



Use of the cyanobacterium *Spirulina platensis* in cattle wastewater bioremediation

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ABSTRACT. In the present study, the microalga *Spirulina platensis* (*Arthrospira*) was grown in two horizontal photobioreactors (HPBR) under two different irradiances (150 and 270 $\mu\text{mol m}^{-2} \text{s}^{-2}$). Anaerobically digested cattle wastewater (ACWW) was used as substrate. The experiment was carried out in batches for a period of 8 days. The maximum specific growth rate of 0.347 day^{-1} and the doubling time of 2.08 days were obtained under the highest illumination of the culture. Dry biomass production reached maximum values between 2.17 g L^{-1} and 6.52 g L^{-1} , with volumetric biomass productivities between 0.0812 and 0.5578 $\text{g L}^{-1} \text{day}^{-1}$. Productivity per area was equal to 47.97 $\text{g m}^{-2} \text{d}^{-1}$, which is the highest value recorded compared to those found in the literature consulted. As for CO_2 biofixation, relevant values for reducing this gas in the atmosphere were obtained, ranging from 128.52 to 882.36 $\text{mg L}^{-1} \text{day}^{-1}$. In terms of organic matter, 16.3-77% of BOD_5 and 12.6-61.6% of COD were reduced. In the reduction of TS, TSS and VSS, values of 71.3-78.5%, 79.5-84.4% and 87.0-88.3%, respectively, were reached. NH_4^+ reduction was 32.5-98.3%, organic nitrogen reduction was 20.3-95.9% and total phosphorus reduction was 33.5-89.9%. The reductions of thermotolerant coliforms were between 71.7% and 99.9%. In view of the results found, it can be considered that the bioremediation of the effluent reached promising efficiencies, with the advantage of producing biomass with potential to obtain bioproducts of relevant economic value.

Keywords: Pollution control; *Spirulina platensis*; CO_2 biofixation; bioresource.

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Introduction

Cattle management and intensive production farms are growing to meet the consumption demand of society, consequently leading to increased generation of cattle wastewater (CWW) (Yu & Kim, 2017). CWW is composed mainly of the water used to wash the floor of the cattle confinement areas and contains feces and urine. If not treated correctly before its disposal in water bodies, CWW can cause severe damage to the environment, such as depletion of dissolved oxygen, increased color, turbidity, eutrophication, and bad odors (Mendonça, Ometto, Otenio, Marques, & Reis, 2018; Silva, Martinez, Pires, Andrade, & Silva, 2012; Souza, Maciel, Otenio, & Mendonça, 2020). According to Mendonça, Ometto, and Otenio (2017), a 1,000-head intensive cattle farming unit has a population equivalent of approximately 41,600 people.

Based on the above, the increase in research related to effluent treatment through new technologies is fundamental to overcome environmental problems. According to Mata, Melo, Simões, and Caetano (2012), the reduction of nutrients from wastewater through microalgae is a promising practice. Through the bioremediation of effluents mediated by microalgae, organic pollutants, nutrients and contaminants can be efficiently reduced, with the advantage of obtaining biomass with high economic value (Santos et al., 2021), contributing to an increasingly greener circular bioeconomy.

Biomass can be used to manufacture important products such as biofuels (biodiesel, bioethanol, bio-oil), biopolymers, biofertilizers, pharmaceuticals, among others. Among the benefits of microalgae cultivation, one can also mention the biological fixation of carbon dioxide (CO_2) (Duarte, Fanka, & Costa, 2020), which helps in air pollution mitigation. The species *Spirulina platensis* is one of the microalgae that most produce oxygen to the planet's atmosphere (Al Hinai, Al Kalbani, Al Rubkhi, Al Kalbani, & Walke, 2019), an important factor for improving air quality.

Microalgae are photosynthetic microorganisms with high CO₂ absorption capacity for transformation into biochemical energy (Ribeiro, Minillo, Silva, & Fonseca, 2019). The fixation of inorganic carbon through photosynthesis is influenced by light intensity, but on the other hand heterotrophic carbon assimilation occurs due to the availability of organic carbon in the culture medium (Andrade & Costa, 2007; Zhang, Zhang, & Chen, 1999). Control of light intensity is fundamental for the cultivation of photosynthetic and also mixotrophic microorganisms, as is the case of microalgae.

Spirulina platensis can assimilate organic compounds as source of energy (mixotrophy). Mixotrophy contributes to the reduction of organic pollutants through biodegradation/bioassimilation (Markou, Chatzipavlidis, & Georgakakis, 2012), causing synergistic effect on cultivation, maximizing biomass production.

When lighting levels are too low (photolimitation) or too high (photoinhibition), there is a reduction in the growth of microorganisms (Andrade & Costa, 2007; Chojnacka & Noworyta, 2004; Grima, Sevilla, Pérez, & Camacho, 1996). For this reason, it is important to verify what is the appropriate illumination for the cultivation of each species of microalgae subject to growth in wastewater such as ACWW.

Bioremediation of wastewater with microalgae is a treatment considered efficient and low cost. These organisms have the characteristic to reduced biochemical oxygen demand (BOD₅), phosphorus, nitrogen, ammonia, sulfate, coliforms and heavy metals from effluents (Mohammadi, Mowla, Esmaeilzadeh, & Ghasemi, 2018). Depending on the methodology adopted, the efficiency in the reduction of pollutants and eutrophic nutrients may be extremely relevant, as verified in the present study.

Another relevant factor is that microalgae grow rapidly (5 to 25 days) with small amounts of water and nutrients compared to terrestrial crops. The amount of water required to produce 1 kg of microalgae biomass is approximately 333 liters, while soybean requires almost 7 times more (2,204 liters) to produce the same amount of green mass (Bhalamurugan, Valerie, & Mark, 2018). The great advantage is that for the cultivation of microalgae clean water can be replaced by wastewater, such as ACWW, making the process even more sustainable, reducing pressure on inputs and raw materials, hence contributing to the conservation and preservation of water resources.

In the present study, the objective was to evaluate the capacity for reduction of organic pollutants, coliforms and nutrients and for CO₂ biofixation of *S. platensis* microalgae cultivated in horizontal photobioreactors (HPBR) operated under two different light intensities. In addition, other objectives were to evaluate the biomass productivity at the end of cultivation in ACWW previously treated by UASB reactor and discuss the main uses of biomass, aiming to contribute to scientific advances in the area.

Material and methods

Microalgae and pre-cultivation

The microalgae used in this study was *Spirulina platensis* (*Arthrospira*), was obtained from the cultivation bank of the Laboratory of Fermentative Processes of the Federal Rural University of Rio de Janeiro (UFRRJ), campus of Seropédica, RJ, Brazil. Pre-cultivation was carried out in Zarrouk medium in 1-L Erlenmeyer flasks, under illumination of 150 μmol m⁻² s⁻² (photoperiod 24h day⁻¹). The pre-cultivation was operated at room temperature 25°C. Agitation was performed using an air compressor (flow rate of 0.5 L min⁻¹) and the biomass concentration obtained during the pre-cultivation stage was 0.88 g L⁻¹ (± 0.15). The biomass produced at this stage was used for inoculation of photobioreactors.

Wastewater used as a culture medium

The cattle wastewater anaerobically digested (ACWW) by upflow anaerobic sludge blanket (UASB) reactor was collected in the “Fazendinha Agroecológica” experimental area of the Federal Rural University of Rio de Janeiro (UFRRJ), campus of Seropédica, RJ, Brazil (coordinates: 22° 45' 21" S; 43° 40' 28" W).

The raw cattle wastewater (CWW) was preliminarily subjected to solids separator (decanter) and primary anaerobic treatment in UASB reactor, operated with hydraulic retention time (HRT) of 7 days. The physical and chemical characterization of the ACWW, which was used as a culture medium, is presented in Table 3, which also contains the post bioremediation parameter data. The analyses were performed according to Standard Methods (American Public Health Association [APHA], 2012) in triplicates.

Experimental design

Two identical horizontal photobioreactors (HPBR) were used on a bench scale with a volume of 7.5 L and a useful area of 0.0872 m². The bottom of each HPBR was equipped with two fine bubble diffusers (20- μ m pore size), connected to an air pump (Aleas, AP-9804 model, China) to promote mixing in the HPBRs. Only air was injected to the HPBRs (0.20 vvm), without additional CO₂ complementation. Both reactors were operated at room temperature 25°C (\pm 2.1), which was measured by a digital thermometer installed in the HPBRs. Illumination was different for each HPBR, being 150 μ mol m⁻² s⁻² in the first reactor (R1) and 270 μ mol m⁻² s⁻¹ in the second reactor (R2) (photoperiod 24h dia⁻¹) (Souza, Valadão, Ribeiro, & Barbosa, & Mendonça, 2021). The lamps used in the experiment is LED, with 100 W and 120 degree angle (Kastello led). Thus, it was possible to compare the growth rate and biomass productivity, in addition to bioremediation and CO₂ biofixation in the cultivation of *S. platensis* under different light intensities. The reactors were separated by a bulkhead (Red Line in Figure 1), so that one lighting does not interfere with the other. Each experiment was reproduced 5 times, for 8 consecutive days (in batch mode). The design layout of the experiment is presented in Figure 1.

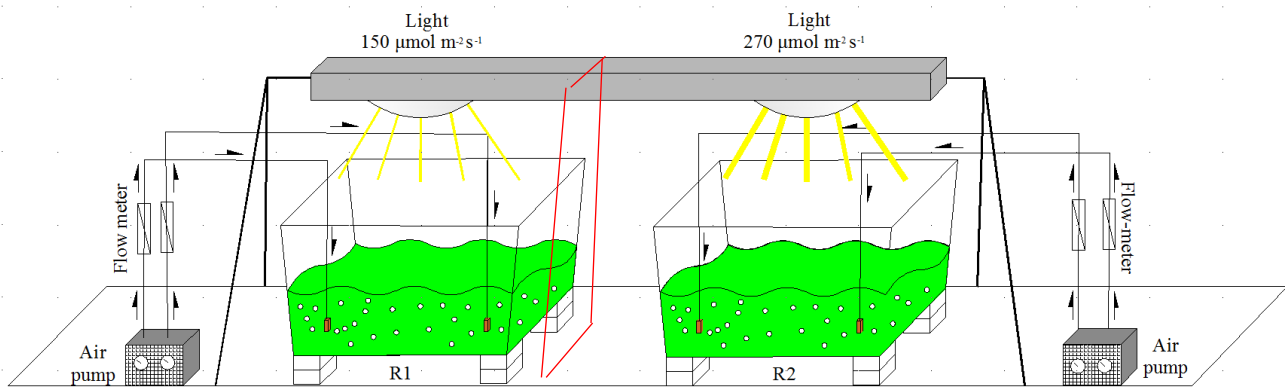


Figure 1. Experimental setup.

Growth parameters and analytical methods

For the growth analysis of *S. platensis* microalgae, the doubling time (Td), the specific maximum growth rate (μ_{max}), dry biomass concentration, volumetric productivity (Pv) and productivity per area (Pa) were calculated.

μ_{max} was calculated by the maximum slope of the curve traced by Neperian logarithms (NL) of the optical density values measured in the exponential growth phase (Log phase).

Doubling time was obtained through Equation 1.

$$Td = \frac{\ln(2)}{\mu_{max}} \quad (1)$$

Volumetric productivity was obtained by Equation 2.

$$Pv = \frac{Xf - Xi}{Tf - Ti} \quad (2)$$

Where, Xf - Xi = Difference between final and initial biomass concentrations (g L⁻¹): and Tf - Ti = Time interval until the end of the process (d).

Biomass production per area (Pa) was calculated using Equation 3.

$$Pa \text{ (g m}^{-2} \text{ d}^{-1}\text{)} = Pv \text{ (g L}^{-1} \text{ d}^{-1}\text{)} \times \frac{\text{Reactor useful volume (L)}}{\text{Reactor surface area (m}^2\text{)}} \quad (3)$$

Biochemical oxygen demand (BOD₅), biochemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS), ammoniacal nitrogen (NH₄⁺), organic nitrogen (ON), total phosphorus (TP), thermotolerant coliforms and pH were determined in triplicates according to Standard Methods (APHA, 2012).

Harvest: biomass separation

The biomass was separated from the treated ACWW by direct filtration (without energy expenditure) in a fine sieve with 0.045-mm mesh (Granutest brand, Brazil).

CO₂ biofixation

CO₂ biofixation (R_{CO_2}) was calculated based on productivity and organic carbon concentration in the biomass ($g\ g^{-1}$), according to Equation 4. Organic carbon concentrations in the biomass were determined by the elemental analysis method (Elementar Vario EL III, German).

$$R_{CO_2} \text{ (mg L}^{-1}\text{d}^{-1}\text{)} = P_V \times C \times \left(\frac{M_{CO_2}}{M_C}\right) \quad (4)$$

P = Biomass productivity ($mg\ L^{-1}\ d^{-1}$); C = Carbon concentration in biomass ($g\ g^{-1}$); M_{CO_2} = CO₂ molar mass ($g\ mol^{-1}$); M_C = Carbon molar mass ($g\ mol^{-1}$).

Ammonia lost by volatilization

The ammonia losses by volatilization to the atmosphere (stripping) were calculated by Equation (5) (Emerson, Russo, Lund, & Thurston, 1975).

$$Vol\ (\%) = \frac{Free\ NH_3}{Total\ NH_4} = \frac{100}{1 + 10^{\left(\left(0.09018 + \frac{2729.92}{T + 273.26}\right) - pH\right)}} \quad (5)$$

Results and discussion

Growth parameters, biomass production and applications

The specific maximum growth rate (μ_{max}) and the minimum doubling time (T_d) in the first reactor (R1) were $0.22\ days^{-1}$ and $4.37\ days$, respectively, while in the second reactor (R2), under the same environmental conditions of cultivation (except for the higher light intensity), the obtained values were $0.35\ day^{-1}$ and $2.08\ days$, respectively (Table 1). As can be observed, due to the higher light intensity ($120\ \mu mol\ m^{-2}\ s^{-1}$ more), the second reactor (R2) had higher μ_{max} and, consequently, lower T_d , compared to the first reactor (R1), thus evidencing better favoring conditions for the growth of *S. platensis* due to increased light supply. Thus, the supply of $270\ \mu mol\ m^{-2}\ s^{-1}$ to the culture prevented self-shadowing and photolimitation problems.

Table 1. Kinetic parameters, volumetric biomass production and CO₂ biofixation.

HPBR	Volumetric biomass productivity ($g\ L^{-1}\ dia^{-1}$)	Maximum specific growth rate μ_{max} (d^{-1})	Doubling time (d)	Carbon in biomass ($g\ g^{-1}$)	CO ₂ biofixation ($mg\ L^{-1}\ d^{-1}$)
R1	0.0812 _(0.002)	0.223 _(0.002)	4.37 _(0.05)	0.38 _(0.1)	128.52 _(5.1)
R2	0.5578 _(0.001)	0.347 _(0.03)	2.08 _(0.02)	0.48 _(0.2)	882.36 _(5.6)

Values in parentheses indicate standard deviation.

Cultivating *Spirulina* sp. in a tubular photobioreactor in the synthetic medium (Zarrouk), under light intensity of $41.6\ \mu mol\ m^{-2}\ s^{-1}$, Duarte et al. (2020) observed a specific maximum growth rate of $0.20 \pm 0.01\ day^{-1}$, a result that agrees with the one found in the first reactor (R1) of the present study, although it is lower than that obtained in the second reactor (R2). In the present study, when *S. platensis* was cultivated in the ACWW, which shows a darker color compared to the synthetic medium, applications of light intensity above the levels found in the cited scientific literature were necessary to achieve an equivalent μ_{max} .

Zhu et al. (2016), growing *Chlorella* sp. in diluted and subsequently filtered livestock waste, illuminated with $240 \pm 10\ \mu mol\ m^{-2}\ s^{-1}$, obtained μ_{max} of $0.375\ day^{-1}$ and T_d of $1.85 (\pm 0.02)\ days$, corresponding to values close to the one obtained in the second reactor (R2) in the present study.

The values of the growth curve of dry biomass produced in the first reactor (R1) were lower than those observed in the second reactor (R2) along the entire experiment (Figure 2). Thus, it was verified that illumination of $270\ \mu mol\ m^{-2}\ s^{-1}$ was favorable to the biomass production of *S. platensis* cultivated in ACWW. On the other hand, illumination of $150\ \mu mol\ m^{-2}\ s^{-1}$ did not provide significant results in terms of dry biomass. The lower light intensity applied was not sufficient for the development of the species, and there was decrease in biomass from the fourth experimental day (Figure 1). The maximum concentration of dry biomass in the first reactor (R1) occurred at 04 days, $2.17\ g\ L^{-1}$, decreasing after this period, which indicates photolimitation, while the maximum concentration of dry biomass in the second reactor (R2) occurred at 7 days of cultivation, $6.52\ g\ L^{-1}$, indicating that the illumination of $270\ \mu mol\ m^{-2}\ s^{-1}$ was sufficient to promote satisfactory development of the culture in ACWW.

The conditions established for obtaining the dry biomass concentration in the second reactor (R2) can be considered promising, indicating that the illumination applied in R2 proved to be an essential factor to meet growth and biomass production, also preventing problems with self-shading and photolimitation during cultivation.

(Hena, Znad, Heong, & Judd, 2017), when cultivating *Arthrospira platensis* in treated dairy farm wastewater, applying $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, recorded a maximum dry biomass production of 5.35 g L^{-1} , an intermediate value between those obtained in the present study, pointing out that for this species higher light intensity is necessary for proportional increments in biomass production when cultivated in cattle wastewater.

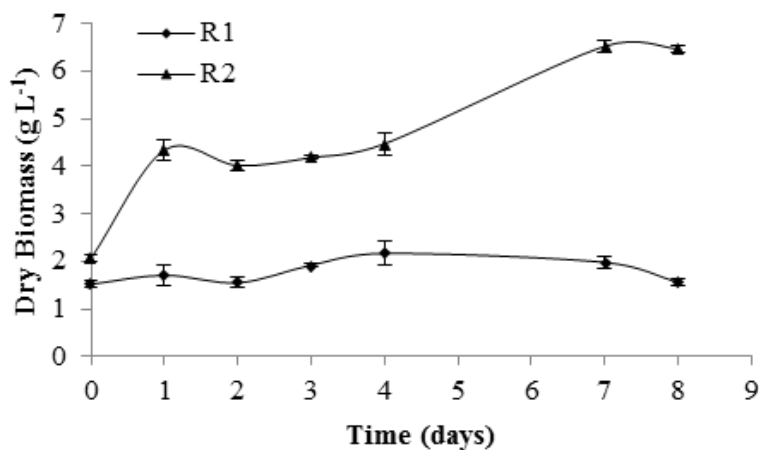


Figure 2. Dry biomass obtained in several moments in experiment.

The present study showed that, in R2, it was possible to obtain considerable volumetric productivities using ACWW as substrate (Table 1). The volumetric productivities recorded in the present study were $0.0812 \text{ g L}^{-1} \text{ day}^{-1}$ in R1 and $0.5578 \text{ g L}^{-1} \text{ day}^{-1}$ in R2 (Table 1), so it is evident that the light intensity in R2 is recommended for cultivation of this species in ACWW using HPBRs.

Qin et al. (2014) used dairy wastewater pretreated with sodium hypochlorite (70 ppm), illuminated with $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, in the cultivation of *Chlorella vulgaris* cultivation, and obtained maximum volumetric productivity of $0.450 \text{ g L}^{-1} \text{ day}^{-1}$, an intermediate result compared to those obtained in the present study.

The conditions established for obtaining productivity per area in the second reactor (R2) can be considered extremely promising, since they led to higher results when compared to those of other studies conducted using different substrates and the same genus/species of microalgae (Table 2). The maximum value found in the present study is approximately 2 times higher than that obtained in the cultivation of the same species of microalgae in Zarrouk medium, a typical culture medium for cultivation of *Spirulina platensis* (*Arthrospira*). This result indicates that ACWW is a potential culture medium, which promotes higher biomass productivity and may replace several other traditional or alternative culture media (Table 2). Another essential factor is that ACWW offers a source of soluble organic carbon that can be assimilated by microalgae via mixotrophy, avoiding complementary expenses with the addition of CO_2 during cultivation, an important fact to boost and encourage the use of this alternative substrate for cultivation in commercial plants (full scale).

This biomass is valuable and can be used for manufacturing various bioproducts. Currently, the most studied bioproduct obtained from biomass is biodiesel (Santos et al., 2021). International studies have shown good results. For example, a system for producing microalgae at approximately 1 g L^{-1} , with about 20% oil content of the biomass for biofuel applications, a total of $\approx 5,000 \text{ L}$ of microalgae culture would need to be processed to generate 1 kg of biofuel (biodiesel or bio-oil) (IEA, 2017). According to dos Santos et al. (2021), if one assumes biomass productivity per area of $20 \text{ g m}^{-2} \text{ d}^{-1}$ ($7,300 \text{ t km}^{-2} \text{ year}^{-1}$), a realistic 20% lipid content in biomass, 60% of saponifiable fraction and a transesterification yield of 98%, a biodiesel output productivity of $858.48 \text{ t km}^{-2} \text{ year}^{-1}$ or $1,031 \text{ m}^3 \text{ km}^{-2} \text{ year}^{-1}$ can be predicted.

Table 2. Biomass productivity per area on different substrates, compared to the present work.

Species	Culture medium	Pa* (g m ⁻² d ⁻¹)	Global region	Reference
<i>Spirulina</i> (<i>Arthrospira</i>)	Zarrouk medium	19.78	South Africa (Musina)	(Grobbelaar, 2009)
<i>Spirulina</i> sp. LEB-18	Mangueira Lagoon Water (MLW) supplemented with Zarrouk (20% MLW + 80% Zarrouk)	21.59	Southern of Brazil (Rio Grande do Sul state)	(Morais et al., 2009)
<i>Spirulina</i> (<i>Arthrospira</i>)	Alkaline lakes in Ordos Plateau region	7.22	China (Inner Mongolia)	(Lu, Xiang, & Wen, 2011)
<i>Spirulina</i> sp. LEB-18	Anaerobic digestion diluted	14.0–14.85	South of Brazil	(Borges et al., 2013)
<i>Arthrospira platensis</i>	Zarrouk medium	22.4	Israel	(Vonshak, Laorawat, Bunnag, & Tanticharoen, 2014)
<i>Arthrospira platensis</i> NIES-39	SOT medium	9.46	Japan (Ishigaki Island)	(Toyoshima, Aikawa, Yamagishi, Kondo, & Kawai, 2015)
<i>Spirulina subsalsa</i>	Seawater enriched with monosodium glutamate residue	5.2	China	(Jiang et al., 2019)
<i>Spirulina platensis</i>	Paoletti synthetic médium	17.7	Northeast of Brazil (Paraíba state)	(Matos, Silva, & Sant'Anna, 2021)
<i>Spirulina Platensis</i>	ACWW	6.99 _(0.2) (R1) 47.97 _(0.01) (R2)	Southeastern of Brazil (Rio de Janeiro)	Current study

*Pa - Biomass productivity by area.

Through the study conducted by the aforementioned authors, it is possible to estimate how much biodiesel could be obtained by the biomass produced in the present study via *S. platensis*. As the productivity per area obtained in R2 was 47.97 g m⁻² d⁻¹, equivalent to 17.509 t km⁻² year⁻¹, knowing that lipid percentage in the *S. platensis* biomass can vary from 4 to 16.6% (Mata, Martins, & Caetano, 2010), it would be possible to obtain from 171.7 to 712.5 t km⁻² year⁻¹ of biodiesel. This would be equivalent to an annual production between 1,703 and 6,925 gallons of biodiesel.

As verified, biodiesel from *S. platensis* biomass can positively contribute to the fuel market. According to recent studies, the price of microalgae biodiesel (including costs of conversion, harvesting, synthetic culture medium and taxes) is approximately USD 2.80 L⁻¹, whereas in the US conventional petroleum-derived diesel is 2.4 times cheaper (USD 1.10 L⁻¹) (Costa et al., 2019). In the present study, using ACWW as culture medium and separating the biomass in a fine mesh of 0.045 mm (direct filtration without energy expenditure), it is estimated that biodiesel production costs can be drastically reduced. As 35% of the production costs come from synthetic culture media (Grima, Belarbi, Ación Fernández, Medina, & Chisti, 2003; Mendonça et al., 2018) and that 20 to 30% more of the production costs come from energy for liquid/biomass separation (Barros, Gonçalves, Simões, & Pires, 2015; Costa et al., 2019), the savings in biomass production is on the order of 55 to 65% (considering ACWW as the culture medium + direct separation in fine mesh). Thus, the cost of biodiesel from *S. platensis* cultivation would be approximately USD 0.98 L⁻¹ to 1.26, making it competitive against petroleum-derived diesel, with the additional benefit of efficiently treating the wastewater tested here.

Another abundant macromolecule in *S. platensis* biomass is carbohydrate, which can be used for bioethanol production through fermentative processes. Carbohydrates from microalgae have additional advantages compared to plant carbohydrates, since they do not contain lignin in their cell composition (Panha et al., 2016). Many studies have recorded that microalgae accumulate a large amount of carbohydrates when grown in wastewater, which can reach 18% for the genus *Spirulina* (Nayak, Karemore, & Sen, 2016). Rempel et al. (2019), cultivating *S. platensis* in residuals recorded a concentration of 46.34% of carbohydrates in dry biomass. These authors recorded energy production via produced bioethanol on the order of 4,664 kJ kg⁻¹, indicating the species as potential for use in the production of this biofuel. According to Costa et al. (2019) and Lam & Lee (2015), the production of bioethanol from microalgae biomass is promising, reaching values from 47,000 to 141,000 L ha⁻¹ year⁻¹, being superior to any other source of raw material for this purpose.

In addition, the energy contained in microalgae biomass can reach values of approximately 35,800 kJ kg⁻¹ for crude oil, 38,100 kJ kg⁻¹ for bio-oil and 39,900 kJ m⁻³ for biogas (Chisti, 2013; de Mendonça et al., 2021; Zewdie & Ali, 2020; Zhou, Schideman, Yu, & Zhang, 2013).

In *S. platensis* biomass, the most abundant macromolecules are proteins (on average 60%), which makes it a potential product for protein supplementation in animal and human feeding (Al Hinai et al., 2019). However, it is important to analyze if the biomass produced from the cultivation of microalgae in effluents can be evil for human and animal health. According to Leech (2018), each 7 grams of dry biomass of *S. platensis* can contain up to 4 grams of protein, 11, 15 and 4% of vitamins B1 (Thiamin), B2 (Riboflavin) and B3 (Niacin), respectively. Other compounds, molecules and chemical elements, such as eight essential amino acids and more than ten non-essential ones, gamma-linolenic acid (GLA), beta-carotene, linoleic acid, arachidonic acid, vitamin B12, iron, calcium, phosphorus, nucleic acids RNA and DNA, chlorophyll and phycocyanin are found in the biomass of this microalga strain (Al Hinai et al., 2019). The market price for selling *Spirulina* (dry biomass as a source of protein / minerals) was € 24 / kg in 2014, growing at a compound annual growth rate of 10% (Garcia, Vicente, & Galán, 2017). As can be seen, the *S. Platensis* biomass has relevant commercial value. The supply of ACWW could supply the demand for nutrients in cultivation. However, the intention of the authors is only to alert researchers and readers about the potential for commercialization of biomass, since studies aimed at human consumption of microalgae biomass need to be studied.

This biomass has valuable potential not only for the production of renewable energy and environmentally friendly bioproducts, but can also be used to fight diseases. Due to the presence of various bioactive compounds in *Spirulina platensis* biomass, its use for medicinal purposes has been increasingly studied for combating and preventing infectious diseases caused by viruses and bacteria such as polio, Zika virus, malaria, Ebola virus (Tang et al., 2020) and also Influenza and COVID-19 (McCarty & DiNicolantonio, 2020).

CO₂ biofixation rate

Carbon (C) concentrations in the biomass of 0.38 (±0.1) and 0.48 (±0.2) g g⁻¹ were recorded in R1 and R2, respectively (Table 1). Since the average C concentration detected in microalgae biomass is 0.50 g g⁻¹ (Duarte et al., 2020), the concentration of this element in R2 was close to that recorded in the literature. This indicates that both the greater illumination and the volume of air per volume of culture per minute (0.20 vvm) were sufficient to maximize C accumulation in the biomass produced in R2, although it is believed that the assimilation of soluble organic C via mixotrophy also helped in the accumulation of this element in the cells.

The CO₂ biofixation of *S. platensis* microalgae reached interesting values in relation to carbon sequestration from the atmosphere. In the first reactor (R1), biofixation was 128.52 mg L⁻¹ day⁻¹ and in the second reactor (R2), with increase in light intensity, the biofixation recorded was ≈ 7 times higher (882.36 mg L⁻¹ day⁻¹), as can be seen in Table 1. In general, the species *S. platensis* can be considered efficient for CO₂ capture, assisting the reduction of this gas from the atmosphere.

In order to obtain a high CO₂ biofixation, it is necessary to observe several factors such as the CO₂ concentration applied, biomass productivity, CO₂ mass transfer, as well as the type of photobioreactor used (Duarte et al., 2020). Therefore, the high biofixation rate found in this study is related to the good performance of biomass productivity achieved by *S. platensis* and also to the operational conditions adopted mainly in the second reactor (R2).

Cultivating *Spirulina* sp. in tubular photobioreactors in the modified Zarrouk medium adding thermoelectric fly ashes, Braga, Moreira, Costa, and Morais (2019) obtained a maximum value of 700 mg L⁻¹ day⁻¹ for CO₂ biofixation. This value achieved by the authors, however, represents an intermediate performance when compared to the results obtained under the two conditions established in the present study.

Cultivating *Spirulina platensis* in wastewater from a family septic tank, illuminated at 180 μmol m⁻² s⁻¹, Almomani et al. (2019) recorded CO₂ biofixation of 378 mg L⁻¹ day⁻¹, which is 3 times higher than that obtained in R1. On the other hand, when comparing the data obtained by the above-mentioned authors with those obtained in R2 in the present study, the biofixation rate was 2 times higher. With this result, it is evident that light intensity interferes in CO₂ biofixation for *S. platensis* when cultivated in ACWW.

ACWW bioremediation

The mean pH values during the last 5 days of cultivation in R1 and R2 were 8.3 and 9.7, respectively (Table 3). The growth process itself altered the pH of the culture medium, keeping it basic, a favorable condition for

the growth of *S. platensis*, which can survive in environments with pH up to 11. The ideal pH for cultivation of this microalgae species is between 9.5 and 9.8 (Sony, Sudhakar, & Rana, 2017), and the pH observed in R2 was within the range considered as ideal for its development. Maintaining the pH within the ideal range also prevents contamination of the culture medium by other species of microalgae and heterotrophic bacteria.

The reduction efficiency of organic pollutants, nutrients and thermotolerant coliforms in R2 were higher than those in R1 (Table 3). The increase in light intensity from 150 to 270 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was fundamental to obtain not only the increase in biomass productivity, but also higher reduction of BOD₅, COD and nutrients.

COD and BOD₅ reduction occurs mainly through the biological assimilation that occurs via mixotrophy (Cheng et al., 2019). As light directly interferes with photosynthesis, the reason for the increase of almost 50% in the reduction of organic pollutants when R1 is compared with R2 is evident. The species *S. platensis* is recognized as mixotrophic (Zhai et al., 2017), being able to assimilate both inorganic carbon (CO₂) and soluble organic carbon contained in ACWW. The mixotrophic mechanism creates an additive and synergistic effect during cultivation, promoting increased biomass productivity, leading at the same time to enhancement of wastewater bioremediation, since it combines the photosynthetic (autotrophic) process with heterotrophy (Bhatnagar, Chinnasamy, Singh, & Das, 2011).

Markou et al. (2012) cultivated *S. platensis* in olive oil mill wastewater treated with sodium hypochlorite and reached COD reduction of 73.18% at 16 days of experiment. In the present study, COD reduction of 61.6% was reached at 8 days of cultivation, half the time used by the aforementioned authors.

After treatment in UASB reactor, the BOD₅ reached the value of 892 mg L⁻¹ and, when the ACWW was subjected to treatment with microalgae, it reached the value of 744.5 mg L⁻¹ (R1 reactor), so there was a reduction of only 145.5 mg L⁻¹ in BOD₅.

In the second reactor (R2), the average reduction in BOD₅ was 685 mg L⁻¹, reaching the final value of 205 mg L⁻¹, with a reduction efficiency of 77% (Table 3). When subjected to higher irradiances, the species *S. platensis* increases its biomass considerably, which aided the mechanisms of BOD₅ reduction from the ACWW.

In terms of reduction of TS, TSS and VSS, the highest reductions were obtained in the second reactor (R2), equal to 78.5%, 84.4% and 88.3%, respectively. However, no disparity of reduction was identified between the two reactors for the reduction of solids (Table 2). This indicates that the method of separation by filtering in fine mesh sieve (20- μm) proved to be efficient for removing both solids and the biomass produced.

NH₄⁺ reduction efficiency reached 98.3% in the second reactor (R2), leaving only 6.16 mg L⁻¹ of ammonia. However, in the first reactor (R1) the efficiency can be considered low, reaching only 32.5%. Again, it is evident that the increase in illumination in the second reactor (R2) was favorable to bioremediation, since the microalgae assimilated this nutrient for their development, under this experimental condition, more efficiently.

Table 3. ACWW bioremediation by *S. platensis* after 8 days of cultivation.

Parameters	ACWW	R1	Removal efficiency (%)	R2	Removal efficiency (%)
pH	7.2 _(0.2)	8.3 _(0.5)	---	9.7 _(0.1)	---
BOD ₅ (mg L ⁻¹)	892 ₍₂₎	744.5 _(7.8)	16.3	205 _(21.2)	77
COD (mg L ⁻¹)	1399 ₍₁₄₎	1224 _(36.8)	12.6	537.5 _(40.3)	61.6
TS (mg L ⁻¹)	651 ₍₁₆₎	186.5 _(89.8)	71.3	140 _(14.1)	78.5
TSS (mg L ⁻¹)	287.5 _(6.2)	59 _(5.7)	79.5	45 _(21.2)	84.4
VSS (mg L ⁻¹)	162.2 _(7.9)	21 _(1.4)	87	19 ₍₀₎	88.3
NH ₄ ⁺ (mg L ⁻¹)	367 _(1.2)	247 ₍₁₇₎	32.5	6.16 _(1.6)	98.3
ON (mg L ⁻¹)	192 _(1.7)	153 _(45.3)	20.3	7.8 _(1.1)	95.9
TP (mg L ⁻¹)	80.5 _(0.5)	52.5 _(0.2)	33.5	8 _(0.2)	90
Thermotolerant Coli. (MPN 100 mL ⁻¹)	3x10 ⁵ ₍₁₀₀₎	8.5x10 ⁴ ₍₄₀₎	71.7	4x10 ² ₍₂₀₎	99.9*

COD (chemical oxygen demand); BOD₅ (biochemical oxygen demand); TSS (total suspended solids); TS (total solids); VSS (volatile suspended solids); NH₄⁺ (ammoniacal nitrogen); TP (total phosphorus); ON (organic nitrogen). Values in parentheses indicate standard deviation. *4 log units.

Cultivating *Chroococcus* sp. in waste from dairy cattle farm, Prajapati, Choudhary, Malik, and Vijay (2014) recorded NH₄⁺ reduction efficiency of 98%, with 16 days of cultivation. In comparison, in the present study, the reduction of NH₄⁺ was higher with 8 days of cultivation, that is, half the time reported by the aforementioned authors. During cultivation, ammoniacal nitrogen is assimilated by microalgae, being converted to organic nitrogen (ON) present in its biomass. When the biomass is separated from the treated wastewater, then ON is reduced in the filtration process, and ON reduction efficiencies of 95.9% in R2 and only 20.3% in R1 were recorded in the present study. The lower values of ON reduction in R1 reinforce that there was low conversion of nitrogen compounds into biomass due to the lower light supply to this reactor.

Another way to remove NH_4^+ from the verified wastewater was through volatilization. NH_4^+ losses of 11.6% in R1 and 37.1% in R2 were calculated (Equation 5). Molinuevo-Salces, Mahdy, Ballesteros, and González-Fernández (2016), studying the treatment of piggery wastewater in photobioreactors reported ammoniacal nitrogen losses between 17 and 29% and the pH reached values of 7.8-8.8, values that corroborate with the present research.

Regarding the reduction of total phosphorus, the maximum value recorded was 89.9% (R2), leaving only 8 mg L^{-1} in the treated ACWW. Phosphorus reduction from wastewater is extremely important to avoid eutrophication of water resources, especially when wastewater is disposed of into lentic environments such as lakes and reservoirs.

Cultivating *Scenedesmus obliquus* in ACWW (from UASB-AF reactor), illuminated with $58 \mu\text{mol m}^{-2} \text{s}^{-1}$, de Mendonça et al. (2018) recorded phosphorus reductions between 69 and 77.5% with 12 days of cultivation. The values obtained were higher than those found in R1 of this study, despite lower illumination used by the authors. On the other hand, in R2, phosphorus reduction values were higher than those recorded by the aforementioned authors, reaching 90%.

Reductions of thermotolerant coliforms greater than 70% were recorded in both HPBRs, reaching 99.9% (R2) (Table 3). The elimination of bacteria of the coliform group is associated with the fact that several metabolites with bactericidal effect are excreted from these microalgae (Kümmerer, 2008), which was also reported de Mendonça et al. (2018) and Gupta, Lee, and Choi (2015). Finally, this bioremediation process mediated by the microalga *S. platensis* in HPBRs can be considered promising as post-treatment of ACWW from UASB reactors, with the advantage of biomass production that has relevant potential for the manufacture of various bioproducts, especially biofuels.

Conclusion

The *S. platensis* demonstrated expressive dry biomass production, reaching a maximum value of 6.52 g L^{-1} . Regarding volumetric productivity, the maximum value obtained was $0.5578 \text{ g L}^{-1} \text{ day}^{-1}$, with productivity per area of $47.97 \text{ g m}^{-2} \text{ d}^{-1}$. This technique can be considered efficient for reducing this CO_2 from atmosphere. The cultivation of *S. platensis* in ACWW showed results of great relevance in the reduction of pollutants, indicating that this technology can be successfully applied in the near future. In addition, the biomass produced can be used to manufacture several bioproducts with high economic value.

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