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CULTIVATION OF *Chlorella vulgaris* IN MEDIUM SUPPLEMENTED WITH DESALINATION CONCENTRATE GROWN IN A PILOT-SCALE OPEN RACEWAY

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Abstract - In this study, we investigated the outdoor production of a microalga *C. vulgaris* cultured in two different media under autotrophic cultivation: Bold Basal Medium (BBM) as the control and BBM supplemented with desalination concentrate (BBM + DC) using open raceway ponds (8 m^2). Data were collected on the growth, biomass productivity and biochemical composition. The culture developed in BBM + DC showed a biomass productivity of 6.8 g m^{-2} day^{-1}, while for the BBM control it was 8.5 g m^{-2} day^{-1}. Intracellular protein was the main algal component (~28.6%), followed by carbohydrate + fiber (~26.0%) and lipids (~5.0%). The predominant fatty acids were mainly α-linolenic (~19.5%), palmitic (~16.5%) and linoleic (~10.0%) acids. This study demonstrates the feasibility of culturing *C. vulgaris* in an alternative medium based on DC in order to valorize the desalination wastewater through its application to algal mass production.
Keywords: Desalination wastewater; Algal cultivation; Autotrophic system; Biochemical composition; Protein.

INTRODUCTION

The northeast region of Brazil, which has a semiarid climate and frequent droughts, is a vast region (982,563 square kilometers) with brackish groundwater. To face the scarcity of good quality water in some parts of the semiarid region of Brazil, brackish groundwater has been desalinized to make it appropriate for use (Ministério do Meio Ambiente, 2004). Desalination through reverse osmosis is the most attractive solution for water supply (Sánchez *et al.,* 2015). Equipment for the desalination of water based on reverse osmosis has been installed in the community of Uruçu, located in São João do Cariri - Paraíba state. In general, the piped groundwater has a flow intake of around 2-3 m^3/h, operating with an average recovery rate of 90%. As a result, a waste stream of brine with a high concentration of sodium, calcium and chlorides is produced.

Extensive research studies have been conducted using many types of wastewater for microalgae cultivation, particularly in raceway ponds. As examples, municipal wastewater has been used to grow many types of algae (Pittman *et al.,* 2011), concentrate from anaerobic digestion for the cultivation of *Scenedesmus* sp. (Tran *et al.,* 2014; Morales-Amaral *et al.,* 2015), effluent from anaerobic digestion to grow *Spirulina*

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sp. LEB-18 (Borges *et al.,* 2013), pig wastewater for the cultivation of *Spirulina* (*Arthrospira*) (Olguín *et al.,* 2003) and dairy wastewater to grow *Chlorella zofingiensis* (Huo *et al.,* 2012). Our focus, however, is to produce algal biomass in a medium combined with desalination concentrate (DC), particularly aimed at developing a system which could be integrated with an inland desalination plant. The current model was designed in the community of Uruçu (Paraíba state, Brazil) as a possible site for algal production due to its favorable climate conditions (low seasonal variation and ample solar irradiance) and geography (local source of brackish groundwater and flat topography).

In an open raceway ponds system, two key factors determine the suitability of a microalga for biomass production: the areal productivity (the amount of biomass per unit area (m^2) per unit time (day)) (Lawton *et al.,* 2015) and the biochemical composition (Batista *et al.,* 2013; Tibbetts *et al.,* 2015). The microalgae commonly cultivated in raceway ponds include *Nannochloropsis* sp., *Chlorella* sp., *Tetraselmis* sp., *Arthrospira* (Spirulina) *platensis*, *Dunaliella salina*, *Scenedesmus* sp. and *Haematococcus pluvialis* (Kumar *et al.,* 2015; Varshney *et al.,* 2015). Most biotechnological research on microalgae has been carried out using Chlorophyta (Varshney *et al.,* 2015). The microalga *C. vulgaris* (Trebouxiophyceae) used in this study is a unicellular alga, 5 to 8 μm in diameter (Graham *et al.,* 2008). In addition, the genus *Chlorella* is widely cultured in open raceway ponds under different wastewater conditions (Pittman *et al.,* 2011; Chu *et al.,* 2015; Lu *et al.,* 2015). Furthermore, the *Chlorella* freshwater microalgae have been targeted for biomass applications due to their high productivity, favorable biochemical composition, cosmopolitan distribution and competitive dominance over other algal species in open culture systems (Wu *et al.,* 2007; Huo *et al.,* 2012). *C. vulgaris* is also a potential source of lipids, which represent a promising pathway to obtain biofuels from microalgae (Halim *et al.,* 2012; El-Sheekh *et al.,* 2013; Skorupskaite *et al.,* 2015).

The present study was carried out following a project designed to evaluate the performance of *C. vulgaris* produced in a medium containing DC. Herein, we describe the outdoor batch growth of *C. vulgaris* in open raceway ponds. The main aim of this research was to investigate the biomass productivity and biochemical composition (*e.g.,* fiber, protein, lipids and fatty acids) of *C. vulgaris* cultivated in two different media: (1) Bold Basal Medium (BBM) as the control; and (2) BBM supplemented with desalination concentrate (BBM + DC) under the natural climatic conditions of the semiarid region of Brazil. As far as the authors are aware, this is the first description of combining desalination wastewater and algal cultivation in the Northeast region. The importance of this study lies not only in the scientific field, but also relates to the practical application of such a system, not only in the community of Uruçu (Paraíba state) but in the rest of the semiarid regions of Brazil.

MATERIALS AND METHODS

Microalgal Species and Inoculum Preparation

The freshwater microalga *C. vulgaris* was obtained from the Laboratory of Food Biotechnology at the Federal University of Santa Catarina. The alga was maintained in autoclaved BBM, which is suitable for freshwater algae (Nichols, 1973). The mineral salt medium composition, per liter of distilled water was: 0.075 g K_2HPO_4, 0.014 g KH_2PO_4, 0.075 g $MgSO_4.7H_2O$, 0.09 g $NaNO_3$, 0.025 g $CaCl_2.2H_2O$, 0.025 g NaCl, 0.05 g EDTA-Na_4, 0.00498 g $FeSO_4.7H_2O$, 0.01142 g H_3BO_3, 0.232 mg $MnCl_2.4H_2O$, 1.41 mg $ZnSO_4.7H_2O$, 0.252 mg $CuSO_4.5H_2O$, 0.192 mg $NaMoO_4.5H_2O$ and 0.080 mg $CoCl_2.6H_2O$. A stock culture was grown at 26°C in a constant-temperature room under 75 μmol photons m^{-2} s^{-1} provided by a combination of cool white (OSRAM Universal, Brazil) and day-light fluorescence lamps on a 12:12 h light/dark cycle. The cultures were first developed in 2.0 L Erlenmeyer flasks, and then scale-up to a 50-L capacity fiber photobioreactor in the Laboratory of Reference in Desalination (LABDES) at the Federal University of Campina Grande (UFCG), Paraíba State, Brazil.

Desalination Concentrate

Desalination concentrate was collected from an inland desalination plant, located in the community of Uruçu, São João do Cariri, Paraíba State, Brazil. The brackish groundwater was pumped through a small tubular (100-mm diameter) well (27-m depth) and fed to the desalination tank. The DC samples were obtained after passing the brackish water through three parallel reverse osmosis membranes (FILMTEC™ TW30 4040, Midland, USA) using desalination equipment. The chemical compositions of the groundwater (from the well) and DC (Table 1) were determined according to standard methods described by APHA (2005).

Ponds, Culture Conditions and Growth Measurements

Two above-ground concrete open raceway ponds (area of 8 m^2, 5.0-m length, 1.6-m width, 0.5-m depth and total capacity of 4,000 L/4 m^3 for each pond) were employed in this study. The outdoor experiments were conducted in 2012 between mid-January and February (austral summer) at an experimental algal farm facility

located in the community of Uruçu, São João do Cariri, Paraíba Sate, Brazil (07°23'27" S, 36°31'58"O) with an altitude of approximately 458 m. According to the Koppen classification, the climate in the study region can be described as semiarid (*BSh* classification) with temperatures varying from 20°C to 35°C during the year.

In order to proceed with the experimental studies in the open raceway ponds, two different media were prepared: (1) BBM (control treatment); and (2) BBM supplemented with 25% of desalination concentrate (BBM + DC) (Figure 1). The ponds were inoculated with exponential-phase inoculum of *C. vulgaris* grown outdoors in fiber photobioreactors (120 L) placed under natural light illumination and temperature, with saturated air-CO_2 (concentration of 0.03% CO_2 by volume with a flow rate of 1.5 L min^{-1}) constantly injected into the photobioreactors.

Approximately 1500 L of fresh culture medium (BBM) and (BBM + DC) was supplied to the open raceway ponds. After 8 days of cultivation, 2000 L of fresh culture medium (BBM) and (BBM + DC) was further added to the two ponds, promoting the microalgal growth until the final working volume (3500 L) was reached. The open raceway ponds were operated in a batch mode (14 days) of cultivation, mixed using a paddle wheel programmed for 10-min periods (*i.e.,* turned on/off at regular intervals) 24 h a day, under the exact same conditions (nutrient replenishment, pH, solar incidence, paddle wheel velocity, etc.). There was no external diffusion of carbon dioxide through the mechanical systems during the experimental cultivation. Evaporation losses were at the rate of 1 to 2 cm/day and were replenished with water every morning. Fresh water was used to replace evaporative losses. When the microalgal culture reached a stationary growth phase after 14 days, all of the culture volume from the open raceway pond was pumped into a 4000-L open-top conical tank. Based on previous laboratory results, as reported by Morioka *et al.* (2014) for *Chlorella* flocculation, the pH of the culture was elevated to 10.5-12.0 by adding (NaOH) + 0.5-1.0 g L^{-1} of flocculant $CaCl_2$. The suspension was manually mixed with a tool (shovel) for 10-15 min and then left to stand overnight (12 h). After the partial sedimentation, the biomass slurry was harvested by continuous centrifugation (Motortronics centrifuge, USA) at 3600 rpm for approximately 3 h. The microalgal pellet was transferred to a dish and dried at 60°C. The dried microalgal biomass samples were transported to the Laboratory of Food Biotechnology (BIOTEC) at the Federal University of Santa Catarina (UFSC) for further analysis to determine the biochemical composition.

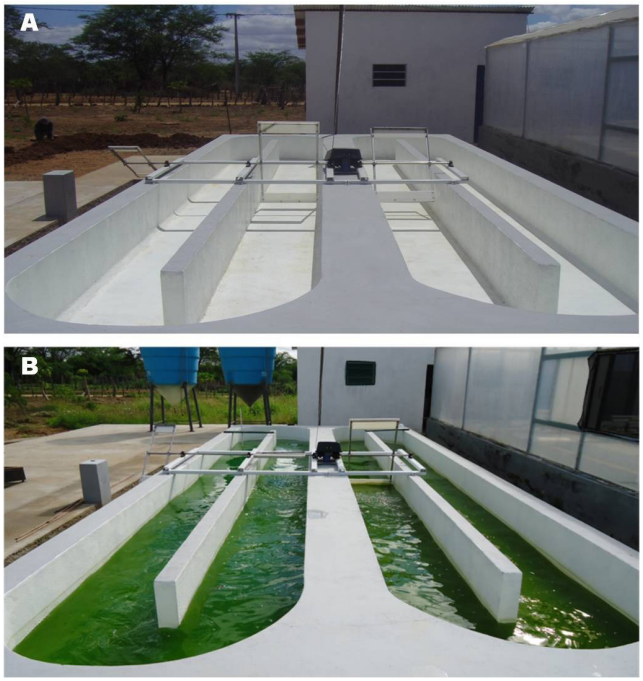

Figure 1. View of the open raceway ponds for microalgae cultivation at an experimental algal farm facility located in the community of Uruçu, São João do Cariri – Paraíba State, Brazil. A – Photograph of the ponds (5.0-m long, 1.6-m width and 0.5-m depth; totaling 8 m^2 and total capacity of 4,000 L/4 m^3 each pond); B – Cultures of *C. vulgaris* in BBM (control, left) and BBM supplemented with DC (BBM + DC, right).

Water temperature, pH, dissolved oxygen (DO) and conductivity were measured on-line and recorded continuously every day, using an HI 9828 Series HANNA Multiparameter probe (HANNA, Romania). The Illuminance was obtained using a digital lux meter LD-500 series (ICEL, Brazil).

Growth parameters were measured every day (for cell density and biomass productivity), using the methods described by Lourenço (2006). Cell counting was carried out using a hemocytometer and a compound microscope (Olympus, Germany). For the biomass production, a sample of the microalgae in the growth medium (10 mL suspension) was filtered through glass microfiber GF/C filter paper (Whatmann, New York, USA) and washed twice with distilled water. The paper with attached cells was dried at 105 °C for 1 h, and then kept over a desiccant in a vacuum desiccator overnight.

The chlorophyll *a* concentration was estimated as described by Ritchie (2006) as an indicator of algal biomass growth. A volume of 25 mL of each sample was filtered through the 0.40 μm glass microfiber GF/C filter paper (Whatman, USA). Chlorophyll *a* was extracted from filters with 90% acetone (Vetec®). Absorbance of the extract was measured at 660 nm (chlorophyll *a*) and 750 nm (turbidity) with a Hach

spectrophotometer (Loveland, CO, USA). Chlorophyll *a* concentration (μg mL^{-1}) was calculated from Eq. 1.

$$C = \left(A_{660} - A_{750}\right) * V / V_s * 11.3 / L / 1000 \quad (1)$$

where C is the chlorophyll *a* concentration (μg mL^{-1}), V is the volume of solvent (mL), V_s is the volume of sample (L), and L is the light path (cm). A is the absorbance and 11.3 is the specific extinction coefficient for acetone.

Biomass productivity calculation (g m^{-2} day^{-1}) was determined according to Bhowmick *et al.* (2014) Eq. (2):

$$AP = \frac{\left(FCD - ICD\right) \times WV}{BRT \times CAO} \quad (2)$$

where AP is the aerial productivity (for carpet area occupied), FCD is the final culture density, ICD is the initial culture density, WV is the working volume, BRT is the batch run time, and CAO is the carpet area occupied.

Analytical Procedures

Moisture - Moisture was determined by drying the sample in an oven at 105°C for 3-4 h (until constant weight) (AOAC, 2005).

Ash content - Total ash content was determined by heating the samples to 550ºC and holding this temperature for 5 h in a carbolite muffle furnace (IAL, 2005).

Fiber content - Total dietary fiber (TDF) content was determined with a total dietary fiber analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland), which includes enzymatic hydrolysis with α-amylase, protease and amyloglucosidade and is approved by the AACC (Method 32-05-01) and the AOAC (Official Method 985.29). Duplicate samples (approximately 1 g) were suspended in 50 mL phosphate buffer and submitted to enzymatic hydrolysis by incubating with 50 μL of α–amylase at 60°C for 30 min. The pH was adjusted to 7.5, 100 μL of protease was added and the samples were incubated at 60°C for 30 min. In the next step, the pH was adjusted to 4.5, 200 μL of amyloglucosidade was added and the samples were incubated at 60°C for 30 min. Lastly, the fiber was precipitated with 95% ethanol at 60°C, filtered through fritted glass crucibles with a Celite filter and the residue in the crucible was dried in an oven at 105°C, cooled in a desiccator and weighed.

Protein content - Total nitrogen was determined by the Kjeldahl method after acid digestion, ammonium addition, steam distillation and titration with 0.1 N HCl (AOAC, 2005). Protein content was calculated using a nitrogen-to-protein conversion factor of N x 4.78 (Lourenço *et al.,* 2004).

Lipid content - Intracellular lipids were extracted by the Soxhlet method with petroleum ether for 6 h, after acid digestion with 4 N HCl, followed by concentration in a rotary evaporator. The samples were then dried in an oven and weighed (AOAC, 2005).

Total carbohydrates - Total carbohydrate contents were calculated as follows: (100% – (moisture + ash + protein + fiber + lipid) (ANVISA, 2003).

Fatty acid analysis - The fatty acids composition was determined after converting the fatty acids to their corresponding fatty acid methyl esters (FAME) (IAL, 2005), by gas chromatography using a GC-2014 (Shimadzu, Kyoto, Japan), equipped with a split-injection port, flame-ionization detector and 105 m-long Restek capillary column (ID = 0.25 mm) coated with 0.25 μm of 10% cyanopropylphenyl and 90% biscyanopropylsiloxane. The injector and detector temperatures were both set at 260°C. The oven temperature was initially set at 140°C for 5 min, then increased at 2.5°C min^{-1} to 260ºC which was held for 30 min. The injection volume was 1 μL, and the split ratio was 10:1. Nitrogen was used as the carrier gas (flow rate 2.2 mL min^{-1}) at a constant pressure of 130.3 kPa. Fatty acid methyl esters were identified by comparison with the retention time of individual standards (Sigma, St. Louis, USA). The proportions of the individual acids were calculated from the ratio of their peak area to the total area of all observed acids and expressed as a mass percentage.

RESULTS AND DISCUSSION

Preliminary Studies of the Optimal DC Concentration for *C. vulgaris* Cultivation

Thirteen microelements were detected in the groundwater and DC (Table 1). The DC is rich in Cl^-, Na^+ and Ca^{2+}. In addition, other nutrients (N and P) and trace elements necessary for microalgae growth, including K^+, Mg^{2+} and Fe^{3+}, were detected in the DC. To assess the applicability of DC as a culture medium for *C. vulgaris* cultivation, the DC was mixed with BBM in different percentages (25%, 35%, 45% and 55% DC). Our previous experimental results obtained in the laboratory (Matos *et al.,* 2015) showed that *C. vulgaris* was able to grow in media with all of the DC percentages studied; however, the biomass concentration and biochemical composition were significantly affected by the cultivation conditions. For example, at high DC concentrations (*i.e.,* 45-55% DC), the biomass concentration was ~ 130 mg L^{-1} and the protein and lipid contents were in the ranges of ~ 20.0 and ~ 3.0%, respectively, while upon growing the

Table 1. Compositions of groundwater and desalination concentrate (DC) from the community of Uruçu, Paraíba State, Brazil.

Parameters	Groundwater	DC
pH	7.3 ± 0.5	8.1 ± 0.4
Electrical conductivity (EC, *m*S cm^{-1})	3.0 ± 0.8	5.6 ± 0.5
Langelier Saturation Index (LSI)	0.08	1.35
Total Dissolved Solids (TDS (mg L^{-1})	1823.8 ± 94	3410.0 ± 126
Cl (mg L^{-1})	720.7 ± 35	1418.2 ± 96
Na (mg L^{-1})	464.0 ± 66	723.8 ± 98
$CaCO_3$ (mg L^{-1})	506.5 ± 97	1080.0 ± 125
Ca (mg L^{-1})	74.0 ± 10	145.0 ± 23
Mg (mg L^{-1})	77.2 ± 21	172.2 ± 14
SiO_2 (mg L^{-1})	48.2 ± 11	82.4 ± 24
SO_4^{2-} (mg L^{-1})	15.6 ± 0.6	102.1 ± 12
K (mg L^{-1})	7.0 ± 2	15.0 ± 5
NO_3^- - N (mg L^{-1})	8.2 ± 0.1	15.4 ± 0.1
NH_4^+ (mg L^{-1})	0.59 ± 0.3	1.35 ± 0.8
PO_4^{3-} - P (mg L^{-1})	0.20 ± 0.1	0.80 ± 0.3
Fe (mg L^{-1})	0.05 ± 0.1	0.08 ± 0.1

microalgae at 25-30% DC the corresponding values were 600 mg L^{-1}, 48.5% and 12.5% (Figure 2). This indicates that *C. vulgaris* undergoes considerable growth inhibition at DC concentrations of 45-55% while it grows relatively well at DC concentrations of 25-30%. According to Kumar *et al.* (2015), the salt concentration influences algae via effects on osmotic stress, salt stress, and cellular ionic ratios. As the ionic concentration of DC is too high for the cultivation of *C. vulgaris*, algae have low capability to tolerate a broader range of DC-salinities with a satisfactory growth. Under this assumption, we supplemented the BBM with 25% DC for *C. vulgaris* cultivation in outdoor experiments.

The most notable differences between BBM and DC are related to the NaCl content and electrical conductivity (EC). DC has a NaCl content of around 2412 mg L^{-1} and EC of 5.6 *m*S cm^{-1}, while the corresponding values for BBM are 25 mg L^{-1} and 1.1 *m*S cm^{-1}, respectively. Regarding the concentrations of the major nutrients that are required for microalgae growth (N and P) there are also differences. BBM

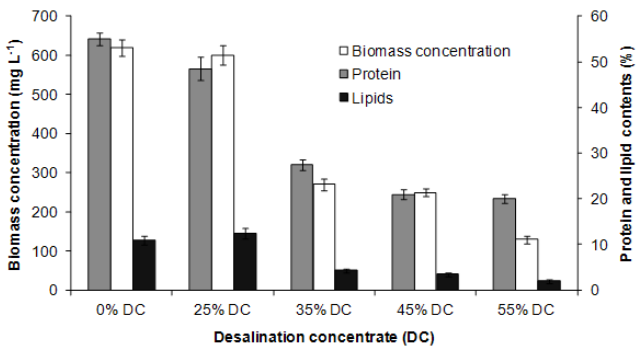

Figure 2. Effect of different concentrations of desalination concentrate (DC) on protein, lipid and biomass concentration of *C. vulgaris* under laboratory conditions.

contains N in the form of nitrate, whereas in the DC, besides nitrate, N is also present as NH_4^+. Concerning the total P, BBM contains 89 mg PO_4^{3-} L^{-1} while the DC content is 0.80 mg PO_4^{3-} L^{-1}. In addition, BBM presents a N/P ratio of 2:1 and for BBM + DC this ratio is 5:1. Park *et al.* (2011) have reported that the N/P ratio in wastewater can range from 4:1 to almost 40:1. Overall, the main difference between BBM and DC is that the latter contains high levels of mineral salts. In addition, a small quantity of ammonia (1.35 mg L^{-1}) was detected in the DC along with sulfate (102.0 mg L^{-1}), compounds which can be toxic to aquatic life (Greenlee *et al.,* 2009). For this reason, *C. vulgaris* has a limit of tolerance, which is a DC concentration of around 25-30% in the culture medium. It is also important to note that chemical pre-treatment and cleaning is a necessity in most desalination plants, which typically includes treatment with chemicals to avoid biofouling, scaling, foaming and corrosion in desalination plants, which may have adverse effects on aquatic organisms (Lattemann and Hopner, 2008; Melián-Martel *et al.,* 2011).

Water Quality, Algal Growth and Biomass Productivity

Over the course of the outdoor experiment (14 days of cultivation), the water quality was recorded continuously on-line every day (Table 2). During the experiments, the average water temperature was around 33.1ºC at mid-day and ~ 23.0°C at night. Maximum dissolved oxygen (DO) values during the cultivation period were between 10.1 and 12.5 mg mL^{-1}, with no significant difference between the two pond cultures. The initial and final pH values of the cultures were 8.10 and 9.40, respectively. Illuminance ranged from 1,000 lux (during sunrise and sunset) to 80,000 lux (at mid-day). The values obtained for the variables

electrical conductivity, alkalinity, total suspended solids (TSS) and turbidity dynamics for the two ponds were statistically different. For the outdoor pond containing BBM + DC, higher electrical conductivity (2.2 *m*S cm^{-1}) and alkalinity (538 mg $CaCO_3$ L^{-1}) were observed compared with the BBM control (1.1 *m*S cm^{-1} and 200 mg $CaCO_3$ L^{-1}, respectively). In addition, TSS and turbidity were also higher in the BBM + DC culture. There were no significant differences in the chlorophyll *a* concentrations for the ponds, with an average of around 350 mg L^{-1}. Concerning the ammonium composition, the NH_4-N content for BBM + DC (1.10 mg L^{-1}) was 4.5 times higher than that for the BBM control (0.25 mg L^{-1}) (Table 2).

Initial results for the growth of *C. vulgaris* were similar in the two ponds containing the BBM control and BBM + DC (Table 3). Based on the maximum cell density (MCD), *C. vulgaris* cells were denser in the BBM control (1.2 x 10^6 cel. mL^{-1}) than in BBM + DC (8.9 x 10^5 cel. mL^{-1}) (Figure 3), while the average specific growth rate was ~0.25 day^{-1}. The cultures grown in BBM control showed a biomass concentration of around 275 mg L^{-1}, while for BBM + DC the result was 220 mg L^{-1}. Actually, the outdoor cultivation of *C. vulgaris* resulted in an average biomass concentration of 250 mg L^{-1} and the data reported herein for the biomass concentration of the outdoor culture are comparable with results obtained by Cuello *et al.* (2015), which ranged from 263 to 403 mg L^{-1} using *Tetraselmis suecica* (Chlorophyta) in open raceway ponds.

The values for the biomass productivity of *C. vulgaris* obtained in the outdoor cultures, *i.e.*, 8.5 g m^{-2} day^{-1} using BBM medium and 6.8 g m^{-2} day^{-1} using BBM + DC, are close to those previously reported for this type of Chlorophyta species cultured

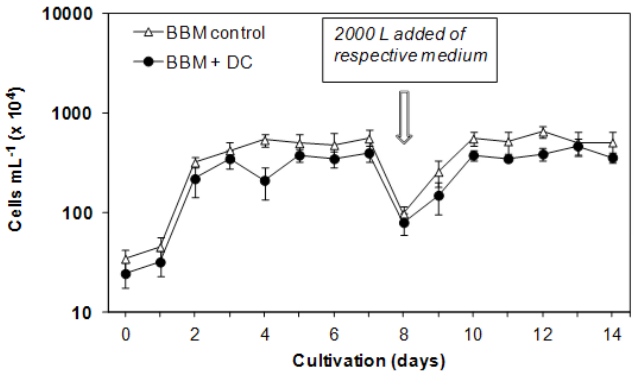

Figure 3. Growth curve of *C. vulgaris* cultivated in open raceway ponds with working volume of 3,500 L during 14 days; (Δ) Control treatment (BBM) and (●) BBM supplemented with DC (BBM + DC).

in raceway ponds, for example, 8.1 g m^{-2} day^{-1} for *Chlorella variabilis* (Bhowmick *et al.,* 2014) and 7.2 g m^{-2} day^{-1} for *Tetraselmis* sp. MUR 233 (Raes *et al.,* 2014). Using wastewater in raceways ponds, the biomass productivity reported generally ranges from 10 to 20 g m^{-2} day^{-1} (*e.g.*, Park *et al.,* 2011). In one case, a biomass productivity of up to 24 g m^{-2} day^{-1} was reported for *Scenedesmus* sp. using 30% concentrate from anaerobic digestion as the nutrient source (Morales-Amaral *et al.,* 2015). In contrast, a biomass productivity of 5.0 g m^{-2} day^{-1} for *Nannochloropsis gaditana* using the same concentrate at 30% in the culture medium was reported by Ledda *et al.* (2015). More specifically biomass productivities in raceway ponds across the globe are well illustrated in Table 4.

Analysis of the Biochemical Composition of *C. vulgaris*

The biochemical compositions of *C. vulgaris* cultivated in the two proposed media (BBM and

Table 2. Median ± standard deviation of water quality variables in the open raceway ponds with respectively culture medium - BBM (control) and BBM supplemented with DC (BBM + DC) – measured during 14 days of operation.

Water quality variable	BBM	BBM + DC
Temperature (°C)	22.0-32.3[a]	23.0-33.1[a]
DO (mg L^{-1})	12.50 ±1.0[a]	10.15 ± 0.5[a]
pH	8.10-9.20[a]	8.12-9.60[a]
EC (*m*S cm^{-1})	1.1 ± 0.1[a]	2.2 ± 0.3[b]
Alkalinity (mg $CaCO_3$ L^{-1})	200.5 ± 24[a]	538.6 ± 45[b]
Turbidity (NTU)	0.2 ± 0.1[a]	0.7 ± 0.2[a]
TSS (mg L^{-1})	57.5 ± 5.2[a]	100.5 ± 3.3[b]
Chlorophyll *a* (µg mL^{-1})	300 ± 20[a]	400 ± 25[a]
NH_4-N (mg L^{-1})	0.25 ± 0.10[a]	1.10 ± 0.15[b]

TSS = Total Suspended Solids. Different letters in the same row correspond to significant differences (p<0.05) by Tukey test.

Table 3. Maximum cell density, the growth rate and biomass concentration of *C. vulgaris* cultured in open raceway ponds in control treatment (BBM) and BBM supplemented with DC (BBM + DC).

Treatment	BBM	BBM + DC
MCD (cel. mL^{-1} x 10^4)	121 ± 10[a]	89 ± 12[a]
Specific growth rate µ (day^{-1})	0.26 ± 0.05[a]	0.25 ± 0.05[a]
Biomass conc. (mg L^{-1})	275 ± 20[a]	220 ± 12[a]

MCD = maximum cellular density; Values shown are averages of three ± the range.

Table 4. Comparison of biomass productivities (g m^{-2} day^{-1}) in open raceway ponds between this study and literature values.

Month	Productivity (g m^{-2} day^{-1})	A* (m^2)	D** (m)	V*** (m s^{-1})	Duration	Species	Location	Temp (ºC)	Reference
Summer	8.56	8	0.3	0.25	Batch 14 days	*Chlorella vulgaris*	Brazil	22-33	This work*
Summer	6.87	8	0.3	0.25	Batch 14 days	*Chlorella vulgaris*	Brazil	22-33	This work**
Summer	8.10	1.5	0.1	0.30	Batch 15 days	*Chlorella variabilis*	India	36	Bhowmich *et al.* (2014)
Summer	26.3	25	0.2	0.20	Semi continuous	*Tetraselmis* sp. MUR 233	Australia	10-28	Sing *et al.* (2014)
Autumn	7.2	1	0.2	0.22	Semi continuous	*Tetraselmis* sp. MUR 233	Australia	12-22	Raes *et al.* (2014)
Summer	14.0	0.08	0.05	17 rpm	Batch 19 days	*Spirulina* sp. LEB-18	Brazil	26-44	Borges *et al.* (2013)
Summer	10.6	25	0.15	0.23	Semi continuous 55 days	*Spirulina (Arthrospira)*	South Africa	20-35	Grobbellar (2009)
Summer	28.0	20		12 rpm	Semi continuous 24 days	*Microcystis aeruginosa*	Malaysia	29-34	Ashokkumar *et al.* (2014)
Spring	6.0-7.9	1192	0.26		Semi continuous	*Nannochloropsis oculata*	China	12-26	Cheng *et al.* (2015)
-	9.0-13.0	100	0.15		Semi continuous	*Haematococcus pluvialis*	United States	16-34	Olaizola (2000)
Summer	6.62	30.37	0.07	0.25	Semi continuous	*Scenedesmus acutus*	United States	12-38	Eustance *et al.* (2016)
Spring	24.0	32	0.12	0.20	Semi continuous	*Scenedesmus* sp.	Spain	-	Morales-Amaral *et al.* (2015)
Annually	4.4-11.5	14,000	0.35	0.20	Semi continuous	Consortium algal/bacterial	New Zealand	13-15	Craggs *et al.* (2012)

* Cultures developed in the control treatment (BBM) and ** cultures developed in the BBM supplemented with DC (BBM + DC); * A = area, ** D = depth of the raceway pond, *** V = velocity of the paddle wheel rotation.

BBM + DC) in the outdoor experiment are shown in Table 5. The dried microalgal biomass presented intermediate moisture content (15.2-18.8%). The ash content observed in the microalgal biomass cultured in BBM + DC (32.8%) was higher than in the BBM control (14.5%), the values being statistically different (P<0.05), which is due to extra precipitated salts, including calcium carbonate, sodium and chlorides that are trapped in the DC-based medium. In addition, these extra salts may interfere in the extraction step for analysis of the proximate composition of the algal biomass (Moheimani *et al.,* 2015).

The organic constituents (*e.g.,* protein, carbohydrate + fiber and lipids) were the major components of the algal biomass studied and differences between the two ponds were observed, with protein being the most abundant component (average 28.6%), followed by carbohydrate + fiber (average 26.0%), and lipid content (average 4.7%). The protein contents of the algal biomass samples were found to be significantly different (P<0.05). The microalgae cultured in the BBM control showed a protein content of 37.2%, almost 2-fold higher than the value for the BBM + DC (20.0%). The reduced protein content in *C. vulgaris* cultured in BBM + DC is most likely due to a redirection of available energy towards processes such as osmoregulation rather than towards the synthesis of proteins (Lawton *et al.,* 2015; Matos *et al.,* 2017).

With regard to the intracellular lipids, the total lipids varied from 5.8% (BBM control) to 3.7% (BBM + DC) (Table 5). Actually, it is expected that *C. vulgaris* cultivated in outdoor conditions would show a lipid content of more than 15-20%, while in this case the lipid content was low (<4.0%), and unsustainable for biodiesel production. It has been noted that environmental factors/conditions such as diurnal, climatic and seasonal variations in temperature, humidity and light impart a profound effect on the biomass in outdoor microalgal cultivation (Varshney *et al.,* 2015). In this regard, the harsh environmental conditions imposed in the present outdoor experiment [*i.e.,* high temperatures (~33°C during the day), high solar radiation (~90 klux mid-day) and nutrient medium containing brine (high salt content)], will have affected the growth and biochemical composition of the *C. vulgaris*. Regarding the salinity, it is expected that BBM and BBM + DC have a NaCl concentration of around 0.4 mM and 10.3 mM NaCl, respectively, that is, the BBM + DC is 25 times more saline than the BBM medium. However, the salinity of BBM + DC can be considered as moderate, being much lower than that of seawater (0.5 M NaCl). Takouridis *et al.* (2015) demonstrated the feasible growth of the freshwater microalga *Chlamydomonas reinhardtii* under high salinity conditions (0.3-0.7 M NaCl) via the selective breeding method of genome shuffling. Nevertheless, only a few microalgal strains can support high salinity, for instance, *Dunaliella*, a green unicellular microalga isolated from high salinity water bodies with NaCl concentrations exceeding 3.0 M NaCl (Borowitzka and Huisman, 1993).

Table 5. Chemical composition of *C. vulgaris* cultivated in open raceway ponds.

Composition (%)	BBM	BBM + DC
Ash	14.5 ± 2.5^a	32.8 ± 3.0^b
Moisture	15.2 ± 0.9^a	18.8 ± 1.4^a
Protein	37.2 ± 2.7^a	20.0 ± 2.1^b
Fiber	7.0 ± 0.5^a	7.1 ± 0.6^a
Carbohydrate	20.3 ± 1.1^a	19.6 ± 1.2^a
Lipids	5.8 ± 0.6^a	3.7 ± 0.5^a

Values shown are averages of three ± the range. Different letters in the same row correspond to significant differences (p<0.05).

The fatty acids (FAs) composition of freshwater *C. vulgaris* after applying the proposed experimental conditions are shown in Table 6. Twelve fatty acids composed of C12:0 to C22:6 ω3 were identified. Based on their percentage of the total FA in *Chlorella vulgaris*, the predominant FAs were α-linolenic acid (C18:3 ω3 ALA, 18.5-21.0%), palmitic acid (C16:0, 16.5-16.6%), linoleic acid (C18:2 ω6, 8.7-11.2%) and oleic acid (C18:1, 7.6-11.8%), representing ~60.6% of

total FA content. The FAs present at moderate levels were pentadecenoic acid (C15:1, 0.1-4.2%), stearic acid (C18:0, 0.5-3.5%) and palmitoleic acid (C16:1, 1.8-2.9%), representing ~10.6% of the total FA content. Fatty acids present at trace levels were lauric acid (C12:0, 0.3-1.2%), myristic acid (C14:0, 0.5-1.1%), γ-linolenic acid (C18:3 ω6 GLA, 0.7-1.0%), dihomo-γ-linolenic acid (C20:3 ω6 DGLA, 0.2-0.4%) and docosahexaenoic acid (C22:6 ω3 DHA, 0.2-0.6%), representing ~4.4% of the total FA contents.

As shown in Table 6, *C. vulgaris* cells that were cultured in BBM tend to produce higher levels of PUFAs (mainly C18:2 ω6 and C18:3 ω3), an average of 32.2%, than culture developed in BBM + DC. Furthermore, the PUFA/SFA ratio was higher for the BBM control (1.67) than for BBM + DC (1.21). On the other hand, *C. vulgaris* cultivated in BBM + DC appears to have a tendency to synthesize more SFAs (mainly C16 and C18:0), consistent with previous data on this fatty acids fraction (Matos et al., 2015). Functionally, higher synthesis of SFAs in *C. vulgaris* can be explained by an adaptive osmoregulatory mechanism to cope with rapid or gradual changes in salinity that are associated with algae-cell-membrane permeability (Takagi and Karseno, 2006; Lawton *et al.,* 2015).

Table 6. Summary of fatty acid methyl esters (FAMEs) composition for *C. vulgaris* cultivated in BBM and BBM + DC.

FAMEs (%)	BBM	BBM + DC
C12:0	1.26 ± 0.2	0.34 ± 0.1
C14:0	0.51 ± 0.2	1.15 ± 0.2
C16:0	16.5 ± 1.2	16.6 ± 1.5
C18:0	0.54 ± 0.1	3.52 ± 0.4
Other SFA	1.21 ± 0.4	2.25 ± 0.8
Σ SFA	20.2 ± 1.5	23.8 ± 1.2
C15:1	4.2 ± 0.8	nd
C16:1	1.8 ± 0.3	2.9 ± 0.5
C18:1	7.6 ± 1.1	11.8 ± 1.1
Other MUFA	0.9 ± 0.1	2.3 ± 0.3
Σ MUFA	14.5 ± 1.3	17.0 ± 1.3
C18:3 ω3 (ALA)	21.0 ± 1.0	18.5 ± 2.3
C22:6 ω3 (DHA)	0.60 ± 0.1	0.28 ± 0.1
Σ PUFA-ω3	21.6 ± 1.3	18.7 ± 1.2
C18:2 ω6	11.2 ± 0.8	8.7 ± 0.6
C18:3 ω6 (GLA)	0.7 ± 0.1	1.0 ± 0.3
C20:3 ω6	0.2 ± 0.1	0.4 ± 0.1
Σ PUFA-ω6	12.3	10.1 ± 0.4
Total PUFA	33.9 ± 1.5	28.8 ± 1.4
PUFA/SFA	1.67	1.21

Values shown are averages of two ± the range. SFA (Saturated Fatty Acid), MUFA (Monounsaturated Fatty Acid), PUFA (Polyunsaturated Fatty Acid), nd (not detected).

CONCLUSIONS

This study was adapted to the community of Uruçu, located in the semiarid region of Brazil, where DC is used for many agricultural purposes. The applicability of DC as a substrate for microalgae cultivation using open raceway ponds was investigated. The microalga *C. vulgaris* was able to grow in DC, but requires a maximum of 25% of DC concentration. Growth of *C. vulgaris* cells was similar in BBM and BBM + DC with a biomass productivity of an average of 7.6 g m^{-2} day^{-1}. Cultures developed in BBM showed a protein content of 37.2% while in BBM + DC the same metabolite was 20.0%, indicating that *C. vulgaris* cultivated in DC shifts its metabolism to osmoregulation rather than towards the synthesis of proteins. Finally, this study demonstrated the use of available DC as nutrient feedstock, which could reduce the need for fresh water and external nutrients. This can be considered as a step forward for the valorization of brine management through algal mass cultivation.

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