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.

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

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

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.* 2019b). 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 $\leq$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 ($\leq$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.* (2019a) 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 ($\lambda_{excitation} = 485$ nm and $\lambda_{emission} = 528$ 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.

$$\text{Chlorophyll } a \ (\mu g\,mL^{-1}) = (12.25 \times Abs_{663}) - (2.79 \times Abs_{647}) \quad (1)$$

$$\text{Chlorophyll } b \ (\mu g\,mL^{-1}) = (21.50 \times Abs_{647}) - (5.10 \times Abs_{663}) \quad (2)$$

$$\text{Total carotenoids } (\mu g\,mL^{-1}) = [(1000 \times Abs_{470}) - (1.82 \times Chl\,a) - (85.02 \times Chl\,b)]/198 \quad (3)$$

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 L min^{-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 ($1{,}351.54 \times 10^6$ 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.* 2019a). The following bands were highlighted: siloxanes and frustules (structure of the siliceous cell wall of diatoms) (υSi-O) at ~1,075 and 900; υ(C-O-C) carbohydrates, saccharides and polysaccharides at ~1,198–1,134; phosphodiesters of nucleic acids and phospholipids ($\upsilon > P = O$) at ~1,240; chlorophyll, CH_2 groups, CH_3 proteins and carboxylic acid groups (υCH_2, υCH_3/CO υCH_2, υCH_3/CO) at ~1,390; amides from $\upsilon(C = O)$ proteins at ~1,637; and lipids and $\upsilon(C = O)$ 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.

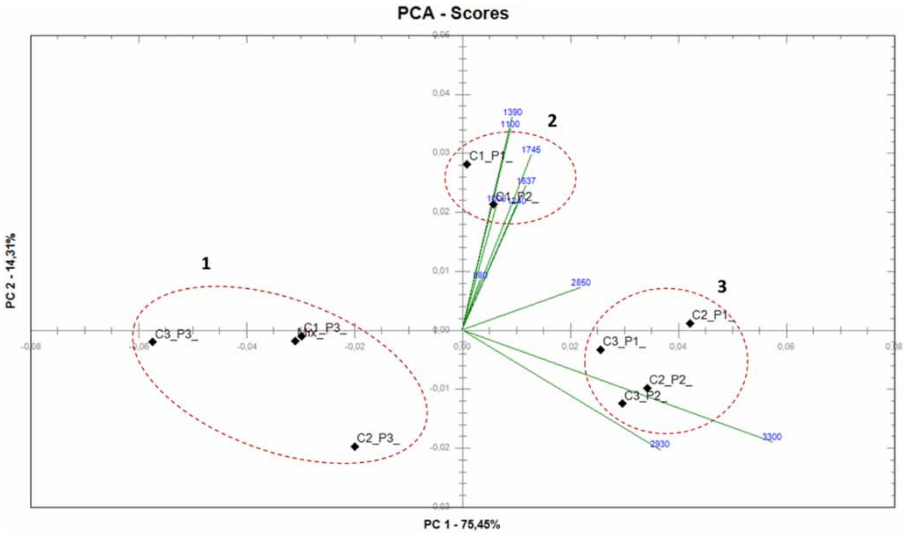

Figure 1 | Scores graphic (PC1xPC2) of the biomass collected (C1: summer, C2: winter, C3: spring, and mix: mix of C1, C2, and C3) in three points (P) ($n = 3$) from the pilot ATS system installed in Dourado Lake.

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.* (2019a). 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.

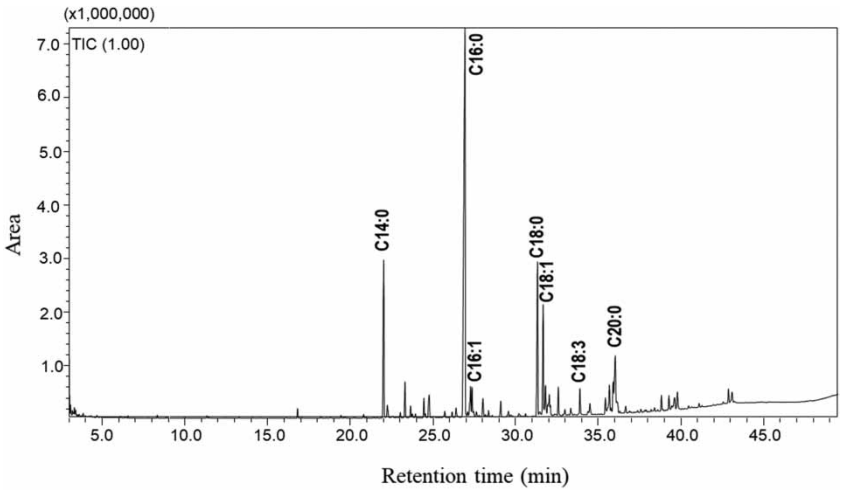

Figure 2 | Chromatogram of fatty acids from periphytic biomass of ATS system obtained by GC-MS analysis.

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 = 1E + 07x +1E + 06	0.9908	0.335	
26.62	C16:0	y = 1E + 07x +2E + 06	0.9916	1.993	
27.09	C16:1	y = 7E + 06x +666418	0.9876	0.303	
31.06	C18:0	y = 1E + 07x +2E + 06	0.9839	0.312	3.65
31.38	C18:1	y = 1E + 07x +2E + 06	0.9861	0.353	
32.29	C18:2	y = 8E + 06x +795091	0.9870	0.212	
33.58	C18:3	y = 7E + 06x +739008	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 analysis of variance (ANOVA) ($p = 0.05$), it was possible to observe that the results are statistically equal since $p > 0.05$ and that variance among replicates is equivalent ($f_{calculated} = 0.06$ and $f_{critical} = 3.35$). 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 predominance (>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 ($p = 0.05$) showed that the results for the different levels are statistically equal since the variance among replicates is equivalent ($f_{calculated} = 9.36$, $f_{critical} = 9.55$). 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.

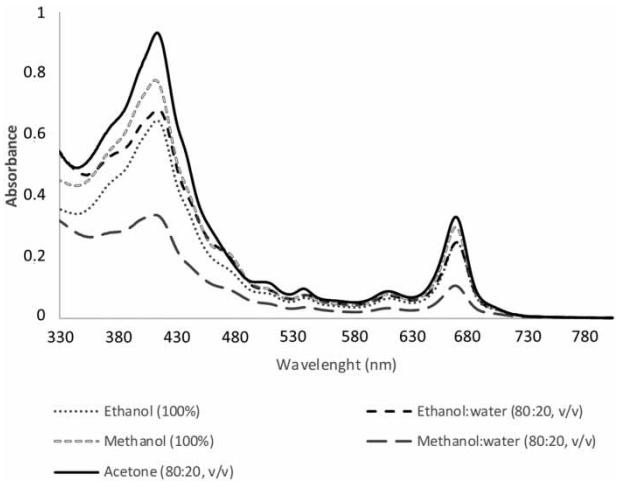

Figure 3 | Comparison of pigment extraction of periphytic biomass by UV-VIS analysis with different solvents.

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 considered the formula established for compounds also extracted in acetone:water (80:20, v/v). The results presented concentrations of 1,719.7, 541.2 and 317.7 $\mu g\ mL^{-1}$ 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% 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 ($p = 0.05$), it was found that the results are statistically equal since $p > 0.05$ and that the variance among replicates was equivalent ($f_{calculated} = 0.008$, $f_{critical} = 3.2$).

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 salmoides* (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), who 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.

REFERENCES

Adey, W. H., Laughinghouse Iv, H. D., Miller, J. B., Hayek, L. A. C., Thompson, J. G., Bertman, S., Hampel, K. & Puvanendran, S. 2013 Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry. *Journal of Phycology* **49** (3), 489–501.

Ahmed, F., Fanning, K., Netzel, M., Turner, W., Li, Y. & Schenk, P. M. 2014 Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. *Food Chemistry* **165**, 300–306.

Algarte, V. M., Siqueira, T., Landeiro, V. L., Rodrigues, L., Bonecker, C. C., Rodrigues, L. C., Santana, N. F., Thomaz, S. M. & Bini, L. M. 2017 Main predictors of periphyton species richness depend on adherence strategy and cell size. *PLoS One* **12** (7), e0181720.

Baierle, F., John, D. K., Souza, M. P., Bjerk, T. R., Moraes, M. S. A., Hoeltz, M., Rohlfes, A. L. B., Camargo, M. E., Corbellini, V. A. & Schneider, R. C. S. 2015 Biomass from microalgae separation by electroflotation with iron and aluminum spiral electrodes. *Chemical Engineering Journal* **267**, 274–281.

Bellinger, B. J., Gretz, M. R., Domozych, D. S., Kiemle, S. N. & Hagerthey, S. E. 2010 Composition of extracellular polymeric substances from periphyton assemblages in the Florida everglades. *Journal of Phycology* **46** (3), 484–496.

Bere, T. & Tundisi, J. 2012 Cadmium and lead toxicity on tropical freshwater periphyton communities under laboratory-based mesocosm experiments. *Hydrobiologia* **680**, 187–197.

Bicudo, C. d. M. & Menezes, M. 2006 *Gêneros de Algas de águas Continentais do Brasil (Algae Genera From Continental Waters of Brazil)*. Rima, São Carlos, Brazil.

Congestri, R., Di Pippo, F., De Philippis, R., Buttino, I., Paradossi, G. & Albertano, P. 2006 Seasonal succession of phototrophic biofilms in an Italian wastewater treatment plant: biovolume, spatial structure and exopolysaccharides. *Aquatic Microbial Ecology* **45** (3), 301–312.

Cui, Y., Jin, L., Ko, S. R., Chun, S. J., Oh, H. S., Lee, C. S., Srivastava, A., Oh, H. M. & Ahn, C. Y. 2017 Periphyton effects on bacterial assemblages and harmful cyanobacterial blooms in a eutrophic freshwater lake: a mesocosm study. *Scientific Reports* **7** (7:7827), 1–11.

de Souza, M. P., Hoeltz, M., Gressler, P. D., Benitez, L. B. & Schneider, R. C. S. 2018 Potential of microalgal bioproducts: general perspectives and main challenges. *Waste and Biomass Valorization* **10**, 1–18.

de Sousa e Silva, A., Sales Morais, N. W., Maciel Holanda Coelho, M., Lopes Pereira, E. & Bezerra dos Santos, A. 2020 Potentialities of biotechnological recovery of methane, hydrogen and carboxylic acids from agro-industrial wastewaters. *Bioresource Technology Reports* **10**, 100406.

Di Pippo, F., Bohn, A., Congestri, R., De Philippis, R. & Albertano, P. 2009 Capsular polysaccharides of cultured phototrophic biofilms. *Biofouling* **25** (6), 495–504.

Duppeti, H., Chakraborty, S., Das, B. S., Mallick, N. & Kotamreddy, J. N. R. 2017 Rapid assessment of algal biomass and pigment contents using diffuse reflectance spectroscopy and chemometrics. *Algal Research* **27**, 274–285.

Gangadhara, B., Keshavanath, P., Ramesha, T. J. & Priyadarshini, M. 2004 Digestibility of bamboo-grown periphyton by carps (Catla catla, labeo rohita, cirrhinus mrigala, cyprinus carpio, ctenopharyngodon idella, and tor khudree) and hybrid red tilapia (Oreochromis mossambicus× O. niloticus). *Journal of Applied Aquaculture* **15** (3–4), 151–162.

Hagerthey, S. E., William Louda, J. & Mongkronsri, P. 2006 Evaluation of pigment extraction methods and a recommended protocol for periphyton chlorophyll a determination and chemotaxonomic assessment. *Journal of Phycology* **42** (5), 1125–1136.

Hill, W. R., Rinchard, J. & Czesny, S. 2011 Light, nutrients and the fatty acid composition of stream periphyton. *Freshwater Biology* **56** (9), 1825–1836.

Hoover, S. W., Marner 2nd, W. D., Brownson, A. K., Lennen, R. M., Wittkopp, T. M., Yoshitani, J., Zulkifly, S., Graham, L. E., Chaston, S. D., McMahon, K. D. & Pfleger, B. F. 2011 Bacterial production of free fatty acids from freshwater macroalgal cellulose. *Applied Microbiology and Biotechnology* **91** (2), 435–446.

Lafarga, T., Clemente, I. & Garcia-Vaquero, M. 2020 Carotenoids from microalgae. In: *Carotenoids: Properties, Processing and Applications* (C. M. Galanakis, ed.). Academic Press, Chania, Greece, pp. 149–187.

Larned, S. T. 2010 A prospectus for periphyton: recent and future ecological research. *Journal of the North American Benthological Society* **29** (1), 182–206.

Li, H.-B., Cheng, K.-W., Wong, C.-C., Fan, K.-W., Chen, F. & Jiang, Y. 2007 Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry* **102** (3), 771–776.

Liu, J., Danneels, B., Vanormelingen, P. & Vyverman, W. 2016 Nutrient removal from horticultural wastewater by benthic filamentous algae *Klebsormidium* sp., *Stigeoclonium* spp. and their communities: from laboratory flask to outdoor Algal Turf Scrubber (ATS). *Water Research* **92**, 61–68.

Lobo, E. A., Schuch, M., Heinrich, C. G., da Costa, A. B., Dupont, A., Wetzel, C. E. & Ector, L. 2015 Development of the trophic water quality index (TWQI) for subtropical temperate Brazilian lotic systems. *Environmental Monitoring and Assessment* **187** (6), 354.

Martini, F. A., Rubert, A., de Souza, M. P., Kist, L. T., Hoeltz, M., Benitez, L. B., Rizzetti, T. M., Gressler, P. D. & Schneider, R. d. C. d. S. 2019a Periphytic biomass composition and exploitation from algae turf scrubber system. *SN Applied Sciences* **1**, 765, 1-9.

Martini, F. A., Rubert, A., de Souza, M. P., Kist, L. T., Hoeltz, M., Benitez, L. B., Rizzetti, T. M., Gressler, P. D. & Schneider, R. d. C. d. S. 2019b Periphytic biomass composition and exploitation from algae turf scrubber system. *SN Applied Sciences* **1** (7), 765.

Mubarak, M., Shaija, A. & Suchithra, T. V. 2015 A review on the extraction of lipid from microalgae for biodiesel production. *Algal Research* **7**, 117–123.

Murdock, J. N. & Wetzel, D. L. 2009 FT-IR microspectroscopy enhances biological and ecological analysis of algae. *Applied Spectroscopy Reviews* **44** (4), 335–361.

Oberholster, P. J., Myburgh, J. G., Ashton, P. J., Coetzee, J. J. & Botha, A. M. 2012 Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. *Ecotoxicology and Environmental Safety* **75** (1), 134–141.

Pacheco, M. M., Hoeltz, M., Moraes, M. S. & Schneider, R. C. 2015 Microalgae: cultivation techniques and wastewater phycoremediation. *Journal Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering* **50** (6), 585–601.

Pandey, L. K. & Bergey, E. A. 2018 Metal toxicity and recovery response of riverine periphytic algae. *Science of The Total Environment* **642**, 1020–1031.

Romanów, M. & Witek, Z. 2011 Periphyton dry mass, ash content, and chlorophyll content on natural substrata in three water bodies of different trophy. *Oceanological and Hydrobiological Studies* **40** (4), 64–70.

Sánchez, M. L., Pérez, G. L., Izaguirre, I. & Pizarro, H. N. 2013 *Influence of Underwater Light Climate on Periphyton and Phytoplankton Communities in Shallow Lakes From the Pampa Plain (Argentina) with Contrasting Steady States*.

Schneider, R. d. C. d. S., de Moura Lima, M., Hoeltz, M., de Farias Neves, F., John, D. K. & de Azevedo, A. 2018 Life cycle assessment of microalgae production in a raceway pond with alternative culture media. *Algal Research* **32**, 280–292.

Souza, L., Zitta, C., Bouzon, Z., Schneider, R., Gressler, P., Miotto, M., Rossi, M. & Rörig, L. 2016 Morphological and ultrastructural characterization of the acidophilic and lipid-producer strain *Chlamydomonas acidophila* LAFIC-004 (Chlorophyta) under different culture conditions. *Protoplasma* **254**, 1–13.

Souza, M. P., Sanchez-Barrios, A., Rizzetti, T. M., Benitez, L. B., Hoeltz, M., Schneider, R. d. C. d. S. & Neves, F. d. F. 2019a Concepts and Trends for Extraction and Application of Microalgae Carbohydrates. In: *Microalgae – From Physiology to Application* (M. Vítová, ed.). IntechOpen, London, UK.

Souza, M. P., Hoeltz, M., Brittes Benitez, L., Machado, Ê. L. & de Souza Schneider, R. d. C. 2019b Microalgae and clean technologies: a review. *CLEAN – Soil, Air, Water* **47** (11), 1–18.

Theriot, E., Herbarium, D., Round, F. E., Crawford, R. M. & Mann, D. G. 1992 The diatoms. Biology and morphology of the genera. *Systematic Biology* **41** (1), 125–126.

Uggetti, E., García, J., Álvarez, J. A. & García-Galán, M. J. 2018 Start-up of a microalgae-based treatment system within the biorefinery concept: from wastewater to bioproducts. *Water Science and Technology* **78** (1), 114–124.

Werlang, E. B., Julich, J., Muller, M. V. G., de Farias Neves, F., Sierra-Ibarra, E., Martinez, A. & Schneider, R. d. C. d. S. 2020 Bioethanol from hydrolyzed Spirulina (*Arthrospira platensis*) biomass using ethanologenic bacteria. *Bioresources and Bioprocessing* **7** (1), 27.

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