Periphytic biomass composition and exploitation from algae turf scrubber system

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Abstract

The algae turf scrubber (ATS) is an excellent system for nutrient removal in wastewater or water bodies. The purpose of this study was to evaluate the production of periphytic biomass in an ATS system installed in a freshwater reservoir and to recognize biomass composition. Mid-infrared spectroscopic analysis was carried out to identify the main composition and variability of biomass using multivariate analysis. The composition was studied for potentiality to use the periphytic biomass for the industry. As a result, the system produced a dry weight of $4.46 \text{ g m}^{-2} \text{ day}^{-1}$ of periphytic biomass. It was determined that the biomass contained 43.71% carbon, 6.03% nitrogen, and 2.87% phosphorus. Protein, lipid, and carbohydrate contents were 19.27%, 5.90%, and 65.9%, respectively. Carbohydrate and protein values indicated that the periphytic biomass has a potential for biofuel, fertilizer, and animal feed, and other products; due to the carbohydrate content, application for fermentative products is relevant.

Keywords Periphyton · Algae turf scrubber · Biomass composition · Carbohydrates · Proteins · Lipids

1 Introduction

Algae turf scrubber (ATS) is a technology used for nutrient removal, mainly nitrogen and phosphorus, from water and wastewater. Regarding the system, biofilm formation represents a poorly explored opportunity for the industry.

In several countries, there are studies on ATS installation in laboratories or on a large scale. In addition, this system can be used to avoid the eutrophication by nutrient consumption through periphytic biofilm formation as preventive technology with high biomass productivity [1–3].

The ATS systems stand out as a potentially cost-effective technology for the removal of nutrients from municipal wastewater, aquariums, liquid animal waste [4], and surface water [2]. This system consists of a downward sloping

flow way in which water, or influent, is pumped. The water flows over the length of the flow way and is then discharged or re-circulated.

The reduction in water pollution associated with the low cost of ATS technology and the co-products it generates can represent a great environmental gain. This advantage can be applied especially in places with technical and operational limitations in the treatment of effluents and/or where the economy depends on water quality, such as mariculture, fishing, public supply, and tourism [5]. The improvement in water quality of the water treated by the ATS system can help in the operation of treatment plants, reducing expenses with chemical products, and consequently improving the quality of life of the population.

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In several countries, ATS has been effectively developed [1, 3, 6, 7].

The ATS is naturally colonized by algae, bacteria, and other micro- and meso-organisms that often form complex microbial consortia (periphyton) that grow attached to surface and spaces on the mesh screen that is fixed on the raceway surface [1, 8].

In biological remediation systems, biomass valorization is an important step, since it can be used for several purposes. Microalgal biomass is rich in many bioproducts such as lipids, carbohydrates, proteins, pigments, and antioxidants. Thus, this biomass could be used as a potential feedstock for biofuel production, food nutrition (human and animal), pharmacology, and cosmetology [6, 9].

The potentiality of the ATS used in Southern Brazil was investigated, in Santa Cruz do Sul city, where there were conditions for the photosynthetic process. ATS was installed in Dourado Lake, which is the main source of water supply of the city and in the last years, presented an increase in algae blooms that caused an unpleasant taste and odor after water treatment. The reservoir occupies an area of 119 ha and has a capacity of up to 3 million cubic meters of water. According to the last bathymetry performed, it has an average depth of approximately 3 m. Dourado Lake receives water from a river that transports nutrients, agricultural fertilization, and urban wastewater. Therefore, surface water sometimes suffers eutrophication and consequently, the likely presence of cyanobacteria proliferation that causes health risks to the community. The performance of an ATS system in this condition provides indications of the bioremediation potential regarding the reduction in water pollution and, indeed, is a preventive technology to avoid algae bloom.

Therefore, considering the efficiency of the ATS system for water treatment and excipient knowledge about periphytic biomass exploitation, this research aimed to evaluate the quantity and composition of periphytic biomass produced in the ATS pilot system. In addition, chemical variability and industrial applicability were analyzed to see if the ATS biomass can be produced in high quantities at various locations.

2 Materials and methods

2.1 ATS operation

A pilot-scale ATS (Fig. 1) was constructed at Dourado Lake, an artificial water reservoir, in the city of Santa Cruz do Sul—RS (29°43′53.7″S 52°27′37.7″W, Brazil).

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 (A) at a flow rate of approximately 2 L min^{-1} via a 5 L surge box at the top of the flow way. The surge box released a pulse of water of an adjustable period between 60 and 120 s. Effluent from the flow way drained through a nylon mesh bag at the bottom of the flow way before draining into the lake (B).

Fig. 1 ATS pilot system used in this study in Dourado Lake

2.2 Harvesting and processing of periphyton

The evaluation of biomass was performed by collecting samples for 7 months, which corresponds to the hot season (March, August, and September) and cold season (April to July) in the southern hemisphere. The average temperatures during the system monitoring were measured at the weather station of the university and at the influent and effluent of ATS system. In the hot season, the maximum temperature was 28.6 °C and the minimum temperature was 15.7 °C. At the influent from the lake, the temperature was 23.2–27.8 °C and the effluent to the lake was 23.8–30.6 °C and in the cold season, the maximum temperature was 23.3 °C, the minimum temperature was 10.4 °C; at the influent from the lake the temperature was 17.7–19.5 °C and at the effluent to the lake was 23.0–25.0 °C.

Periphyton was harvested manually at intervals of 1–3 weeks, and the results were presented in mass per m² per day for the periphyton growth rate. Before each harvest, water flow to the ATS was turned off, and the scrubber was allowed to drain for 4 h. The residual biomass fixed on the ramp was used as an inoculum for the next growth cycle.

Periphyton was harvested from the 5 m of each flow way. 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. In this step, the biomass yield was accomplished through gravimetric measurements until constant mass. After drying, the biomass was milled with a Willey-type cryogenic knife mill (Tecnal TE-680) with a milling time of 6 min, holding time of 2 min and 20 min of connected time with a frequency of 20 Hz. The biomass was maintained in polypropylene (PP) tubes and stored in a freezer at -20°C . The biomass was quantified concerning the production time in the ATS system (days) and surface area (m²).

2.3 Analysis of periphytic biomass

2.3.1 Determination of lipids and fatty acids

The total lipids were determined from the adapted Bligh and Dyer method using ultrasound for cell disruption and chloroform (Sigma-Aldrich, USA); and methanol (J.T. Baker, USA) as extraction solvents [10, 11]. Gravimetric assays were performed until constant weight to calculate lipid yield. To determine fatty acids, 6 mL of methanolic solution of NaOH (J.T. Baker, USA) was added to the volumetric flask and refluxed for 20 min (saponification). Then, 7 mL of a 14% (w/v) boron trifluoride (Sigma-Aldrich, USA) in methanol (derivatizing) was added through the reflux condenser by dripping, then refluxed for 4 min. Likewise, 5 mL

of heptane was added to the reaction medium, remaining at reflux for 2 min. After reaching room temperature, 2 mL of saturated aqueous NaCl solution was added. Finally, the upper heptane phase was separated and dried with anhydrous sodium sulfate (before injection).

Chromatographic analyses were performed using the GC–MS system (Shimadzu, Japan) equipped with GC 2010 gas chromatography and MS-QP 2010 Plus detector. The GC–MS system was operated with a 30 m capillary column ZB-WAX (polyethylene glycol, Phenomenex, USA) with an internal diameter of 0.25 mm and 0.25 µm of film thickness. The column oven temperature program was 70 °C (4 °C min⁻¹) to 240 °C for 5 min and elevated to 250 °C (35 min). The injector temperature was 250 °C with an injection volume of 1 µL, and injection mode was split (1:10). Transfer line temperature was set at 260 °C; ion source was electron ionization (EI) at 70 eV and the temperature was set at 270 °C. Helium was used as a carrier gas with a flow of 1 mL min⁻¹. The methyl ester of fatty acids was identified and quantified by comparison with spectra library and with the reference standard of the Fatty Acid Methyl Esters (Unsaturated Kit and Saturated Straight Chains Kit; Sigma-Aldrich, USA).

2.3.2 Elemental analysis (CHNSO)

Elemental composition of C, H, N, and S was determined using a PE-2400 CHNS elemental analyzer (model PE-2400, PerkinElmer, USA). The instrument was standardized, and then the samples and blanks were analyzed. The analysis was performed in duplicate, using 2 mg of sample weighed in a microbalance (model AD 6000, PerkinElmer, USA). The reference standard used was acetanilide (C = 71.09%, H = 6.71%, N = 10.36%). The N values of the elemental analysis were multiplied by the correction factor of 4.78 to determine the protein content [12]. The oxygen content (%) was obtained by difference of the content (%) of C, H, N, S, and ashes [13].

2.3.3 Determination of phosphorus

The amount of 1 g of dry biomass was treated in a Kjeldahl flask with 25 mL of concentrated HNO₃ (Merck, German). The solution was heated for 35 min at 83 °C to oxidize all organic matter and cooled to room temperature. A 15 mL of HClO₄ (72%, v/v) (Dinâmica, Brazil) was added to the extract and boiled again for a period of 1 h at 170 °C until the solution became colorless. After this step, the solution was cooled for 15 min, and 50 mL of H₂O was added and later boiled for 10 min.

The solution was cooled to room temperature and transferred to a 250 mL volumetric flask. After homogenization, the solution was filtered with filter paper where

reading was done through inductively coupled plasma—optical emission spectrometry (ICP-OES, model Optima 8300, PerkinElmer, USA). An analytical curve was constructed from a standard solution of phosphate by external standardization in the concentration of 0.25–20 mg L⁻¹, and the analysis was carried out in triplicate.

2.3.4 Determination of ash and total carbohydrates

Ash determination and total carbohydrate quantification were performed following the methodology recommended by the National Renewable Energy Laboratory (NREL) [14]. Chromatographic conditions for quantification of carbohydrates (glucose, xylose) were performed using the high-efficiency liquid chromatography (Shimadzu, Japan) with refractive index detection (RID). For chromatographic separation, a RHM monosaccharide H⁺ column (300 × 7.8 mm) with an oven temperature at 85 °C was employed. The mobile phase was ultra-pure water with a flow rate of 0.8 mL min⁻¹ and a sample injection of 20 µL. An analytical curve was constructed from a standard solution of glucose and xylose by external standardization in the concentration of 0.005–0.075 mg mL⁻¹.

2.3.5 Infrared analysis spectroscopy (FT-IR)

A mid-infrared spectrometer (model Spectrum 400, PerkinElmer, USA) configured on the system FT-IR using the attenuated total reflectance (ATR) accessory was used to obtain spectra from the dried biomass. The spectra were recorded in the frequency range of 650–4000 cm⁻¹ at a resolution of 4 cm⁻¹ and eight scans. For FT-IR measurement, approximately 3.0 mg of biomass was finely milled and applied to the surface of the ATR crystal to obtain the spectra.

In order to classify the samples obtained in the ATS in relation to the different times of the year and from the three flow ways in which they were collected, the multivariate analysis using the infrared data was employed. For this, the spectra were preprocessed in the Chemostat® 1.0.0.0 software, and the single linkage method was used for the Hierarchical Clustering Analysis (HCA).

2.4 Statistical methods

Data were reported as mean ± standard deviation. Statistical analyses were performed using variance analysis (Graph Pad Instat v.3) with a 5% level of probability ($p < 0.05$) selected in advance to sufficiently demonstrate a statistically significant difference. The data were submitted for analysis of variance using the Kruskal–Wallis test with Dunn's posttest for comparison of the columns.

3 Results and discussion

3.1 Production and characterization of periphytic biomass

Biomass production was $4.46 \pm 1.60 \text{ g m}^{-2} \text{ day}^{-1}$ ash-free, and the organic phase of biomass was 2.87% phosphorus, 6.03% nitrogen, 43.71% carbon, 0.89% sulfur, 4.79% hydrogen, and 11.23% oxygen with relative standard deviations less than 3%. Considering the potential of fixing these elements in the periphyton of ATS system, we calculated that it could be fixed as follows: 467.2 kg P he⁻¹ year⁻¹, 981.6 kg N he⁻¹ year⁻¹, and 7115.5 kg C he⁻¹ y 45 45⁻¹.

The inorganic composition of the biomass was determined by the ash content at an average of $33.35 \pm 5.36\%$ with higher values during the rainiest periods due to the increase in the turbulence of the water that moves the sediment, which is deposited on the ATS surface. This high sediment concentration led to a reduction in periphyton productivity. Besides, the amount of light available for photosynthesis, water depth is also a factor. The light that reaches the periphyton through particulate matter can be absorbed or scattered [2].

In 2015, 119 ha of water from Dourado Lake was treated with 12 tons of Phoslock® (95% bentonite-5% lanthanum/<http://phoslock.com.br/>) for phosphorus sequestration. Therefore, a large amount of phosphorus can be found in the sediment and there is the possibility of sediments being a source of nutrients to favor algae growth [15]. Besides that, higher temperatures and intense light incidence for extended periods are factors that directly influence periphytic biomass production [5].

This was less when compared to the productivity of other pilot systems as seen in the results obtained by Sandefur et al. [16] in a 9-month operating time ($26 \text{ g m}^{-2} \text{ day}^{-1}$) and similar to Kangas et al. [1] with $4.3 \text{ g ash-free DW m}^{-2} \text{ day}^{-1}$.

Based on this, the biomass formation in the ATS system is promising for the fixation of elements such as C, N, and P. In addition, it is possible to obtain biomass for several applications that depend mainly on majority composition (carbohydrates, lipids, and proteins) as presented in Table 1. The results did not show a significant difference between each harvest and each flow way channel ($p > 0.05$).

The values found are similar to those observed for some types of algae, the most abundant species of the periphyton [1]. According to Huang et al. [17] less than 25% of the periphyton consists of other organisms.

As for protein content, isolated microalgae from freshwater presented 13–15% protein as obtained in

Table 1 Main constituent analysis of the biomass harvested in the ATS system

Composition	Average of different time (%), <i>n</i> = 3			Average $\pm$ standard deviation (%)
	Harvest 1	Harvest 2	Harvest 3	
Proteins	17.48	21.79	18.53	19.27 $\pm$ 2.24
Carbohydrates	74.21	57.70	63.38	65.09 $\pm$ 8.39
Lipids	6.29	6.22	5.22	5.90 $\pm$ 0.59

the study by Tibbetts et al. [18]. For Santhakumaran et al. [19], the levels were higher for many microalgae; however, they used an *N* conversion factor of 6.25 for protein, and in this work, 4.78 was used, as proposed by Templeton, Laurens [12].

Total carbohydrates in the ash-free biomass were high. This was probably because in the biofilm there is mucilaginous extracellular polymeric substance (EPS) formation that can consist of 10–90% biofilm. EPS are mainly polysaccharides secreted due to the presence of bacteria and microalgae association with the substrate, thus ensuring the survival of aggregates [8, 20].

In less proportion, there was lipid content in the periphyton. Therefore, this biomass is a feedstock for biofuel with low lipid content typically laden with high levels of ash that can be converted by hydrothermal liquefaction and fermentation due to carbohydrate and protein content [21].

3.1.1 Monosaccharides and fatty acids through chromatography analysis

The carbohydrates identified in the ash-free biomass were composed mainly of glucose (77–86%) and xylose (16–23%). In addition to EPS, a wide variety of algae species have rigid cell walls containing large amounts of polysaccharides. The variation in the structural composition of carbohydrates of the various species happens with changes in sunlight intensity, nutrients, and harvesting periods [22]. In this study, a significant difference between monosaccharides separated by chromatography in each harvest was not found.

Through gas chromatography (GC–MS), we determined the fatty acids in the lipid fractions. In this study, we did not find differences between the lipid content of the dried biomasses of each flow way channel, but significant differences ($p = 0.05$) were present among the samples when analyzed seasonally.

Table 2 shows the fatty acid (FA) profile of lipids contained in the periphyton biomass. A higher synthesis of unsaturated fatty acids (44.93%) in the hot season was observed under better growing conditions, and a higher

Table 2 Fatty acid profile from periphyton dried biomass from relative area in the GC–MS

Fatty acid identification		Hot season (%)	Cold season (%)
Miristic	C14:0	5.82	5.66
Pentadecylic	C15:0	0.53	0.83
Palmitic	C16:0	26.96	47.26
Palmitoleic	C16:1	4.41	3.71
Margaric	C17:0	0.62	0.97
Estearic	C18:0	6.63	6.77
Oleic	C18:1	13.09	6.30
Lionleic	C18:2	6.79	4.80
Linolenic	C18:3	8.70	5.43
Araquidic	C20:0	0.00	0.64
Araquidonic	C20:4	0.00	0.38
Eicosapentaenoic (EPA)	C20:5	7.09	0.93
Behenic	C22:0	0.00	0.89
Docosahexaenoic (DHA)	C22:6	4.85	0.00
Others compounds	nd	14.51	15.43
Saturated total		40.56	63.02
Unsaturated total		44.93	21.55

amount of saturated fatty acids (63.02%) was seen when cultivated in the cold season. The samples present a polyunsaturated composition that is important as essential fatty acids.

Essential lipids from periphyton are crucial for fish feed. When the trophic chain is evaluated, naturally this periphytic biomass is food for fish since it has high amounts of polyunsaturated fatty acids (PUFAs) [23]. We highlight that the periphyton presents the essential fatty acid (EPA and DHA) as well as $\omega 3$, $\omega 6$, and $\omega 9$ that make up around 35–40% of unsaturated FA. Furthermore, DHA was not found, and EPA was less during the cold season when there was less growth. Makulla [24] suggests that for microalgae (*Scenedesmus obliquus*) that higher growth rates promoted higher EPA concentrations.

According to Maazouzi et al. [25], in the diet of a shrimp species, it is likely that periphyton is a dietary source of the essential fatty acids.

3.1.2 Infrared spectroscopy analysis of dried periphytic biomass

The biochemical composition evaluation of the periphytic biomass should be thorough since the identification of the infrared bands is complex. The complexity is due to a macromolecular overlap of the proteins, lipids, nucleic acids, carbohydrates, among other compounds [26].

Based on the overlapping of the infrared spectra, the profile of the generated spectra was the same for all

Fig. 2 Infrared spectra overlapping from biomass harvest in the pilot ATS system installed in Dourado Lake

Table 3 Infrared bands characterization of the periphytic biomass harvest in the pilot ATS system installed in Dourado Lake

Wave length (cm ⁻¹)	Band characterization	Functional groups	References
~ 3400–3200	$\nu\text{O-H}/\nu\text{N-H}$	Water, protein	[27]
~ 2930	νCH_2	CH_2 of methylene group	[28]
~ 2850	$\nu\text{CH}_2, \nu\text{CH}_3/\text{C-O}$	CH_3 e CH_2 methyl and methylene group	[28]
~ 1745	$\nu(\text{C=O})$	Lipids, esters, and fatty acids	[27, 28]
~ 1637	$\nu(\text{C=O})$	Amide from protein	[27]
~ 1390	$\nu\text{CH}_2, \nu\text{CH}_3/\text{C-O}$	Chlorophyll, CH_2 and CH_3 of protein and carboxylic acids	[29]
~ 1240	$\nu(>\text{P=O})$	Phosphodiester of nucleic acids, phospholipids	[28]
~ 1198–1134	$\nu(\text{C-O-C})$	Carbohydrates, polysaccharides	[27]
~ 1075 e 900	$\nu\text{Si-O}$	Siloxanes, siliceous frustules	[30]
~ 900–690	$\nu\text{C-H}$	Aromatic compounds and kaolinite	[31]

harvests, as shown in Fig. 2. The bands are characterized in Table 3.

The spectra confirm the results previously found concerning the presence of carbohydrates, lipids, and proteins. In addition, the presence of diatom was identified due to band relativity for siliceous frustules (~ 1075 e 900 cm^{-1}) and phospholipids due to the double linkage between P and O ($\sim 1240\text{ cm}^{-1}$) observed in the spectra.

Although overlapping bands have shown the similarity between harvests, band intensity may vary, and small bands may bring relevant chemical information. Therefore, the multivariate analysis assists in the comparison of the samples. For this purpose, the infrared spectra were treated with the Hierarchical Cluster Analysis (HCA) method.

The use of the HCA in the similarity study allows for the identification of possible variability between the harvests since it is an indicator for the applicability of the biomass. Figure 3 shows three main groups with a similarity of approximately 0.95. The formation of groups indicates that all triplicates were properly grouped into subgroups. The first group consisted of three points—flow ways (P) of the harvests performed in summer (H1), in winter (H2), in spring (H3) and the mix of all these samples analyzed through infrared spectroscopy.

This behavior can be explained by the possibility that the biofilm formation is not uniform in all of the flow way channels due to sedimentation, which is influenced by water movement and preferential direction of the wind. The data also show that stretching bands of

HCA

Similarity

0.50
0.60
0.70
0.80
0.90
1.00

H2P3_3
H2P3_1
H2P3_2
H3P3_1
H3P3_2
H3P3_3
H1P3_1
H1P3_2
H1P3_3
Mix_1
Mix_2
Mix_3
H1P1_1
H1P1_2
H1P1_3
H1P2_3
H1P2_1
H1P2_2
H2P1_1
H2P1_2
H2P1_3
H2P2_1
H2P2_2
H2P2_3
H3P2_1
H3P2_2
H3P2_3
H3P1_1
H3P1_2
H3P1_3

Group 1 Group 2 Group 3

The multivariate chemometric approach from mid-infrared spectra assumes that the samples show high similarity even though the samples were collected from different seasons. In the samples, variation in the composition of saturated and unsaturated fatty acids was observed. This variation would be responsible for sample discrimination in different groups [32]. However, the lipid content in the samples is low, and the variation in the mid-infrared spectra was not preponderant to other chemical information from cellular components of the present species.

In biological remediation systems, the periphytic community is an excellent bioindicator of water quality and its trophic state. In addition, microalgal biomass is known for its richness in lipids, carbohydrates, proteins, pigments, and antioxidants. In this context, the biomass valorization is very important, since it can be used for several purposes, for example, in the production of biofuels, food nutrition (human and animal), pharmacology, and cosmetology. The biomass composition found in this study indicated relatively low lipid content and high levels of carbohydrates and proteins.

For soil fertilization, the periphytic biomass captures a high amount of carbon, as well as nitrogen and phosphorus. Research has shown that the organic component in the periphyton delays the leaching of nutrients, further increasing the value of the fertilizer product [4]. Studies have reported that the use of dry ATS biomass as a slow-release organic fertilizer is equivalent to commercial organic fertilizer in regards to plant mass and nutrient content [36].

We highlight that such eutrophic water remediation technology is also a way of capturing carbon from the atmosphere since more than $7115.5 \text{ kg C he}^{-1} \text{ year}^{-1}$ can be fixed.

Adey et al. [5] evaluated the potential for biofuel generation from the biomass of algae grown in an ATS system. The study provided evidence that the biomass showed promise for ethanol production when compared to other biofuel feedstocks. Using data from the US Department of Agriculture, which reported corn production averages of about $973 \text{ g m}^{-2} \text{ day}^{-1}$, it is a possible yield of $0.40 \text{ L ethanol m}^{-2} \text{ year}^{-1}$. Since the carbohydrate content measured in the harvested biomass was approximately 24%, Adey et al. [5] assumed an equivalent yield of ethanol from the fermentation of algal carbohydrates. They had predicted that an ATS facility with 3D screens could produce about $2.24 \text{ L m}^{-2} \text{ year}^{-1}$ ethanol or about 5.6 times greater than from corn feedstock.

Another by-product of periphytic biomass is pigment, which was not evaluated in this study. But it is known that microalgae produce a variety of pigment molecules like chlorophyll, carotenoids, and carotene that are used as colorants in food and the cosmetic industry [9].

4 Conclusions

The composition of the periphyton developed in the ATS system highlights the potential for the use of this biomass. The biomass contains high concentrations of carbohydrates (65%) and proteins (19%) and is a good feedstock for industries. The lipid content is less, but the composition is rich in essential unsaturated fatty acids.

For the potentiality of the periphytic biomass, it is crucial to maintain the higher production of biomass of periphyton. For this, we highlight that the productivity can be improved when the ATS system is installed in large scale.

Thus, the use of periphytic biomass from ATS can be promising on a large-scale proposal once there are little variability and high applicability. The composition is useful for producing animal feed, fertilizer, and biofuel. In addition, the high concentration of carbohydrates is appropriate for use in the fermentation process. Therefore, the application of this community as a natural substrate can contribute to fermentative processes with less added nutrients, which makes for a more economical and environmentally friendly alternative.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Kangas P, Mulbry W, Klavon P, Laughinghouse HD, Adey W (2017) High diversity within the periphyton community of an algal turf scrubber on the Susquehanna River. *Ecol Eng* 108:564–572. <https://doi.org/10.1016/j.ecoleng.2017.05.010>
- Sutherland DL, Craggs RJ (2017) Utilising periphytic algae as nutrient removal systems for the treatment of diffuse nutrient pollution in waterways. *Algal Res* 25:496–506. <https://doi.org/10.1016/j.algal.2017.05.023>
- Yan Z, Kangas PC, Yuan X, Chen Y, Zhang Y, Li J, Su Y, Gao X, Chen N (2018) Flow conditions influence nutrient removal at an artificial lake and a drinking water reservoir with an algal floway. *Algal Res* 35:245–252. <https://doi.org/10.1016/j.algal.2018.08.016>
- Mulbry W, Kondrad S, Pizarro C, Kebede-Westhead E (2008) Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresour Technol* 99(17):8137–8142. <https://doi.org/10.1016/j.biortech.2008.03.073>
- Adey WH, Laughinghouse HDT, Miller JB, Hayek LA, Thompson JG, Bertman S, Hampel K, Puvanendran S (2013) Algal turf scrubber (ATS) flowways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry(1). *J Phycol* 49(3):489–501. <https://doi.org/10.1111/jpy.12056>
- Choudhary P, Prajapati SK, Kumar P, Malik A, Pant KK (2017) Development and performance evaluation of an algal biofilm reactor for treatment of multiple wastewaters and characterization of biomass for diverse applications. *Bioresour Technol* 224:276–284. <https://doi.org/10.1016/j.biortech.2016.10.078>
- Bohutskiy P, Chow S, Ketter B, Fung Shek C, Yacar D, Tang Y, Zivojnovich M, Betenbaugh MJ, Bouwer EJ (2016) Phytoremediation of agriculture runoff by filamentous algae poly-culture for biomethane production, and nutrient recovery for secondary cultivation of lipid generating microalgae. *Bioresour Technol* 222:294–308. <https://doi.org/10.1016/j.biortech.2016.10.013>
- Sun R, Sun P, Zhang J, Esquivel-Elizondo S, Wu Y (2018) Microorganisms-based methods for harmful algal blooms control: a review. *Bioresour Technol* 248(Pt B):12–20. <https://doi.org/10.1016/j.biortech.2017.07.175>
- de Souza MP, Hoeltz M, Gressler PD, Benitez LB, Schneider RCS (2018) Potential of microalgal bioproducts: general perspectives and main challenges. *Waste Biomass Valoriz*. <https://doi.org/10.1007/s12649-018-0253-6>
- Gressler PD, Bjerk TR, Schneider Rde C, Souza MP, Lobo EA, Zappe AL, Corbellini VA, Moraes MS (2014) Cultivation of *Desmodesmus subspicatus* in a tubular photobioreactor for bioremediation and microalgae oil production. *Environ Technol* 35(1–4):209–219. <https://doi.org/10.1080/09593330.2013.822523>
- Souza LD, Simioni C, Bouzon ZL, Schneider RC, Gressler P, Miotto MC, Rossi MJ, Rorig LR (2017) Morphological and ultrastructural characterization of the acidophilic and lipid-producer strain *Chlamydomonas acidophila* LAFIC-004 (Chlorophyta) under

- different culture conditions. *Protoplasma* 254(3):1385–1398. <https://doi.org/10.1007/s00709-016-1030-7>
12. Templeton DW, Laurens LML (2015) Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel. *Algal Res* 11:359–367. <https://doi.org/10.1016/j.algal.2015.07.013>
 13. Leite E, Protásio T, Rosado S, Trugilho PF, Valle MLA, Siqueira H (2015) Elemental chemical composition of wood and charcoal from *Coffea arabica* for bioenergy use. *Coffee Sci* 10(4):10
 14. Van Wychen S, Laurens LML (2016) Determination of total carbohydrates in algal biomass: laboratory analytical procedure (LAP). National Renewable Energy Lab., Golden
 15. Izagirre O, Serra A, Guasch H, Elosegi A (2009) Effects of sediment deposition on periphytic biomass, photosynthetic activity and algal community structure. *Sci Total Environ* 407(21):5694–5700. <https://doi.org/10.1016/j.scitotenv.2009.06.049>
 16. Sandefur HN, Matlock MD, Costello TA (2011) Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecol Eng* 37(10):1476–1480. <https://doi.org/10.1016/j.ecoleng.2011.04.002>
 17. Huang W, Liu X, Peng W, Wu L, Yano S, Zhang J, Zhao F (2018) Periphyton and ecosystem metabolism as indicators of river ecosystem response to environmental flow restoration in a flow-reduced river. *Ecol Ind* 92:394–401. <https://doi.org/10.1016/j.ecolind.2017.11.025>
 18. Tibbetts SM, Whitney CG, MacPherson MJ, Bhatti S, Banskota AH, Stefanova R, McGinn PJ (2015) Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications. *Algal Res* 11:435–447. <https://doi.org/10.1016/j.algal.2014.11.011>
 19. Santhakumaran P, Kookal SK, Ray JG (2018) Biomass yield and biochemical profile of fourteen species of fast-growing green algae from eutrophic bloomed freshwaters of Kerala, South India. *Biomass Bioenergy* 119:155–165. <https://doi.org/10.1016/j.biombioe.2018.09.021>
 20. Mantzourou A, Ververidis F (2019) Microalgal biofilms: a further step over current microalgal cultivation techniques. *Sci Total Environ* 651(Pt 2):3187–3201. <https://doi.org/10.1016/j.scitotenv.2018.09.355>
 21. Aston JE, Wahlen BD, Davis RW, Siccardi AJ, Wendt LM (2018) Application of aqueous alkaline extraction to remove ash from algae harvested from an algal turf scrubber. *Algal Res* 35:370–377. <https://doi.org/10.1016/j.algal.2018.09.006>
 22. Skriptsova AV, Shevchenko NM, Zvyagintseva TN, Imbs TI (2009) Monthly changes in the content and monosaccharide composition of fucooidan from *Undaria pinnatifida* (Laminariales, Phaeophyta). *J Appl Phycol* 22(1):79–86. <https://doi.org/10.1007/s10811-009-9438-5>
 23. Hussain B, Sultana T, Sultana S, Al-Ghanim KA, Al-Misned F, Mahboob S (2019) Influence of habitat degradation on the fatty acid profiles of fish, microalgae, and zoobenthos in a river ecosystem. *Process Saf Environ Prot* 123:24–32. <https://doi.org/10.1016/j.psep.2018.12.024>
 24. Makulla A (2000) Fatty acid composition of *Scenedesmus obliquus*: correlation to dilution rates. *Limnologia* 30(2):162–168. [https://doi.org/10.1016/S0075-9511\(00\)80011-0](https://doi.org/10.1016/S0075-9511(00)80011-0)
 25. Maazouzi C, Masson G, Izquierdo MS, Pihan JC (2007) Fatty acid composition of the amphipod *Dikerogammarus villosus*: feeding strategies and trophic links. *Comp Biochem Physiol A Mol Integr Physiol* 147(4):868–875. <https://doi.org/10.1016/j.cbpa.2007.02.010>
 26. Duppeti H, Chakraborty S, Das BS, Mallick N, Kotamreddy JNR (2017) Rapid assessment of algal biomass and pigment contents using diffuse reflectance spectroscopy and chemometrics. *Algal Res* 27:274–285. <https://doi.org/10.1016/j.algal.2017.09.016>
 27. Tan S-T, Balasubramanian RK, Das P, Obbard JP, Chew W (2013) Application of mid-infrared chemical imaging and multivariate chemometrics analyses to characterise a population of microalgae cells. *Bioresour Technol* 134:316–323. <https://doi.org/10.1016/j.biortech.2013.01.060>
 28. Duygu DY, Udoh AU, Ozer TB, Akbulut A, Erkaya IA, Yildiz K, Guler D (2012) Fourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus obliquus* (Turpin) Kützing 1833. *Afr J Biotechnol* 11(16):3817–3824
 29. Neault JF, Tajmir-Riahi HA (1999) Structural analysis of DNA-chlorophyll complexes by Fourier transform infrared difference spectroscopy. *Biophys J* 76(4):2177–2182. [https://doi.org/10.1016/S0006-3495\(99\)77372-8](https://doi.org/10.1016/S0006-3495(99)77372-8)
 30. Jiang Y, Yoshida T, Quigg A (2012) Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. *Plant Physiol Biochem* 54:70–77. <https://doi.org/10.1016/j.plaphy.2012.02.012>
 31. Stuart B (2004) Infrared spectroscopy: fundamental and applications. Analytical techniques in the science. Wiley. <https://doi.org/10.1002/0470011149>
 32. Vendruscolo RG, Facchi MMX, Maroneze MM, Fagundes MB, Cichoski AJ, Zepka LQ, Barin JS, Jacob-Lopes E, Wagner R (2018) Polar and non-polar intracellular compounds from microalgae: methods of simultaneous extraction, gas chromatography determination and comparative analysis. *Food Res Int* 109:204–212. <https://doi.org/10.1016/j.foodres.2018.04.017>
 33. Lamminen M, Halmemies-Beauchet-Filleau A, Kokkonen T, Jaakkola S, Vanhatalo A (2019) Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. *Anim Feed Sci Technol* 247:112–126. <https://doi.org/10.1016/j.anifeedsci.2018.11.005>
 34. Pamplona-Silva MT, Goncalves LC, Marin-Morales MA (2018) Genetic toxicity of water contaminated by microcystins collected during a cyanobacteria bloom. *Ecotoxicol Environ Saf* 166:223–230. <https://doi.org/10.1016/j.ecoenv.2018.09.090>
 35. Romero Garcia JM, Acien Fernandez FG, Fernandez Sevilla JM (2012) Development of a process for the production of L-amino acids concentrates from microalgae by enzymatic hydrolysis. *Bioresour Technol* 112:164–170. <https://doi.org/10.1016/j.biortech.2012.02.094>
 36. Mulbry W, Westhead EK, Pizarro C, Sikora L (2005) Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. *Bioresour Technol* 96(4):451–458. <https://doi.org/10.1016/j.biortech.2004.05.026>

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