], the lipid content also depends on the environmental conditions, such as the high ratio between carbon and nitrogen (C/N) since higher C/N ratios can increase the lipid content. In unfavorable environmental conditions or stress, many microalgae alter their biosynthetic pathways to form and accumulate lipids. It is possible to increase lipid productivity by stress conditions such as by nitrogen inhibition, higher temperatures, changes in pH and high salt concentrations.

Triacylglycerols are the major constituent of lipid molecules [58]. The most common fatty acid composition in the triacylglycerols of the microalgal lipids is a mixture of unsaturated fatty acids, palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic (18:3), and saturated fatty acids, palmitic (16:0) and stearic (18:0) [59].

Antioxidants

Antioxidants are biological macromolecules that protect organisms or biological compounds against oxidative radicals. The advantages of consuming antioxidants have been disseminated to the public in general and are related to the improvement of life quality and to mortality and morbid- ity prevention. These molecules may prevent or minimize the oxidative damage caused by reactive oxygen species in lipids, proteins and nucleic acids. Thus, antioxidants can delay aging and several chronic conditions, such as

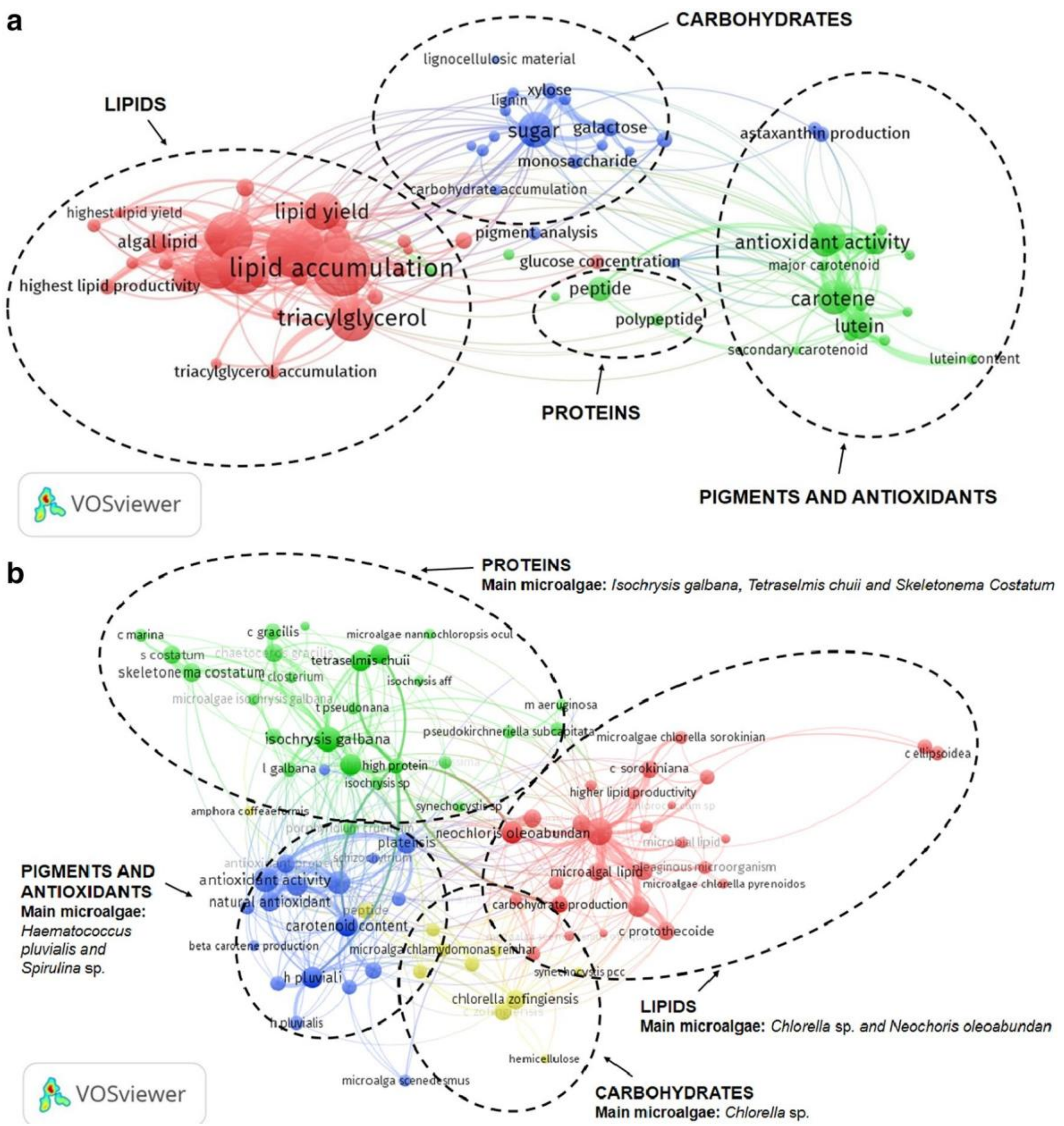

Fig. 2 Combined mapping of the most frequently cited keywords that appeared in the field of bioproducts from microalgae in the period 2007–2017 using the Web of science database. Publications are labeled according to the **a** relationship between microalgae and their main bioproducts and **b** the main microalgae studied and their respective bioproducts

heart disease, atherosclerosis and cancer, induced by free radicals [60, 61].

Among the sources of natural antioxidants, microalgal biomass is considered a promising alternative to sustain the growing demands, especially in the food and pharmaceutical industries. This can be justified by the great variety of physiological and pharmacological effects [62]. According to Li et al. [63], microalgae represent an almost unexplored source of natural antioxidants due to their enormous biodiversity, which is considered higher than that of some plants.

Several compounds can be potentially used as antioxidant sources such as pigments (including carotenoids), phenolic compounds, sulfated polysaccharides and long-chain fatty acids [10]. According to Marxen et al. [64], 2,2-diphenyl-1-picrylhydrazyl is a common example of that enables the screening of microalgae as promising candidates in a commercial sense. These authors tested microalgae *Anabaena* sp., *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Porphyridium purpureum*, and *Synechocystis* sp. PCC6803 in their ability to reduce the DPPH radical.

Table 1 Microalgae and main bioproducts considering cultivation medium and system, extraction procedure, detection and the bioproducts content

Microalgae	Bioproduct	Cultivation medium	Cultivation system	Extraction procedure	Determination	Bioproducts content	References
Arthrospira platensis	Proteins	Zarrouk	10 L indoor tubular airlift photobioreactor	High pressure nization homogei- nization	Elemental analysis	78.0 ± 2.8%	[49]
Chlorella vulgaris	Proteins	Sueoka	10 L indoor tubular airlift photobioreactor	High pressure homogei-	Elemental analysis	52.8 ± 0.6%	[49]
Chlorella fusca	Proteins, carbohydrates, lipids, pigments	f/medium	Lab-scale with photo- period flasks	Bradford, Dubois, Bligh and Dyer and Jeffrey–Humphrey, respectively	Spectrophotometry	14.7 ± 3.4 μg mg^{-1} dw 47.2 ± 4 μg mg^{-1} dw 192 ± 6 μg mg^{-1} dw 3.12 ± 0.6 mg L^{-1}	[50]
Coelastrum cf. pseu-domicroporum	Pigments	Wastewater	250 mL erlenmeyer	Solvent extraction	Spectrophotometry	0.17–9.12 mg L^{-1} 79 ± 3 μg mg^{-1} dw	[51]
Dunaliella salina	Proteins, carbohydrates, lipids, pigments	Johnson	Lab-scale with photo- period bioreactors	Bradford, Dubois, nization Bligh and Dyer and Jeffrey–Humphrey, respectively	Spectrophotometry	29.2 ± 1 μg mg^{-1} dw 155 ± 2 μg mg^{-1} dw 0.65 ± 1 mg L^{-1} dw	[50]
Haematococcus	Proteins drates, pigments and proteins	Basal wastewater	Tubular airlift photo- photobioreactor	High pressure all by-products	Elemental analysis tometry, HPLC–UV and spectrophotom-etry, respectively	41.7 ± 3.7% 5.3%, respectively	[49]
Nannochloropsis sp.	Vitamins	f_2 medium	2 L erlenmeyer flasks with photoperiod	Solvent extraction	HPLC–UV	0.25–3200 μg g^{-1}	[11]
Nannochloropsis oculata	Proteins	Conway medium	Tubular airlift photo-bioreactors	High pressure homogei-nization	Elemental analysis	52.3 ± 0.6	[49]
Pavlova pinguis	Vitamins	f_2 medium	2 L erlenmeyer flasks with photoperiod	Solvent extraction	HPLC–UV	0,25-1300 μg g^{-1}	[11]
Phormidium autumnale	Pigments	BG11 medium	Incubation with photo-period	Solvent extraction	HPLC-PDA	714.3–2.14 × 10^5 μg g^{-1} (dry wt)	[44]
Porphyridium cruentum	Proteins	Hemerick medium	Tubular airlift photo-bioreactors	High pressure homogei-nization	Elemental analysis	90.0 ± 2.4%	[49]
Shizochytrium limacinum	Lipids	–	–	Supercritical fluid ectraction	GC–MS	33.9%	[54]
Stichococcus sp.	Vitamins	f_2 medium	2 L erlenmeyer flasks with photoperiod	Solvent extraction	HPLC–UV	0.25–2500 μg g^{-1}	[11]

Table 1 (continued)

Microalgae	Bioproduct	Cultivation medium	Cultivation system	Extraction procedure	Determination	Bioproducts content	References
Spirulina sp.	Proteins, carbohydrates, lipids, pigments	BG-11 medium	Lab scale with photoperiod	Bradford, Dubois, Bligh and Dyer and Jeffrey–Humphrey, respectivey	Spectrophotometry	52.3 ± 2 μg mg^{-1} dw 150 ± 13 μg mg^{-1} dw 58.6 ± 1 μg mg^{-1} dw 2.54 ± 0.4 mg L^{-1}	[50]
Tetraselmis sp.	Vitamins	f_2 medium	2 L erlenmeyer flasks with photoperiod	Solvent extraction	HPLC–UV	0.35–3000 μg g^{-1}	[11]

The example of the antioxidant activity of DPPH is shown in Fig. 3.

Proteins

Proteins are composed of different amino acids, and the nutritional quality of these bioproducts is related basically to their content, proportion and the availability of amino acids [65]. Microalgal biomass composition can contain generally approximately 50–70% of proteins, and they are of fundamental importance in human and animal feed [22]. Examples of different microalgae with the potential of protein obtention include: *Ankistrodesmus falcatus* (5.2%) and *Aphanothece microscopica Nägeli* (0.5%) [66], *Aphanizomenon flos-aquae* (62%), *Chlorella pyrenoidosa* (57%), *Dunaliella salina* (57%) and *Scenedesmus obliquus* (50–55%) [65], *Chlorella fusca* (52%) and *Spirulina* sp. (52%) [50].

The proteins contained in the microalgal biomass compete favorably, in terms of quantity and quality, with proteins presented in conventional foods, such as soy, eggs and fish [34]. The amounts of proteins accumulated in these microorganisms depend upon the species, growth phase and light. Modification of the protein content can be accomplished through nutritional adjustment and environmental stress [67]. Another interesting application is the BioProtein development. BioProtein is produced by a mixed methanotrophic and heterotrophic bacterial culture or other microorganisms using natural gas as the main source of energy [68]. The production of BioProteins is a great alternative since carbon dioxide can be used for faster growth of bacteria and microalgae with a high protein content of 60–70% compared to 30–36% in soy and 20% in meat [69].

Carbohydrates

Carbohydrates are molecules composed of carbon, hydrogen, and oxygen, such as sugars, sugar derivatives and their polymers [70, 71]. The main representatives of this group are glucose, starch, cellulose and polysaccharides (Fig. 4). Glucose or starch are conventionally used for the production of biofuels, such as bioethanol and biohydrogen. Polysaccharides have biological functions as protection and storage and are structural molecules. In addition to these advantages, they can also modulate the immune system to inflammatory reactions, making them highly favorable to act as sources of active molecules for insertion in cosmetics, food ingredients and as natural therapeutic agents [22].

Microalgae can have approximately 50% of their dry weight as carbohydrates depending on the species [22]. For these organisms, carbohydrate production has two main purposes: acting as structural components in the cell wall and as storage components inside the cells, thereby providing the energy required for their metabolism [71].

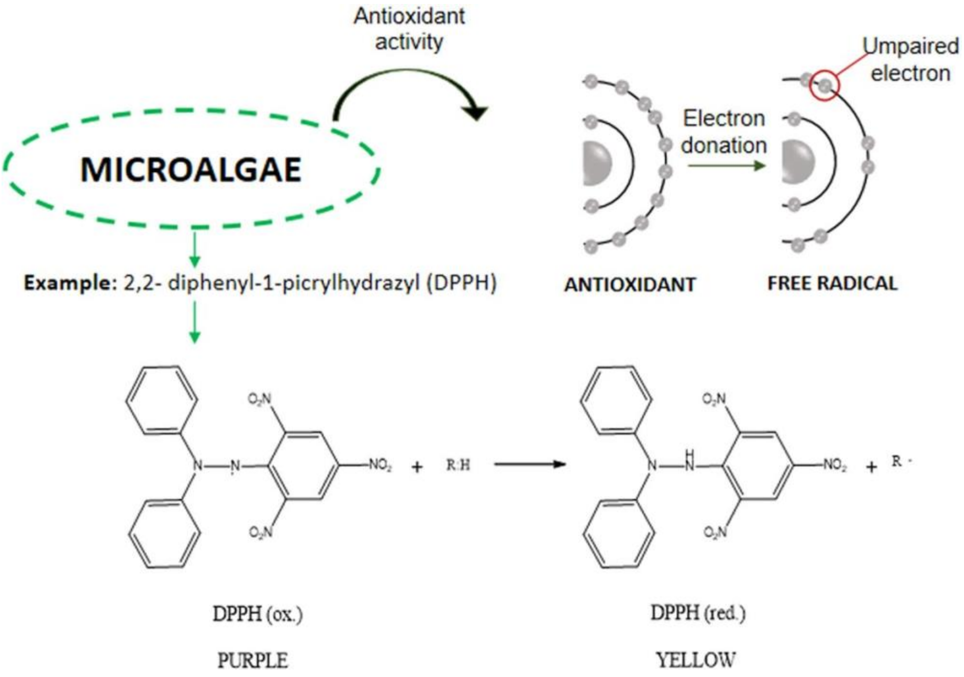

Fig. 3 Example of the antioxidant activity of DPPH in microalgae

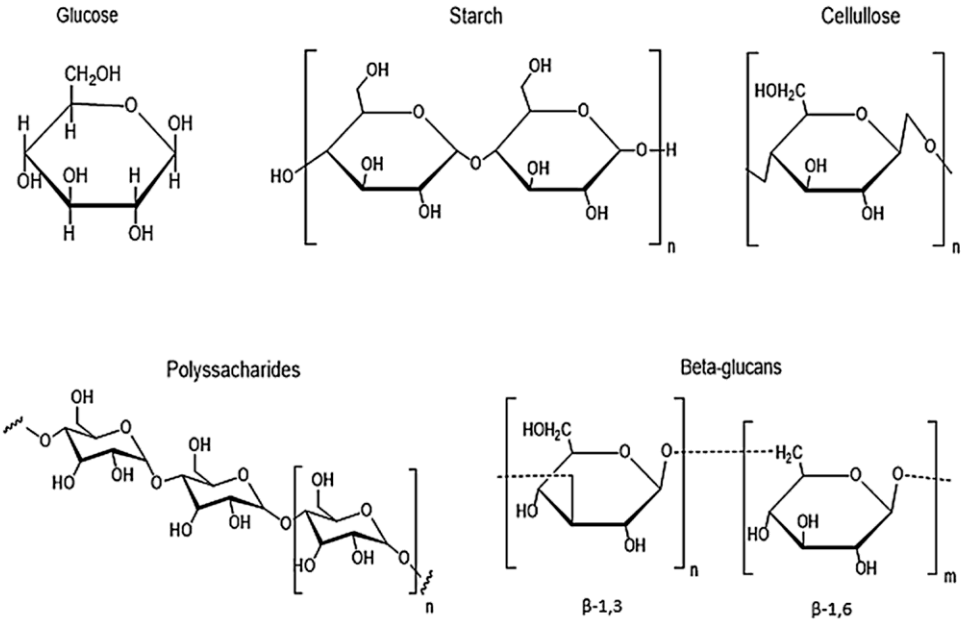

Fig. 4 Main representative groups of carbohydrates present in microalgal biomass

Beta-glucans are one of the most important representative carbohydrate classes, since they are responsible for initiating host defense reactions in response to the molecules of pathogens. *Chlorella* sp. is recognized as the microalgae that has the most beta-glucans in its composition [72]. In addition, other microalgae can produce carbohydrates, such as: *Chlorella fusca, Dunaliella salina* [50] and *Kirchneriella* sp. [46].

Pigments

Color, which is one of the most visible characteristics of microalgae, is determined by the pigments present in its structure. Pigments are chemical substances that present different colors and are part of the photosynthetic material of the microalgae system [73]. Natural pigments are essential in photosynthetic metabolism and algae pigmentation. In addition to these factors, they have biological activities as antioxidants, anticancer, anti-inflammatory, anti-obesity and neuroprotective agents [74]. Different environmental parameters that may influence the content of individual pigments of microalgae include temperature, irradiation, wavelength, photoperiod, pH, nutrient limitation, nitrogen, salinity, pesticides and heavy metals [75]. As the result of the influence of environmental conditions, the carotenoid-to-chlorophyll ratio (Car/Chl) can be cited as example for preferable indicator of carotenogenesis in microalgae since increases with combined stress of high irradiance and nitrogen deprivation due to the accumulation of secondary carotenoids [76].

Carotenoids, chlorophylls and phycobiliproteins are considered to be the main natural pigments present in microalgae. These pigments are usually applied in human and animal feed, additives, cosmetics, the pharmaceutical industries, food colorants and biomaterials [22]. Chlorophyll, carotenoids and phycobiliproteins present colors that range from green, yellow and brown to red. These colors vary according to each microalga, for example, blue pigment from *Spirulina* (provided by phycocyanins), yellow pigment from *Dunaliella* (by β-carotene presence) and yellow to red pigments from *Haematococcus* (due to astaxanthin) [77]. The main pigments and their different applications are shown in Fig. 5.

Rodrigues et al. [44] identified twenty-four carotenoids, three phycobiliproteins and two chlorophylls in the microalga *Phormidium autumnale*. The major pigments found in the biomass were all-trans-β-carotene (225.44 μg g^{-1}), all-trans-lutein (117.56 μg g^{-1}) and all-trans-zeaxanthin (88.46 μg g^{-1}), chlorophyll a (2.700 μg g^{-1}) and C-phycocyanin (2.05 × 105 μg g^{-1}). When the microalga *Coelastrum* cf. *pseudomicroporum Korshikov* was cultivated in urban wastewater and under salt stress, this species accumulated carotenoids in the range of 1.73–91.2 pg cell^{-1}.

Vitamins

Vitamins are nutrients required for normal physiological functioning. A bioavailability study of these components is fundamental to the evaluation of their feed quality. Microalgae present high levels of essential vitamins and have suitable availability of these compounds. Vitamin production from microalgae may be directly related to the presence of nitrogen in the medium [22]. Several vitamins can be found in microalgae such as: vitamins A (retinoids and carotene), B1 (thiamin), B2 (riboflavin), B3 (niacin), B6 (pyridoxine), B7 (biotin), B9 (folic acid), B12 (cobalamin), C (ascorbic acid) and E (alpha-tocopherol).

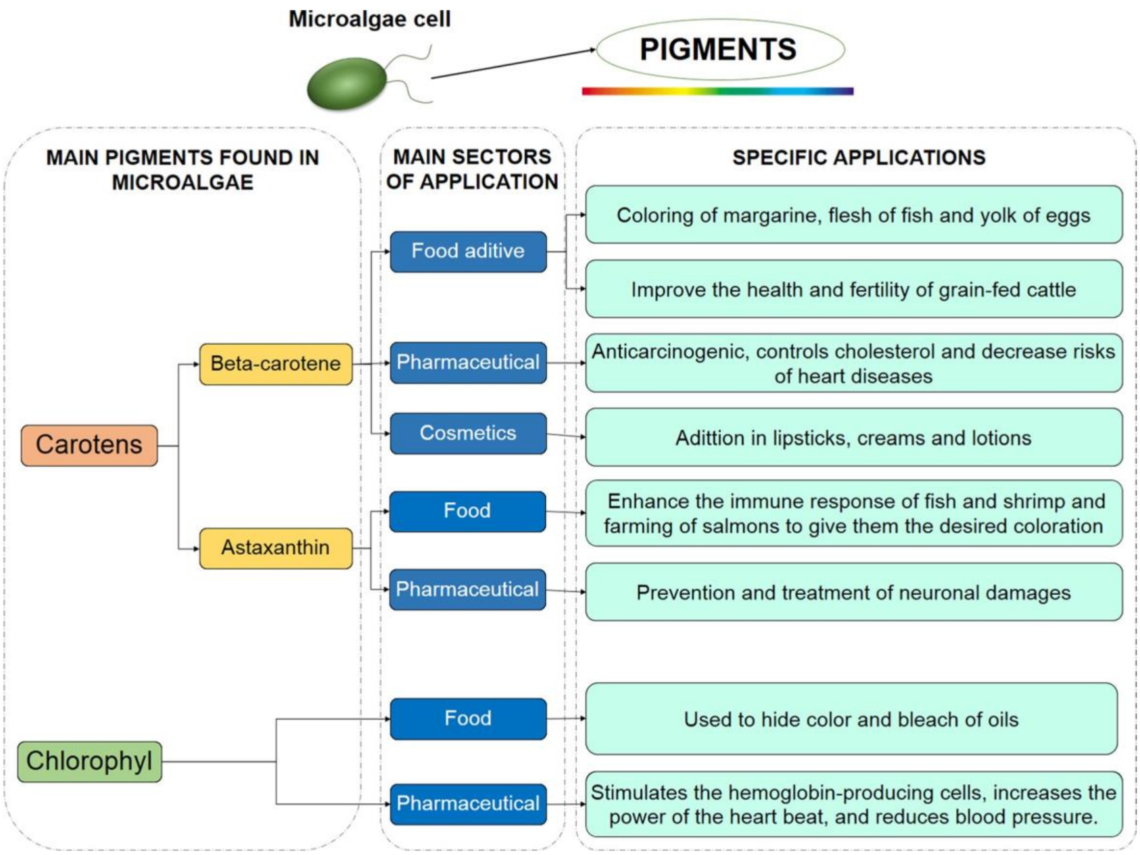

Fig. 5 Main pigments obtained from microalgae and their main areas of application

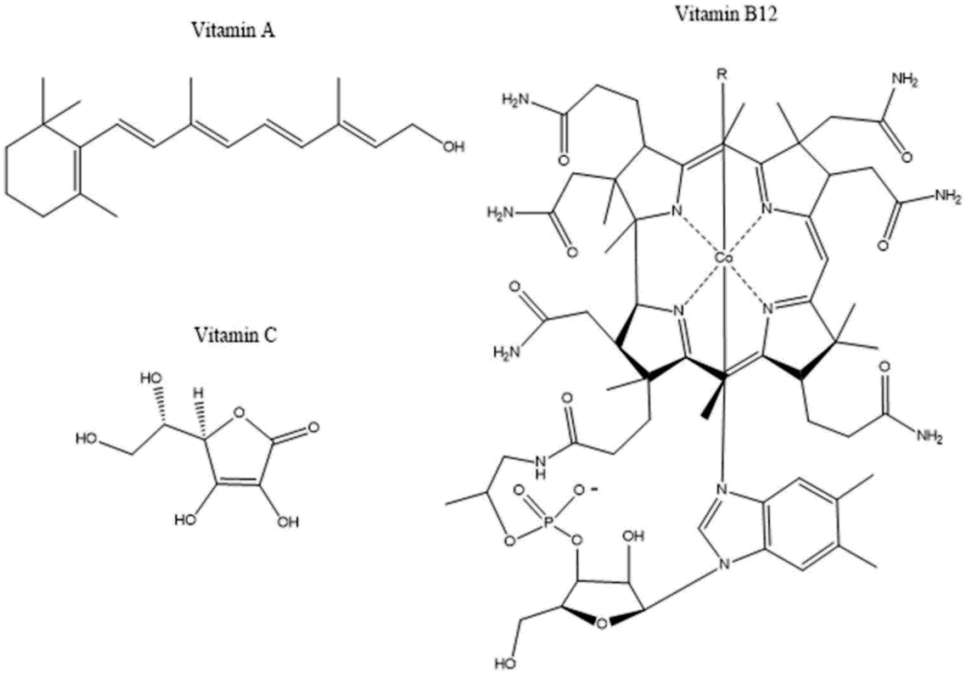

Fig. 6 Main representative vitamins structures presented in microalgal biomass

According to Tang and Suter [54], *Spirulina, Chlorella* and *Dunaliella* are concentrated sources of provitamins A, C and B12 (Fig. 6). These microalgae are produced worldwide and have several health benefits, acting as dietary supplements of macro- and micronutrients. In addition, it is important to emphasize that the use of these microalgae for the production of high-value products is generally safe; however, care must be taken to cultivate them in secure environments to avoid contamination by cyanobacteria, for example.

Potential of Microalgae Bioproducts

According to recent data, more than 30,000 species of microalgae have been identified; however, fewer than 10 are commercially produced. Despite the fact that some of these species are used as feed, current industrial production still does not reach the expected level due to the difficulty of producing at in larger scale as well as the stage of biomass biorefining, which demands high costs [16]. In studies, several microalgae have been cultivated annually to produce high value compounds. According to Pulz and Gross [78], the annual average production of some microalgae is approximately *Spirulina* (3000 t/year), *Chlorella* (2000 t/year), and *Dunaliella* (1200 t/year).

To highlight the major sectors in which microalgae have been inserted in recent years, bibliometric mapping was performed using a combination of the word "microalgae" related to the application sectors that were available in the results of keywords obtained by the software. The bibliometric research of the main sectors in which microalgae have been studied are shown in Fig. 7.

According to the bibliometric mapping, it was possible to verify the separation of three main groups, divided into biofuels (with emphasis on biodiesel, biogas, bioethanol and biomethane), pharmacy and cosmetology (with progress of studies about pharmaceutical industry, cosmetic application and anti-inflammatory action) and human and animal nutrition (specially as a potential food source in diets and dietary supplementation).

Biofuels

Biofuel production from microalgae has been the target subject of several studies. The use of microalgae was extensively tested and reported in studies to produce different types of biofuels, such as biodiesel [79–83], bioethanol [84–87], biogas [88–93], biohydrogen [94, 95], bio-oil [96, 97] and biokerosene [98].

The main advantages related to the production of biofuels from microalgae are higher productivity per unit area, high photosynthetic efficiency and non-competition with food crops [18]. Furthermore, depending on the species involved, microalgae are supposed to be in a better position to produce biodiesel due to their lipid content, which may be considerably higher than other biomasses used for this purpose [99]. However, although it has been stated that biofuel production from microalgae is relatively close to being economically feasible, the data reported by [100, 101] demonstrate that technologies must be optimized to substantially reduce

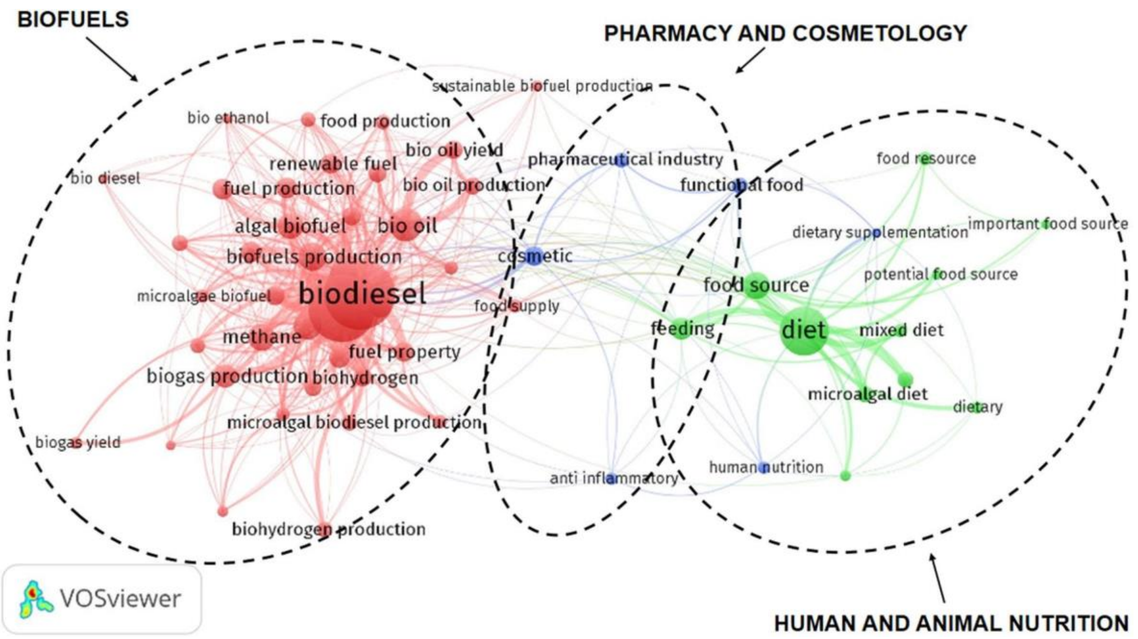

Fig. 7 Combined mapping of the most frequently cited keywords in the studies that appeared in the field of the main sectors in which microalgae were studied in the period 2007–2017 using the Web of Science database

the operating costs. Additional research is required in this field since several parameters have not yet been adequately assessed to understand the potential for macroalgae-based production. These parameters include: appropriate cultivation step, species of seaweed and seaweed yield per hectare, time and method of harvesting, carbon balance, costs of the harvested seaweed and cost of the produced biofuel [102]. According to the Environment Committee of the European Parliament, "advanced biofuels sourced from seaweed or certain types of waste should account for at least 1.25% of energy consumption in transport by 2020" [103]. The costs associated with oil extraction, biodiesel processing and the variability of algal biomass production should focus on methods of the oil-rich algae itself [104].

Despite the presence of the bottlenecks to produce biofuels from microalga, some proposals are forthcoming to produce biofuels from microalgae at larger scales. The Government of Japan wants to introduce bio-jet fuel for commercial flights in 2020, and the volume of production is ambitiously estimated to be 100,000 to 1 million liters [105]. The Bioenergy Technologies Office (BETO) estimates that by 2017, this model would be able to supply 1 million metric tons of ash-free dry weight cultivated algal biomass. By 2022, the sustainable model would supply 20 million metric tons, and in 2030, the production of 5 billion gallons per year of algal biofuels would be possible [106].

Human and Animal Nutrition

The estimate of annual microalgal biomass production until 2016 is approximately 5000–7500 tons, which generates an annual average income of US$1.25 billion. *Spirulina* and *Chlorella* are the main microalgae that have already been inserted on the market. The annual worldwide sales volume of *Chlorella* sp. is generally over US$38 billion for human nutrition, animal feed and food additives. In addition, approximately US$10 billion are intended for the production of nutritional supplements that can be produced by microalgae such as *Schizochytrium* sp. and *Crypthecodinium* sp. [107].

One of the main advantages of using microalgae in nutrition is the possibility to offer several compounds of interest simultaneously. According to Blecker and Barka [16], some microalgae such as *Spirulina*, *Chlorella*, *Dunaliella* and *Scenedesmus*, when correctly processed, present an attractive flavor and can be incorporated into several types of food. In addition to their high protein content, microalgae present fatty acids, a favorable amino acid content, pigments and vitamins that are essential for use in the feed industry and to add value to milk [41, 108].

The study of microalgal bioproducts as food sources is increasing due to the several benefits that can be obtained, such as the ability to improve nutrition due to probiotic effects that positively affects human and animal health. Microalgae can also be used to increase product shelf life and to act as a source of natural dyes in foods such as pasta, snacks, chocolate and gums. In addition, they may be inserted into drinks and supplements in the form of tablets and capsules [109].

Functional foods or nutraceuticals produced by microalgae present health benefits and are an attractive option for consumers. In 2004, [78] predicted that the functional food market would be one of the most dynamic sectors in the food industry and could constitute up to 20% of the entire food market. Although in recent years there has been an increase of the consumption of nutraceuticals, this sector is still under development [110]. Algae Technology Limited [111] related that a wide range of algae and microalgae are commercially produced for use in human health and food supplement applications and that the global market in 2015 was estimated to be US$800 million. In addition, more than 40 different species have been developed for nutraceutical applications; however, *Arthrospira* (*Spirulina*), *Chlorella, Dunaliella*, *Nannochloropsis*, *Haematococcus* and *Schizochytrium* represent over 95% of the current market. The nutraceutical prices can range from US$15 to over US$100 per kilogram, depending on the algal species, product quality and nutritional value. Another important detail is that more than 90% of the total nutraceutical algae production worldwide is based on open cultivation systems.

According to Rocca et al. [104], approximately 9200 dry wt tons of microalgae are produced annually, worldwide. The application focus is in diet or health food for human consumption and feed additives for aquaculture. It is highlighted that in Asia, the US and Israel, the microalgae *Arthrospira platensis* and *Haematococcus pluvialis* are the most abundant strains for this purpose, with production of approximately 3000 dry wt tons each.

Microalgae as a source of food are widely discussed and are a suitable alternative for future sustainability. In 2030, the estimate is that there will be 9 billion people on Earth, of which 1 billion will suffer from hunger. More critical opinions also relate that this will not be remedied with fish or shrimp but with microalgae [112].

Pharmacy and Cosmetology

The use of microalgae in pharmacology and cosmetology has also attracted considerable attention due to their beneficial effects on human health. 1,3-β-Glucan is one of the most important substances in the pharmaceutical industry and can be obtained from *Chlorella*. This compound acts as an active immunostimulator, eliminates free radicals and has the ability to reduce blood lipids. In addition, other effects of this microalga have been found to promote health (efficacy against gastric ulcers, wounds and constipation and preventive action against atherosclerosis and antitumor action) [8].

Microalgae contain omega-3 fatty acids, which are one of major types of compounds that can be sold in pharmaceutical and cosmetic markets [113] The strain of *Schizochytrium* sp. is widely studied to produce omega-3 fatty acids. In addition, this microalga can produce eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in high concentrations as well as being able to obtain its energy from dextrose [114]. Pages [115] presented the following valuable market calculation data about omega-3 production showing that the *Schizochytrium* product available in industry has a long chain (LC) omega 3 content of approximately 20% of the total dry whole cell algae. These data give the following market size indicators of LC omega 3 and indicates an annual requirement of 40,000 tons of actual LC omega 3, which translates to 200,000 tons of *Schizochytrium* whole-cell dry algae.

Pigments obtained from microalgae are a suitable alternative to be inserted in the pharmaceutical and cosmetic sectors due to the toxic effects of synthetic dyes [116]. The estimated worldwide market for astaxanthin production by *Haematococcus pluvialis* was approximately US$200 million in 2016 [107]. In addition, according to Zegarac [117], the global astaxanthin market is projected to reach $1.1 billion by 2020.

The bioactive substances derived from microalgae have functional roles that can act as secondary metabolites, and consequently, can be applied in the development of cosmetics [118]. Although the relation of microalgae and these sectors are being discussed, the information about the economic aspects in this context are very scarce and difficult to find.

Challenges of Microalgae in Industrial Applications

In general, microalgal growth seems to be simple since it is possible to verify the natural presence of these microorganism in several environments; however, conversion of biomass into bioproducts at an industrial level is challenging. It is known that microalgae require light energy and CO_2 sources for autotrophic growth, organic sources for heterotrophic growth or the intermediate between these two levels, which is the mixotrophic condition. According to Grobbelaar [12], between these two extremes, there are several intermediate trophic routes. The cultivation in photobioreactors implies the necessity of autotrophic growth, nutrient additions, carbon source insertion, temperature control and a suitable pH.

According to Acién et al. [101], it is necessary to reduce the cost of cultivation systems to be inserted at commercial and industrial scales. The use of photobioreactors are a suitable alternative; however, the design simplification and the materials used must be optimized. Another option is to use wastewater and combustion gases, thereby reducing energy and labor consumption in production. All the aforementioned aspects involve direct economic expenses, so the alternatives of production must be well studied. Accord- ing to Walker [119], these aspects are essential to plan and start a business involving microalgae to avoid unnecessary expenses as well as the loss of money by investors.

Microalgae cultivation requires large amounts of water and nutrients, often resulting in high production costs and consequently making it difficult for the insertion of microalgal bioproducts on a commercial scale. However, a good option to solve this problem is the development of the association of microalgae cultivation in freshwater, effluent or another medium containing available organic compounds while performing bioremediation [99].

Another option to reduce costs and increase productivity is the use of open pound systems such as the Algae Turf Scrubber [120]. This system is modeled on algae communities found in coral reefs. The source of light used for cultivation may be either natural or artificial. The high rate of biomass production is one of the main advantages of this system since they present higher production yields than the values recorded in other cropping systems. Many pollutants are absorbed in the algae biomass and can be removed by the system itself. If the absence of toxic compounds is ensured, the biomass can be converted into products of commercial interest, such as fertilizers and animal feed [121]. However, according to Michel [122], the main drawback of open pond systems is their relatively low biomass yield of 10–25 g/(m^2d) compared to closed systems which presents 25–50 g/(m^2d). In addition, another disadvantage is that a limited number of microalgae can be cultivated in open ponds and some species are very vulnerable to contamination and evaporative water loss.

In addition to the possibilities for cultivation step, photobioreactors can be a suitable option since appropriate configurations lead to effective photosynthesis, higher biomass yield and also have the advantage of optimal removal efficiency of nitrogen and phosphorus. Currently, many photobioreactors have been invented and developed for microalgae cultivation and some of them have achieved large scale commercial production. Besides, it is an option for axenic cultivation of microalgae and to generates high-value added products [123].

Other parameters must be considered as challenges to be overcome for the application of microalgae on an industrial scale. In this context Spolaore et al. [8] highlights the fact that microalgae with high fatty acid content, rapid growth and resisting performance, combined with the oil-containing organisms, which have gone through genetic engineering, will be sorted out. This can be explained since the use of transgenic microalgae for commercial applications has not been reported until now, however holds significant promise considering that these modified strains could overproduce traditional or newly discovered algal compounds. Advances in technological developments, especially related to cultivating oil-containing microalgae as a biofuel, the large-scale production technology associated with the decrease in greenhouse gases must be improved to produce liquid fuel from oil-containing microalgae.

In relation to microalgae as a source of bioproducts, there are some studies that have attempted to improve the production to reduce costs. Malik et al. [124] investigated whether algal bio-crude production is environmentally, economically and socially sustainable using a life cycle assessment. Bio-crude, also known as bio-oil, is a suitable alternative which consist process semi-dry biomass by hydrothermal liquefaction and can later be distilled into its fractions at a refinery [124]. The results indicate that the production of 1 million tons of bio-crude would be possible to generate almost 13,000 new jobs and US$4 billion worth of economic stimulus. Benvenuti et al. [125] presented a projection for a two-step 100-ha-scale process involving microalgal triacylglycerides (TAGs). As a result, the suggested improvements could decrease production costs to 3.3€ per kg of biomass containing 60% TAG (w/w) within the next 8 years. In 2017, a company in Japan announced that it was doubling production capacity of food-use microalgae *Euglena* to 160 tons. This production facility will increase from 80 to 160 tons. This improvement will meet the increasing demand in a market as well as allow the company to respond to the diversifying needs of its customers [126].

Finally, to visualize the perspectives between microalgae and economy, bibliometric mapping was performed with a combination of the keywords “microalgae” that were related to “costs”, “economies”, “obstacles and challenges” and “industrial and commercial scale” (Fig. 8).

Figure 8 presents the change of the search profile results in relation to microalgae and economy over the last 10 years. After bibliometric analysis it was possible to verify that the expression “production cost” is the main challenge of microalgae in industrial applications. This expression was interconnected to valuable parameters that must be taken into consideration in this field, such as “industrial scale”, “commercialization”, “economy”, “high cost”, “economic viability”, “major bottleneck” and others.

These results demonstrate that the future prospect for microalgae bioproducts is more optimistic about the parameters that could enable the commercialization of bioproducts on an industrial scale. However, considering the demonstrated data, the aspects that aim at economic viability and the reduction of energy cost cannot be left aside, and these are the main challenges that must be discussed and overcome.

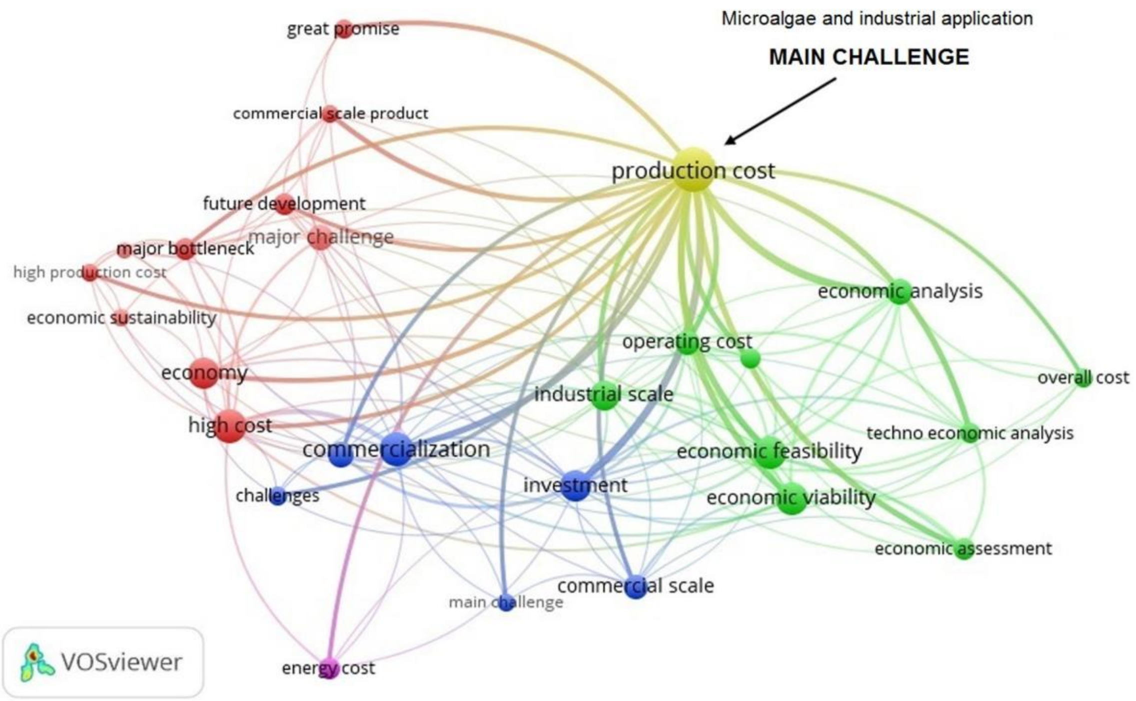

Fig. 8 Combined mapping of the most frequently cited keywords related to the combination of microalgae associated with the production cost and economic viability in the period 2007–2017 using the Web of Science database

Outlook and Future Prospects

Studies on microalgae and bioproducts must be updated, since the scenarios to develop high-value compounds are constantly changing. The current production of bioproducts through these microorganisms can be unsustainable if the process is not well established. Hence, economic study and processing are required to improve commercialization aspects. As demonstrated in this review, microalgae can synthesize lipids, pigments, vitamins, antioxidant and others compounds.

Despite the large number of studies that were described in this review, microalgae biomass is still considered for many researchers as not viable for commercial biofuels production due to the extensive energy input. This can be justified since the demand of biofuels in commercial scale exceeds the accessibility of these kinds of feedstock. However, there are some industries that related promising perspectives in this field, but some parameters must be simplified to obtain long-term sustainability and environmental benefits.

As shown in the bibliometric mapping performed in our study, new markets for high value compounds should be explored. The demanding market of bioproducts from microalgae and their subsequent utilization in food nutrition may be a suitable option in the next years to support the goals to establish microalgae at industrial scale.

Considering the increasing concern to have healthy lifestyles and as the economy increase, more consumers can afford the costs of pharmaceuticals, cosmetics and nutritious feed from microalgae, and this sectors can be another option to promote bioproducts in the near future. As most of the microalgae are being consumed as health food or supplements, there is a high expectation with respect to the quality and the market sustainability for commercial scale, then high-value products must be considered. In this context, several concerns need to be addressed such as the relatively small market for different bioproducts, culture growth conditions to avoid losses caused by product degradation and long term stability studies of the microalgae products.

Cultivation technologies are a suitable option to promote new strategies to commercialize the desired final product and to achieve economic progress. As shown in the topic “Challenges of microalgae in industrial applications” some barriers must be overcome to provide suitable technologies of design for culture conditions, light control and to induce and activate the accumulation of bioproducts. In addition, genetically modified microalgae (considered as a positive promise in this context) can also help to improve the targeted bioproducts in industrial scale.

Cost-effectiveness assessment is necessary to evaluate the economics options of microalgal commercialization. The association between microalgae and technologies, especially through life cycle assessment tool (LCA), can be a suitable alternative for future developments since it is possible to obtain an inventory of input and output mass and energy. This allow the quantification of generated environmental impacts of valuable parameters to increase viability for industrial applications. Also, appropriate policy environment and sufficiently low costs are required for these technologies to be introduced and recognized as a promising alternative to commercialize bioproducts from microalgae.

Conclusions

This review presented several application possibilities of the bioproducts obtained from microalgae and that could be valuable to intensify industrial applications; however, overcoming the high costs involved is one of the crucial factors to boost the production at larger scales. Studies are still needed in this area to select the best species to achieve good productivity and to design the best processes. The constant search for new technologies is fundamental to solve these challenges and to find efficient ways to commercialize bioproducts. In addition, it is essential to find means of obtaining and characterizing bioproducts to use smaller amounts of solvents and to avoid unnecessary energy costs in order to reduce environmental impacts and costs. The data obtained through bibliometric mapping allowed a general evaluation of the items that were most searched in the last 10 years. This made possible an overview of the studies that were being carried out and the future trends involving microalgae. The study of all these aspects are fundamental for microalgae to enter the market with greater force and to boost the commercialization of products of interest to generate production on an industrial scale.

Acknowledgements The authors would like to thank CAPES for scholarships, the University of Santa Cruz do Sul (UNISC), TecnoUNISC, through the Center for Research in Chemical and Biotechnological Processes and Products and MCTIC (01.0144.00/2010) as well as Secretary of Development in Economy, Science and Technology of the state of Rio Grande do Sul (SDECT-RS).

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4.2 MANUSCRIPT 2 - Microalgae and Clean Technologies: a review

This review article shows the association between microalgae and clean technologies in order to minimize impacts associated with the stages of cultivation, harvesting, biomass drying and bioproducts extraction. In this article, the tools used in the area for this purpose stand out, emphasizing Life Cycle Analysis. This article was published in 2019 in the periodic “CLEAN - Soil Air Water” with Qualis CAPES A2 (researched in 2021).

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Microalgae and Clean Technologies: AReview

*Maiara Priscilla de Souza, Michele Hoeltz, Lisianne Brittes Benitez,
Ênio Leandro Machado, and Rosana de Cassia de Souza Schneider**

The search for clean technologies needs to be continued to offer alternatives for achieving sustainable energy production and a sustainable economy. This concern is particularly related to the demands of both producing enough renewable energy to meet future needs and reducing greenhouse gas (GHG) emissions. Microalgae are recognized for several benefits they offer, and in recent years, the use of life cycle assessment (LCA) to evaluate the benefits resulting from microalgae cultivation, harvesting/dewatering, biomass drying, extraction, and byproduct development has stimulated research in this area. Considering the importance of microalgae and clean technologies and the increasing number of publications on these subjects, this review aims to perform bibliometric mapping of such studies from 2008 to 2018. Web of Science and Scopus databases are used to identify leading trends.

Visualization of similarities viewer (VOSviewer) software is applied to analyze the interactions among keywords. The results of this study indicate an association of microalgae and clean technologies and demonstrate that LCA is one of the most common tools used for such analyses. Bibliometric mapping provides relevant data to reinforce this association and understand the main bottlenecks that must be overcome in this field for future progress to be made.

1. Introduction

Problems associated with global warming and greenhouse gas (GHG) emissions are becoming a major technological issue associated with concerns about social and political challenges.[1] Economic studies recognize that a transition to clean technology can control and reduce fossil fuel emissions, and empirical evidence suggests that innovation can lead to a shift from dirty to clean technologies in response to changes in prices and policies.[2]

Clean technology is a general concept under which sustainable ways to provide for human needs are sought. The benefits of this technology can include reduced resource use and environmental damage compared to alternative technologies while remaining economically competitive.[3,4] The implementation of these technologies at larger scales, especially to reduce GHG emissions, can reduce severe pollution threats. It is possible to increase the economic performance of industries and potentially save water, energy, raw materials, and waste using clean technology.[1,5]

New technological and environmental proposals must be cost-effective to be a competitive alternative to conventional technologies and fossil fuels. Additionally, the technologies being developed to facilitate the use of renewable energy or reduce energy consumption and pollution must first go through a stage of acceptance and be commercialized and positively diffused before they generate positive environmental outcomes.[6]

There are some aspects that must be analyzed before the adoption of a clean technology. This process is not instantaneous; innovations tend to occur in a gradual manner because the adoption of a new technology will depend on its costs and benefits. In addition, there are many factors that must be considered before adoption, such as differences regarding organization, products, the type of manufacturing process, the economic lifetime of manufacturing processes, technological capabilities, and management and financial situations.[7]

In this context, microalgae have become an alternative to clean technologies due to their numerous benefits. These microorganisms present simple requirements for their development, such as water, light, CO_2, and nutrients. In addition, they can develop in a number of habitats, at extreme temperatures and under pH variations.[8,9] These microorganisms have been used for wastewater treatment and as an alternative energy source. The use of microalgae for bioremediation is a valuable alternative for contaminant removal because in most cases, existing technologies cannot achieve effective treatment of these substances. These microorganisms can assimilate inorganic nitrogen and phosphorus for their own development and can remove potentially toxic metals and organic substances.[10,11] The combination of microalgae with wastewater can be a suitable alternative for improving clean technologies. This combination is possible because many microalgal species can be cultivated in this medium, where they remove contaminants from wastewater and reduce the use of drinking water for cultivation while also producing biomass.[12]

Water recycling in microalgal cultivation is important to decrease water demand and improve economic feasibility due to nutrient and energy savings.[13] The cultivation of *Chlorella*

M. P. de Souza, Prof. M. Hoeltz, Prof. L. Brittes Benitez,
Prof. Ê. L. Machado, Prof. R. de C. de Souza Schneider
Environmental Technology Postgraduate Program
University of Santa Cruz do Sul
Santa Cruz do Sul, Rio Grande do Sul 96815-900, Brazil
E-mail: rosana@unisc.br

DOI: 10.1002/clen.201800380

vulgaris using recycled water reduced nitrogen and phosphorus levels by 55% when 100% of the water was recycled.[14] With the use of this same microalgae,[15] a 45% reduction in energy consumption was associated with nitrogen and phosphorus production.

In addition to their use in bioremediation, microalgae can also be a potential source of clean technology for biofuel production due to the presence of high-value products in their biomass composition. Biomass is the oldest known source of energy and is an attractive option for many reasons: it is renewable (if properly managed); it is more evenly distributed over the Earth's surface than conventional and finite energy sources; and it can be exploited using more environmentally friendly technologies and provides an opportunity to optimize local, regional, and national energy self-sufficiency.[1,16] It is estimated that biomass can provide approximately 25% of our global energy requirements. In addition, it can serve as a source of chemicals, pharmaceuticals, and food additives.[17]

Studies have shown that microalgae can be used to produce a variety of active compounds; however, industrial-scale cultivation of these microorganisms is challenging due to difficulties in achieving economic feasibility and low levels of biomass production. Therefore, microalgal cultivation in natural environments is an interesting option to associate the production of bioproducts with wastewater treatment and decrease total costs.[18]

The association between microalgae and clean technologies, especially when examined using the life cycle assessment (LCA) tool, may be a suitable alternative for future developments in the environmental sector. LCA is a useful option for verifying potential environmental benefits. With the use of this tool, the definition of the goal and scope are established, and it is possible to obtain an inventory of input and output mass and energy through the quantification of generated environmental impacts.[19] For microalgae, many production steps have been studied by LCA, such as cultivation,[20,21] harvesting/dewatering,[19,22] and extraction,[23] as have related high-value products[24,25] and biofuels, such as biodiesel,[26–28] biogas,[29] and biojet fuel.[30] After LCA, the conclusion with respect to the defined goals can be discussed to determine the best scenario and understand the impacts associated with each production step.[19]

Considering the importance of these technologies in this context, there are still relatively few studies that describe the use of microalgae and cleaner technologies; however, the information available on this topic to date highlights the importance of these technologies in dewatering, in methods to produce more biomass, and, especially, in studies on oils, which are also related to biorefineries and biodiesel.

To facilitate associations and trends in this field, bibliometric analysis can be a useful approach for identifying many research areas or topics and the levels of interactions among them. This tool has advanced considerably in recent years and usually applies statistical analysis and data techniques involving analyses of the literature, patents, citations, terms, and keywords.[31] According to McMillan and Hamilton Iii,[32] bibliometry considers that a particular paper or patent exhibiting greater merit, influence, or importance will be cited more frequently. In 2010, van Eck et al.[33] introduced VOSviewer, a freely available computer program that automates term identification and constructs bibliometric maps. The aim of these maps is to present the dynamic and structural keywords of scientific studies that enable easier visualization of general aspects, reinforcing the current situation and future trends in a number of fields.[34] VOSviewer has been successfully applied in medical research,[35] marketing,[36] informatics, and engineering[37] and may be a suitable alternative for use in environmental sciences.

Maiara Priscilla de Souza is a Ph.D. student focusing on biofuels, microalgae (bioremediation and bioproducts), and analytical instrumentation with emphasis on chromatographic techniques and method validation for residues and contaminants in food and environmental samples.

Lisianne Brittes Benitez is associated with biology and pharmaceutical research with experience in applied microbiology with emphasis on bioremediation, phytoremediation, and biotechnology in the production and characterization of bioactive compounds from agro-industrial residues.

Rosana de Cassia de Souza Schneider is associated with chemistry research focusing on oil extraction, transformation, and analysis. From microalgae, she develops bioproducts with emphasis on biofuels, starting from phytoremediation systems.

The focus of this review was confined to 10 years, considering that the increase in the number of publications about microalgae and clean technologies started in 2008. The use of databases Scopus and Web of Science was necessary to complement information in this field. Therefore, this study aimed to present information about studies involving clean technologies and microalgae via bibliometric mapping covering the last 10 years. The main steps that should be considered are described in this review, as is the importance of LCA in this process.

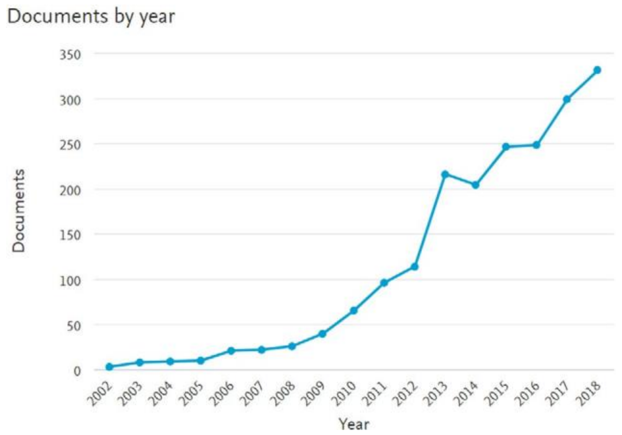

Figure 1. Results obtained from a search via Scopus with the combination of "microalgae" and "technology" in recent years.

2. Research Methodology

The research for this review focused on the main aspects of microalgae and related advances to disseminate studies about clean technologies. The Science Direct, Scopus, and Web of Science databases were used to build bibliometric maps of keywords, citations, co-citations, co-authorship, and co-occurrence of terms over the last 10 years. VOSviewer software version 1.6.6 was applied for bibliometric analysis using the Web of Science database. It is important to highlight that the searches were restricted to 10 years, considering that the preliminary research in Scopus (using the keywords microalgae and technology) showed that the first publications on this subject appeared in 2008, as shown in **Figure 1**.

The search was also conducted using Web of Science; however, this database showed that publications in this field started in 2013. After the analysis of each article, it was possible to determine that there were publications about this subject before 2013; however, the articles did not necessarily include the expression "clean technology," which was the format of the keyword selected for this search. Considering this, the analysis was confined to the years 2008–2018 to avoid losing relevant information. The use of both databases was necessary because Web of Science projected the best bibliometric maps in terms of keywords, and Scopus was used to complement information in this field.

The first search in Web of Science was conducted with the search title of "clean technology" between 2008 and 2018, and as a result, 485 original publications were found. Then, the combination of "microalgae" and "clean technology" was searched, but no results were received. This could be explained by the fact that many studies about microalgae found in bibliometric analyses are associated with the term "clean technologies"; however, the most common expression found in articles was "technology." Considering the explanation for this absence, the keywords "microalgae" and "technology" were selected to gain perspective on the existing topics in this field, via a search in the Web of Science database between 2008 and 2018. The combination of "microalgae" and "technology" resulted in 961 original publications. Keywords from Web of Science were extracted from the "title and abstract" using a minimum of ten occurrences of a term in binary counting for both searches.

After the bibliometric analysis, the search for papers in this field revealed the necessity to explore topics that could be relevant to the development of clean technologies, for example, cultivation, harvesting/dewatering, biomass drying, and bioproduct extraction. During the description of these items, it was possible to verify that one of the most important tools for describing all of these items was LCA. Considering the results and the importance of LCA, a search in the Scopus database was carried out using the title–abstract–keyword combination of the terms "life cycle assessment" and "microalgae" with publication years between 2008 and 2018. The total number of studies found on this subject was 219. Then, to complement and aggregate the information available in this context, a general search of studies was structured by publication year to illustrate studies on microalgae and clean technologies. It was also possible to visualize the main microalgae being studied, the predominant stage in which the LCA was being applied, the software or methodologies used for data analysis, and the main problems and how clean technologies may help to solve or minimize these problems.

A search performed in Scopus and Web of Science with the keywords "clean technology," "microalgae," and "future" to visualize future trends about this subject with a result of 214 publications is described in Section 6.

Finally, in Section 7, the final considerations about this review are described by the authors to demonstrate the main aspects discussed herein and the critical analysis for revealing alternatives for the development of strategies combining microalgae and clean technologies.

3. Microalgae and Clean Technology: Bibliometric Mapping

To obtain more information about studies involving clean technologies, bibliometric mapping was used to evaluate research trends and developments in this field over the last 10 years. The database was generated from publications in Web of Science, and VOSviewer software was used. The article search was performed by searching for the phrase "clean technology" including results from 2008 to 2018. The generated data were exported and saved for later data processing in relation to the keywords selected for this study. Based on the data obtained by searching for the phrase "clean technology," software and keywords related to items involving "technologies" and "biotechnology" were selected. The generated graph is shown in **Figure 2**.

In Figure 2, it is possible to distinguish three main groups. One group, marked in red, includes the main aspects that should be taken into account for the application of clean technologies. The main items in this group include words related to economics, prices, projects, strategies, management, environmental policy, and legislation, among others.

A second group (blue) provides the main study topics involving clean technologies, such as studies on emissions (of gases in general), CO_2 emissions, climate change, energy demand, fossil fuels, and renewable energy. In this group, the most commonly searched items and the most cited studies were related to "emissions," and this term is surrounded by words that are related

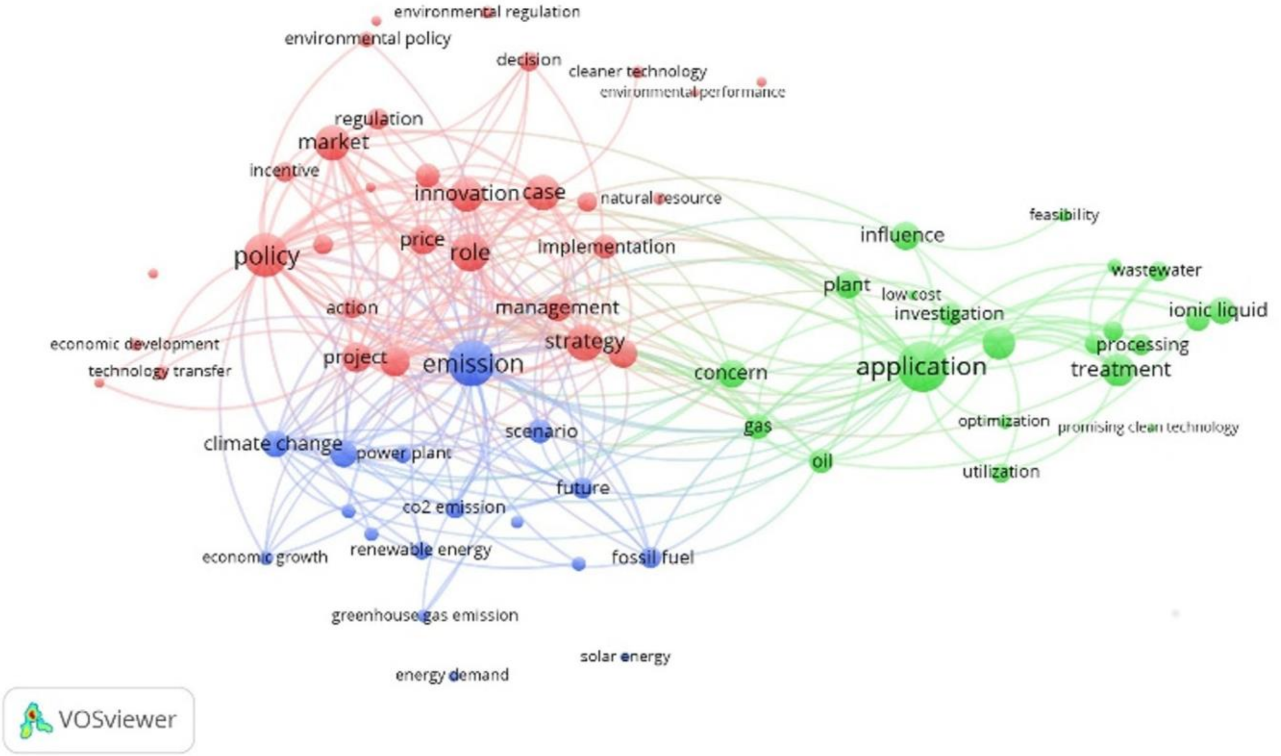

Figure 2. Cluster graphic obtained through bibliometric mapping using VOSviewer and the Web of Science database for studies from the last 10 years involving keywords related to "clean technologies," "technology," and "biotechnology."

to strategies, management, projects, legislation, innovations, incentives, implementation, actions, and case studies. This result demonstrates the growing attention in recent years focused on studies that aim to minimize GHG emissions and demonstrates all items needed to enable studies in this sector, including the analysis of viable strategies and the requirement to consider environmental policies and compliance.

In the third group (green), the main concerns that should be considered in studies of these technologies included investigations, applications, feasibility, processes, optimizations, and other keywords related to alternatives in this context. In addition, there was a dense area around the topic "application," demonstrating that there are many investigations on this topic that are trying put concepts into practice; these studies mainly include keywords related to wastewater treatment.

The results of bibliometric mapping were in accordance with the information found in the literature. According to del Río González,[38] the major obstacles to introducing clean technologies on the market include economic, institutional, and social barriers. In addition, perspectives associated with fossil-based energy combustion and emissions can be "internalized" through policy interventions. In addition, higher prices can regularly cause consumers to use less fossil fuels.[39]

After analysis of the general perspectives regarding clean technologies, a search using a combination of keywords related to "microalgae," "technology," and "biotechnology" was performed. The data generated in this search are provided in **Figure 3**.

Figure 3a reveals the generation of three main clusters. In one group (green), the main relationships between microalgae are shown with an emphasis on clean technologies, involving words such as "microalgae technology," "new technology," "sustainability," "economic viability," "prices," "energy balance," and "microalgae production," related to environmental problems such as "greenhouse gas emissions" and concerns with "bioenergy" and "commercial production." Another group (blue) presents perspectives about microalgal technologies, involving words such as "future," "prospect," "progress," "recent development," and "future development." The third group (red) includes microalgae that are related to studies on clean technologies, among which it is possible to highlight *Chlorella* sp., *Scenedesmus* sp., *Chlamydomonas* sp., *Nannochloropsis* sp., and *Dunaliella salina*; there are also words related to aspects of "algal growth," "microalgae harvesting," "flocculation," and "wastewater treatment."

In Figure 3b, the keyword "microalgae technology" is highlighted, and the main links to the keywords are shown. The technologies involving these microorganisms are especially related to subjects such as "wastewater treatment," "microalgae harvesting," and "commercialization." In general, this result shows that the demand for microalgal technologies is particularly related to a focus on "life cycle assessment," "economic analysis," "sustainability," "environmental impact," "energy balance," "wastewater treatment," "microalgae harvesting," and "climate change." It is also possible to visualize that "life cycle assessment" and "case studies" are necessary tools for improving these technologies. In

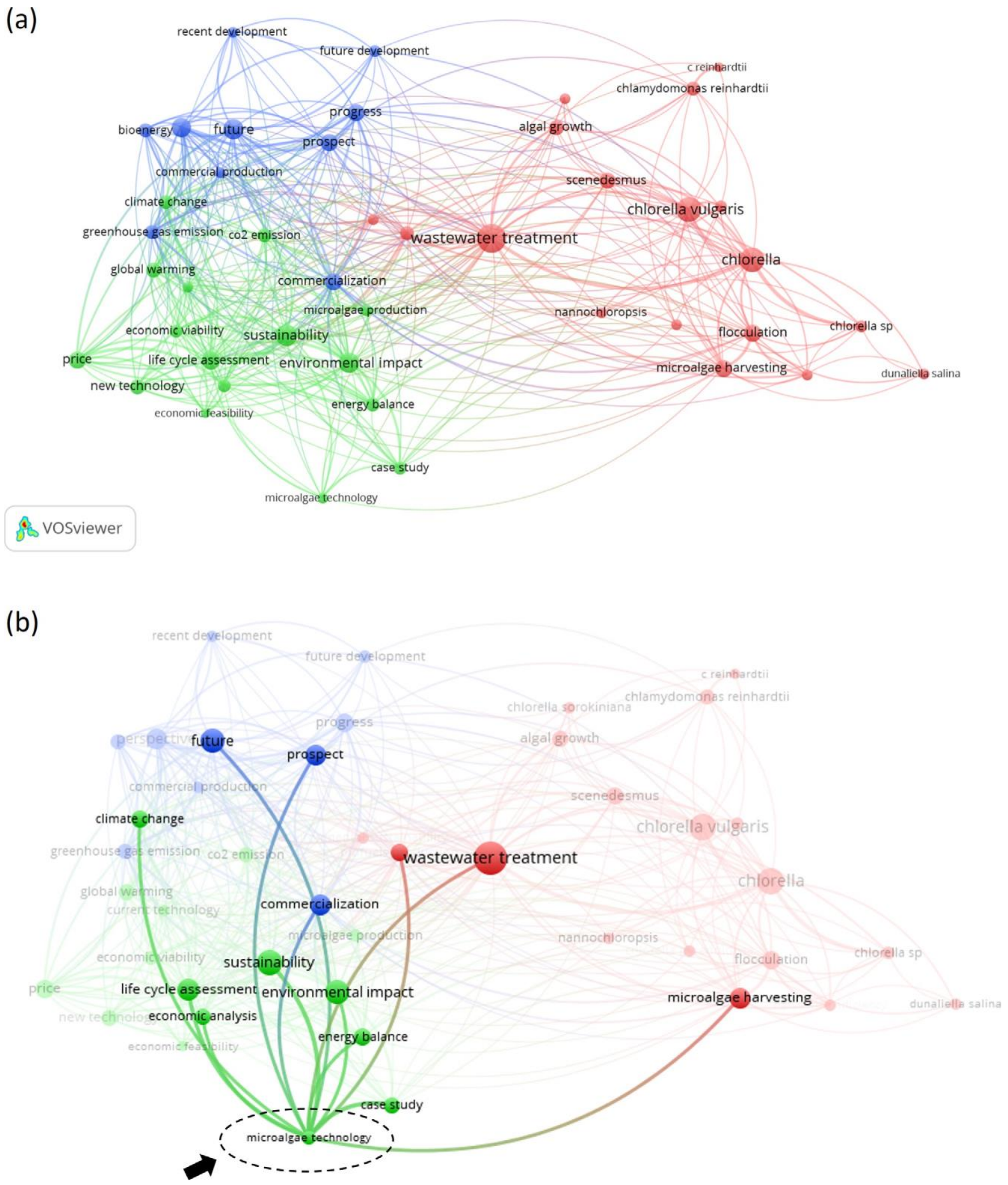

Figure 3. Cluster graphic obtained through bibliometric mapping using VOSviewer and the Web of Science database for studies from the last 10 years involving the keywords "microalgae," "clean technologies," "technology," and "biotechnology" (a) and highlighting the relationship of "microalgae technology" with the keywords (b).

addition, new technologies involving microalgae present future prospects related to commercialization.

4. Main Steps That Should Be Considered to Develop Cleaner Technologies from Microalgae

The production of microalgae depends on main steps such as cultivation, harvesting/dewatering, drying, and extraction. All of these parameters must be chosen to generate less GHG emissions and reduce energy use and residues. In **Figure 4**, it is possible to see the steps involved in microalgal production and their main inputs and outputs. However, to improve clean technologies involving microalgae, these steps must be evaluated (**Table 1**).

4.1 Cultivation

To enable microalgal cultivation, input parameters, such as CO_2, light, and nutrients (nitrogen, phosphorus, potassium, and organic compounds), must be taken into consideration to provide favorable conditions, as shown in Figure 4. Because microalgae consume large amounts of CO_2, which is posteriorly converted into biomass, the main output is the release of oxygen (O_2) into the atmosphere through photosynthesis.[46]

Microalgae can be cultivated in various systems with different volumes, such as open raceway systems, natural waters (lakes and ponds), artificial ponds, tanks, turf scrubber systems, or closed systems, such as photobioreactors. One of the major advantages of cultivation in open systems is their simplicity compared to closed systems. However, some factors should be taken into consideration when choosing the most appropriate system, such as microalga biology, implantation areas, energy, water, nutrients, climate, and the desired bioproduct.[47]

In general, photobioreactors may be a suitable alternative for cleaner production because these systems exhibit 85% lower energy consumption than indoor cultivation systems. However, negative aspects regarding energy balance have been found in bubble column photobioreactors.[48] Additionally, the choice of cultivation systems is one of the most important steps for large-scale development, because high costs are involved in most cases.[49]

On the other hand, the algal turf scrubber (ATS) system is a suitable option to reduce costs and increase productivity. The high rate of biomass production in this system is its main advantage, because it presents higher production yields than cropping systems. Many pollutants are absorbed in algae biomass and can be removed by the system itself. If it is ensured that there are no toxic compounds present, biomass can be converted into products of commercial interest, such as fertilizers and animal feed.[50]

4.2 Harvesting/Dewatering

Harvesting/dewatering is also a great challenge due to the low densities and suspended cells present in culture medium.[51] Unfortunately, the costs related to the harvesting and dewatering of microalgal biomass are still high and must be reduced for the process to become commercially viable.[52,53] Microalgal biomass is diluted within cultures (0.3–0.5 g dry biomass L^{-1}), resulting in difficulties in harvesting and dewatering. Additionally, microalga harvesting typically accounts for up to 20–30% of the total biomass production cost, which makes the harvesting process one of the major obstacles to the introduction of microalgae to industries.[54]

The recovery of microalgal biomass is essential for producing bioproducts that can later be applied at commercial and industrial scales. Several factors must be considered when choosing an efficient alternative to harvesting/dewatering, such as the nature of microalgal cells (size, charge, and morphology), the low concentration of biomass, and the high cost of necessary equipment,[41] as shown in Table 1. According to Soomro et al.,[22] several methodologies can be applied to promote harvesting/dewatering (Figure 4), including chemical (mostly flocculation), mechanical (centrifugation, filtration, natural sedimentation, flotation, and foam separation), electrical (electrocoagulation), and biological (autoflocculation and microbial flocculation) methods.

Chemical dewatering approaches, such as flocculation, are considered to be simple methods that can be performed with many potential variations; however, these procedures are expensive, because performance increases when the amount of flocculant increases.[42,55] Among the main chemical products tested as flocculants, the inorganic multivalent metal salts (aluminum sulfate, ferric chloride, ferric sulfate, etc.) and organic polymers/polyelectrolytes (chitosan) are described well in publications. Two of the major disadvantages related to inorganic flocculants are that most of the time, this process demands high quantities of flocculants, and biomass can be contaminated with aluminum, iron, or other metals. However, compared to other flocculants, chitosan is nontoxic and biodegradable.[56]

In mechanical separation, centrifugation is advantageous in microalga dewatering because it is rapid, easy, and effective; however, shear forces during the process can disrupt cells, which is economically unattractive because energy consumption is higher in mechanical separation than in other methods, such as chemical separation.[55,57,58] In addition, according to Pacheco et al.,[46] centrifugation presents one of the highest levels of life cycle energy consumption (700 MJ per dry ton of algae), high GHG emissions (50 kg CO_2 eq. per dry ton of algae), and high equipment costs.

Filtration is also an efficient alternative due to its high efficiency, low energy input, low cost, and recycling and reuse of water.[59,60] In contrast, one of the major disadvantages of this approach is that it is difficult to filter biological feed due to the compressible nature of biomass cake.[22] Another option is flotation followed by mechanical or filtration removal because this combination can achieve high efficiency in a short period. Although flotation is recognized as a favorable technique for microalga harvesting, there are some associated limitations, including the use of surfactants with different dosages to improve performance. This surfactant use can subsequently increase processing costs and may compromise effluent reuse.[61]

Within the electrical separation group, electrocoagulation can be highlighted; however, the main drawbacks of this approach are

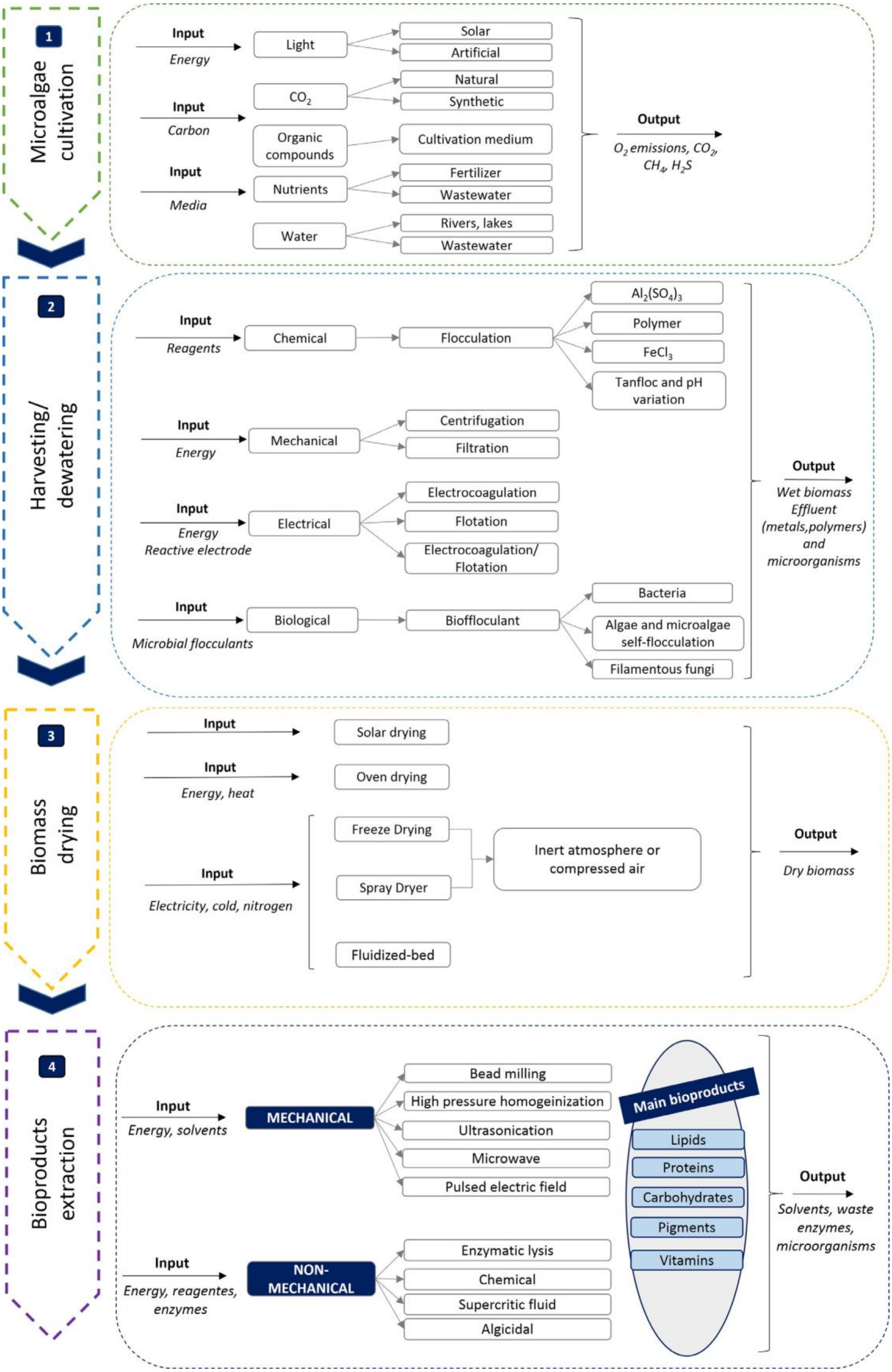

Figure 4. Main steps involved in microalgal biomass production.

Main step of microalgal production	Important parameter	Alternative to be considered to optimize these parameters	Reference
Cultivation	Photosynthetic efficiency	Average area production rates, light intensity and quality, intermittent light, and light acclimation	[40]
	Turbulence	Degree of mixing, hydrodynamic stress, and flow	
	Nutrients	Salt content, major ionic components (K^+, Mg^+, Na^+, $Ca_2{}^+$, $SO_4{}^{2-}$, and Cl^-), nitrogen sources (nitrite, ammonium and urea), carbon source, pH, trace elements, and vitamins	
	Growth rate	Water, carbon source, light intensity, nutrient concentrations (dissolved CO_2 or $HCO_3{}^-$), and favorable temperature and pH	
Harvesting/dewatering	Nature of microalgae cells	Particle size, gravity, and morphology	[41]
	Surface charge	For pH > 6, surface charge is dominated by negatively charged carboxylate ions and neutral amine groups. With lower pH, the surface charge of the cells was reduced, and they became unable to form large flocks. With surface charge neutralized at pH 4, flocculation efficiencies can reach their maximum	[42]
	Biomass	Increase concentration	
Biomass drying	Properties of the microalgae suspension	Content of target component, cell size, shape and surface charge, salt concentration, pH, etc.	[43]
	Drying method	Selection of an appropriated drying method in order to isolate biomass properties	
Bioproduct extraction	Biomass preparation	Concentrating and dewatering microalgae before undergoing extraction	[44]
	Cell wall disruption	Thick and robust algal cell walls need appropriate methods for disruption (e.g., ultrasonication and microwave) and solvents (or solvent-free)	
	Extraction/recovery	Associated with cell wall disruption	
	Safety and environmental concerns	Apply green chemistry concepts	[45]

the cost of electricity, fouling of cathodes, and periodic changes in anode materials.[53,62] On the other hand, the combination of electrocoagulation–flotation offers an attractive alternative for this purpose with relatively low energy consumption (0.3 kWh m^{-3}).[62] In this case, effluent reuse during the process may also be compromised, considering that microorganisms can remain in the medium, affecting the production of microalgae that will be cultivated later.

The most common biological separation approach is bioflocculation because the microalga dewatering costs under this technique can be reduced, no chemical costs are involved, and the process requires lower energy consumption. For example, microbial flocculants can be used for this purpose due to their high harvesting efficiency and biodegradability.[63,64]

Finally, considering the consumption of chemical or biological agents, the generation of effluents to be discarded or reused, and energy consumption, some of the most commonly employed techniques are still centrifugation and filtration because they are solely mechanical processes. Regarding these two methods, it should be noted that the use of clean technologies, such as photovoltaic methods, can guarantee better economic and environmental benefits than traditional approaches.

4.3 Drying

Biomass drying is also an important step in the process, and although it entails high energy consumption, it provides better conditions for the next step, which is extraction.[65] Numerous drying methods can be found in the literature, among which freeze drying, sun drying, oven drying, belt drying, and spray drying are the most common, as shown in Figure 4.[66–70]

Parameters such as pressure and temperature should be taken into consideration as well, because these conditions can degrade important cellular components of microalgae (Table 1). High temperatures are recognized to decrease the physical properties of microalgal cells, especially their ability to retain lipids, compromising the quality of the final product. On the other hand, low-temperature methods, such as freeze drying, ensure greater stability regarding the lipids produced but can also disrupt cells and destabilize lipids during freezing.[43,71]

The choice of drying technique is related to energy costs, processing time, and the application of dry biomass. Although freeze drying is one of the main techniques for maintaining cell stability, an oven furnace is energetically more cost-effective; thus, a spray dryer is often applied for this purpose, especially on an industrial scale.[71]

Spray drying is one of the predominant techniques in the dairy industry and is characterized by a lower specific energy cost and higher productivity than other techniques such as freeze drying.[72] Spray dryers are used in industry particularly for aseptic pharmaceutical processing and feed processing, removing anywhere from a few kilograms of liquid per hour to well over 100 tons per hour. Another advantage of this approach is the versatility conferred by its flexible suitability for meeting biotechnological requirements, including the use of low-heat treatments to avoid loss of activity.[73]

The use of spray dryers is common in industries due to their ability to convert liquid feedstocks into dry solid products;

however, this type of drying consumes large quantities of energy.[74] According to Baker and McKenzie,[74] after evaluating the energy consumption of typical spray dryers, it was possible to verify that spray dryers exhibit evaporation rates ranging from 0.1 to 12 t h^{-1}, specific energy consumption (Es) varying from 3 to 20 GJ t^{-1} water evaporated, and a fuel-to-electricity consumption ratio of approximately 27.

Lin[75] subjected *Chlorella* and *Spirulina* to spray drying and freeze drying under several different conditions. The results showed that spray drying destroys toxic substances much more effectively than freeze drying and produces a thicker powder (3.5–5.5 kg), whereas freeze drying produces a powder that is almost half as hard (2.5 kg).

4.4 Bioproduct Extraction

Another obstacle to obtaining high-value products is associated with extraction because the microalgal plasma membrane is protected by a complex cell wall that presents complexity and rigidity.[76] In addition to these factors, combinations of extraction methods that are economically and environmentally friendly are continuously being investigated.

Microalgal cell walls present diversity mainly in terms of their molecular components, intra- and intermolecular linkages, and overall structure.[77] Many compounds can be extracted from microalgae, such as fine chemicals, polyunsaturated fatty acids, oil, pigments, sugars, antioxidants, bioactive compounds, and proteins.[78] According to research by Lardon et al.[26] using LCAs, lipid extraction is one of the most studied processes and accounts for 90% of process energy consumption (70% when considering wet extraction).

This statement demonstrates the need to improve the economic viability of the process. In contrast, microalgae present an unprecedented potential to supply future energy demands, and commercial process viability is related to the costs incurred, particularly in lipid extraction.[79,80]

Several mechanical (bead milling, high-pressure homogenization, ultrasonication, microwave, and electrochemical) and nonmechanical (enzymatic lysis, chemical extraction, and supercritical fluid) cell wall disruption methods are used for microalga extraction, as shown in Figure 4. However, the microalgal species involved and their biology and cell wall characteristics must be considered to select appropriate cell disruption and extraction methods, avoiding methods that can cause environmental impacts,[81] as shown in Table 1. After the evaluation of all of these parameters, the sector in which the microalgae will be used must be considered because there are many sectors in which microalgal biomass could be an interesting source of high-value products, such as lipids, proteins, pigments, carbohydrates, and vitamins.[82]

The most traditional methods of lipid extraction from microalgae were developed by Folch et al.[83] and Bligh and Dyer,[84] who demonstrated rapid lipid extraction. The first of these two methods demands the use of mineral salts, such as NaCl, and large volumes of solvent, and both methods use chloroform and methanol, which are toxic and flammable solvents that affect health and the environment. Additionally, these solvents can affect the quality of the final product because they can dissolve undesired products (chlorophyll) during the extraction process.[79]

For microalgal proteins, Bradford[85] described the most common methods for measuring the total protein concentration in a sample and performing high-pressure homogenization,[86] which can increase protein treatment and the degree of hydrolysis.[87] To extract carbohydrates from microalgae to produce simple sugars, it is necessary to convert polysaccharides into fermentable sugars, and this process involves enzymatic or slightly acidified hydrolysis.[88] Additionally, the pretreatment of microalgae with acids as an important step in this process causes cell disruption and an increase in the sugar levels available for conversion. Enzymes can be used to hydrolyze cellulose and hemicellulose into simple sugars, including glucose, and when combined with acid hydrolysis, this process is environmentally acceptable.[89]

Pigment extraction is usually performed with solvents, such as acetone or methanol,[90] and can be improved with ultrasound.[91,92] The most common method for vitamin extraction from microalgae involves the use of solvents, such as ethanol and chloroform,[93] or supercritical fluid extraction.[94]

4.5 Management in Crop Protection

The previously mentioned steps are the steps that are most commonly found in the literature in relation to the production of microalgae; however, in recent years, the selection of microalgal strains and the concept of crop protection have been increasing in importance. The analysis of these parameters is essential because they can influence the stage of cultivation, bioproduct obtention, and the application of derivatives for crop protection. The management of microalgal productivity can reduce environmental impacts, reinforcing the concept of clean technologies.

Strain selection is one of the requirements for the improvement of production, and if this choice is not performed properly, it can impact productivity and applicability. Management of the production of microalgae leads to the improvement of yield in biomass productivity. Therefore, strain selection is one of the main management measures that leads to a better relationship between output and input in this process.

As shown in **Table 2**, light/dark cycles, pH, CO_2, and medium composition can directly affect microalgal productivity. All of these factors may vary, and the microalgal strain must be carefully selected. Another parameter that must be considered in future studies is the progress on crop protection in microalgae.

Crop protection is a promising alternative to avoid economic losses and deal with pests without causing damage to people or the environment. This strategy is very challenging considering the multiple existing pests, such as insects, plant pathogens, and weeds.[99]

Algae are more productive than other terrestrial biofuel crops because they may present a better oil yield, and bioproducts from microalgae exhibit considerable potential in many fields. In this context, research on crop protection has been increasing in recent years because there are grazing pests (e.g., rotifers, ciliates, and cladocerans) and pathogens (e.g., bacteria, viruses, and fungi) that could interfere with the production of microalgal biomass and bioproducts.[100]

Table 2. Main steps of microalgal production, the most important associated parameters, and alternatives to optimize these steps.

Strain	Study focus	Parameter tested to improve strain conditions	Best condition	Yield	Reference
Chlamydomonas reinhardtii	Starch	Sulfur-replete, and sulfur-depleted medium	Sulfur-deprived medium	49% w/w	[95]
Chlorella vulgaris	Biomass and lipid	Total inorganic carbon (CO_2) to total organic carbon (glucose) and total carbon to total nitrogen	Total inorganic carbon to total organic carbon (20:1) Molar ratio of total carbon to total nitrogen (72:1)	Maximum growth rate (32 g m^{-2} per day) and lipid (47.53%)	[96]
Desmodemus sp.	Biomass and biodiesel	pH	pH 3	Biomass productivity of 0.25 g dry wt. L^{-1} and fatty acid methyl ester (13%)	[97]
Heterochlorella sp.	Biomass and biodiesel	pH	pH 3	Biomass productivity (0.45 g dry wt. L^{-1}) and fatty acid methyl ester (15%)	[97]
Nannochloropsis gaditana	Biomass, lipid, and protein	Light/dark regimes (L/D), autotrophic, mixotrophic, and heterotrophic	Biomass productivity: 16L/08D, mixotrophic Lipid: 16L/08D, autotrophic Protein: 12L/12D, mixotrophic	Biomass productivity (142 mg L^{-1} per day), lipids (16.7%), and proteins (44.8%)	[98]

The development of methods for crop protection is essential to inhibit pests without affecting microalgae and to increase the commercialization of bioproducts from these microorganisms on an industrial scale. Some of the promising crop protection agents for sustainable algaculture are related, such as ammonia, copper, rotenone, hypochlorite, and quinine.[100,101] According to El-Sayed et al.,[101] rotenone can be used as a crop protector for algae because it is preventative against grazing predators, avoids algal open pond crashes, presents a low cost, and stimulates the algal biofuel industry. Thomas et al.[100] studied the effects of free ammonia as an algal crop protector in defined media and dairy wastewater in the presence of *Brachionus plicatilis* rotifers. The use of ammonia was shown to be advantageous, because it requires neither input costs nor the application of chemical compounds.

The development of cost-effective methods is necessary to protect algal crops against predators and is therefore vital for the successful production of microalgae on an industrial scale. Crop protection strategies associated with low costs are vital to sustainable production. Studies associating crop protection and microalgae are still incipient; however, this approach may be a suitable alternative for overcoming barriers in microalgal production and improving clean technologies.

5. Importance of LCA to Improve Clean Technologies Using Microalgae

LCA is a useful methodology for quantifying the environmental impacts and energy requirements of a particular product from the extraction of raw materials to its production, use and recycling, and the final disposal of its wastes. The major advantage of LCA is the possibility of determining the total environmental performance of a process, which is useful for decision-making aspects of environmental management, monitoring and policy-making related to energy, and emission "bottlenecks."[27] According to Benemann et al.,[102] LCA can take into consideration all environmental impacts of a process from "cradle to grave," and the use of this tool is now a major requirement for all studies on renewable energy processes.

To Gerbens-Leenes et al.,[103] cradle-to-cradle boundary is another option to be considered, for example, for the production of microalgae fuels. This system can evaluate the production of microalgae with different cultivation systems and to convert routes at varied climate situations. Besides, Solé-Bundó et al.[104] demonstrated that recovery of wastewater in high-rate algal ponds is a prerequisite for technological development of a cradle-to-cradle bio-based economy. Considering all the studies related to LCA and its importance in the microalgal field, the connection between these studies could be a starting point for developing clean technologies. For better visualization of studies on LCA, a new search was carried out in the Scopus and Web of Science databases to obtain additional information using the title–abstract–keyword combination "life cycle assessment" and "microalgae" with publication dates between 2008 and 2018, and 214 documents were found in this search. The information generated was in the form of published documents by year and per year by source, author, affiliation, country or territory, and type. The results are provided in **Table 3**, where it can be seen that microalgal production associated with LCA is a current topic among researchers, especially in the last 5 years.

After this preliminary analysis, it was possible to observe that the number of documents per year has increased over the last 10 years, with an emphasis on the fields of energy and environmental sciences. The main country generating publications on this subject in recent years was the United States, and the types of publications (which initially only included articles) included reviews, conference papers, books, and book chapters. Through the individual analysis of each parameter, it was possible to obtain a clearer idea about the studies involving microalgae, clean technology, and LCA. It was observed that there is a close relationship between LCA and microalgae, with a focus on biodiesel production, as shown in **Figure 5**. The analysis of the publications focusing on their content revealed that LCA is a useful option for evaluating microalgal cultivation, harvesting and biomass drying, and the extraction of

Table 3. Results obtained from a Scopus search using the combination of "life cycle assessment" and "microalgae" over the last 10 years in documents published by year, number of publications, country or territory, area, and type of publication.

Year	Number of publications	Main country	Main area	Publication type
2008	1	China	Energy	Article
2009	3	China	Environmental Science	Article and review
2010	7	United States	Chemical Engineering	Article and conference paper
2011	13	United States	Environmental Science	Article and conference paper
2012	15	United States	Energy	Article, review, and conference paper
2013	23	United States	Environmental Science	Article, review, and book
2014	27	United States	Environmental Science	Article, review, conference paper, and book
2015	32	United States	Energy and Environmental Science	Article, review, and book
2016	31	United States	Energy	Article, review, conference paper, and book
2017	32	United States	Energy	Article, review, conference paper, and book
2018	28	Brazil, India, and Malaysia	Energy	Article, review, conference paper, and book

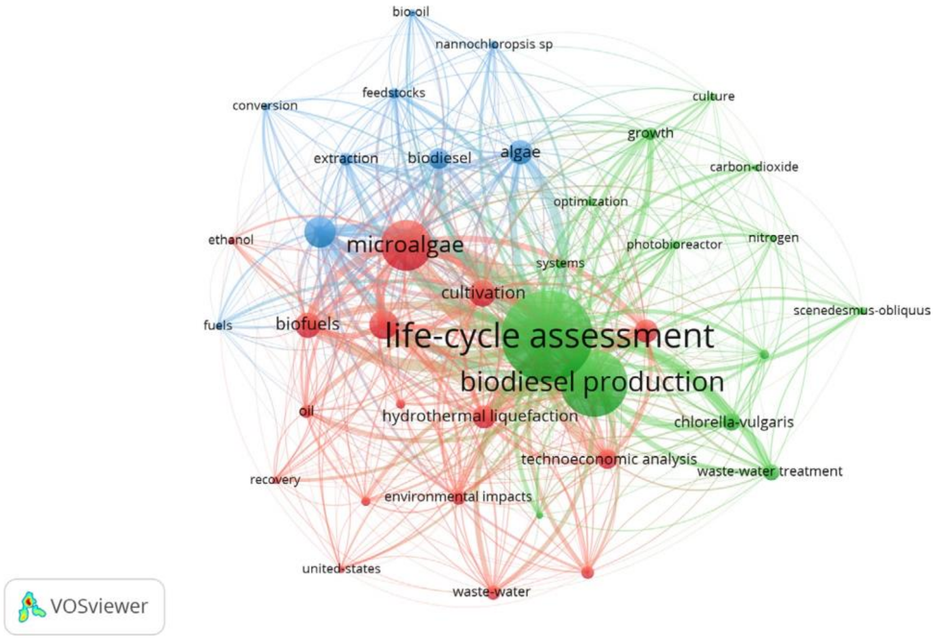

Figure 5. Cluster graphic obtained through bibliometric mapping using VOSviewer and the Web of Science database for studies from the last 10 years showing co-occurrence of the keywords "microalgae" and "life cycle assessment."

high-value products. In addition, LCA enables viable technological innovation, reducing the intensity of energy use and improving environmental performance in general.[102,105] It is important to highlight that despite the major advantages of LCA, the relationships between goal and scope, system boundaries, functional units and life cycle impact assessment (LCIA) methods, and the items required to appropriately evaluate necessary steps for the production of microalgae are difficult to determine.[106]

Microalgal cultivation can be optimized by LCA, and many studies have described the potential of this tool, especially for the production of higher biomass content. The main studies that have used microalgae to develop clean technologies are shown in **Table 4**.

Jorquera et al.[20] performed an analysis of the energy life cycle for the production of biomass in raceway ponds and tubular and flat-plate photobioreactors (PBRs) using *Nannochloropsis* sp. and showed that PBRs are less economically feasible than raceway pond systems. However, Silva et al.[49] showed that these systems are an efficient option for cultivation because they can guarantee atmospheric CO_2 fixation, do not contribute to increases in GHG emissions, and qualify as a true renewable biomass source for biofuel production. In the same context, Zaimes and Khanna[21] affirmed that many studies have indicated that PBRs exhibit high initial capital and operating costs, hindering their commercial viability. These authors proposed a study to examine critical life cycle energy and environmental drivers in algae

Table 4. Life cycle assessment studies of microalgal products and major steps that are problematic or show potential for improvement in processes using clean technologies.

Microalga	Clean technology examined	Problem	Impact analysis evaluation for LCA	How did the clean technology help?	Reference
Chlorella vulgaris	Biodiesel	There are many obstacles and limitations about biodiesel from microalgae	CML method	LCA is a relevant tool to evaluate new technologies for energy production. This tool was capable identifying technological bottlenecks and therefore supports the eco-design of an efficient and sustainable production chain	[26]
Chlorella vulgaris	Biodiesel	More complete LCAs of biodiesel production from microalgae are required	Gabi 4 software	Cultivation in raceways would be significantly more sustainable than closed air-lift tubular bioreactors because they present a global warming potential ~80% lower than fossil-derived diesel	[108]
Nannochloropsis sp.	Biomass production in photobioreactors and open pounds	High costs associated with biomass production	Gabi software	The net energy ratio of ponds and flat-plate photobioreactors could be significantly higher if the lipid content of biomass was increased to 60% dry weight/cell dry weight	[20]
Nannochloropsis sp.	CO_2 analysis of microalgae-to-biodiesel	Microalgae are known to be environmentally friendlier alternatives to conventional fuels; however, debate about its ecological benefits or drawbacks still exists	ISO 14000 series (ISO 14041–43)	LCA was helpful to visualize that biodiesel production does not necessarily reduce greenhouse gas emissions. There is also a relationship between the amount of CO_2 emitted and total energy requirements	[109]
Chlorella vulgaris	Water and nutrients to biodiesel production	Biodiesel production from microalgae may consume potable water and too much nutrients becoming the process unfeasible	Reports from literature	Recycling harvest reduces water and nutrients by 84% and 55%, respectively. Using sea/wastewater decreases water requirement by 90% and eliminates the need for all the nutrients except phosphate	[14]
Chlorella vulgaris	Biogas	To achieve progress in this sector, costs and circulation between different production steps must be decreased	CML method	Methane production with biodiesel and palm biodiesel presents a better option in terms of land use (24.5% of the impact of rape biodiesel), photochemical oxidation, eutrophication, and acidification	[29]
Microalgae mix	Biodiesel	The need for a high production rate to make algal biodiesel economically attractive	SimaPro 7 software	Favorable soil conditions can achieve high annual growth rates, which is an economically viable way to reduce greenhouse gas emissions by growing algae and processing it into biodiesel	[110]
Microalgae mix	Oil	Essentially, algae oils are produced in small amounts for fuel testing	–	Based on LCA, microalgae may be sustainably produced with a relatively small greenhouse gas footprint	[102]
Chlorella vulgaris	Biomass	Biomass production must be analyzed to make algae a viable and more efficient alternative for biofuel production	Cradle-to-grave analysis	A systems approach with life cycle thinking can test, ground the claims, and assess the environmental sustainability of emerging technologies	[21]
Tetraselmis chui	Biomass and bio-oil	Until this study, LCA was not available to assesses pyrolytic conversion of microalgae biomass into various co-products	SimaPro 7.3 software	Application of the LCA method showed that biological carbon capture and storage via microalgae-derived biochar is not currently ideal due to the material and energy intensity of the overall value chain	[105]
Haematococcus pluvialis	Carotenoid astaxanthin	Particular interest in producing astaxanthin depends on different cultivation technologies to improve productivity and yield	SimaPro 7.3 software	It was possible to propose a two flat-panel photobioreactor system with artificial illumination that reduced between 62% and 79% of the impact, depending on the considered category	[25]

(*Continued*)

Table 4. Continued.

Microalga	Clean technology examined	Problem	Impact analysis evaluation for LCA	How did the clean technology help?	Reference
Nannochloropsis oculata	Biodiesel	The environmental benefits obtained from microalgae are still unclear	ReCiPe midpoint method	This analysis led to a new insight on energy production by microalgae that includes systems to support local energy consumption	[28]
Microalgae mix	Biojet fuel	Conventional jet fuel presents high emissions of greenhouse gases	SimaPro 7.3.3 software	The GHG emissions of algal biojet fuel can be reduced by 76% compared to those of conventional jet fuel	[30]
Chlorella vulgaris	Biodiesel	Biofuels are related to the level of microalgae biomass and lipid content, and these aspects depend on the cultivation process	OpenLCA 1.3 software	Quantification of the environmental impact of microalgal-based biodiesel production was calculated, and the global warming potential and fossil energy requirement savings were 42% and 38% if compared to fossil-derived diesel, respectively	[27]
Species in general	Biomass production in photobioreactors	To reach industrial-scale microalgae biomass production, studies are necessary to find the issues that prevent this energy source from being a reality	SimaPro 7 software	The use of compact PBRs for cultivation of microalgae has the potential to cause less of an impact on the environment (land, sea, and air resources) from a life cycle point of view than cultivation in raceway ponds	[49]
Scenedesmus dimorphus	Thermochemical process to biofuel production	A need exists to evaluate and compare the environmental impacts of thermochemical process technologies applied to microalgae	GREET model	The pyrolysis pathway was not energetically or environmentally favorable. Excess energy in the pyrolysis process can be used in other processing steps, such as drying	[23]
Microalgae mix	Dewatering process	The significant cost of the dewatering process and difficulty applying microalgae at the commercial scale	ISO 14040	A comparison of the two-step dewatering processes was proposed, and centrifugation was suggested as a single dewatering technique because it presented less energy, low	[22]
Neochloris oleoabundans *Chlorella sorokiniana* *Tetraselmis suecica* *Nannochloropsis oculata*	Biorefinery co-products	Valorization of co-products must be evaluated to achieve economic and environmental goals	GREET model	The growth cycle times that maximize a single fraction do not necessarily result in the most favorable environmental performance on a life cycle basis, underscoring the importance of designing biorefinery systems that simultaneously optimize lipid and nonlipid fractions	[24]
Scenedesmus dimorphus	Biodiesel	The overall impact of biodiesel production from microalgae under multiple scenarios has not been completely explored	GaBi 6.5.1.8 software	Among the scenarios, cultivation in a raceway pond was the most energy intensive process with the mode of culture mixing and biomass productivity being the principal determinants. The impacts were found to be directly linked to energy demand and had an inverse relationship with biomass productivity	[11]
Chlorella vulgaris	Harvesting	LCAs specifically focusing on the assessment of microalgae harvesting have to be presented	SimaPro 7.3 software	Centrifugation appeared to be the worst alternative, whereas flocculation has a lower impact in most of the relevant environmental categories	[19]

cultivation in open raceway ponds. The results indicated that the proposed scenarios and technologies may have the potential to reduce the critical demands of biomass production and should be considered to make algae a viable and more efficient biofuel alternative.

Yang et al.[14] examined water and nutrient life cycles to evaluate the production of the microalga *C. vulgaris* using harvested water recycling and sea/wastewater as a water source, and the results showed that the use of sea/wastewater decreases water requirements and eliminates the need for all nutrients except for phosphate. A comparative LCA was conducted by Collotta et al.[19] using scenarios based on the application of two harvest- ing technologies: 1) flocculation and centrifugation and 2) direct centrifugation (without flocculation). The results indicated that scenario 1 prevailed over scenario 2 due to its better results in environmental impact categories.

Concerning dewatering, the evaluation of an effective framework for assessing the performance of different technologies was studied by LCA to establish a two-stage dewatering system that could decrease energy consumption, CO_2 emissions, and costs. The LCA results presented viable information about the energy input, environmental impacts, and costs related to a two-step microalga dewatering technique, showing the advantages of this method in comparison to conventional dewatering with a single step.[22] Another option for the improvement of processes that might reduce overall energy use and GHG emissions is the enzymatic degradation of algal biomass in aqueous solutions.[107] This option could eliminate the need for energy-intensive dewatering/dehydration, and CO_2 emissions would be reduced by 45%.

Another important aspect to highlight is the impact categories evaluated in LCA, which are parameters that refer to a wide range of categories, such as climate change, resource depletion, and ecotoxicity. All of these potential impacts should be considered to provide an integrated approach for complete impact assessment.[112]

The impact categories of abiotic depletion, potential acidification, eutrophication, global warming potential, ozone layer depletion, human and marine toxicity measurement impacts, land competition, emission of ionizing radiation, and photochemical oxidation were included in an LCA of biodiesel[26] and biogas[29] production using the microalga *C. vulgaris* and biodiesel production using *Scenedesmus dimorphus*.[111] These impacts were also evaluated for *Haematococcus pluvialis* to verify the best scenario for astaxanthin production.[25]

The LCA parameters for biodiesel production using the microalga *Nannochloropsis oculata* were evaluated through an analysis of fertilization, culture, dewatering, transformation, combustion, infrastructure, fertilizer production, other chemical production, process emissions, electricity production, heat production, and combustion emissions.[28] For biojet fuel production from a microalgal mixture, several impact categories have been evaluated, such as transportation to the refinery, transportation of aqueous coproducts to a wastewater treatment plant, hydrothermal liquefaction, upgrading processes, and al- gal production at a wastewater treatment plant, among other processes.[30]

The impact categories of energy consumption for air pumping and water pumping (cooler), water consumed for cooling, the flow rate of sea water to maintain the dilution rate, and caloric content equipment were evaluated for the microalga *Nannochloropsis* sp. to verify the best conditions for its cultivation and for achieving higher yields of biomass.[20] Global warm- ing, abiotic resource depletion, land transformation and use, water resource depletion, eutrophication, acidification, ecotoxi- city, human toxicity, photochemical smog, ozone depletion, ion- izing radiation, and respiratory effects were studied in relation to biomass and bio-oil production by the microalga *Tetraselmis chui*.[105]

Regarding the use of microalgae to convert biomass into biofuels, the thermochemical process of pyrolysis has been indicated proven to be suitable for this purpose according to an LCA evaluation. However, there are some challenges associated with the use of microalgae as feedstock, especially in the step of microalgal drying, because it is an energy-intensive process.[23]

Another step that must be considered is the biorefinery of coproducts. Montazeri et al.[24] studied the importance of coproduct valorization for achieving energy and environmental goals in biorefineries using the microalgae *Neochloris oleoabun- dans*, *Chlorella sorokiniana*, *Tetraselmis suecica*, and *N. oculata*. The results indicated that per kilogram of biodiesel produced, *C. sorokiniana* harvested at day 12 was most favorable in terms of both reducing GHG emissions and its cumulative energy demand, despite exhibiting a lipid content below 20%. For the production of astaxanthin, Pérez-López et al.[25] performed LCA following ISO 14040, and ten impact categories were considered: abiotic depletion, acidification, eutrophication, global warming, ozone layer depletion, human toxicity, freshwater aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity, and photochemical oxidant formation. According to their results, these categories are related to electricity requirements as a major contributor to the environmental cost of activities involved in astaxanthin production.

The concept of liquid biofuel production compensating for fossil fuel production is problematic because it would require a long-term reduction in GHG emissions.[102] Instead, an increase in the biofuel supply might intensify fuel consumption, including that of fossil fuels. According to Benemann et al.,[102] one of the major disadvantages of producing biofuels from microal- gae is high costs. Lardon et al.[26] stated based on LCA that production, harvesting, and oil extraction from microalgae demand high-energy consumption, compromising the overall energetic balance. It has also been noted that biodiesel produced from microalgae is not environmentally competitive under current feasibility assumptions and that environmental and energetic analysis to improve energy balance is clearly the key priority for making microalgal cultivation sustainable.[28] As an alternative to decreasing these difficulties, Togarcheti et al.[111] suggested that a cultivation system involving culture mixing and higher biomass productivity may make algal biodiesel production viable and sustainable.

Biogas from microalgae is another renewable source of energy that is being studied. Collet et al.[29] used LCA to evaluate the potential impacts of methane and its combustion using *C. vulgaris* biomass. The results suggested that the impacts generated by the production of biogas from microalgae are mainly correlated with electricity consumption.

The production of algal biojet fuels was also evaluated us- ing LCA. This study indicated that biojet fuel produced from algal feedstocks does not result in higher life cycle greenhouse gas (LC-GHG) emissions than conventional jet fuel production and that this approach could be a suitable alternative for biofuel production.[30]

According to most studies involving LCA and microalgae, the main tools used for impact analysis are chain manage- ment by life cycle assessment (CMLCA),[26,29] which is a soft- ware tool that supports the technical steps of the LCA procedure; GaBi (Ganzheitliche Bilanz),[20,108,111] which aids in LCA and cost evaluations in a working environment; SimaPro,[25,28,49,105,110] a tool for collecting data and analyzing the environmental per- formance of products, services, and LCA; the ReCiPe mid- point method[28] for evaluating impact assessment (LCIA) in LCA; the GREET MODEL[23,24] for performing LCA simu- lations of alternative transportation fuels and vehicle tech- nologies; and ISO 14040,[22] which is also used for LCA studies.

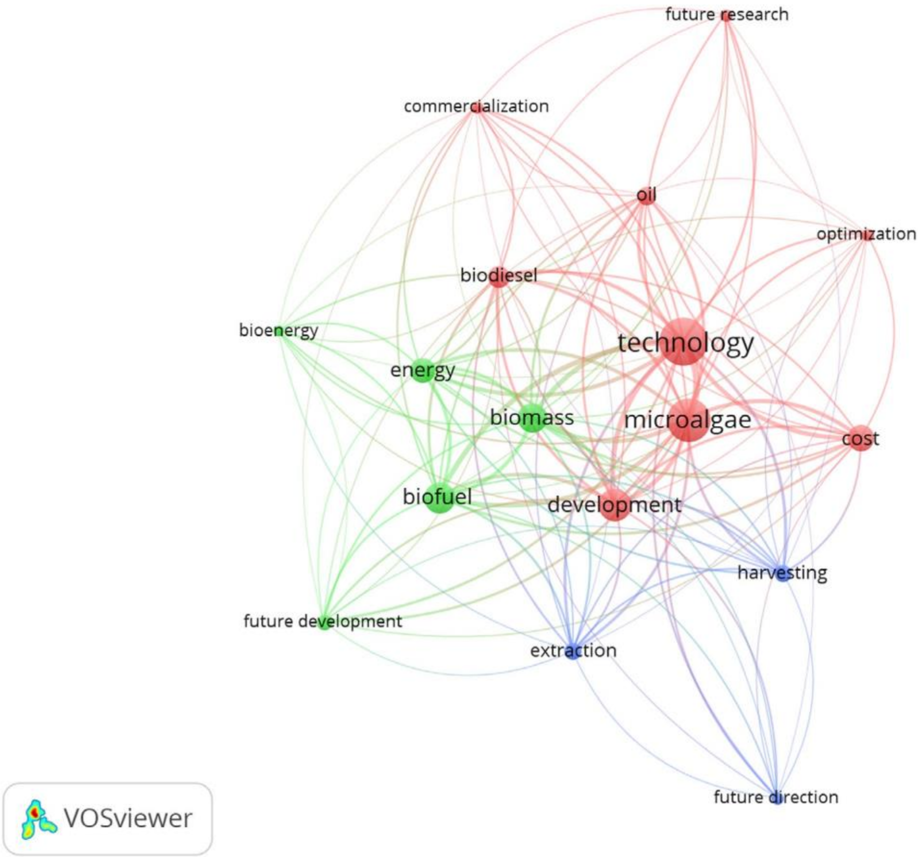

Figure 6. Cluster graphic obtained through bibliometric mapping using VOSviewer and the Web of Science database for studies from the last 10 years showing keywords related to "technology," "microalgae," and "future."

6. Future Trends

As shown in this review, the association between clean technologies and microalgae is increasingly being studied. Thus, this combination may bring about future advances in several steps in the production of microalgae, such as the obtention of bioproducts, bioremediation, or reduction of environmental impacts caused by GHG emissions. To verify the future trends in this field, a final bibliometry analysis was conducted with the keyword combination "microalgae," "technology," and "future." The results of this search are presented in **Figure 6**.

It should be noted that the results obtained through bibliometric mapping showed that the development of technologies associated with microalgae is one of the most cited topics in stud- ies in this field (red cluster). The costs and commercialization associated with this cluster, especially in the biofuel sector, can be considered to be among of the main bottlenecks in making the use of these technologies to more widespread. In the blue cluster, it is possible to observe that the biomass extraction and harvesting steps must be studied further. Future research on the development (green cluster) of biofuel, biomass, and bioenergy production from microalgae must continue to advance because these topics are challenging.

The steps mentioned during this review, especially those related to harvesting and extraction, are still considered problematic. In these cases, clean technologies are important to solve these bottlenecks because they are a promising alternative for future development in this area. Recent studies have shown that harvesting is still one of the most critical steps in microal- gal production, considering the expensive operational costs and high energy dependence of this process.[113] According to Nguyen et al.,[114] the cost of harvesting can account for 20–30% of the to- tal cost of microalgal production and may even reach 60% of the total cost in some circumstances when postproduction is necessary. In this context, organic biopolymers can become an attractive option for use as flocculants in microalga industries.[115] In addition, bioflocculation (especially using autoflocculating microorganisms and filamentous fungi) can be an option for the harvesting of microalgal biomass by flocculation, because the associated energy consumption is low. Automating this step could represent a great step forward in facilitating future innovations in this field.[116]

For the extraction step, it is essential to choose methods that minimize the use of solvents and consequently reduce environmental impacts. The search for clean technologies that improve the relationship between microalgal cultivation and biomass production is necessary for future development in this field, as are alternatives to increase productivity and efficiency and reduce process inputs. According to Souza et al.,[117] bioproducts from microalgae may be unsustainable if the production process is not well established. The commercial production of biofuels and the costs involved are nonviable in many studies, and one of the main bottlenecks is the extensive energy input required. Al-Ameri and Al-Zuhair[118] indicated that simultaneous wet microalgal cell disruption and oil extraction, together with transesterification and biodiesel separation in one step, may be an effective innovation because it avoids energy-intensive drying and other steps. Additionally, wet microalgal cells can be used directly for biofuel production after pretreatment. The extraction of lipids from dried microalgal cells demands a great deal of energy during the dewatering process.[119]

All of these parameters can be analyzed in further studies to facilitate the main steps in microalgal production. New technologies are constantly being studied to optimize future processes; for example, bioelectrogenesis with microbial fuel cells (MFCs) consists of treating different types of wastewater and producing electrical energy,[120] and microalgae can potentially be used in bioelectrochemical systems for power generation and removal of unwanted nutrients.[121] These ideas are of great interest for the future development in processes with long-term sustainability and environmental benefits.

The use of LCA for process optimization is a useful alternative for improving clean technologies in this field. In general, there is concern related to the study of new technologies in all areas of microalgal production. Although many studies have reported increases in this area, many steps should be improved in terms of cost and efficiency for the production of bioproducts and the removal of pollutants. All of these concerns must be addressed to develop clean technologies, because these technologies will gradually be applied in the future. Clean technologies for energy production may represent strong candidates for competing with fossil fuel technologies; however, an appropriate policy environment and sufficiently low costs are required for these technologies to be introduced and recognized as promising alternatives.

7. Final Considerations about Microalgae and Clean Technologies

Studies on microalgae and clean technologies are still scarce; however, there are many aspects described in the literature that could improve these technologies and make them feasible. As shown in this review, the energy costs associated with microalgal cultivation, harvesting, drying, and compound extraction represent major obstacles and must be studied carefully. It is also emphasized that although studying individual steps initially offers relevant information, for future applications, all aspects must be studied together.

The cultivation step should be improved to obtain a higher yield of biomass and concomitantly reduce the costs associated with energy, water, and nutrient requirements. In this context, the use of wastewater is a suitable alternative because microalgae can bioremediate the medium, while water and nutrient expenses are simultaneously minimized. Regarding biomass harvesting, the applied methods should be adequate for harvesting, minimize the loss of biomass, and present lower environmental impacts, as is the case for bioflocculation.

The choice of drying method is mainly related to energy cost and processing time, but no LCA studies related to this step were found. However, it can be highlighted that all three main methods for biomass drying (oven drying, spray dryer, and freeze drying) involve high-energy requirements. Although freeze drying is considered one of the best techniques for maintaining cell stability, its energetic cost and time requirements are much higher than those of spray drying.

In the extraction step, one of the most troubling factors is the use of harmful solvents with potential environmental impacts and adverse effects on human health; solvent use is especially troubling in the extraction of compounds of commercial interest, such as lipids. Nonmechanical extraction employing solvents for cell disruption (especially chloroform, methanol, and dichloromethane) is the most common extraction method used for this purpose. This option is frequently employed for extraction due to the lower initial capital equipment investment required.

Thus, clean technologies should target alternatives considering the principles of green chemistry to reduce solvent use or replace solvents with less harmful alternatives. It has noted in the literature that the use of a single extraction method may not be sufficient for achieving maximum yields of high-value products from microalgae; however, pretreatment methods may be feasible in this regard, such as the use of ultrasonication and microwave-assisted methods combined with solvent extraction. Additionally, enzymatic extraction may be one of the most environmentally friendly options due to the nontoxicity of enzymes and the possibility of recovering enzymes for further experiments. The major disadvantage of enzymatic extraction is the high cost of the enzymes themselves. All of these methods should be investigated as alternatives; however, energy costs must be improved to achieve appropriate cost benefits.

Extracted bioproduct destinations should also be the target of studies. It can be seen from this review that the major coproduct of interest is biodiesel; however, there are still many bottlenecks to overcome to make bioproducts feasible. In the production of microalgal bioproducts at an industrial scale, the high energy demand and costs of microalgal cultivation and biorefineries represent major limitations. As an alternative, in situ technologies can be developed to eliminate unnecessary steps, such as the need to extract oil from biomass. In addition, biomass could be applied in several other fields, for example, in animal feed, the pharmaceutical industry, or food nutrition.

The suggestion of adopting wastewater treatment to obtain biomass is an interesting option for reducing contaminants and GHG and represents a possibility for producing bioproducts such as animal feed, biochar, and biofuels.

8. Conclusions

The association between microalgae and clean technologies is still considered an uncommon area of research; however, LCA is already a well-established tool for the evaluation of several steps required in microalgal production and can be very useful for technological development. Individual evaluation of the main steps involved is essential for a detailed analysis; however, these concepts must be aggregated for the development of methodologies that are more viable and to take into account the reduction of environmental impacts and costs, mainly associated with energy balance. The data obtained in this study through bibliometric mapping allow a general evaluation of the most studied topics in the last 10 years. Studying all of these aspects was fundamental to the development of this review and demonstrated all of the possible ways to improve the association between microalgae and clean technologies.

The application of new technologies that enable simplifications and cost reductions may play a major role in the development of technologies using microalgae. Finally, it should be highlighted that clean technologies do not specifically need to be new but, rather, must be an alternative that surpasses and is more efficient than existing alternatives and that the use of microalgae associated with these technologies may be a sustainable alternative in the near future.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors would also like to thank the University of Santa Cruz do Sul (UNISC), TecnoUNISC, through the Center for Research in Oleochemical and Biotechnological Processes and Products for MCTIC (01.0144.00/2010) and CNPq (309652/2016-2).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

bibliometric mapping, biomass, clean technology, life cycle assessment, microalgae

Received: September 4, 2018
Revised: April 1, 2019

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4.3 MANUSCRIPT 3 - Bioproducts characterization of residual periphytic biomass produced in an algal turf scrubber (ATS) bioremediation system

This research article shows the exploratory analysis of the periphytic biomass composition, especially for lipids, carbohydrates, proteins, pigments, and antioxidants. This article was published in 2020, in the periodic “Water Science & Technology” with Qualis CAPES A3 (researched in 2021).

Bioproducts characterization of residual periphytic biomass produced in an algal turf scrubber (ATS) bioremediation system

Maiara P. de Souza, Tiele M. Rizzetti, Michele Hoeltz, Mainara Dahmer,
João A. Júnior, Gisele Alves, Lisianne B. Benitez
and Rosana C. S. Schneider

ABSTRACT

The transformation of residual biomass from bioremediation processes into new products is a worldwide trend driven by economic, environmental and social gain. The present study aimed to evaluate the potential for obtaining bioproducts of technological interest from the remaining periphytic biomass formed during a bioremediation process with an algal turf scrubber (ATS) system installed in a lake catchment. Different methodologies were used according to the target bioproduct. Analyses were performed by high performance liquid chromatography with diode array detector (HPLC/DAD), gas chromatography mass spectrometry (GC-MS), ultraviolet–visible spectroscopy (UV-VIS) and inductively coupled plasma optical emission spectrometry (ICP-OES). The results demonstrated that the periphytic biomass presented potential since protein (17.7%), carbohydrates (22.4%), total lipids (3.3%) with 3.6 mg mL^{-1} of fatty acids, antioxidants (144.5 μmol Trolox eq. g^{-1}) and chlorophyll a, chlorophyll b and carotenoids (1,719.7 μg mL^{-1}, 541.2 μg mL^{-1} and 317.7 μg mL^{-1}, respectively) were obtained. Inorganic analysis presented a value of 42.3 ± 2.58% of total ash and metal presence was detected, indicating bioaccumulation. The properties found in periphyton strengthen the possibility of its application in different areas, ensuring bioremediation efficiency.

Maiara P. de Souza
Tiele M. Rizzetti
Michele Hoeltz
Lisianne B. Benitez
Rosana C. S. Schneider (corresponding author)
Environmental Technology Postgraduate Program and Center of Excellence in Oilchemistry and Biotechnology,
University of Santa Cruz do Sul,
Av. Independência 2293, Bloco 53, CEP 96815-900 Rio Grande do Sul,
Brazil
E-mail: rosana@unisc.br

Mainara Dahmer
João A. Júnior
Gisele Alves
Center of Excellence in Oilchemistry and Biotechnology,
University of Santa Cruz do Sul,
Av. Independência 2293, Bloco 53, CEP 96815-900 Rio Grande do Sul,
Brazil

Key words | antioxidants, carbohydrates, lipids, metals, pigments, proteins

HIGHLIGHTS

- Periphytic residual biomass from an ATS bioremediation system have been exploited to produce bioproducts;
- Organic (lipids, proteins, carbohydrates, antioxidants, pigments) and inorganic (ash and metals) analysis were performed to evaluate periphytic biomass;
- The possible transformation of residual periphytic biomass into scalable bioproducts was discussed;
- Biofuels and biofertilizer production could be a suitable alternative to periphytic biomass valorization.

INTRODUCTION

Periphyton is composed of a variety of autotrophic and heterotrophic organisms that grow on surfaces in aquatic environments. It can be formed by freshwater benthic photoautotrophic algae and prokaryotes, heterotrophic and chemoautotrophic organisms, fungi, protozoa, metazoans and viruses (Larned 2010). The species that compose

doi: 10.2166/wst.2020.343

the periphyton in freshwater ecosystems are usually algal species. Studies also demonstrate that the habitat and regional richness of microalgae species have a strong influence on periphytic algal communities (Algarte *et al.* 2017). In the natural environment, the periphyton is very important in the functioning of these ecosystems, as the primary producers provide a high-quality food source for macroinvertebrates and play a crucial role in the oxygen, carbon and nutrient cycles in aquatic ecosystems. The periphytic configuration ensures element fixation and assists in the nutrient composition of the biomass (Cui *et al.* 2017).

Algal turf scrubber (ATS) systems are widely used for periphyton formation. These systems are controlled ecosystems that aim to perform bioremediation through wastewater draining over a sloping surface that is covered by periphyton (biofilm) (Liu *et al.* 2016). The high rate of biomass production followed by harvesting when filtering power is finished (saturated) are the main advantages of the ATS system because it presents higher yields compared to values recorded in other cultivation systems. The combined use of the ATS system to remove nutrients from eutrophic waters and to enhance biomass can reduce the costs involved in the production of products of commercial interest. The development of new bioproducts from microalgae is a suitable alternative for this use. Moreover, microalgal biomass production in an ATS system is considered economically viable. If it is ensured that no toxic compounds are present, biomass can be transformed into products of commercial interest (de Souza *et al.* 2018).

Water treatment through an ATS system followed by the use of biomass to produce different bioproducts can contribute to minimizing the impacts, which are mainly related to microalgae blooms due to the eutrophication of water bodies. This problem is reflected in the quality of water consumed by the population. Thus, the ATS structure, which is efficient, requires that biomass is used adequately and contributes to the economic and environmental sustainability of this treatment method (Uggetti *et al.* 2018). In addition, Adey *et al.* (2013) demonstrates the possibility of increased productivity with annual averages that could reach 150 t ha^{-1} year^{-1}.

The use of biomass composed of different microorganisms to produce biofuels and other products of commercial interest is becoming increasingly popular, as it provides the possibility of reducing environmental impacts and diversifying energy sources. It is estimated that biomass could provide approximately 25% of global energy requirements. In addition, the presence of a biomass consortium stimulates the production of metabolites or bioproducts and may be a source of chemicals, pharmaceuticals, and food additives (de Souza *et al.* 2018).

Analyzing the physicochemical characteristics of wastewater biomass from a water treatment system can show its potential applications in different areas, enabling the development of new products or adding properties to those already existing in the market. The enhancement of biomass to produce biofuels and other products of commercial interest is becoming increasingly popular, as it provides the possibility of reducing environmental impacts and diversifying energy sources. In this context, this work aims to evaluate the lipids, proteins, carbohydrates, antioxidants, pigments and metals in a periphytic biomass produced in an ATS pilot system installed in a lake catchment, in order to recognize the potential for bioproducts obtention

METHODS

Periphyton identification and biomass preparation

The pilot-scale ATS system was placed in Dourado Lake, which is an artificial water reservoir in the city of Santa Cruz do Sul, RS, Brazil (29°43′53.7′′S 52°27′37.7′′W). The lake receives water from Pardinho river, which is surrounded by crops and other agricultural production. From these locations there is an uncontrolled wastewater drainage from crops and soil leaching. The lake area is 119 hectares with maximum depth of 3 m and can accumulate 3 million cubic meters of water. The ATS system (5-m long and 1-m wide) was constructed with an iron structure and a layer of 0.27-mm nylon mesh screen for periphyton attachment. The received water from Dourado Lake to the ATS system presented a flow rate of approximately 2 L min^{-1} (Martini *et al.* 2019). Periphyton harvesting was accomplished by scraping the biomass with a plastic spatula at three selected points (0.25 × 0.25 m surface area), for seven months, in different seasons (summer, winter and spring). Briefly, biomass preparation consisted of drying the biomass in an oven with air circulation followed by milling with a Wiley-type cryogenic knife mill (Tecnal-TE 680, Brazil). Then, the biomass was maintained in polypropylene tubes and stored in a freezer at ≤20 °C until analysis.

For the analysis of the species composition of the periphytic community, biomass of each collected point was homogenized and fixed with formaldehyde at a final concentration of 2% The aliquots were stored in a freezer until analysis (≤20 °C). Samples were examined in triplicate at 100× magnification under a light microscope (Motic

BA410) with a micrometer lens and a photographic camera. The identification of taxa was performed with the help of a specialized bibliography with identification keys (Theriot *et al.* 1992; Bicudo & Menezes 2006).

Organic composition analysis of periphytic biomass

Exploratory analysis by infrared spectroscopy and determination of proteins, lipids and carbohydrates in biomass

For the exploratory analysis of biomass by infrared spectroscopy and the determination of proteins, lipids, and carbohydrates, the sample preparation and analysis conditions were performed according to Martini *et al.* (2019) using CHNS elemental analysis (PE-2400, Perkin Elmer, USA), gas chromatography with mass spectrometry (GC/MS) (MS-QP 2010 Plus, Shimadzu, Japan) and high-performance liquid chromatography with diode array detector (HPLC/DAD) (LC-20AD Shimadzu, Japan), respectively.

The first biomass analysis consisted of the analysis of all biomass samples by infrared spectroscopy. The infrared data were evaluated by multivariate analysis through principal component analysis (PCA) using Chemostat® 1.0.0.0 software. Thus, this exploratory study made it possible to evaluate possible bioproducts present in the sample and to assess the variability among samples from different collection points. Therefore, it was possible to combine the biomass, and this biomass mixture was employed for the determination of proteins, lipids, carbohydrates, antioxidants and pigments.

Antioxidants

Antioxidants were determined with the oxygen radical absorbance capacity assay (ORAC) method. Stock solutions of Trolox (4,000 µmol L^{-1}), fluorescein (407 µmol L^{-1}) and AAPH (152 mmol L^{-1}) were prepared in phosphate buffer (75 mM, pH 7.4). An analytical curve with Trolox was prepared at a concentration of 4 to 100 mol L^{-1}. Sample preparation was performed by dilution of the periphytic biomass in methanol to obtain concentrations of 100, 200 and 500 mg L^{-1}. The standards and samples were placed in the wells of the microplates. Fluorescence measurements were carried out using SpectraMax® M3 equipment. Samples were incubated for 10 min at 37 °C, after which the equipment plate was removed and 25 µL of AAPH solution was added, and homogenization was conducted for another 30 s. The fluorescence intensity ($\lambda_{excitation}$ ¼ 485 nm and $\lambda_{emission}$ ¼ 528 nm) was monitored each min at 37 °C for 90 min.

Pigments

To analyze the pigment profile, 0.1 g of biomass was weighed, and 5 mL of different solvents was added: (1) acetone:water (80:20, v/v); (2) methanol (100%); (3) methanol:water (80:20, v/v); (4) ethanol (100%); and (5) methanol:water (80:20, v/v). The mixture was vortexed for 1 min. Then, the samples were sonicated in an ultrasound bath for 20 min and centrifuged for 15 min at 3,400 rpm. The chosen best solvent for pigment extraction as well as for the verification of the pigment profile was used with an Analytik Jena UV/Vis Specord 210 Plus spectrophotometer with a double-beam monochromator. Scanning was performed from 300 to 800 nm. The analyses were performed on an optical path of 1 cm. Due to the high concentration of pigments, the samples had to be diluted ten times before the analyses.

The determination of chlorophyll *a* and *b* and total carotenoids was performed using Equations (1)–(3). Absorbance values at 470, 647 and 663 nm were recorded.

$$\text{Chlorophyll } a\ (\mu\text{g mL}^{-1})\ \text{¼}\ (12{:}25 \times \text{Abs}_{663}) - (2{:}79 \times \text{Abs}_{647}) \quad (1)$$

$$\text{Chlorophyll } b\ (\mu\text{g mL}^{-1})\ \text{¼}\ (21{:}50 \times \text{Abs}_{647}) - (5{:}10 \times \text{Abs}_{663}) \quad (2)$$

$$\text{Totalcarotenoids } (\mu\text{gmL}^{-1})\ \text{¼}\ [(1000 \times \text{Abs}_{470}) - (1{:}82 \times \text{Chl}\,a) - (85{:}02 \times \text{Chl}\,b)] = 198 \quad (3)$$

Inorganic composition analysis of biomass

Ash and metals

Ash content was determined using gravimetry. Initially, the samples were calcined in a muffle furnace at 575 °C for 12 h. Then, the samples were transferred to a desiccator until they reached a constant weight. Finally, the crucibles were placed in the muffle at 575 °C for 24 h and transferred to the desiccator until a constant weight was reached. The ash content (%) was calculated in relation to dry biomass.

For metal determination, sample digestion was performed with a CEM brand microwave oven, model MARS Xpress, with 24 digestion vessels and a temperature ramp. A total of 250 mg of sample was weighed, and 3 mL of HNO_3, 2 mL of H_2O_2 and 2 mL of water were added to each tube. The power used was 1,600 W, and the heating program involved a 20 min ramp and a gradual increase to 200 °C, and then the samples remained at this temperature. The metal analysis was performed in a Perkin Elmer ICP-OES Optima 8,300 model with a Cross Flow GemTIp® nebulizer, Scott nebulizer chamber and 1.8 mm-internal-diameter alumina injector. The analysis conditions were 10 L min^{-1} plasma, 0.5 Lmin^{-1} auxiliary plasma, 0.8 mL min^{-1} nebulization.

RESULTS AND DISCUSSION

Periphyton identification

Through the analysis of the micrograph images, it was possible to identify the main taxonomic groups that occurred with relatively high frequency in the periphytic samples. A considerable number of green algae (*Chlorella* sp., *Desmodesmus* sp., *Pediastrum* spp. Meyen and *Spirogyra* sp) and Bacillariophyceae (diatoms) were found. In the periphyton, algae gain prominence because they play a fundamental role as primary producer systems (Lobo *et al.* 2015) and consequently assume a key position in the continental aquatic food chain. Congestri *et al.* (2006) analyzed the major components of biofilms in different seasons. The results demonstrated that biofilms were essentially composed of cyanobacteria, diatoms and green algae. Maximum total biovolume (1,351.54 × 10^6 µm^3 cm^{-2}) was recorded in spring with a co-dominance of raphid diatoms. Summer and autumn assemblage were also dominated by diatoms that constituted up to 75% of total biomass and cyanobacteria were prevalent in winter. In addition to cyanobacteria and diatoms, it should be noted that algae from the Chlorophytes taxon were also found, such as *Chlorococcum* sp., *Desmodesmus* sp., *Pseudococcomyxa* sp., *Sphaerocystis* sp. and *Stigeoclonium* sp.

Organic composition analysis of biomass

Exploratory analysis of biomass by Fourier-transform infrared spectroscopy

In previous studies, we presented the infrared band characterization of the periphytic biomass harvested (Martini *et al.* 2019). The following bands were highlighted: siloxanes and frustules (structure of the siliceous cell wall of diatoms) (Si-O) at ~1,075 and 900; (C-Oν-C) carbohydrates, saccharides and polysaccharides at ~1,198–1,134; phosphodiesters of nucleic acids and phospholipids (ν > *P* ¼ O) at ~1,240; chlorophyll, CH_2 groups, CH_3 proteins and carboxylic acid groups ($\nu$$CH_2$, $\nu$$CH_3$/CO $\nu$$CH_2$, $\nu$$CH_3$/CO) at ~1,390; amides from ν(C ¼ O) proteins at ~1,637; and lipids and ν(C ¼ O) fatty acid esters at ~1,745.

Peaks at approximately 1,450 cm^{-1} were mainly associated with shear and flexion of –CH_3 and –CH_2— bonds. The absorbance bands in the region of 1,200 to 1,000 cm^{-1} were attributed to the C-O-C strain vibrations of cellulosic compounds. The presence of these bands may be strongly associated with green algae belonging to Cladophorales or other organisms that compose the periphyton (Hoover *et al.* 2011).

According to Murdock & Wetzel (2009), major macromolecular bands and vibrational frequency assignments can be associated with different types of algae. Green algae, diatoms and cyanobacteria have distinct chemical compositions. In general, green algae have a relatively high starch and cellulose content (cell walls and energy storage products) of ~1,100 to 900 cm^{-1}, while diatoms have a distinct type of silicate (absorption at ~1,100–1,060 cm^{-1} and ~800 cm^{-1}) due to silica (cell wall) frustules, whereas cyanobacterial spectra are dominated by proteins and lipids, with less abundant carbohydrates than green algae, but the proportions of these macromolecules may vary substantially among the species of these groups according to nutrient availability.

Through the analysis of infrared spectroscopy for the identification of the main bands, it was possible to evaluate the general profile of the periphyton. The characteristics and main similarities among the bands were analyzed using the PCA model. The PCA results were obtained following the preprocessing of the mean-centered PCA. By analyzing the results of the data decomposition through the mean-centered PCA, it can be observed that 98.18% of the total variance was explained by the three main components (PCs). Figure 1 shows the graph of the scores of PC1 (75.45%) versus those of PC2 (14.31%). The graph shows that PC1 separates three main groups: 1, which groups all samples from point 3; 2, which groups the samples from points 1 and 2 from collection 1; and 3, which is defined by points 1 and 2 and collections 1 and 2.

From the generation of the principal components, a score graph was created to distinguish some common parameters among the samples, verifying their grouping according to the similar components among them, as shown in Figure 1.

In this case, PC1 × PC2 was used to demonstrate the grouping of the main collections and sampling points. The presence of outliers was not observed during the analysis.

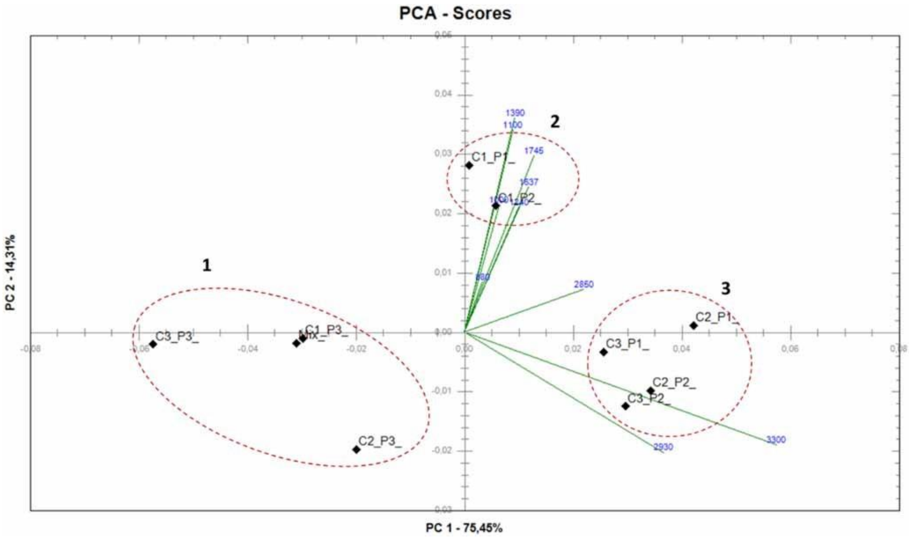

Figure 1 | Scores graphic (PC1 x PC2) of the biomass collected (C1: summer, C2: winter, C3: spring, and mix: mix of C1, C2, and C3) in three points (P) (n¼3) from the pilot ATS system installed in Dourado Lake.

Using the PCA with the biplot tool, it was possible to associate the three main clusters with the respective bands that influenced the separation of these groups. In cluster 1, the selected bands were not evident, and other bands are possibly responsible for this behavior. For cluster 2, bands related to methyl groups, water and proteins were observed. Cluster 3 showed the greatest potential for bioproducts since the remaining bands were grouped in this interval, noting the presence of bioproducts such as lipids, carbohydrates, proteins, and pigments, among others.

Based on the profile of the bands and with an aim of representativeness, all samples were mixed and homogenized. Thus, only a single mixed biomass sample was used for the bioproduct analyses.

Proteins

Through the analysis with CHNS equipment, values of carbon (28.46 ± 0.19), hydrogen (5.07 ± 0.99%), nitrogen (3.70 ± 0.60%) and sulfur (0.98 ± 0.02%) were obtained. For protein determination through the CHNS analysis, the value found for nitrogen was converted from total N to total protein. Thus, the protein value obtained for the biomass was 17.70%, similar to the 19.27% obtained by Martini *et al.* (2019). The protein content found can be considered adequate because it is residual biomass and can be reused for other purposes, such as for animal feed or biogas production. Compared to the average protein content found in other studies for aquaculture (25–35%) (Gangadhara *et al.* 2004), the value found in our study indicates that the biomass could be an option for animal feed; however, toxicity must be previously evaluated.

Considering these values, the use of the periphytic biomass can also be an alternative for BioProtein obtention, which is an interesting trend for commercialization. This kind of protein is produced by methanotrophic and heterotrophic bacterial culture or other microorganisms, using natural gas as the main source of energy, and can be a suitable alternative for supplements (de Souza *et al.* 2018).

Lipids

The total lipid content in the periphyton was 3.3 ± 0.28%. This yield is considered very low compared to that found in green algae, including those found in the analyzed periphyton (*Desmodesmus* sp., *Pediastrum* spp. Meyen and *Spirogyra* sp). In this study, the low lipid content can be explained by the high levels of ash, which are further described in inorganic analysis. In addition, due to the low nutrient levels available in Dourado Lake, the microalgae found in the periphyton did not present favorable conditions to accumulate high lipids concentrations (Pacheco *et al.* 2015; Souza *et al.* 2016).

After this step, the samples were derivatized to convert the extracts into methyl esters for further GC-MS chromatographic analysis. The fatty acid profile of the periphyton biomass as well as the content and composition of the total fatty acids can be seen in Figure 2 and Table 1, respectively.

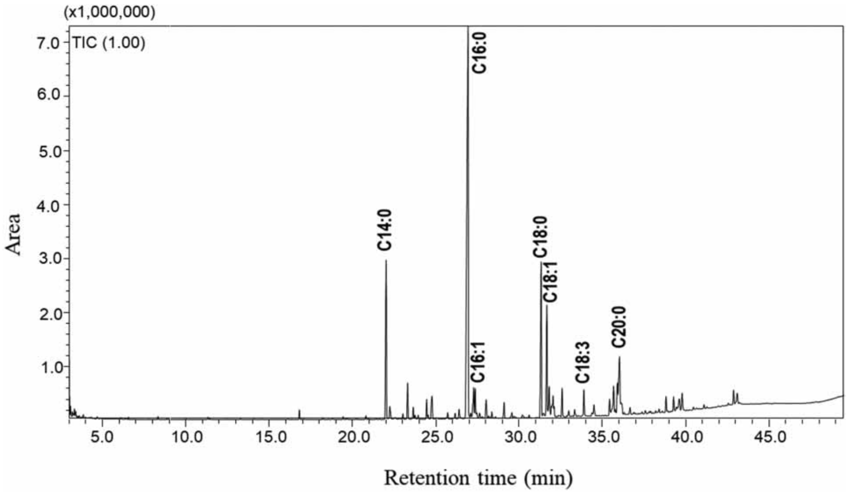

Figure 2 | Chromatogram of fatty acids from periphytic biomass of ATS system obtained by GC-MS analysis.

Table 1 | Fatty acids found in biomass obtained in Dourado Lake by GC-MS with the retention time (min), equation with r^2, sample concentration (mg mL^{-1}) and identified total fatty acids (mg mL^{-1})

Retention time (min)	Fatty acid	Equation	r^2	Sample concentration (mg mL^{-1})	Identified total fatty acids (mg mL^{-1})
21.77	C14:0	y ¼ 1E þ 07x þ1E þ 06	0.9908	0.335	
26.62	C16:0	y ¼ 1E þ 07x þ2E þ 06	0.9916	1.993	
27.09	C16:1	y ¼ 7E þ 06x þ666418	0.9876	0.303	
31.06	C18:0	y ¼ 1E þ 07x þ2E þ 06	0.9839	0.312	3.65
31.38	C18:1	y ¼ 1E þ 07x þ2E þ 06	0.9861	0.353	
32.29	C18:2	y ¼ 8E þ 06x þ795091	0.9870	0.212	
33.58	C18:3	y ¼ 7E þ 06x þ739008	0.9800	0.146	

As shown in Table 1, palmitic acid (C16:0) was the predominant fatty acid in the periphytic samples. Through statistical analysis by ANOVA (p ¼ 0.05), it was possible to observe that the results are statistically equal since $p >$ 0.05 and that variance among replicates is equivalent ($f_{calculated}$ ¼ 0.06 and $f_{critical}$ ¼ 3.35). It is important to highlight that other compounds were separated by gas chromatography that have not been identified, since the lipid fraction extracted by the Bligh-Dyer method contains pigments that are in the lipid phase.

Hoover *et al.* (2011) analyzed periphyton with a predominance of *Cladophora glomerata* collected from Mendota Lake, WI, USA. After fatty acid analysis, it was found that C16:0 represented a predo%minance (>80) of the detected fatty acids. Hill *et al.* (2011) performed an analysis of the parameters capable of influencing the fatty acid profile, such as light and nutrients, in periphyton formed in freshwater. The periphyton had a predominance of diatoms, and its fatty acid profile included palmitic (C16:0), palmitoleic (C16:1) and eicosapentaenoic (C20:5) acids, which were the main fatty acids found, representing saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, respectively. Linoleic (C18:2) and linolenic (C18:3) acids, characteristic of chlorophytes and cyanophytes, comprised <2% of the total fatty acids.

An important application of lipids extracted from periphytic biomass microalgae is the conversion into biodiesel and, in the nutraceutical area, as essential oils and functional compounds; however, the lipid yield must be optimized in further studies in order to be suitable for this type of application (Mubarak *et al.* 2015).

Carbohydrates

The total carbohydrate content was 22.4% in relation to the dry biomass, with 16.8% and 5.6% represented by glucose

Table 2 | Sugars found in periphytic biomass with concentration (mg mL^{-1}), yield (%) and total carbohydrate content (%)

Sugars	Average concentration (mg mL^{-1})	Standard deviation ($n = 5$)	Yield (%)	Total carbohydrates (%)
Glucose	0.6	0.03	16.8	22.4
Xylose	0.2	0.02	5.6	

and xylose, respectively. Monosaccharides from the hydrolysated periphytic biomass in terms of concentration (mg mL^{-1}), yield (%) and total carbohydrate content (%) are shown in Table 2.

The results do not discriminate the origin of carbohydrates in terms of whether they are intra- or extracellular. Bellinger *et al.* (2010) evaluated extracellular polymeric substances secreted by algae and bacteria. The isolated fraction of periphyton presented carbohydrate values that ranged from 8.6 to 43.8%. Glucose was the predominant saccharide residue (19.9–65.1%). Other sugars were galactose (7.4–22.1%), fucose (5.7–25.8%), mannose (4.5–1.2%) and xylose (4.3–19.4%). Congestri *et al.* (2006) analyzed the monosaccharide composition of capsular polysaccharides by HPLC in biofilm obtained after cold and hot extraction in different seasons. As a result, it was observed that glucose yield was similar to our study, since the values ranged from 5.3 to 20.2%. In another study, Di Pippo *et al.* (2009) evaluated the capsular polysaccharides of cultured phototrophic biofilms by HPLC, and the results demonstrated that the values for glucose and xylose were
27.8 and 5.2%, respectively.

It is important to highlight that glucose or starch are conventionally used for biofuels production such as bioethanol and biohydrogen. The investigation of other polysaccharides could be also interesting for commercial applications considering that they can have biological functions of protection and storage. Besides these advantages, they can be highly promising as sources of active molecules, which can be a great option for cosmetics, food ingredients and natural therapeutic agents (Souza *et al.* 2019a).

Antioxidants

Antioxidant analysis was performed with the ORAC test at three concentrations levels of periphytic biomass in methanol (Table 3) to provide a starting point considering the unknown characteristics of the sample.

Considering that another similar evaluation of periphyton was not found, this result is extremely relevant for biomass exploitation. At the three concentration levels, it was possible to observe the presence of antioxidants in the sample.

Table 3 | Antioxidant (µmol eq g^{-1}) obtained in different concentrations (mg L^{-1}) found in the periphytic biomass by ORAC methodology

Sample	Concentration (mg L^{-1})	Antioxidants (µmol eq g^{-1})	Average	Standard deviation ($n = 3$)
1	100	128.0 133.2 130.9	130.7	2.6
2	200	130.9 163.5 155.2	149.8	16.9
3	500	142.4 150.8 166.1	153.1	12.0

The statistical analysis by ANOVA (p ¼0.05) showed that the results for the different levels are statistically equal since the variance among replicates is equivalent ($f_{\text{calculated}}$¼9.36, f_{critical}¼9.55). Thus, the antioxidants are another alternative bioproduct that could be obtained from the periphytic biomass under study, and the comparison with microalgae highlights the functionality that can be found in the production of this biomass. For instance, the carotenoid profile of 12 commercial microalgae collected from brackish and marine subtropical waters was analyzed to evaluate their applicability in the aquaculture industry. From the carotenoid extracts, which were more concentrated than the biomass, the results showed that the antioxidant value obtained from the ORAC method ranged from 45 to 577 µmol eq g^{-1} DW, demonstrating the potential for using these microalgae strains for human health as food additives or as dietary supplements (Ahmed *et al.* 2014).

The antioxidant capacity and the total content of phenolic compounds from the different fractions of 23 microalgae were evaluated using the Trolox equivalent total antioxidant capacity test. For the hexane fractions, the antioxidant capacities ranged from 0.01 to 11.41 µmol Trolox g^{-1}; for the ethyl acetate fractions, the antioxidant capacities ranged from 0.01 to 16.00 µmol Trolox g^{-1}; and for water, the antioxidant capacity was 0.01 to 9.23 µmol Trolox g^{-1} (Li *et al.* 2007).

Therefore, with the results found with periphytic biomass and with the biomass or compounds from microalgae, bacteria or fungi presented here, it can be verified that the antioxidant content found in our study (130–153 µmol Trolox eq g^{-1}) is suitable for exploiting the antioxidant potential of periphyton. This indicates that this bioproduct should be further studied to evaluate the applicability of this biological activity. After obtaining this information safely, the use of this biomass could be a promising alternative to sustain the growing demands in food and pharmaceutical industries. These applications are possible since natural antioxidants may prevent or minimize the oxidative damage caused by reactive oxygen species and also delay aging and several chronic conditions (heart diseases, atherosclerosis and cancer) (de Souza *et al.* 2018)

Pigments

The profile of the spectrum of absorbance for the different extraction solvents evaluated can be seen in Figure 3.

According to Figure 3, it is possible to observe that acetone:water (80:20, v/v) presented the best extraction potential, followed by 100% methanol. Overall, predominant bands were observed at 418, 555, 596 and 671 nm. According to Sánchez *et al.* (2013), autotrophic organisms collect light energy from the underwater light field through photosynthetic pigments. The absorption of these molecules generally ranges from 400 to 700 nm, which is the region known as photosynthetically active radiation. Photosynthesis can be divided into two main light reaction sets: photosystem I and photosystem II. Each photosystem consists of a nucleus formed by chlorophyll *a* or light-absorbing molecules, which in addition to chlorophyll *a* can be chlorophyll *b*, chlorophyll *c*, carotenoids or phycobiliproteins. The composition of these photosystems may vary depending on the taxonomic group of algae, cyanobacteria, or other photosynthetic organisms of interest.

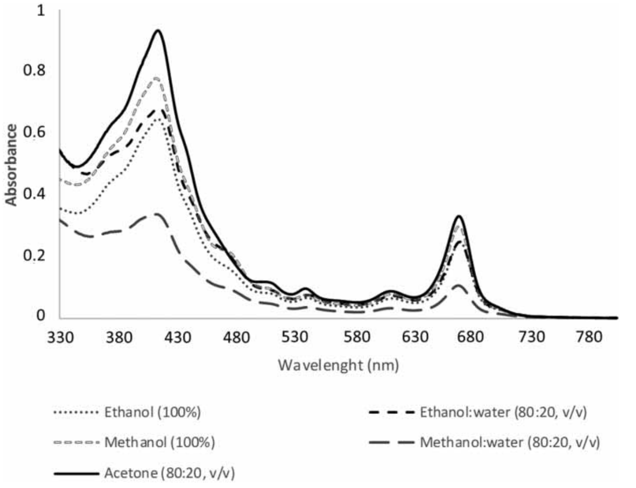

Figure 3 | Comparison of pigment extraction of periphytic biomass by UV-VIS analysis with different solvents.

Several parameters of a biochemical nature must be taken into account, such as the method speed, toxicity and availability of extraction solvents, reproducibility, efficiency and selectivity. The most commonly used techniques for extracting pigments are those using a solvent in combination with maceration (soaking), percolation, pressurized liquid extraction and microwaves; however, these methods often involve high costs and are destructive (Duppeti *et al.* 2017). Bioproduct extraction from periphytic biomass is very complex. First, this is due to the high difficulty of separating algae or other organisms from the substrate. Second, the periphyton generally forms thick biofilms and may be composed of dense matrices, such as periphyton composed of filamentous cyanobacteria or benthic diatoms. Third, dense periphyton may contain very high water contents, which could reduce the solvation and penetration properties of the extraction solvent (Hagerthey *et al.* 2006).

After analyzing the main bands and identifying acetone as the best extraction solvent, the chlorophyll and carotenoid contents were calculated. Thus, it was possible to quantify chlorophyll *a*, chlorophyll *b* and total carotenes in the periphytic biomass. The calculations applied con- sidered the formula established for compounds also extracted in acetone:water (80:20, v/v). The results pre- sented concentrations of 1,719.7, 541.2 and 317.7 μg mL^{-1} for chlorophyll *a*, chlorophyll *b* and total carotenoids, respectively.

Safe carotenoids and chlorophylls obtention from biomass could be a great alternative in human and animal feed, additives, cosmetics, pharmaceutical industries, food colorants and biomaterials. However, further studies must be done in order to certify that no toxicity is present in the extract (Lafarga *et al.* 2020).

Inorganic composition analysis of biomass

Ash and metals

The inorganic composition of the biomass as determined according to the ash content presented a value of 42.3 ± 2.58%. Romanów & Witek (2011) studied periphytic communities with macrophyte predominance (*Phragmites australis*, *Potamogeton lucens*, and *Nuphar lutea*) in three different types of lakes. The results showed that this content ranged from 8 to 76%. The authors also highlighted in this study that the sample that presented 42% of ash was collected in July in a eutrophic lake.

Microwave digestion was performed for sample preparation to analyze the metal content, considering the complexity of breaking the cell walls in periphytic biomass. This methodology proved to be effective for this purpose. According to Table 4, high concentrations of aluminum, barium, calcium, iron, magnesium, manganese and potassium can be observed in the results. Through statistical analysis by ANOVA (p ¼ 0.05), it was found that the results are statistically equal since $p > 0.05$ and that the variance among replicates was equivalent ($f_{calculated}$ ¼ 0.008, $f_{critical}$ ¼ 3.2).

After metal analysis, it was possible to verify a high amount of aluminum and iron in the periphytic biomass obtained in Dourado Lake. It was observed that these metals were also found in the lake water before the analysis of the periphytic biomass. These results can be associated considering the uncontrolled wastewater drainage from crops and soil leaching caused by rivers that flow into the lake. Another explanation is that periphyton can easily accumulate heavy metals, which can be adsorbed and transferred to other organisms (Cui *et al.* (2017).

According to Cui *et al.* (2017), this process can influence the structure of the food chain and may affect members of higher trophic levels. In this context, more research on the process of metal distribution throughout the food chain is needed to adequately explain heavy metal toxicity at higher trophic levels. Aluminum (Al) and iron (Fe) concentrations were evaluated at Loskop Lake in Africa over a four-month period in samples of phytobenthos, phytoplankton, macroinvertebrates, amphibians and fish. The highest Al and Fe concentrations were measured in the filamentous algae *Spirogyra fluviatilis* (Hillse) and *Spirogyra adnata* (Kutz) (Al 18,997.5 mg kg^{-1} dry weight and Fe 22,054.2 mg kg^{-1} dry weight). Al concentrations in the macroinvertebrate families collected ranged from 140.6 to 385.7 mg kg^{-1} dry weight, with the highest values measured for Al and Fe in the *Gomphidae* family (385.7 and 1,710.0 mg kg^{-1} dry weight, respectively) in comparison to those in the other sampled macroinvertebrate families. Al and Fe concentrations (2,580 and 10.697 mg kg^{-1} dry weight, respectively) in the stomach content of adult fish of the species *Oreochromis mossambicus* were much higher than those in adult fish of the species *Micropterus sal- moides* (98.5 and 439.6 mg kg^{-1} dry weight, respectively) (Oberholster *et al.* 2012).

Some studies show the possibility of verifying alternatives to minimize metals that bioaccumulate in periphytic biomass. Bere & Tundisi (2012) showed the importance of

Table 4 | Metals found in water from Dourado Lake and in the periphytic biomass from ATS system

		Assays in periphytic biomass (mg kg^{-1})				
Metals	Original metal concentration in Dourado Lake (mg L^{-1})	1	2	3	Average	Standard deviation
Aluminum	0.59	7,512.74	7,908.71	7,452.81	7,624.75	247.73
Antimony	<0.005	0.14	0.17	0.14	0.15	0.02
Barium	<0.200	501.82	543.55	462.06	502.47	40.75
Cadmium	0.001	0.03	0.03	0.03	0.03	0.00
Calcium	10.98	1,281.71	1,378.89	1296,00	1,318.86	52.47
Lead	0.007	6.92	6.83	6.66	6.80	0.13
Cobalt	<0.001	3.75	3.84	3.61	3.73	0.11
Copper	<0.020	11.48	9.24	8.40	9.71	1.59
Total chrome	<0.050	6.22	6.30	6.16	6.23	0.07
Iron	0.65	5,302.72	5,649.12	5,087.93	5,346.59	283.15
Magnesium	2.91	812.38	882.95	772.61	822.65	55.88
Manganese	<0.02	404.93	439.37	379.73	408.01	29.94
Nickel	<0.02	5.04	4.76	5.60	5.13	0.43
Potassium	2.70	772.05	846.82	792.22	803.70	38.68
Silver	<0.001	0.03	0.03	0.03	0.03	0.00
Sodium	5.43	252.03	282.55	272.75	269.11	15.58
Zinc	0.10	18.76	18.48	17.64	18.30	0.58

developing experiments that better mimic field conditions for metal toxicity in periphyton and enable improved accuracy in the extrapolations from laboratory scale assays to responses in natural systems. Pandey & Bergey (2018) demonstrated that it is possible to reduce metal toxicity in the periphyton. According to the authors, diatom communities, which are present in the periphyton, integrate habitat conditions and respond faster to environmental and anthropogenic instabilities. For these reasons they are excellent biological indicators for many types of pollution in aquatic systems. However, metal toxicity of periphyton must be analyzed to understand recovery response of the periphyton in different ecosystems. These structures are directly visible in live frustules and can be globally assessed with simple protocols. Then, the authors demonstrate that it is possible to provide insight into bioremediation potential, monitoring options and restoration approaches to decrease metals concentration in periphyton.

Considering these data for metal evaluation, the results of Al and Fe could affect biomass use in animal feed application or for pharmaceutical and cosmetology uses. Then, new evaluations must be done in terms of toxicity and possibility of biorefining processes. At this moment, it would be interesting to test biomass efficiency for biogas and biochar production (Souza *et al.* 2019b) or study the use of biomass containing high amounts of Al and Fe in other feeding sources or additives that allows these metal concentrations.

Potential for transformation of residual periphytic biomass into scalable bioproducts

The industry demand for food, bioenergy and compounds with high added value associated with population increase demands new alternatives to expand the bioeconomy. The idea of this work allows the possibility of using residual biomass to produce high added-value compounds. This is a great alternative; however, the viability and sustainability of converting residual biomass into suitable scalable bioproducts depends especially on the development of biorefinery process. This step is still considered delicate and requires more studies due to the bottlenecks faced in the high cost of production.

The valorization of bioproducts that can be obtained from periphytic biomass presents the opportunity to be applied in different areas. For this, biomass analysis in terms of security with constant monitoring of toxicity is necessary since biomass production in surface waters, such as Dourado Lake, tends to suffer impacts from wastewater emissions from villages and crops leaching around the river. According to the metal analysis in the periphyton, the toxicity of aluminum in the biomass cannot be neglected, as discussed by Baierle *et al.* (2015), wgi used aluminum electrodes for biomass separation, obtaining unwanted residue effects on biomass and water.

The commercialization of these bioproducts will be possible; however, all the production steps must be individually evaluated, including a study of the environmental impacts of the steps required to make commercial production feasible. In this context, the association between microalgae and tools such as life cycle assessment (LCA) are suitable for the evaluation of cultivation, harvesting, drying, extraction and commercialization of these bioproducts (Schneider *et al.* 2018; Souza *et al.* 2019b). This strategy can be very useful for technological development. As a complement, it is also necessary to constantly search industries data in order to demonstrate the probability of bioproducts growth in different areas, production costs, yields, and trends for the next years. The previous information helps to assess the choice of bioproducts that are gaining attention in the market and that may be a better investment alternative. Considering the previous assessments performed by our research group, a more focused study on pigments would be interesting based on our results and current demands. Another alternative would be the production of biochar or a sequence of bioproducts utilization, such as the production of energy and carbon consumption.

Biofuels production from periphytic biomass can also be a business opportunity, especially for bioethanol, biohydrogen and biogas. The production of ethanol from periphyton is an interesting path mainly using metabolically modified microorganisms that make suitable use of monosaccharides produced in hydrolysis. As an example, the use of *Arthrospira platensis* biomass using metabolically engineered *Escherichia coli* strain MS04 showed excellent results for converting the hydrolysate into ethanol. The best results for ethanol production may be associated to the nutrient availability that supports bacterium needs, which can be supplied by microalgae biomass (Werlang *et al.* 2020).

Methane production can be integrated with the production of biofertilizers. In this case, there are still precautions with toxicity to dispose of this treated biomass in the soil. With full use of biomass, there can be an optimized carbon cycle.

Furthermore, continuing this work, a critical analysis is being performed for each of these bioproducts in order to check which of them would be the most viable to be introduced in the market. For this, surveys of materials inputs and outputs, biorefining, toxicity, economic evaluation and better statistical strategies are being carried out considering the sector and location in which these bioproducts will be inserted. The possible prospects to optimize periphytic biomass valorization will help to achieve more desirable compounds in microalgae-based biotechnology.

In order to guarantee the nutrient removal from a reservoir with Dourado Lake dimensions, it is possible to maintain a business for biomass use and, if necessary, to use this biomass associated with agro-industrial residues in biotechnological processes (de Sousa e Silva *et al.* 2020). The nutritional composition of periphytic biomass is relevant for several processes and the continuity of this research predicts the delivery of an engineering project, with information on the potential products to be developed, based on lipids, proteins, carbohydrates, antioxidants, pigments and metals characterization. This can be seen as a positive investment for companies that manage the water reservoir for public supply, which can consider this type of treatment system as an opportunity and not a cost.

CONCLUSIONS

Bioremediation with the ATS system followed by the utilization of residual biomass proved to be a promising strategy. The main bioproducts found in this study were lipids, carbohydrates, antioxidants, proteins and pigments. The current challenge is to turn this potential into real products with a scalable process, transforming traditional water bioremediation into a low-cost and high-added-value circular technology. Regarding the levels found in the organic composition, lipids presented low yields and would not be a suitable option for biodiesel production or other applications that require a high lipid content. The other bioproducts showed fair values when compared to those found in the literature. The protein, carbohydrate and antioxidant content could also have potential uses, e.g., the antioxidants could be included in food and cosmetic products. Primary products were also found, highlighting innovative knowledge regarding the presence of antioxidants and pigment concentrations in the periphytic biomass from ATS systems installed to treat surface water in a lake to provide the most appropriate applicability.

The inorganic composition showed a high aluminum and iron content. These metals could limit biomass exploitation. These results demonstrate that periphytic biomass could be used in the production of high-added-value compounds of commercial interest if safety with regard to toxicity is demonstrated. Considering this present study and after a preliminary analysis of the main bioproducts found in periphytic biomass, it is possible to focus the remaining periphytic biomass to be an alternative for biogas, bioethanol or biochar production. After a better understanding of the characteristics of these microorganisms and with new studies for system optimization in order to decrease the levels of aluminum and iron, it will be possible to destine these bioproducts for an appropriate market.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors would also like to thank the University of Santa Cruz do Sul (UNISC), TecnoUNISC, through the Center for Research in Oleochemical and Biotechnological Processes and Products for MCTIC (01.0144.00/2010) and CNPq (310228/2019-0).

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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First received 12 December 2019; accepted in revised form 14 July 2020. Available online 27 July 2020

4.4 MANUSCRIPT 4 - Multicriteria decision analysis of bioproducts from microalgae: an industrial approach

This work presents a study case applications of high added value molecules produced from periphytic biomass obtained in an algal turf scrubber (ATS) system. Through multicriteria decision analysis, it was possible to predict which bioproducts from microalgae have more chances to be inserted in industries. This article was not submitted yet.

5 FINAL CONSIDERATIONS

The PhD thesis was divided into 5 manuscripts that initially added relevant information for the knowledge about the bioproducts that can be obtained from periphyton and the mains sectors that they can be inserted. Clean technologies and their importance for the development of environmentally friendly methods and viable for the main microalgae production stages were also discussed.

In manuscript 1, the bibliometric analysis was extremely relevant to analyze the studies carried out so far, emphasizinglipid, protein, carbohydrates, pigments, vitamins, and antioxidants. The main application sectors were biofuels, human and animal food, pharmacology, and cosmetology. It was also possible to observe that the food and feed sector can be considered a future trend. And finally, it could be highlighted that the high cost of production is the main obstacle faced for these bioproducts to be commercialized.

In manuscript 2, clean technologies were considered the basis for developing and optimizing the main steps for microalgae production. Through bibliometric analysis, it was possible to observe that Life Cycle Assessment proves to be an essential tool for economically viable and environmentally methods optimization. In addition, it was possible to observe that clean technology is necessary mainly to optimize the stages of biomass harvesting and bioproducts extraction.

Manuscript 3 presented an exploratory analysis of periphytic biomass as an option to valorize this biomass obtained in eutrophic surface waters. The initial infrared spectroscopy analysis was essential to have a biomass screening of the main bioproducts of commercial interest in its composition. According to the results, it was possible to verify that the periphytic biomass presented several bioproducts that were individually evaluated. Thus, it is emphasized that the value of proteins, carbohydrates and antioxidants was suitable if compared to the values already existing in studies. It is noteworthy that the lipid content was low, which indicates that this bioproduct would not be suitable for applying this biomass in sectors that demand this raw material. Finally, the potential for the use of pigments was verified, finding a high yield of this bioproduct. However, the identification and quantification of these molecules are not yet concluded.

In manuscript 4, a survey about industries data using scientific articles, industry reports and ongoing projects was performed. Subsequently, a case study was carried out with data obtained from experiments already published by our research group combined with industry reports data. Then, it was verified which bioproduct would be more viable to be produced on an industrial scale through multi-criteria decision analysis (MCDA). As a result, it was possible to verify that pigments were the bioproduct with the greatest chance for successful market entry, considering the studied periphytic biomass, followed by antioxidants, proteins, carbohydrates, and lipids.

Manuscript 5 demonstrates the possibility of applying a pilot-scale system for pigment production. This study enabled a complete analysis of how the system proposed in the current study could strengthen the knowledge obtained on a real scale. The main results were that the construction and operation of the ATS system did not have significant impacts. Drying was the most impactful step in the process, followed by extraction. These impacts could be minimized or even avoided through solar drying and acetone reuse in the extraction stages.

In addition to the development of the manuscripts, an overview of the breadth of the market for bioproducts involving microalgae was possible. In general, the vision of entrepreneurship in this field becomes possible, since the possibility of obtaining bioproducts is clear. However, the best strategies must be considered, such as, clean and easy-to-insert technologies, the possibility of isolating a microalga with greater chances of being inserted in the market, analyzing the cost-benefit of cultivation until the development of the final product, among others.

This thesis demonstrates the countless possibilities and scenarios in different sectors in which microalgae can be inserted. In future studies, it is necessary to have a continuous overview of the market so that these bioproducts can be successfully inserted since the applicability and market potential is already proven in the literature. In general, each stage developed so far has been shown to be fundamental for the theoretical basis and to assist in the development and choice of bioproducts of interest, in order to give them an appropriate destination for future studies.

6 FUTURE PERSPECTIVES

- To characterize the microbiota present in the periphytic biomass through metagenomic analysis;
- To develop a method for pigments analysis by LC-MS in order to identify them through the interpretation of mass spectra;
- To optimize a simultaneous extraction method for the target bioproducts;
- To study the production on a pilot scale for all bioproducts through Life Cycle Assessment in order to evaluate the environmental impacts and alternatives to reduce them.