Enhanced *Arthrospira platensis* Biomass Production Combined with Anaerobic Cattle Wastewater Bioremediation

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Abstract

Microalgae biomasses offer important benefits regarding macromolecules that serve as promising raw materials for sustainable production. In the present study, the microalgae *Arthrospira platensis* DHR 20 was cultivated in horizontal photobioreactors (HPBR), with and without temperature control, in batch mode (6 to 7 days), with anaerobically digested cattle wastewater (ACWW) as substrate. High dry biomass concentrations were observed (6.3–7.15 g L^{-1}). Volumetric protein, carbohydrate, and lipid productivities were 0.299, 0.135, and 0.108 g L^{-1} day^{-1}, respectively. Promising lipid productivities per area were estimated between 22.257 and 39.446 L ha^{-1} $year^{-1}$. High CO_2 bio-fixation rates were recorded (875.6–1051 mg L^{-1} day^{-1}), indicating the relevant potential of the studied microalgae to mitigate atmospheric pollution. Carbon concentrations in biomass ranged between 41.8 and 43.6%. ACWW bioremediation was satisfactory, with BOD_5 and COD removal efficiencies of 72.2–82.6% and 63.3–73.6%. Maximum values of 100, 95.5, 92.4, 80, 98, and 94% were achieved concerning the removal of NH_4^+, NO_3^-, P_t, SO_4^{2-}, Zn, and Cu, respectively. Total and thermotolerant coliform removals reached 99–99.7% and 99.7–99.9%. This microalgae-mediated process is, thus, promising for ACWW bioremediation and valuation, producing a microalgae biomass rich in macromolecules that can be used to obtain friendly bio-based products and bioenergy.

Keywords Bioproducts · Macromolecules · Lipid production · CO_2 biofixation · Bioresource

Introduction

In order to escalate milk production, intensive cattle farming has been increasingly applied worldwide [1]. Wastewater generation in intensive farming can reach up to 130 L $animal^{-1}$ day^{-1}, with this waste containing high nutrient and organic matter concentrations [2, 3]. Cattle wastewater (CWW) displays a BOD_5 between 2000 and 30,000 mg L^{-1}, total nitrogen ranging from 200 to 2,055 mg L^{-1}, ammonia between 110 and 1650 mg L^{-1}, and total phosphorus varying from 100 to 620 mg L^{-1} [4]. These concentrations are alarming and can cause dissolved oxygen depletion in water courses and eutrophication.

However, after undergoing pre-treatments, CWW can become a potential culture medium for microalgae [5–8]. Besides removing nutrients from wastewater, some microalgae species are also able to assimilate soluble organic carbon contained in substrates through mixotrophy [9]. *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Arthrospira platensis* are recognized as mixotrophic [10]. The mixotrophic mechanism creates an additive and synergistic effect during cultivation, resulting in increased biomass productivity while at the same time promoting wastewater bioremediation [5].

Fossil energy sources like oil, gas, and mineral coal emit approximately 6 billion t of CO_2 into the atmosphere [11]. In this context, microalgae exhibit relevant CO_2 bio-fixation rates, allowing for increased biomasses, thus ensuring higher culture productivity and growth rates [10], consecutively leading to decreased CO_2 emissions. Therefore, microalgae cultivation aiming at waste treatment is favorable in mitigating this environmental problem regarding two aspects, namely the reduction of greenhouse gases (GHG), mainly through CO_2 bio-fixation, and wastewater bioremediation.

A general history of the energy matrix reveals that the energy consumed worldwide in recent years was in the order of 14,279,569 ktoe, of which ~ 14% originated from renewable

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sources, such as solar, hydropower, wind, biomass, and waste [12]. Despite the recent advancements of renewable energy alternatives, their use is still limited concerning their potential [13] and, mainly, the urgent need for a paradigm change in this sector.

Within this scenario of necessary changes, the use of microalgae for the production of 3rd generation biofuels has recently gained more attention from the scientific community. Algal biomasses can be used to produce different biofuels, such as biodiesel/bio-oil, biogas, and bioethanol. Microalgae exhibit high photosynthetic rates compared to higher plants [14], leading to high biomass productivity. In addition, they display the ability to develop in unsuitable agriculture areas [15], avoiding food security-associated conflicts, and can be produced during the wastewater treatment, thus categorized as a nutrient recycling process, requiring no potable water for cultivation [16].

Given the above, new microalgae cultivation techniques are essential in order to increase the productivity of biomasses that contain important macromolecules for the production of friendly bio-based products, focused on bioenergy.

In this context, the aims of the present study were to assess the potential of Arthrospira platensis DRH 20 for the bioremediation of CWW previously treated by an UASB reactor, obtain CO_2 biomass bio-fixation rates and culture kinetic parameters at different temperatures, improve the quantitative and qualitative production of the Arthrospira platensis biomass, evaluate macromolecular components, such as lipids, proteins, and carbohydrates contained in the produced biomass, and finally, discuss potential biomass uses for commercial and energy purposes.

Material and Methods

Microalgae Strain

The Arthrospira platensis DHR 20 microalgae used herein were obtained from the Federal Rural University of Rio de Janeiro (UFRRJ) Fermentation Laboratory culture bank, Seropédica campus, RJ, Brazil. Pre-cultivation was performed in a Zarrouk medium in 1-L flasks, illuminated with 150 μmol m^{-2} s^{-2} by means of fluorescent white lamps (18 W), at 24.1 °C (± 1.2). Agitation was performed by injecting air from the atmosphere to the bottom of the flasks using an air compressor at a 0.5 L min^{-1} flow rate. The biomass concentration obtained during the pre-cultivation stage was 0.88 g L^{-1} (± 0.15), used for the inoculation of horizontal photobioreactors (HPBRs). All procedures were performed according to Mendonça et al. [5].

Wastewater Used as a Culture Medium

The CWW was generated at the experimental UFRRJ "Fazendinha Agroecológica" farm, Seropédica campus, RJ,

Brazil (coordinates: 22° 45′ 21″ S; 43° 40′ 28″ W). Cattle in this area are raised in confinement and fed only organic food items, produced on the farm itself, without the use of agrochemicals. Prior to collection, the CWW underwent preliminary treatment in a solid-liquid separator (decanter) and primary anaerobic treatment in a UASB reactor, operated with a hydraulic retention time (HRT) of 7 days. The physicochemical characterization of the anaerobically digested cattle wastewater (ACWW) is presented in Table 1. All analyses were performed according to Standard Methods [17].

Horizontal Photobioreactors (HPBRs) and Experimental Setup

Two identical bench-scale HPBRs, with a usable volume of 7.5 L and usable surface area of 0.08721 m^2 (Fig. 1), were used to cultivate the Arthrospira platensis DHR 20 microalgae in ACWW, displaying the characteristics presented in Table 1.

Table 1 ACWW physical-chemical characterization (used as a culture medium)

Parameters	Concentration
pH	$7_{(0.15)}$
EC (μS cm^{-1})	$1496_{(2.2)}$
COD (mg L^{-1})	$1400_{(3.1)}$
BOD_5 (mg L^{-1})	$890_{(0.2)}$
BOD_5/COD	0.64
TOC (mg L^{-1})	$351.3_{(3.1)}$
TS (mL L^{-1})	$650_{(22)}$
TSS (mL L^{-1})	$288_{(8)}$
VSS (mL L^{-1})	$162_{(3)}$
VS (mL L^{-1})	$490_{(13)}$
TKN (mL L^{-1})	$558_{(4)}$
NH_4^+ (mg L^{-1})	$366_{(0.9)}$
NO_3^- (mg L^{-1})	$77_{(0.1)}$
P_t (mg L^{-1})	$79_{(0.2)}$
SO_4^{2-}	$116_{(9)}$
Ca^{+2} (mg L^{-1})	$90_{(0.2)}$
Mg^{+2} (mg L^{-1})	$63_{(0.01)}$
Na^+ (mg L^{-1})	$114_{(1.1)}$
K^+	$195_{(0.51)}$
Zn	$0.56_{(0.001)}$
Cu	$0.35_{(0)}$
Total coliforms (MPN/100 mL)	$6 \times 10^{+5}{}_{(4.5 \times 10^{+1})}$
Thermotolerant coliforms (MPN/100 mL)	$3 \times 10^{+5}{}_{(3 \times 10^{+1})}$

EC, electrical conductivity; COD, chemical oxygen demand; BOD_5, biochemical oxygen demand; TS, total solids; TSS, total suspended solids; VSS, volatile suspended solids; TOC, total organic carbon; TKN, total Kjeldahl nitrogen; VS, volatile solids; NH_4^+, ammoniacal nitrogen; NO_3^-, nitrate; P_t, total phosphorus; SO_4^{2-}, sulfate; N.D., not detected. Number of repetitions, N = 7. Values in parentheses indicate standard deviation
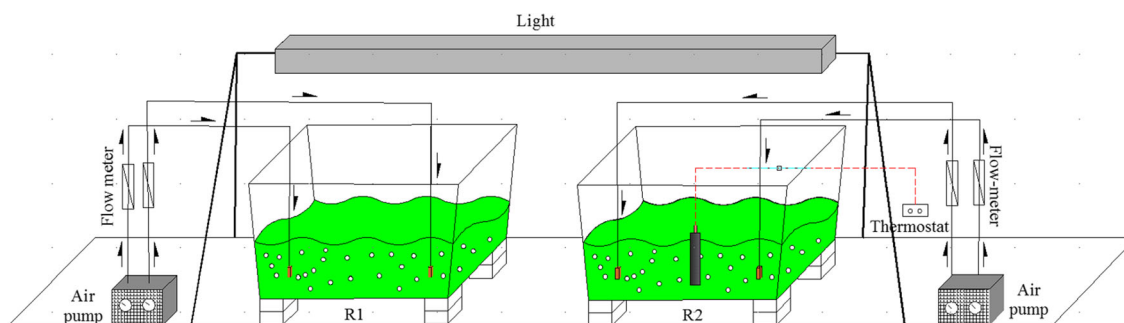
Fig. 1 Experimental setup: R1 (HPBR at room temperature), R2 (HPBR heated at 35 °C)

To promote a complete mix in the reactors and prevent self-shading during cultivation, atmospheric air was injected to the bottom of the HPBRs through two fine bubble diffusers (20-μm pore size). Both reactors were operated in batches. Air from the local atmosphere was pumped to the diffusers by a 4-W air pump (Aleas, AP-9804 model, China) at a 1.5-L min⁻¹ flow rate and pressure of 0.002 MPa. The local atmosphere CO_2 concentration was 0.0401% (± 0.0001), determined by gas chromatography. The air volume per culture volume of per minute (VVM) was 0.40. To avoid HPBR surface foam formation, 10 mL (10%) of a silicone-based defoamer were added daily [1].

Illumination was maintained constant (24 h day⁻¹ photoperiod) at 265 μmol m⁻² s⁻¹, measured using a Lux-Meter-Phywe, Germany. The lamps were positioned horizontally 10 cm above each HPBR (Fig. 1).

One HPBR was operated under two different room temperature ranges, termed R1. The mean fluid temperature was 30 °C (± 2.6 °C) in experiment 1 and 25 °C (± 1.9 °C) in experiment 2. When conducting both experiments, one HPBR (R2) was operated in parallel with heating heated to 35 °C (± 1 °C), considered ideal for the cultivation of the studied species [18]. In both experiments, R2 was considered the control. Both R2 heating and temperature were controlled by a thermostat (Hopar, H386-75 W model, China) directly immersed in the fluid (ACWW + microalgae). To ensure data stability and reliability, each experiment was repeated four times, always in pairs (Fig. 1). All data were collected in triplicate.

Batch Experiment

The growth curves of *Arthrospira platensis* in ACWW were determined as a function of dry biomass and optical density determined at 670 nm on a 1105 Bel Photonics spectrophotometer (Italy), in triplicates. The time between the reactor inoculation and biomass harvest was defined by culture growth stabilization (steady state).

After measuring the dry biomass in an oven (105 °C), a linear correlation was performed between the dry biomass-Y (g L⁻¹) and optical density (OD_{670}). The biomass calibration equations for experiments 1 (at 30 °C ± 2.6) and 2 (at 25 °C ±

1.9) were Y_{R1} (g L⁻¹) = 1.4932 (OD_{670}) + 3.8299 and Y_{R1} (g L⁻¹) = 1.164 (OD_{670}) + 3.3488, with correlation coefficients of $R^2 = 0.93$ and $R^2 = 0.95$, respectively. The calibration equation determined for R2 (control at 35 °C ± 1) was Y_{R2} (g L⁻¹) = 4.8139 (OD_{670}) − 1.2045, with a correlation coefficient of $R^2 = 0.97$.

Biomass production per area (Pa) was calculated using Eq. 1.

$$Pa\left(g\,m^{-2}day^{-1}\right) = Pv\left(g\,L^{-1}day^{-1}\right) \times \frac{\text{Reactor volume (L)}}{\text{reactor area } (m^2)} \quad (1)$$

where Pv = volumetric biomass production.

The expected raw oil (total lipid) yield L ha⁻²year⁻¹ (BP) was calculated using Eq. 2.

$$BP\left(L\,ha^{-2}year^{-1}\right) = \left[\frac{Pv\,\left(g\,L^{-1}day^{-1}\right)}{\text{Lipid density } (g\,L^{-1})} \times \frac{\text{n°of days in operation}}{\frac{\text{reactor area } (m^2)}{\text{reactor volume (L)}}} \right] \times 10,000\,(m^2) \quad (2)$$

CO_2 Bio-Fixation

The CO_2 bio-fixation rate (R_{CO2}) was calculated through the relationship between biomass productivity and carbon concentration ($C\%$) (Eq. 3). Carbon (C) biomass concentrations were determined by an elemental analysis (Elementar Vario EL III, German).

$$R_{CO2}\left(mg\,L^{-1}\,day^1\right) = P \times C \times (M_{CO2}/M_C) \quad (3)$$

where P = biomass productivity, mg L⁻¹ day⁻¹; C = carbon concentration in biomass, g g⁻¹; M_{CO2} = molar mass of CO_2, g mol⁻¹; M_C = molar mass of carbon, g mol⁻¹.

All procedures were performed according to Duarte et al. [13] and Mendonça et al. [5].

Analytical Methods

Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total organic carbon (TOC), total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS),

ashes (fixed solids - FS), ammoniacal nitrogen (NH_4^+), total Kjeldahl nitrogen (TKN), pH, electrical conductivity (EC), total phosphorus (P_t), nitrate (NO_3^-), potassium (K^+), copper (Cu), zinc (Zn), calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^+), sulfate (SO_4^{2-}), and coliforms were determined in triplicate according to standard methods [17].

The treated ACWW biomass was separated using a 0.045-mm-mesh sieve (Granutest, Brazil) and freeze-dried using a Liotop L 101 lyophilizer connected to a pump (Vacuum Technology SRL, Bologna, Italy). After freeze-drying, the biomass was sprayed in a mill (Shymsen, IKA A11 basic, Germany). Subsequently, protein concentrations were quantified by the Kjeldahl method [17]. Carbohydrates were determined according to Dubois et al. [19]. Lipids were quantified by Soxhlet extraction using hexane (130 mL) and ethanol (130 mL), in round-bottom distillation flasks, with a solubilization period for 6 h with hexane and 3 h with ethanol, using the same biomass-containing cartridge. After each extraction, the solvent was evaporated using a rotary evaporator (Buchi Waterbath B-480, Germany) with a thermostatically controlled bath at 50 °C. The pressures used for hexane and ethanol were 500 mbar and 350 mbar, respectively [5].

The local atmosphere CO_2 concentration was determined by gas chromatography using a Varian 430-GC cromatograph equipped with a thermal conductivity detector and a Varian Capillary Column SelectTM Permanent Gases/CO_2 HR - Malsieve 5 A Parabond Q Tandem #CP7430 column. Helium gas was used as the drag gas (52 mL mn^{-1}). A 0.5-mL air injection for all 18 analyzed samples was used in the chromatograph.

Statistical Analyses

The experimental results were evaluated by growth curve comparisons, and Tukey test was applied to kinetic parameters, biomass production, and pollutant removal. Significant values were obtained when $p \leq 0.05$. Prior to performing the parametric tests, the data normality was confirmed by the Shapiro–Wilk test using the PAST software.

Results and Discussion

Specific Growth Rate and Doubling Time

The maximum specific rate growth (μ_{max}) and doubling time (Td) observed in experiment 1 for reactors R1 and R2 were 0.41 day^{-1} and 1.67 day and 0.20 day^{-1} and 3.39 day, respectively. In experiment 2, the values for R1 and R2 were 0.27 day^{-1} and 2.48 day and 0.22 day^{-1} and 3.22 day, respectively. It is

interesting to note that there was little difference in μ_{max} and Td values during the experiments for the heated reactor (R2). In general, the R2 reactor exhibited higher Td compared to the experiments conducted at room temperature. Td was lower at 30 °C in the reactor operated at room temperature (R1), indicating higher culture growth speed at this temperature.

Regarding Td and μ_{max}, only the biomass produced in R1 at 30 °C displayed a significant difference ($p \leq 0.05$) in comparison to the same biomass grown at 25 and 35 °C. This indicates that the investigated *Arthrospira platensis* DHR 20 strain grows favorably under the established R1 conditions of experiment 1. This is an important result, as the ideal temperature for cultivating this species ranged from 34 to 35 °C.

De Mendonça et al. [5] recorded μ_{max} and Td of 0.27 day^{-1} and 2.5 day under CO_2 addition conditions and 0.15 day^{-1} and 4.7 day without f CO_2 addition when cultivating *Scenedesmus obliquus* in ACWW from a UASB-AF reactor. The best results found by these authors under CO_2 addition conditions were very close to those observed in experiment 2 of the present study. During experiment 1 (in R1), the Td for *Arthrospira platensis* was lower than that reported for *Scenedesmus obliquus* described by the aforementioned authors.

Zhu et al. [20] cultivated the microalga *Chlorella sp.* in livestock waste diluted and then filtered in paper filter (to remove non-soluble particles) and recorded μ_{max} between 0.275 and 0.375 day^{-1} and Td between 2.52 and 2.85 days, values close to those of the present study.

Kim and Kim [6] cultivated *Chlorella emersonii* in CWW from a tertiary treatment mixed with BG-11 culture medium and obtained $\mu_{max} = 0.61\ day^{-1}$ and Td = 1.2 days. The growth rate and doubling time in the present study that most approached the values reported by these authors are those obtained in experiment 1 (R1).

Cardoso et al. [21] cultivated *Spirulina sp.* in aquaculture wastewater (AWW) and recorded specific growth rates ranging from 0.18 day^{-1} (raw AWW) to 0.47 day^{-1} (25% AWW + 75% of Zarrouk medium). The values obtained in the present study were higher than the μ_{max} obtained by the authors for raw AWW and lower than the AWW μ_{max} with the addition of 75% Zarrouk medium.

Based on the results of the aforementioned studies, *Arthrospira platensis* growth is comparable to those of other microalgae that have already been successfully cultivated in wastewater, especially from cattle farming.

In general terms, *Arthrospira platensis* (DHR 20) displayed satisfactory growth in ACWW. In comparison to other studies in CWW using other species, *Arthrospira platensis* was able to adapt and grow satisfactorily in these liquid wastes without the need for either water dilution or the addition of synthetic culture media. This is valuable information, as the wastewater proposed herein can serve as an alternative culture medium for biomass growth and production regarding the studied strain.

Biomass Concentration and Volumetric Productivity

High dry biomass concentrations were achieved (Fig. 2a, b). Maximum concentrations of 7.15 g L^{-1} and 6.30 g L^{-1} were recorded in R1, while maximum productivities of 6.55 to 6.6 g L^{-1} were recorded in R2.

In all experiments, the maximum dry biomass value was reached on the 7th experimental day, except in experiment 1 in R1, where the maximum value was recorded on the 6th day, when Td was also lower. In general terms, the maximum productivity recorded in the present study (7.14 g L^{-1}) was obtained in R1 at an average room temperature of 30 °C. Considering the maximum concentration obtained in R2 heated at 35 °C (6.6 g L^{-1}), 0.54 g L^{-1} more biomass was produced by the HPBR operated at room temperature (R1) during experiment 1. This indicates that the ideal temperature for this species when cultivated in ACWW is of 30 °C, with an acceptable standard deviation of up to ± 2.6 °C. However, regardless of temperature, the dry biomass concentrations were very similar at the end of each experiment (Fig. 2).

Therefore, under the cultivation conditions proposed herein, high *Arthrospira platensis* biomass concentrations can be obtained without the need for HPBR heating, which is positive, as no heating systems are required, reducing production costs.

Dry biomass values close to those recorded in the present study were also reported by Hena et al. [22], who used treated dairy farm wastewater as a culture medium (Table 2) and *Arthrospira platensis* in PBR with an illumination of 300 μmol m^{-2} s^{-1}.

The maximum volumetric biomass productivities were recorded in experiment 1—R1 (0.664 g L^{-1} day^{-1}). The second highest productivity was recorded in experiment 2—R2 (0.610 g L^{-1} day^{-1}). These productivities can be considered high compared to values reported in other studies conducted with agro-industrial wastewater. Values closer to those observed in the present study were obtained with the microalgae *Arthrospira platensis* (*Spirulina platensis*), *Chroococcus sp.* (cyanobacteria), and the green microalgae *Chlorella emersonii* and *Chlorella vulgaris* (Table 2).

Zhu et al. [20] obtained a dry biomass concentration of 2.9 g L^{-1} cultivating *Chlorella sp.* in diluted and filtered livestock waste. Zhou et al. [28] obtained a dry biomass of 0.81 g L^{-1} when cultivating *Spirulina platensis* in saline wastewater. Almomani et al. [27] cultivated *Arthrospira platensis* in flasks containing sewage treated in a septic tank, obtaining 0.246 g L^{-1} of dry biomass. The lower biomass concentrations detected in these three studies are attributed to lower NH_4^+ and P concentrations in the wastewaters used as the culture media compared to the present study.

Yu and Kim [8] recorded 2.6 g L^{-1} of dry biomass when cultivating *Botryococcus braunii* in continuous mode in SBR reactor. In the present study, higher values were recorded due to higher nutrient concentrations and illumination rates (Table 2). Furthermore, higher biomass concentrations can be obtained in batch operating modes compared to continuous modes [5].

Hena et al. [23] cultivated a microalgae mix (*Chlorella accharophila*, *Chlamydomonas pseudococcum*, *Scenedesmus sp.*, and *Neochloris oleoabundans*) in CWW treated by activated sludge system and obtained a biomass concentration of 3.02 g L^{-1}.

De Mendonça et al. [5] cultivated the microalga *Scenedesmus obliquus* in CWW after anaerobic digestion in a hybrid reactor and achieved a volumetric productivity of 0.213–0.358 g L^{-1} day^{-1} and maximum dry biomass of 3.7 g L^{-1}. Although *Scenedesmus obliquus* exhibited exceptional adaptability and growth in ACWW with pollutant and nutrient concentrations similar to those of the present study, the microalga *Arthrospira platensis* displayed better performance concerning biomass production.

In addition, *Arthrospira platensis* (present study) achieved a higher volumetric biomass productivity (0.664 g L^{-1} day^{-1}) when compared to other cyanobacteria, such as *Chroococcus sp.* (0.558 g L^{-1} day^{-1}) [24]. These authors obtained a relevant productivity when cultivating this cyanobacterium in CWW without primary treatment. The results reported in the present compared to other studies presented herein indicate that ACWW is an adequate culture medium, as the anaerobic digestion process preserves the nutrients in the solution while at the same time clarifying the effluent, allowing for more light

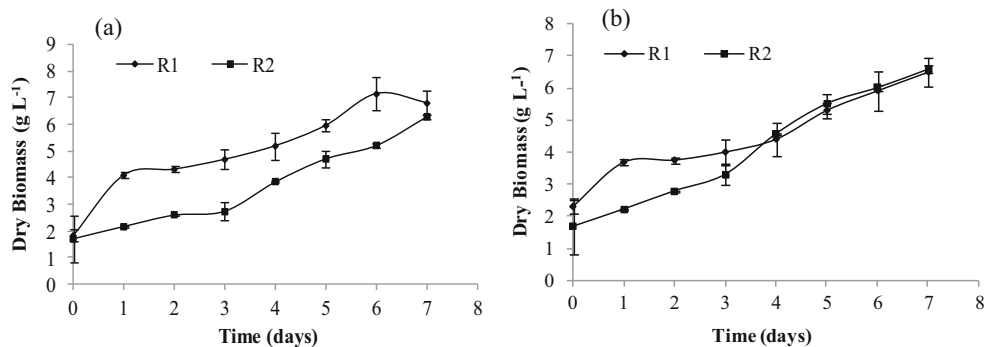**Fig. 2** Dry biomass. **a** Experiment 1. **b** Experiment 2

Table 2 Microalgae biomass concentration and volumetric productivity in agro-industrial wastewater

Substrate	Reactor	Strain	Light (μmol m^{-2} s^{-1})	Dry Biomass (g L^{-1})	Volumetric biomass production (g L^{-1} day^{-1})	Reference
Treated dairy farm wastewater	HPBR	[1]Mix	80	3.02	0.276	[23]
Treated dairy farm wastewater	PBR HRP	Arthrospira platensis	300	5.35 4.01	0.41 0.38	[22]
Cattle wastewater	PBR	Scenedesmus obliquus	58	3.7	0.213–0.358	[5]
Treated livestock wastewater	SBR	Botryococcus braunii	$\approx$ 180	2.6	0.316	[8]
Waste from dairy cattle farm	Flasks	Chroococcus sp.	$\approx$ 1480*	4.34	0.558	[24]
Dairy wastewater	PBR	Chlorella vulgaris	300	NR	0.450	[25]
Livestock wastewater	PBR	Chlorella emersonii	160	1.46	0.61	[6]
Livestock waste	Flasks	Chlorella sp.	240	2.88	0.288	[20]
Olive mill wastewater	PBR	Spirulina platensis	$\approx$ 135	1.69	NR	[26]
Wastewater from a family septic tank	Flasks	Spirulina platensis	$\approx$ 180	NR	0.246	[27]
Saline wastewater	Flasks	Spirulina platensis	90	0.81	NR	[28]
ACWW	HPBR	Arthrospira platensis DHR 20	265	7.15$_{(0.08)}$	0.664$_{(0.06)}$	Present study Exp. 1 (R1)
				6.3$_{(0.1)}$	0.572$_{(0.1)}$	Present study Exp. 1 (R2)
				6.5$_{(0.1)}$	0.524$_{(0.08)}$	Present study Exp. 2 (R1)
				6.6$_{(0.2)}$	0.610$_{(0.18)}$	Present study Exp. 2 (R2)

PBR, photobioreactor; *HRP*, high rate pond; *SBR*, sequencing batch reactor; *HPBR*, horizontal photobioreactor; values in parentheses indicate standard deviation; *Maximum value of natural sunlight

to enter the system. Thus, the CWW pre-treatment in the UASB reactor may have favored the significant dry biomass concentrations and volumetric productivities observed herein.

No significant differences ($p \geq 0.05$) between the experiments and reactors in terms of biomass concentration or volumetric productivity were observed.

The productivity per area (Pa) observed in the present study was relevant compared to synthetic culture media (Table 3). The R1 value (experiment 1) indicates a Pa 2.55-fold higher than that obtained in Zarrouk medium, the traditional *Arthrospira* culture medium. In this case, the Pa was higher than the synthetic media values reported in the literature in all experiments.

The average productivity values per area were not significantly different ($p \geq 0.05$) between reactors in all tests.

CO$_2$ Bio-Fixation Rate

The CO$_2$ bio-fixation and carbon percentages contained in the obtained biomass are presented in Table 4.

Comparing the carbon (C) percentages between the experiments and reactors, concentrations were very similar and lower than the typical concentration detected in microalgae, of 50% [13]. Lower C concentrations in cells can be attributed to two factors. The first is the fact that *Arthrospira platensis* is an

efficient protein accumulator with lower cellular C concentrations, especially when exposed to high substrate N concentrations, as in the present study. This was also reported by de Mendonça et al. [5], who increased N culture supply by changing the operating mode from batches to continuous, with C concentration depletion from 43.9 to 35.7%. Another factor is associated to the low concentration of this element comprises the low CO$_2$ supply in the present study, from only a local atmosphere air application, without any additional CO$_2$ source.

Almomani et al. [27] recorded C concentrations between 46.5 and 55.5% in an *Arthrospira platensis* biomass with the addition of 10% CO$_2$ to the air injected to flasks containing sewage treated in a septic tank as a culture medium.

High bio-fixation values were observed in all experiments, especially in R1 in experiment 1 (1,051 mg L^{-1} day^{-1}). These significant values result from the high biomass productivity achieved during this experimental stage. Although HPBRs are not as efficient as airlift PBR, high bio-fixation rates were still possible.

A study conducted with *Spirulina* sp. LEB 18 cultivated in Zarrouk medium (inorganic) recorded CO$_2$ fixation rates ranging from 165 to 183 mg L^{-1} day^{-1} in tubular PBRs and from 110 to 123.8 mg L^{-1} day^{-1} in raceway bioreactors [13].

De Mendonça et al. [5] recorded a maximum fixation rate of 547 mg CO$_2$ L^{-1} day^{-1} in a *Scenedesmus obliquus* culture

Table 3 Comparison of biomass productivity per area between ACWW and synthetic culture media

Strain	Culture medium	Pa (g m^{-2} day^{-1})	Reference
Spirulina (Arthrospira)	Zarrouk medium	19.78	[29]
Spirulina sp. LEB-18	Mangueira Lagoon Water (MLW) supplemented with Zarrouk (20% MLW + 80% Zarrouk)	21.59	[30]
Arthrospira platensis	Zarrouk medium	22.4	[31]
Arthrospira platensis NIES-39	SOT medium	9.46	[32]
Spirulina platensis	Paoletti synthetic medium	17.7	[33]
Arthrospira platensis DHR 20	ACWW	57.14$_{(0.2)}$	Present study Exp. 1 (R1)
		49.16$_{(0.5)}$	Present study Exp. 1 (R2)
		45.1$_{(0.1)}$	Present study Exp. 2 (R1)
		52.89$_{(0.8)}$	Present study Exp. 2 (R2)

Pa, biomass productivity per area

in PBRs with ACWW from a hybrid reactor (UASB-AF) substrate. The bio-fixation rates of the present study were approximately twofold higher than those reported in that study. Thus, *Arthrospira platensis* exhibits considerable potential to mitigate atmospheric CO_2.

In another study carried out with an organic substrate (sewage treated in a septic tank), Almomani et al. [27] recorded a bio-fixation rate of 378 mg L^{-1} day^{-1}, almost threefold lower than that observed in R1 in experiment 1. Although the authors used the same microalgae species as the present study, volumetric productivities were fourfold lower, explaining the lower bio-fixation rates reported.

The relevant biomass production and CO_2 bio-fixation values were also associated with the operational characteristics of the HPBRs, indicating that the adopted agitation and illumination conditions were appropriate for the efficient cultivation of the studied microalgae. According to Mata et al. [34] and Duarte et al. [13], the photobioreactor is key to achieving carbon fixation efficiency, and Ouyang et al. [35] emphasize that, for successful CO_2 fixation, adequate illumination is required in the performed experiments. These requirements were met in the present study.

Table 4 Carbon percentage in dry biomass and CO_2 bio-fixation rate (R_{CO2})

HPBR	Experiment 1		Experiment 2	
	C (%)	R_{CO2} (mg L^{-1} day^{-1})	C (%)	R_{CO2} (mg L^{-1} day^{-1})
R1	43.6$_{(1.5)}$	1,051$_{(12.5)}$	42.7$_{(0.75)}$	829.4$_{(7)}$
R2	41.8$_{(1.1)}$	875.6$_{(8.8)}$	41.4$_{(1.3)}$	942$_{(8.4)}$

Finally, it is important to note that the bio-fixation rates reported herein are those that would be obtained if all carbon were assimilated by exclusively photoautotrophic nutrition and do not take into account the fraction assimilated from organic carbon (via mixotrophy).

ACWW Bioremediation

The pH values were maintained between 8.5 and 9 during the experiments (Supplementary material). During cultivation, the pH remained basic, a favorable condition for *Spirulina platensis* growth, which can survive in environments with pH of up to 11 [36].

The obtained NH_4^+ removal was satisfactory, reaching 98% and 98.6% in R1 in experiment 1 (6 days) and experiment 2 (7 days), respectively. Regarding the heated reactors (R2), removal reached 100% in both experiments at 7 days of operation. Although NH_4^+ was completely removed in the heated HPBRs, the final concentrations after treatments in the HPBRs operated at room temperature were very low, of 5–7.3 mg L^1 (Supplementary material). In Brazil, for example, the maximum NH_4^+ limit for treated effluent disposal into watercourses is of 20 mg L^{-1}. In this case, R1 reactors would result in the safe disposal of the treated CWW into watercourses at concentrations three- to fourfold lower than those allowed by the Brazilian legislation.

Lv et al. [7] recorded NH_4^+ removals ranging from 83.16 to 94.27% in a CWW treatment with the microalgae *C. vulgaris* during 5 days of experiment, close to the values observed in the present study. Hena et al. [23] reported an NH_4^+ removal of 100% from CWW, as in the present experiment (in R2), but after 10 days of cultivation (Table 5).

**Table 5** Agro-industrial wastewater bioremediation by microalgae

Substrate	Strain	RT (day)	COD (%)	TOC (%)	NH_4^+ (%)	Phosphorus (%)	Reference
Dairy farm wastewater	[1]Mix	10	98.8	NR	100	98.8	[23]
Cattle farm wastewater	*C. vulgaris*	5	91.24–92.17	NR	83.16–94.27	90.98–94.41	[7]
Cattle wastewater	*Scenedesmus obliquus*	12	65–70	NR	98–99	69–77.5	[5]
Dairy wastewater	*C. vulgaris*	4	51.5–74.8	NR	99.3–99.8	91.6–99.7	[25]
Waste from dairy cattle farm	*Chroococcus* sp.	16	80	NR	98	86.4	[24]
Dairy farm wastewater	[2]Mix	8	84.18	NR	99.26	89.92	[37]
Olive oil mill wastewater	*Spirulina platensis*	16	73.18	NR	NR	100	[26]
Aquaculture wastewater	*Spirulina platensis*	7	89.34	NR	NR	99.97	[21]
Anaerobically digested distillery wastewater	*Spirulina platensis*	5	15–23	1–8	48–72	18–100	[38]
Wastewater form a family septic tank	*Spirulina platensis*	6	39.5–82.6	NR	NR	85.7–99	[27]
Saline wastewater	*Spirulina platensis*	10	90.02	NR	NR	93.35	[28]
ACWW	*Arthrospira platensis* DHR 20	6	$59.6_{(0.1)}$	$59.3_{(0.2)}$	$98_{(0.0)}$	$92.4_{(0.03)}$	Present study Exp. 1 (R1)
		7	$72.3_{(1.2)}$	$72_{(0.15)}$	$100_{(0.0)}$	$87.6_{(0.2)}$	Present study Exp. 1 (R2)
			$63.6_{(0.5)}$	$63.4_{(0.1)}$	$98.6_{(0.02)}$	$87.3_{(0.1)}$	Present study Exp. 2 (R1)
			$73.6_{(0.01)}$	$73.3_{(0.1)}$	$100_{(0.0)}$	$91.1_{(0.01)}$	Present study Exp. 2 (R2)

P_t removals were higher than 87% in all experimental conditions, reaching a maximum value of 92.4% in R1 (experiment 1). Markou et al. [26] cultivated *Arthrospira platensis* in the wastewater from an olive oil factory and were able to obtain 100% phosphorus removal after 16 days of cultivation, a further 9 days compared to the present study.

In general, microalgae play a key role in the bioremediation of wastewater containing nutrients and organic matter (Fig. 3).

Fig. 3 Removal of organic matter, solids, nutrients, and coliforms from ACWW. **a** Experiment 1. **b** Experiment 2
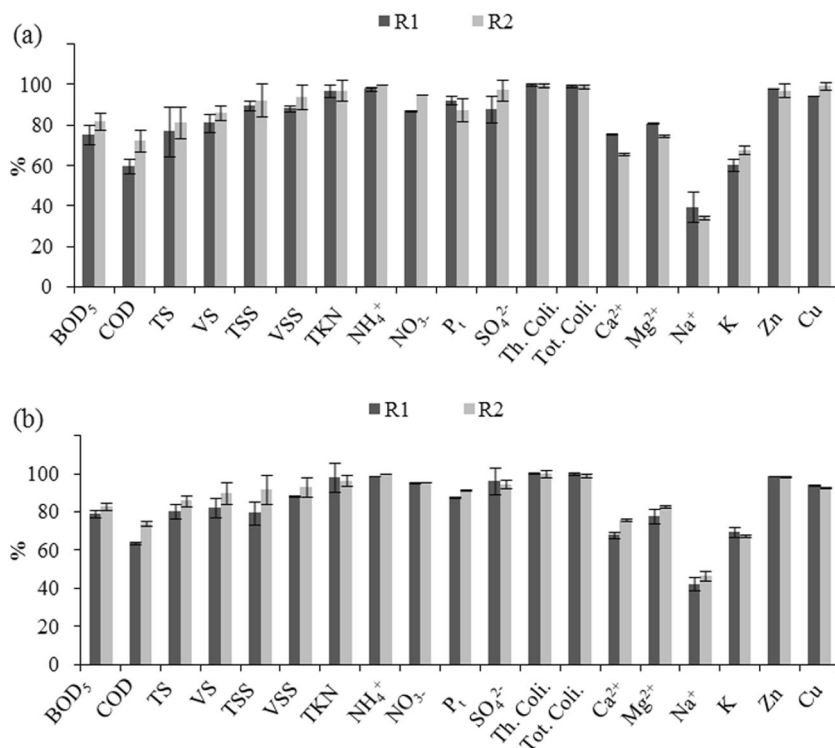
Regarding NO_3^- removal, a maximum value of 95.5% was obtained in R2 of experiment 2, and no difference was observed compared to its removal among the other treatments (Fig. 3). Nayak et al. [39] recorded a maximum NO_3^- removal of 70.2% when cultivating the microalgae *Scenedesmus* sp. in sewage, slightly lower than that observed in the present study.

Concerning SO_4^{2-}, removal values above 80% were verified in all experiments (Fig. 3). This indicates that the studied microalgae is able to result in relevant removal values of this anion. Sulfate (SO_4^{2-}) removal has received significant attention in recent years due to its water resource polluting potential, which can pose environmental degradation risks for both ecosystems and human health. In the present experiment, sulfate values after the HPBR treatment ranged between 3 and 14 mg L^{-1} (Supplementary material). Both the reactors operated at room temperature (R1) and heated (R2) were able to efficiently remove this molecule.

Molecules containing sulfur (S) participate in the formation of amino acids essential to cell energy metabolism. Sulfur and nitrogen are a relevant part of protein composition, abundant macromolecules in *Arthrospira platensis* biomass, which explain the high SO_4^{2-} removal observed herein.

The removals obtained in the present study ranged between 61 and 77% for K^+, 66 and 75.6% for Ca^{2+}, 76 and 81% for Mg^{+2}, and 34.2 and 46.5% for Na^+. The final concentrations of all analyzed macronutrients are reported in the Supplementary material.

The micronutrients Zn and Cu, despite being present at low concentrations in the ACWW (Table 1), were satisfactorily assimilated by the investigated microalgae. Removal efficiencies above 98% and 94% were noted for Zn and Cu, respectively. Cu plays a key role in photosynthesis and an important role in nitrogen fixation, while Zn is an enzymatic activator and growth promoter. The presence of both is crucial for the development not only of terrestrial crops, but also of microalgae.

In terms of organic matter, COD removals for reactors R1 and R2 were 59.6 and 72.3% in experiment 1 and 63.3 and 73.6% in experiment 2, respectively. In parallel, BOD_5 removals were equal to 75.3 and 82% in reactors R1 and R2 of experiment 1 and to 78.7 and 82.6% in reactors R1 and R2 of experiment 2 (Fig. 3 and Table 5). Thus, COD and BOD_5 removals were higher in the HPBRs with heating. TOC removals between 59.3 and 73.3 were obtained. A comparison of COD and TOC removals with those obtained in other studies is displayed in Table 5. Figure 3a and b display the efficiencies of organic matter, macro and micronutrient, solids, and coliform removals from the ACWW at the end of the experiments.

The total and thermotolerant coliform removals were 99–99.7% and 99.7–99.9%, respectively (Fig. 3). Gupta et al. [40] reported total coliform removals between 99.93 and 99.97% from sanitary sewage when cultivating the microalgae *Scenedesmus obliquus*. Therefore, relevant microorganism

reductions can be achieved during microalgae cultivation. The elimination of bacteria belonging to the coliform group may be associated to the fact that several metabolites displaying bactericidal activities are excreted from these microalgae [41].

Values higher than 77 and 88% were obtained for average TS and VS removals in the experiments. In the heated reactors (R2), solid removals were higher (Fig. 3). In the same figure, TSS and VSS removals greater than 80% are observed in both experiments, indicating that the adopted filtration mechanism was efficient to reduce the solid loads contained in the wastewater along with the produced biomass. Fine-mesh filtration (0.045-mm-mesh sieve) is considered efficient for separation of *Scenedesmus obliquus* biomasses, while also not requiring the use of electricity as in the case of centrifuge separation, leading to production process savings.

Although the experiments conducted with heating at 35 °C led to greater organic pollutant and nutrient removals, no significant difference ($p \geq 0.05$) between the reactors operated at room temperature was observed.

Macromolecular Composition of Biomass and Bioproducts

In terms of macromolecule production, no significant differences ($p \geq 0.05$) between the experiments or reactors were verified. Concerning macromolecular composition, protein concentrations were the most abundant, as expected. The maximum value was recorded in R1, of 45% (Fig. 4a).

Ogbonda et al. [42] studied the relationship between temperature and both biomass production and amino acid biosynthesis in *Spirulina* sp. and concluded that the highest amounts of proteins were obtained at 30 °C, corroborating the results reported in the present study. Biomasses with significant protein concentrations can be used for animal feeding as a complementary protein source or for agricultural use as nitrogen biofertilizers.

Morais et al. [43] cultivated *Spirulina* sp. at different glycerol concentrations and obtained a protein percentage of 69.78% (0.05 mol L^{-1} of glycerol), carbohydrate percentage of 12.41% (0.01 mol L^{-1} of glycerol), and lipid percentage of 13.34% (0.01 mol L^{-1} of glycerol).

Ash concentrations (fixed solids) were above 5% in experiment 1 and below 5% in experiment 2, without great differences (Fig. 4a, b).

When heated, the HPBRs (R2) enabled the production of higher carbohydrate concentrations by the microalgae. The maximum obtained value was of 24% (Fig. 4a). The obtained carbohydrate values are considered relevant and are associated with two factors: (1) The adoption of constant illumination (24 h photoperiod, at 265 μmol m^{-2} s^{-1}), as increased lighting hours is a driving force that intensified carbohydrate synthesis [44]; (2) The high CO_2 bio-fixation rates, along with the
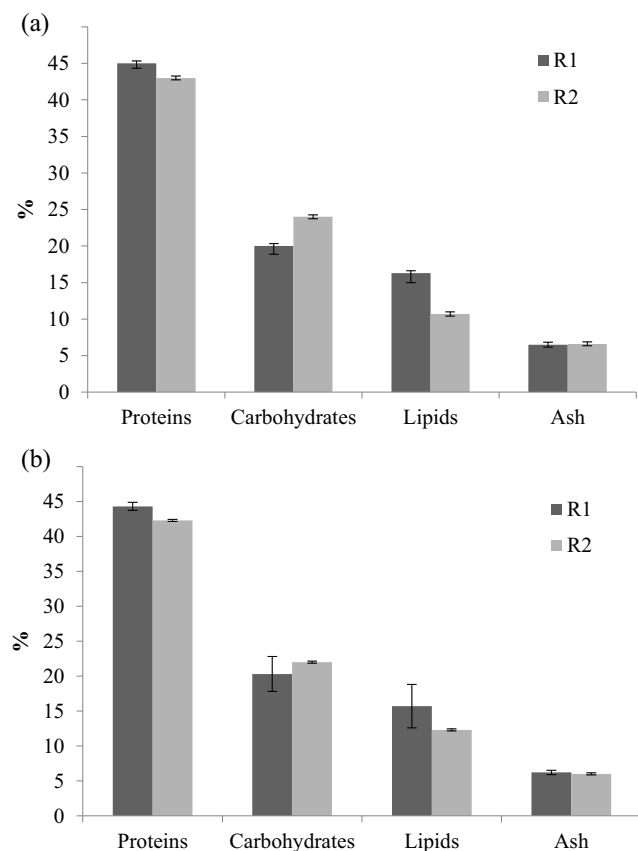
(a)

(b)

Fig. 4 Macromolecules and ashes from dry biomass of *Spirulina Platensis* DHR 20. **a** Experiment 1. **b** Experiment 2

species' ability to perform mixotrophy, resulting in carbohydrate production. Biomasses containing relevant concentrations of this macromolecule can be used in the production of biofuels such as biobutanol, bioethanol, biohydrogen, and methane/biomethane [45]. Furthermore, according to Molinuevo-Salces et al. [46], higher carbohydrate accumulations seem to be achieved in batch operations, the operating mode adopted in the present study. As the batch process with wastewater progresses, nitrogen compound concentrations decrease (and are no longer replaced), approaching zero in the last days, when the culture begins a more intense lipid accumulation process. Microalgae tend to accumulate lipids under stress conditions, such as nutrient limitations. Lipid microalgae accumulation depends on several conditions, such as lack of nitrogen and phosphate, high salinity, light intensity, and temperature, in addition to the carbon source concentration [47].

Lipid concentrations were higher in the unheated reactors (R1) in both experiments, with maximum values of 16.3% in experiment 1 and 15.7% in experiment 2 (Fig. 4). These values are considered high for this species, since the literature reports lipid concentrations ranging from 4 to 16.6% in *Arthrospira platensis* [34].

De Jesus et al. [48] cultivated *Arthrospira platensis* outdoors in both northern and southern Brazil in Zarrouk synthetic medium and obtained a maximum lipid percentage of 12%, close to that of the experiment conducted herein.

In this context, ACWW was proven a valuable culture medium to maximize lipid production in *S. platensis*. This points to the potential use of this species in biodiesel production, which is often neglected by the scientific community. The high dry biomass production of *Arthrospira platensis* (6.3 to 7.15 g L^{-1} day^{-1}), when grown in ACWW compensates its lower lipid concentrations when compared to other species. For example, *Chlorella* sp., a microalgae exhibiting potential for biodiesel production, can reach lipid concentrations between 10 and 48% of its biomass, but reaching productivity values between 0.02 and 2.5 g L^{-1} day^{-1} [34]. Comparing the data obtained by the aforementioned authors with those of the present study, *Chlorella* sp. reaches an average lipid productivity of 0.0421 g L^{-1} day^{-1}, whereas *Arthrospira platensis* DHR 20 reaches productivities between 0.061 and 0.108 g L^{-1} day^{-1} (Table 5). On the other hand, Kuo et al. [49] cultivated *Chlorella* sp. in swine wastewater and recorded a volumetric lipid productivity of 0.155 g L^{-1} day^{-1}, close to that obtained in R1 (experiment 1).

De Mendonça et al. [5] cultivated *Scenedesmus obliquus* in PBR with ACWW from a hybrid reactor as a culture medium and recorded a maximum dry biomass lipid accumulation of 29%, while the maximum volumetric productivity was 0.064 g L^{-1} day^{-1}, 1.7-fold lower than that reported in the present study. Kumar et al. [50] cultivated *Ascochloris* sp. in dairy wastewater and obtained a lipid productivity of 0.094 g L^{-1} day^{-1}, close to the maximum detected in the present study.

Another hypothesis to justify a higher lipid production is that the biomasses in microalgal-based wastewater treatment comprise a mixture of algae, bacteria, zooplankton, and detritus (ALBAZOD). In this case, part of the assessed biomass was probably not composed of *Arthrospira platensis* DHR 20, contributing to a higher lipid content.

Therefore, when grown in ACWW (after a UASB reactor treatment) under the conditions proposed in the present study, *Arthrospira platensis* DHR 20 exhibits the potential for significant lipid production, and low cellular lipid concentrations are compensated by high biomass productivity.

Lipid productivities per area between 5.24 and 9.29 g m^{-2} day^{-1} were observed herein (Table 6). These values allowed for the calculation of an expected total crude oil production from biomass per hectare between 22,257 and 39,446 L ha^{-1} $year^{-1}$ (Table 6). This is considered promising, especially compared to terrestrial plant oil sources, like sunflower (1,190 L ha^{-1} $year^{-1}$), canola (1,892 L ha^{-1} $year^{-1}$), coconut (2,689 L ha^{-1} $year^{-1}$), and oil palm (12,000 L ha^{-1} $year^{-1}$) [34, 45].

Based on the observed productivities, it would be possible to obtain 7.864 to 9.900 gallons ha^{-1} $year^{-1}$ of total lipids

Table 6 Lipid productivity and expected raw oil (total lipid) yield from the microalgae *Arthrospira platensis* DHR 20

Experiment	Reactors	Lipid productivity (g L^{-1} day^{-1})	Areal lipid productivity (g m^{-2} day^{-1})	Expected raw oil yield (L ha^{-1} year^{-1})	Expected raw oil yield in American gallons (gallons ha^{-1} year^{-1})
1	R1	0.108$_{(0.01)}$	9.29$_{(1.1)}$	39,446$_{(1.2)}$	9813$_{(1)}$
	R2	0.061$_{(0.0)}$	5.24$_{(0.54)}$	22,257$_{(0.48)}$	7804$_{(0.5)}$
2	R1	0.082$_{(0.02)}$	7.06$_{(0.15)}$	29,986$_{(0.2)}$	8900$_{(0.16)}$
	R2	0.076$_{(0.015)}$	6.52$_{(0.22)}$	27,685$_{(0.2)}$	8395$_{(0.21)}$

(Table 6). As a realistic projection, considering that 68% of total lipids are saponifiable [51] and considering 2% transesterification process losses [45], about 5.201 to 6.540 gallons per hectare per year of biodiesel can be produced via *Arthrospira platensis* DHR 20 biomasses.

It is a noteworthy that, as certain essential amino acids, some lipids contained in *Arthrospira platensis* biomasses are also essential, including a-linolenic acid and linoleic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), omega-3, omega-6, and other long-chain essential polyunsaturated fatty acids [52, 53]. The potential use of microalgae as a lipid supplement in the feeding of lactating cows was assessed by Stamey et al. [54], who reported fatty acid profile changes in milk, especially due to increased omega-3 contents, indicating that the lipid traces contained in microalgae are also beneficial when used in ruminant feeding. This is a valuable feature, as the proximity of the microalgae production units to intensive cattle production facilities leads to transportation and logistics savings and ease in obtaining effluents for cultivation. In addition, the biogas generated during the primary wastewater treatment process (by anaerobic digestion) can be converted into energy for biomass drying and separation without additional electricity costs.

The carbohydrate and protein productivities (volumetric and per area) contained in the dry *Arthrospira platensis* biomass are presented in Table 7.

Carbohydrate productivities were similar in all experiments, and the lowest value was obtained in the R1 cultivation in experiment 2 (Table 7).

The highest productivity macromolecular compounds were proteins. In terms of volumetric productivity, values between 0.232 and 0.299 g L^{-1} day^{-1} indicate potential dry biomass use as a cattle protein supplement, for example. Production values per area reached up to 25.75 g m^{-2} day^{-1} (Table 7).

Despite the operational simplicity of the HPBRs used in the present study, they were efficient for *Arthrospira platensis* cultivation, converting potential environmental threats into an opportunity to obtain high economic value bioproducts.

Finally, the use of microalgae biomass grown in anaerobic cattle wastewater will soon become a valuable animal feed source and biofertilizer production, as well as biofuel, especially biodiesel production. It is important to highlight that, with the use of ACWW as a culture medium, about 35% of biomass production costs can be saved [5]. It is also important to point out that, due to the presence of various bioactive compounds contained in *Arthrospira platensis* biomasses , further studies are required concerning medicinal purposes to combat and prevent autoimmune, degenerative, and infectious diseases caused by viruses, parasitic bacteria or fungi, such as lupus, Alzheimer's disease, HIV/AIDS, polio, influenza, Zika virus, malaria, Ebola virus [55], and COVID-19. These authors suggest that further research should be carried out to improve both upstream and downstream processes to produce bioactive compounds from microalgae biomasses and their conversion for low-cost commercialization in different industrial and agricultural sectors mainly using wastewater as culture medium.

Conclusions

Arthrospira platensis (DHR 20) cultivation in HPBR displays high potential for dry biomass production, with the highest value obtained at 30 °C. Relevant protein, carbohydrate, and

Table 7 Carbohydrate and protein productivity of *Arthrospira platensis* DHR 20 biomass

Experiment	Reactors	Carbohydrate productivity (g L^{-1} day^{-1})	Areal carbohydrate productivity (g m^{-2} day^{-1})	Protein productivity (g L^{-1} day^{-1})	Areal protein productivity (g m^{-2} day^{-1})
1	R1	0.1351$_{(0.01)}$	11.61$_{(0.2)}$	0.299$_{(0.02)}$	25.71$_{(0.8)}$
	R2	0.1353$_{(0.02)}$	11.64$_{(0.33)}$	0.244$_{(0.01)}$	20.98$_{(0.55)}$
2	R1	0.1066$_{(0.0)}$	9.17$_{(0.11)}$	0.232$_{(0.01)}$	19.99$_{(0.23)}$
	R2	0.1350$_{(0.05)}$	11.60$_{(0.2)}$	0.260$_{(0.03)}$	22.39$_{(0.2)}$

lipid concentrations were recorded, displaying the high potential of this species to produce macromolecules exhibiting relevant economic value. Heating the HPBRs did not significantly alter protein, carbohydrate, and lipid production, but the doubling time was shorter at 30 °C, indicating a higher culture growth rate. High CO_2 bio-fixation rates were observed, indicating a relevant potential of the studied microalgae in mitigating air pollution. Macro- and micronutrients contained in the ACWW were satisfactorily assimilated by the assessed microalgae, resulting in improved and intensified biomass production. An intensive biomass production lead to considerable thermotolerant and total coliforms reductions. Finally, the macromolecular *Arthrospira platensis* composition displays the potential to produce important bioproducts such as biodiesel, bioethanol, nitrogen biofertilizers, and animal feed, displaying environmental and economic importance, reducing the pressure on raw materials and contributing to an increasingly green and circular bioeconomy.

Data Availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics Approval and Consent to Participate Not applicable

Consent for Publication Not applicable

Conflict of Interest The authors declare no competing interests.

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