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Spirulina platensis in livestock wastewater bioremediation: pollution control by obtaining macromolecules

Spirulina platensis na biorremediação de águas residuais da pecuária: controle da poluição por meio da obtenção de macromoléculas

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ABSTRACT: This study evaluated the cultivation of the microalga Spirulina platensis DRH 20 in horizontal batch photobioreactors for 8 days under two light intensities (150 and 300 µmol m^{-2} s^{-1}). Higher irradiation resulted in better cultivation performance, with a specific growth rate of 0.35 day^{-1} and a doubling time of 2.1 days. Dry biomass ranged from 2.2 to 6.5 g L^{-1}, with productivity ranging from 0.08 to 0.56 g L^{-1} day^{-1} and productivity per area ranging from 50 g m^{-2} day^{-1}. CO_2 biofixation ranged from 128 to 882 mg L^{-1} day^{-1}, demonstrating the potential of microalgae for emissions mitigation. Regarding effluent treatment, removals of 16.3 to 77% of BOD_5 and 12.6 to 61.6% of COD were obtained; for ST, SST, and SSV, removals were 71 to 80%, 79 to 84%, and 87 to 88%, respectively. Nutrient removal was also significant, with 33 to 98% of NH_4^+, 20 to 96% of organic nitrogen and 35 to 90% of Pt. Thus, the cultivation of S. platensis proved to be efficient in the bioremediation of effluent, allowing simultaneous production of biomass with economic potential for the synthesis of macromolecules of industrial interest.

Keywords: Bioremediation; Bioresource; Carbon assimilation; Photosynthesis.

RESUMO: Esta pesquisa avaliou o cultivo da microalga *Spirulina platensis* DRH 20 em fotobiorreatores horizontais operados em batelada por 8 dias sob duas intensidades luminosas (150 e 300 µmol m^{-2} s^{-1}). A maior irradiação resultou em melhor desempenho do cultivo, com taxa de crescimento específico de 0,35 dia^{-1} e tempo de duplicação de 2,1 dias. A biomassa seca variou de 2,2 a 6,5 g L^{-1}, com produtividade de 0,08 a 0,56 g L^{-1} dia^{-1} e produtividade por área de 50 g m^{-2} dia^{-1}. A biofixação de CO_2 apresentou valores entre 128 e 882 mg L^{-1} dia^{-1}, demonstrando o potencial da microalga para mitigação de emissões. Em relação ao tratamento do efluente, foram obtidas remoções de 16,3 a 77% de DBO_5 e de 12,6 a 61,6% de DQO; para ST, SST e SSV, as remoções foram de 71 a 80%, 79 a 84% e 87 a 88%, respectivamente. A remoção de nutrientes também foi expressiva, com 33 a 98% de NH_4^+, 20 a 96% de nitrogênio orgânico e 35 a 90% de Pt. Dessa forma, o cultivo de *S. platensis* demonstrou ser eficiente na biorremediação do efluente, permitindo simultânea produção de biomassa com potencial econômico para síntese de macromoléculas de interesse industrial.

Palavras-chave: Assimilação de carbono; Biorremediação; Fotossíntese; Recursos biológicos.

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1 INTRODUCTION

The farms for intensive management and production of cattle farming are growing to meet society's consumption demands, consequently leading to an increase in cattle wastewater (CWW) generation (Souza *et al.* 2023). CWW mainly consists of wash water from cattle confinement areas, containing feces and urine. If CWW is not properly treated before being discharged into water bodies, it can cause severe environmental damage, such as depletion of dissolved oxygen, increased color, turbidity, eutrophication, and unpleasant odors (Yu and Kim 2017; Mendonça *et al.* 2018; Souza *et al.* 2020). According to (Mendonça *et al.* 2017), an intensive cattle farming unit with 1000 head has a population equivalent of approximately 41,600 people.

Given the above, the increase in research related to effluent treatment through new technologies is crucial to address environmental issues. According to (Mata *et al.* 2012), the removal of nutrients from wastewater through microalgae is a promising practice. Through effluent bioremediation mediated by microalgae, organic pollutants, nutrients, and contaminants can be efficiently removed, with the advantage of obtaining biomass with high economic value (Santos *et al.* 2021).

The biomass can be used to produce important products such as biofuels (biodiesel, bioethanol, bio-oil), biopolymers, biofertilizers, pharmaceuticals, among others. Among the benefits of microalgae cultivation, the biological fixation of carbon dioxide (CO_2) (Duarte *et al.* 2020) can also be mentioned, aiding in the mitigation of air pollution. The species *Spirulina platensis* is one of the microalgae that produces more oxygen for the planet's atmosphere (Al Hinai *et al.* 2019), an important factor for improving air quality.

Microalgae are photosynthetic microorganisms with a high capacity for CO_2 absorption for transformation into biochemical energy (Ribeiro *et al.* 2019). Inorganic carbon fixation through photosynthesis is influenced by light intensity, while heterotrophic carbon assimilation occurs due to the availability of organic carbon in the culture medium (Andrade and Costa 2007). Control of light intensity is essential for the cultivation of photosynthetic microorganisms as well as mixotrophic organisms, such as microalgae. *Spirulina platensis* can assimilate organic compounds as a source of energy (mixotrophy). Mixotrophy contributes to the removal of organic pollutants through biodegradation/bioassimilation (Markou *et al.* 2012), causing a synergistic effect in cultivation, maximizing biomass production.

When light levels are too low (photo-limitation) or too high (photo-inhibition), the growth of microorganisms decreases (Molina Grima *et al.* 1996; Chojnacka and Noworyta 2004; Andrade and Costa 2007). For this reason, it is important to determine the appropriate light conditions for cultivating each species of microalgae subjected to growth in wastewater, such as CWW.

The bioremediation of wastewater using microalgae is considered an efficient and cost-effective treatment. These organisms have the ability to remove biochemical oxygen demand (BOD5), phosphorus, nitrogen, ammonia, sulfate, coliforms, and heavy metals from effluents (Mohammadi *et al.* 2018; Aragaw and Asmare, 2018). Depending on the adopted methodology, the efficiency of pollutant and eutrophying nutrient removal can be extremely relevant (Dagnaisser *et al.* 2022).

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 needed to produce 1 kg of microalgae biomass is approximately 333 liters, compared to soybeans, which require nearly 7 times more (2,205 liters) to produce the same amount of green mass (Bhalamurugan *et al.* 2018). The significant advantage is that for microalgae cultivation, clean water can be replaced with wastewater, such as CWW, making the process even more sustainable, reducing pressure on inputs and raw materials, and contributing to the conservation and preservation of water resources.

In this study, the objectives were to assess the capacity for removal of organic pollutants, coliforms, nutrients, and CO_2 biofixation through the cultivation of the microalga *S. platensis* in horizontal photobioreactors (HPBRs) operated under two different light intensities. Another objective was to evaluate the biomass productivity at the end of cultivation in CWW that had been previously treated by a UASB reactor and to discuss the primary uses of the biomass, aiming to contribute to scientific advancements in the field.

2 MATERIALS AND METHODS

2.1 PRE-CULTIVATION

The microalga used in this research was *Spirulina platensis* DRH20, extracted from the cultivation bank of the Bioenergy and Environmental Technologies Laboratory at the Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica campus, RJ, Brazil. The pre-cultivation was carried out in Zarrouk synthetic medium (Zarrouk, 1966) in 1 L Erlenmeyer flasks, under illumination (100 µmol m^{-2} s^{-1}). Agitation was achieved using an air compressor (flow rate of 0.5 L min^{-1}). The biomass produced in this stage was used for inoculation of the photobioreactors.

2.2 WASTEWATER USED AS A GROWING MEDIUM

The anaerobically digested wastewater from bovine farming, treated by a UASB reactor (CWW), was collected from the experimental area of the Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, Brazil (coordinates: 22º 45' 21" S; 43º 40' 28" W). The raw bovine farming wastewater (CWW) underwent preliminary treatment, including solid separation (settling) and primary anaerobic treatment in a UASB reactor operated with a hydraulic retention time (HRT) of 10 days. The physicochemical characterization of the treated wastewater (CWW), which was used as the cultivation medium (CWW after UASB), is presented in Table 4. Each experiment with reactor pairs was repeated 10 times, and all analyses were quantified in triplicates, following the Standard Methods (APHA 2012).

2.3 EXPERIMENTATION

Two identical bench-scale horizontal photobioreactors (HPBRs) with a volume of 8 L and a useful area of 0.087 m² were used. Two fine bubble diffusers (20-µm) were placed at the bottom of each HPBR, connected to an air pump (Aleas, model AP-9804, China), to promote mixing within the HPBRs. Only air was injected into the HPBRs (0.20 vvm), with no additional CO_2 supplementation. Both reactors were operated at room temperature, 26°C (±4°C), which was measured using a digital thermometer. The illumination was different for each HPBR. In the first reactor (HPBR1), it was set at 150 µmol m^{-2} s^{-1}, and in the second reactor (HPBR2), it was set at 300 µmol m^{-2} s^{-1}. The reactors were separated by an opaque partition to prevent any influence from cross-illumination or external environment. This setup allowed for the comparison of growth rate, biomass productivity, as well as bioremediation and CO_2 biofixation in the cultivation of *S. platensis* under different light intensities. Each experiment was replicated four times under the conditions to collect the data.

The electrical energy used for continuous illumination in the experiment was obtained from a solar energy panel (2 meters by 1 meter), connected to a frequency inverter and a battery. After the battery, the power wiring directly reached the lighting lamps of both HPBRs (Figure 1).

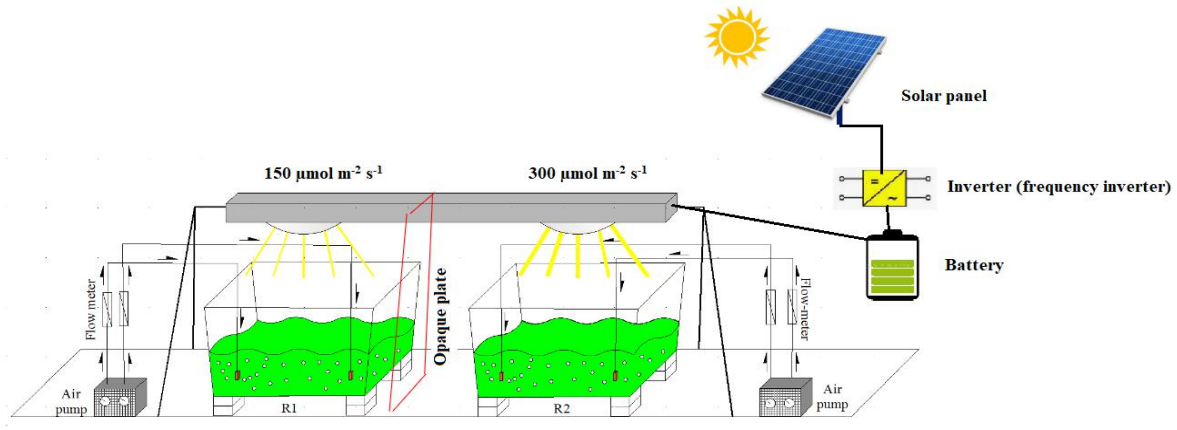

Figure 1. Photobioreactors with different illuminations (R1 150 µmol m^{-2} s^{-2} and R2 300 µmol m^{-2} s^{-2}) generated from solar panel

The experiment's synthesis can be observed in the flowchart presented in Figure 2. Each experiment was repeated 10 times, and all analyses were performed in triplicate.

2.4 GROWTH AND PRODUCTIVITY PARAMETERS AND CO_2 BIOFIXATION

For the analysis of *S. platensis* microalgae growth, the following parameters were calculated: doubling time (Dt), maximum specific growth rate (µmax), concentration of dry biomass, volumetric productivity (Pv), area-based productivity (Pa) and CO_2 fixation, were obtained according to recommendations by de Souza *et al.* (2023).

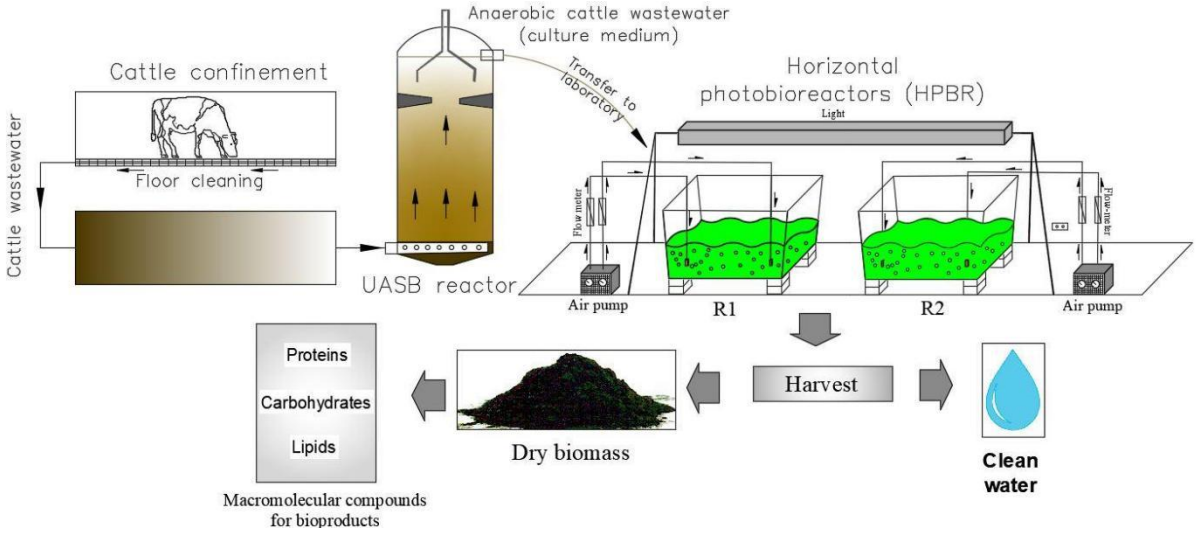

Figure 2. General synthesis of the experiment from the production of wastewater in the stable to the biomass production with subsequent generation of industrial interest macromolecules such as lipids, carbohydrates, and proteins

2.5 BIOREMEDIATION CONTROL PARAMETERS

Biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS), ammonia nitrogen (NH_4^+), organic nitrogen (Norg), total phosphorus (TP), thermal-tolerant coliform, and pH were determined in triplicates according to the Standard Methods (APHA, 2012). The separation of biomass from the treated CWW was carried out through direct filtration (without energy consumption) using a fine mesh sieve with a mesh size of 0.045 mm (Granutest brand, Brazil).

2.6 DETERMINATION OF MACROMOLECULES: TOTAL LIPIDS, CARBOHYDRATES, AND PROTEINS

Total lipids were quantified by extraction in a Soxhlet extractor with hexane solvents (130 mL) in round-bottom distillation flasks, with solubilization for 4 hours, using the same cartridge containing the biomass. After each extraction, the solvent was evaporated in a rotary evaporator (Buchi Waterbath B-480, Germany) with a thermostatically controlled bath at 50°C. The pressures used were 500 mbar for hexane. Lipid samples were taken in the 2nd, 3rd, 5th, and 7th experimental weeks, considering the maximum and minimum peaks of produced biomass. The carbohydrate analyses were conducted following the methodology of (Dubois et al. 1956). The methodology involves a colorimetric method, preceded by acid hydrolysis. After this process, 2 mL of the sample, 0.05 mL of 80% phenol solution, and 5 mL of concentrated sulfuric acid are added to a tube. Once the solution has cooled to room temperature, it is measured at 490 nm. Proteins were quantified using the Kjeldahl method (APHA, 2012).

2.7 DETERMINATION OF FATTY ACIDS

For sample preparation, 100 mg aliquots were used, individually saponified in 2.0 mL of methanolic sodium hydroxide solution (0.5 mol L^{-1}), which was placed in a thermal bath with stirring at a temperature of 77.5 °C (± 3.5°C) for 25 minutes. The resulting solution was transferred to a 5.0 mL test tube and completed with methanol. The samples were analysed by capillary electrophoresis (CE) according to the work by (Lomeu et al. 2023). Before EC injection, each sample was diluted in methanol 1:1 (v/v), in triplicates.

3 RESULTS AND DISCUSSION

3.1 GROWTH PARAMETERS, BIOMASS PRODUCTION AND APPLICATIONS

The maximum specific growth rate (μmax) and minimum doubling time (Dt) in the first reactor (HPBR1) were 0.20 day^{-1} and 4.4 days, respectively. In the second reactor (HPBR2), under the same environmental cultivation conditions (except for the higher applied illumination), values of 0.39 day^{-1} for μmax and 2 days for Dt were obtained (Table 1). As observed, due to the higher light intensity (an additional 120 μmol m^{-2} s^{-1}), the second reactor (HPBR2) exhibited a higher μmax and consequently a lower Dt compared to the first reactor (HPBR1). This indicates that increased light supply favored the growth of *S. platensis*, avoiding issues of self-shading and photoinhibition by providing 300 μmol m^{-2} s^{-1} to the cultivation.

Cultivating *Spirulina* sp. in a tubular photobioreactor using synthetic medium (Zarrouk) under a light intensity of 41.6 μmol m^{-2} s^{-1}, (Duarte *et al.* 2020) observed a maximum specific growth rate of 0.20 ± 0.01 day^{-1}, which is consistent with the result obtained in the first reactor (HPBR1) of this study, although it is lower than the result obtained in the second reactor (HPBR2). In the present research, when cultivating *S. platensis* in CWW, which has a darker coloration compared to synthetic medium, higher light intensity applications were required than those reported in the cited scientific literature to achieve an equivalent μmax.

The authors (Zhu *et al.* 2017), cultivating *Chlorella* sp. in diluted and filtered corral water, illuminated with 240 ± 5 μmol m^{-2} s^{-1}, achieved a μmax of 0.38 day^{-1} and a Dt of 1.9 (±0.02) days, which corresponds to values similar to those achieved in the second reactor (HPBR2) in the present study.

The dry biomass growth curve produced in the first reactor (HPBR1) obtained lower values than those observed in the second reactor (HPBR2) throughout the experiment (Figure 3). Thus, it was observed that an illumination of 300 μmol m^{-2} s^{-1} favored the production of S. platensis biomass cultivated in CWW. On the other hand, the illumination of 150 μmol m^{-2} s^{-1} did not yield significant results in terms of dry biomass. The lower applied light intensity was not sufficient for the species' development, leading to a decline in biomass from the fourth experimental day onwards (Figure 2). The maximum concentration of dry biomass in the first reactor (HPBR1) occurred on the 4th day, reaching 2.17 g L^{-1}, and decreased thereafter, indicating photo-

inhibition. In contrast, the maximum concentration of dry biomass in the second reactor (HPBR2) was achieved after 7 days of cultivation, reaching 6.5 g L-1, indicating that an illumination of 300 µmol m^{-2} s^{-1} was sufficient to promote satisfactory culture development in CWW.

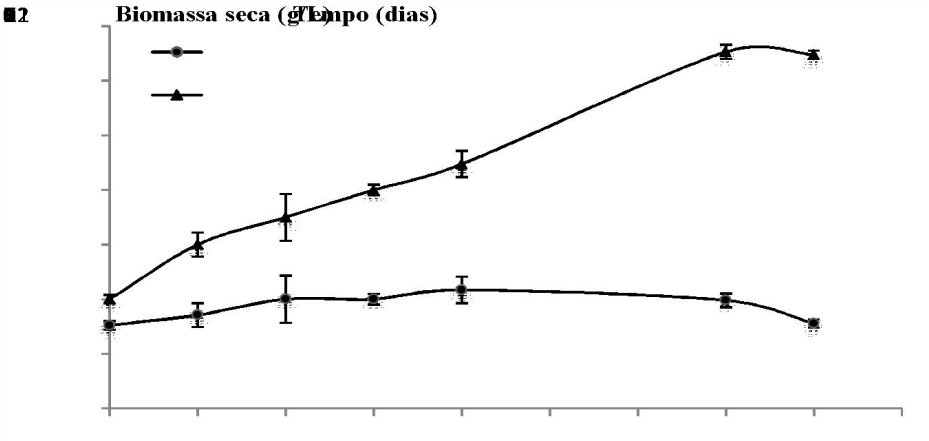

Figure 3. Biomass Growth Curve: R1 150 µmol m^{-2} s^{-2} and R2 300 µmol m^{-2} s^{-2}.

The conditions established in obtaining the concentration of dry mass in the second reactor (HPBR2) can be considered promising, indicating that the illumination applied in HPBR2 proved to be an essential factor in supporting biomass growth and production, thereby addressing issues related to self-shading and photoinhibition during cultivation.

Hena *et al.* (2018), when cultivating *Arthrospira platensis* in treated cattle wastewater, applying 300 µmol m^{-2} s^{-1}, recorded a maximum dry biomass production of 5.35 g L^{-1}, an intermediate value compared to the results achieved in this study. This suggests that for cultivations of this species, higher light intensities are required to achieve proportional increases in biomass production when cultivated in wastewater derived from cattle farming.

The present study demonstrated that in HPBR2, significant volumetric productions could be achieved using CWW as a substrate (Table 1). The volumetric productivities recorded in this research were 0.080 g L^{-1} day^{-1} in HPBR1 and 0.56 g L^{-1} day^{-1} in HPBR2 (Table 1), clearly indicating that the light intensity in HPBR2 is suitable for cultivating the species in CWW using HPBRs.

Qin *et al.* (2014) used pre-treated CWW with sodium hypochlorite (70 ppm), illuminated with 300 µmol m^{-2} s^{-1}, in the cultivation of *Chlorella vulgaris*, obtaining a maximum volumetric productivity of 0.45 g L^{-1} day^{-1}, which is an intermediate result compared to the findings of the present study.

Table 1. Kinetic Parameters, Volumetric Biomass Production (VBP), and CO_2 Biofixation (RCO2) by Microalga *S. Platensis*.

HPBR	PV (g L^{-1} dia^{-1})	$\mu_{máx}$ (dia^{-1})	Dt (dias)	Carbon (g g^{-1})	RCO_2 (mg L^{-1} dia^{-1})
1	$0.08_{(0.2)}$	$0.22_{(0.02)}$	$4.3_{(0.3)}$	$0.38_{(0.1)}$	$130_{(3)}$
2	$0.55_{(0.01)}$	$0.34_{(0.3)}$	$2.1_{(0.2)}$	$0.48_{(0.2)}$	$882_{(2)}$

Values in parentheses indicate standard deviation.

The conditions established for obtaining productivity per in the second reactor (HPBR2) can be considered extremely promising, as they yielded higher results compared to other studies using different substrates and the same genus/species of microalgae (Table 2). The maximum value found in this study is approximately 2 times higher than the cultivation of the same species of microalgae in Zarrouk medium, a typical medium for cultivating *Spirulina platensis (Arthrospira)*. This indicates that CWW is a potential cultivation medium that results in higher biomass production, and it could potentially replace several other traditional or alternative cultivation media (Table 2). Another essential factor is that CWW provides a source of soluble organic carbon that can be assimilated by the microalgae through mixotrophy, eliminating the need for additional CO_2 supplementation during cultivation. This is an important aspect that can promote and encourage the use of this alternative substrate for cultivation in commercial-scale plants (full scale).

Table 2. Biomass of *Spirulina platensis* cultivated on different substrates and locations worldwide: Highlighting the HPBR2's area productivity, which has the highest value recorded in the consulted literature

Species	Medium	Pa* ($g\ m^{-2}\ d^{-1}$)	Region	Reference
Spirulina (Arthrospira)	Zarrouk medium	19.8	Africa	(Grobbelaar, 2009)
Spirulina (Arthrospira)	Lake water	7.2	China	(Lu et al., 2011)
Spirulina (Arthrospira platensis)	Zarrouk medium	22.4	Israel	(Vonshak et al., 2014)
Spirulina (Arthrospira platensis NIES-39)	SOT medium	9.5	Japan	(Toyoshima et al., 2015)
Spirulina platensis	synthetic Paoletti	17.7	Brazil (Paraíba)	(Matos et al., 2021)
Spirulina Platensis DRH 20	CWW	$6.9_{(0,1)}$ (HPBR1) $50_{(0,.3)}$ (HPBR2)	Brazil (Rio de Janeiro)	Present study

*Pa - Biomass production per area.

This biomass is valuable and can be used to produce various bioproducts. Currently, the most studied bioproduct obtained from this biomass is biodiesel (dos Santos *et al.* 2021). International studies have shown promising results. For instance, a microalgae production system at approximately 1 g L^{-1}, with around 20% lipid content in the biomass for biofuel applications, would require processing of approximately 5,000 L of microalgae culture to generate 1 kg of biodiesel or bio-oil (IEA 2017). According to dos Santos et al. (2021), assuming a biomass productivity per area of 20 g m^{-2} d^{-1} (7,300 t km^{-2} $year^{-1}$), with 20% lipid content, 60% saponifiable fraction, and 98% transesterification yield, it is possible to produce 858.48 t km^{-2} $year^{-1}$ or 1,031 m^3 km^{-2} $year^{-1}$ of biodiesel.

The projections are consistent with the data obtained in this research, as the concentrations of total lipids obtained from the biomass cultivated in HPBR2 were 20.3%. In contrast, the lipid concentration obtained in the biomass of HPBR1 was only 6%. The higher lipid production in HPBR2 was attributed to two factors: 1) higher light

intensity increases carbohydrate synthesis and consequently lipid synthesis; 2) the longer illumination time promoted the growth of other opportunistic microalgae that have a greater capacity for lipid accumulation. In this case, the following species were identified: *Chlorella* sp, *Nannochloropsis* sp, *Scenedesmus* sp. In contrast, no invasive species were identified in the less illuminated reactor.

Considering the achieved biomass productivity in HPBR2 of 48 g m^{-2} d^{-1}, equivalent to 17,509 t km^{-2} $year^{-1}$, and considering that the lipid content in biomass can vary between 6 to 20.3% (Mata *et al*. 2010), biodiesel production could range from 190 to 800 t km^{-2} $year^{-1}$. This would correspond to an annual production of between 2,000 and 7,100 gallons of biodiesel.

As for the quality of the biodiesel produced, we can show that lighting significantly affects the characteristics of fatty acids (Table 3).

Table 3. FAME composition of oil from both photobioreactors

Fatty Acid	Name	HPBR 1 (%)	HPBR 2 (%)
(C10:0)	Capric acid	2.1(±0.1)	3.9(±0.0)
(C13:0)	Tridecylic acid	3.3(±0.2)	2.2(±0.02)
(C16:0)	Palmitic acid	5.1(±0.01)	20(±1.2)
(C18:1)	Oleic acid	70(±2)	30.2(±0.1)
(C18:2)	Linoleic acid	19(±0.5)	15.6(±1.1)
(C18:3)	Linolenic	5.2(±0.1)	12.4(±0.02)
(C20:0)	Arachidic acid	6.6(±0.5)	Not detected

In HPBR2, the concentrations of the main fatty acids were better distributed, with emphasis on Palmitic acid. In HPBR, however, the fatty acid produced in greater quantity was Oleic. Another important highlight is that in the cultivation conditions reviewed in the reactor with more lighting, there was a marked production of Linolenic acid, above 12%, indicating high explosive power. Therefore, this biodiesel must be used with caution or mixed with other types of biofuels so as not to impair the performance of Diesel cycle engines.

As observed, biodiesel derived from *S. platensis* biomass could have a positive impact on the fuel market. Recent studies indicate that the price of microalgae-derived biodiesel (including conversion costs, harvesting, synthetic cultivation media, and taxes) is approximately USD 2.80 per liter, while conventional petroleum-derived diesel in the United States is 2.4 times cheaper at USD 1.10 per liter (Costa *et al*. 2019). In this research, using CWW as the cultivation medium and fine mesh separation of 0.045 mm (direct filtration without energy consumption), it is estimated that biodiesel production costs could be significantly reduced. Considering that 35% of production costs come from synthetic cultivation media (Molina Grima *et al.* 2003; de Mendonça et al. 2018), and an additional 20 to 30% of production costs come from energy for liquid/biomass separation (Barros *et al*. 2015; Costa *et al*. 2019), there is potential for a production cost reduction ranging from 55% to 65% (considering CWW as the cultivation medium + direct fine mesh separation). Consequently, the cost of biodiesel derived from *S. platensis* cultivation could be approximately USD 0.98 to 1.26 per liter, making it competitive with petroleum-derived diesel, with the added benefit of efficient treatment of wastewater.

Another abundant macromolecule in *S. platensis* biomass is carbohydrates, which can be used for bioethanol production through fermentation processes. Carbohydrates from microalgae have additional advantages compared to plant carbohydrates, as they do not contain lignin in their cellular composition (Pancha *et al*. 2016). Many studies have reported that microalgae accumulate a significant amount of carbohydrates when cultivated in wastewater, reaching up to 18% for the *Spirulina genus* (Nayak *et al.* 2016). In the present research, carbohydrate concentrations of 15% (±2%) and 27% (±1.2%) were detected in HPBR1 and 2, respectively. Higher carbohydrate yields were obtained under greater lighting.

Rempel *et al*. (2019) cultivated *S. platensis* in waste materials and recorded a carbohydrate concentration of 46.34% in the dry biomass. These authors reported energy production through bioethanol of approximately 4.664 kJ kg^{-1}, indicating the species' potential for bioethanol production. According to (Lam and Lee 2015; Costa *et al*. 2019), bioethanol production from microalgae biomass is promising and could reach values between 47,000 and 141,000 L ha^{-1} $year^{-1}$, surpassing any other raw material source for this purpose.

In addition, the energy content in microalgae biomass can reach 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; Zhou *et al.* 2013; Zewdie and Ali 2020; Vieira de Mendonça *et al.* 2021).

As for protein production, the values detected were 45% (±3%) for HPBR1 and 40% (±1%) for HPBR2. The high protein accumulations in microalgae always give them great use as a nitrogen fertilizer or as a food source, which is also rich in other nutrients.

Completely, 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 microalgae 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, with a compound annual growth rate of 10% (García *et al.* 2017).

In the supplementary material, prices and companies producing *S. platensis* biomass worldwide are presented, providing readers with a perspective on the selling costs of the biomass used as a protein supplement.

This biomass has valuable potential not only to produce renewable energy and eco-friendly bioproducts but also for disease prevention and treatment. 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), as well as Influenza and COVID-19 (McCarty and DiNicolantonio 2020).

3.2 CO_2 BIOFIXATION

Carbon (C) concentrations in the biomass were recorded as 0.39 (±0.1) and 0.47 g g^{-1} (±0.2) in HPBR1 and HPBR2, respectively (Table 1). Considering that the average C concentration detected in microalgae biomass is 0.50 g g^{-1} (Duarte *et al.* 2020), the

concentration of this element in HPBR2 was close to what is reported in the literature. This indicates that both the higher illumination and the volume of air per volume of culture (0.20 vvm) were sufficient to maximize the accumulation of C in the biomass produced in HPBR2, although it is believed that the assimilation of soluble organic C through mixotrophy also contributed to the accumulation of this element in the cells.

The carbon dioxide (CO_2) biofixation by the microalga *S. platensis* reached significant values in relation to carbon sequestration from the atmosphere. In the first reactor (HPBR1), the biofixation was 130 mg L^{-1} day^{-1}, and in the second reactor (HPBR2), with the increased light intensity, approximately 7 times higher biofixation was recorded (882 mg L^{-1} day^{-1}). In general, the species *S. platensis* can be considered efficient for CO_2 capture, aiding in the reduction of this gas from the atmosphere.

To achieve high CO_2 biofixation, several factors need to be considered, such as the applied CO_2 concentration, biomass productivity, CO_2 mass transfer, and the type of photobioreactor used (Duarte *et al*. 2020). Therefore, the high biofixation rate found in this research is related to the good performance of biomass productivity achieved by *S. platensis*, as well as the operational conditions adopted in the second reactor (HPBR2), primarily.

Cultivating *Spirulina* sp. in tubular photobioreactors in modified Zarrouk medium with added thermoelectric fly ashes, (Braga *et al*. 2019) achieved a maximum value of 700 mg L^{-1} day^{-1} for CO_2 biofixation. However, this value obtained by the authors presented intermediate performance when compared to the results obtained under the two conditions established in the present study. Cultivating *S. platensis* in wastewater from a family septic tank, illuminated at 180 µmol m^{-2} s^{-1}, (Almomani *et al*. 2019) recorded 378 mg L^{-1} day^{-1} of CO_2 biofixation, a value 3 times higher than that obtained in HPBR1. On the other hand, when comparing the data from the authors with the values obtained in HPBR2 in the present research, the biofixation rate was 2 times higher. This result clearly demonstrates that light intensity affects CO_2 biofixation for *S. platensis* cultivation when grown in CWW.

3.3 BIOREMEDIATION

The average pH values during the last 5 days of cultivation in HPBR1 and HPBR2 were 8.0 and 10, respectively (Table 4). The growth of the culture itself altered the pH of the culture medium, keeping it alkaline, which is a favorable condition for the growth of *S. platensis*, as it can survive in environments with a pH as high as 11. The optimal pH for the cultivation of this microalga species is between 9.5 and 9.8 (Soni *et al.* 2017), and notably, the pH observed in HPBR2 fell within the range considered ideal for the species' development. Maintaining the pH within the ideal range also helps prevent contamination of the culture medium by other species of microalgae and heterotrophic bacteria.

The efficiencies of removal of organic pollutants, nutrients, and thermotolerant coliforms in HPBR2 were higher when compared to HPBR1 (Table 4). The increase in light intensity from 150 to 300 µmol m^{-2} s^{-1} was crucial to achieve not only an increase in biomass productivity but also higher removals of BOD_5, COD, and nutrients.

The removal of COD and BOD_5 primarily occurs through biological assimilation, which happens via mixotrophy (Cheng *et al.* 2019). Considering that light directly affects

photosynthesis, it becomes evident why there is an almost 50% increase in the removal of organic pollutants when comparing HPBR1 with HPBR2. The species *S. platensis* is recognized as mixotrophic (Zhai *et al.* 2017), capable of assimilating both inorganic carbon (CO_2) and soluble organic carbon present in the CWW. The mixotrophic mechanism creates an additive and synergistic effect during cultivation, leading to increased biomass productivity while simultaneously enhancing the remediation potential of wastewater. This is achieved by combining the photosynthetic (autotrophic) process with heterotrophy.

The authors Markou *et al*. (2012) cultivated *S. platensis* in olive oil mill wastewater treated with sodium hypochlorite and achieved a removal rate of 73.18% for COD over a 16-day experiment. In the present study, a COD removal rate of 61.6% was achieved in 8 days of cultivation, half the time used by the previously mentioned authors.

The BOD_5 after treatment in the UASB reactor reached a value of 892 mg L^{-1}, and when the CWW was subjected to treatment with microalgae, it reached a value of 745 mg BOD_5 L^{-1} (HPBR1 reactor), resulting in a reduction of only 145 mg L^{-1} of BOD_5. In the second reactor (HPBR2), an average reduction of 685 mg L-1 of BOD_5 was recorded, reaching a final value of 205 mg L^{-1}, with an efficiency of 77% removal (Table 4). When exposed to higher irradiances, the species *S. platensis* significantly increases its biomass, which facilitated the mechanisms of BOD_5 removal from the CWW.

Regarding the removal of TSS, SST, and VSS, the highest removal rates were achieved in the second reactor (HPBR2) with 80%, 84%, and 88% respectively. However, no significant differences in solid removal were identified between the two reactors, indicating that the fine mesh sieve filtration method (20-µm) proved to be efficient for the removal of both solids and biomass.

Table 4. Data Before and After bioremediation of CWW

Parameters	CWW after UASB	HPBR1	Removal (%)	HPBR2	Removal (%)
pH	$7.2_{(0.2)}$	$8.3_{(0.5)}$	---	$9.7_{(0.1)}$	---
BOD_5 (mg L^{-1})	$892_{(2)}$	$744_{(7.8)}$	15	$204_{(21)}$	77
COD (mg L^{-1})	$1399_{(14)}$	$1124_{(36)}$	13	$537_{(40)}$	62
TS (mg L^{-1})	$651_{(16)}$	$186.5_{(8)}$	71	$140_{(14)}$	79
TSS (mg L^{-1})	$300_{(6.2)}$	$59_{(5.7)}$	79	$45_{(21)}$	84
VSS (mg L^{-1})	$160_{(7.9)}$	$21_{(1.4)}$	87	$19_{(0)}$	88
NH_4^+ (mg L^{-1})	$377_{(1.2)}$	$247_{(17)}$	32	$6.16_{(1.6)}$	98
N_{org} (mg L^{-1})	$192_{(1.7)}$	$153_{(45.3)}$	20	$7.8_{(1.1)}$	96
TP (mg L^{-1})	$81_{(0.5)}$	$52_{(0.2)}$	33	$8_{(0.2)}$	90

COD (Chemical Oxygen Demand); BOD_5 (Biochemical Oxygen Demand); TSS (Total Suspended Solids); TS (Total Solids); VSS (Volatile Suspended Solids); NH_4^+ (Ammonium Nitrogen); TP (Total Phosphorus); Norg (Organic Nitrogen). Values in parentheses indicate standard deviation.

The efficiency of NH_4^+ removal reached 98.3% in the second reactor (HPBR2), leaving only 6 mg L^{-1} of ammonia. However, in the first reactor (HPBR1), the efficiency can be considered low, reaching only 33%. Once again, the increased illumination in the second reactor (HPBR2) favored bioremediation, as the microalgae efficiently assimilated this nutrient for their growth under these experimental conditions. Cultivating *Chroococcus* sp. in waste from a dairy cattle farm, (Prajapati *et al.* 2014) recorded 98% efficiency in NH_4^+ removal over 16 days of cultivation. In comparison, in

the present study, NH_4^+ removal was superior in just 8 days of cultivation, half the time taken by the authors. During cultivation, ammoniacal nitrogen is assimilated by microalgae and converted into organic nitrogen (Norg) present in their biomass. Upon separating the biomass from the treated wastewater, the Norg is removed during the filtration process. In this study, a removal efficiency of 96% in HPBR2 and only 20% in HPBR1 was recorded. The lower organic N removal values in HPBR1 reinforce the limited conversion of nitrogen compounds into biomass due to lower light supply in this reactor.

Regarding total phosphorus removal, the maximum recorded value was 90% (HPBR2), leaving only 8 mg L^{-1} in the treated CWW. The removal of phosphorus from wastewater is crucial to prevent eutrophication of water resources, especially when wastewater is discharged into lentic environments such as lakes and reservoirs. Cultivating *Scenedesmus obliquus* in CWW (from primary treatment in a UASB-AF reactor), illuminated with 58 µmol m-2 s-1, de Mendonça *et al.* (2018) recorded phosphorus removal rates between 69-78% over 12 days of cultivation. The values obtained were higher than in HPBR1 of this study, even with lower illumination used by the authors. On the other hand, in HPBR2, the phosphorus removal values were higher than those recorded by the authors, reaching 90%.

Thermal-tolerant coliform removal rates greater than 70% were observed in both HPBRs, reaching a value of 99.9% in HPBR2 (Table 4). The elimination of coliform bacteria is associated with the excretion of various metabolites with bactericidal effects by these microalgae, as reported by (Kümmerer 2008; Gupta *et al.* 2015; de Mendonça *et al.* 2018). In conclusion, this bioremediation process mediated by *S. platensis* in HPBRs can be considered promising as a post-treatment method for CWW originating from UASB reactors, with the added benefit of producing biomass that has significant potential to produce various bioproducts, particularly biofuels.

4 CONCLUSIONS

The studied microalga exhibited a high capacity for phytoremediation of CWW under the higher irradiance applied to the cultivation system. The values of CO_2 biofixation rates by the microalga indicate potential resources to aid in air pollution mitigation. The biomass produced can be used to generate various sustainable bioproducts with high added value in the market, especially in the food and biofuels sectors.

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