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Pilot-scale outdoor production of *Scenedesmus* sp. in raceways using flue gases and centrate from anaerobic digestion as the sole culture medium

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ABSTRACT

This work investigated the production of *Scenedesmus* sp. in semi-continuous mode in three pilot-scale outdoor raceways (7.2 m^2) using flue gas for CO_2 supply and centrate from the anaerobic digestion of urban wastewater as the sole nutrient source. Experiments were performed at different culture depths, 5, 10 and 15 cm, while evaluating two centrate concentrations (30% and 45%) at dilution rates of 0.2 and 0.3 d^{-1}. Under optimal conditions of 30% centrate, 0.3 d^{-1} dilution rate and a 15 cm culture depth, a maximum biomass productivity of 22.9 g m^{-2} d^{-1} was obtained. The optical properties of the cultures were studied and the results showed a photosynthetic efficiency of up to 2.0% and a quantum yield of 0.3 g biomass E^{-1}. Nitrogen and phosphorus removal rates of 3 g N m^{-2} d^{-1} and 0.6 g P m^{-2} d^{-1} were recorded, respectively. Lipid productivity of 2.3 g m^{-2} d^{-1} was determined possessing a suitable fatty acids profile for biofuel production.

1. Introduction

Producing treated water that can be safely released into water bodies in the environment is currently an important issue for urban wastewater treatment plants. Conventional wastewater treatment technologies are mainly based on the activated sludge process, which often has drawbacks such as inefficiency, with only a fraction of the total nitrogen and phosphorus present in the effluent being removed (Cabanelas et al., 2013a; Pittman et al., 2011) as well as high operational costs of up to 0.2 €/m^3(Arbib et al., 2014; Pittman et al., 2011). Considering the detrimental environmental impact of wastewater released into aquatic ecosystems, principally the eutrophication risk (Pittman et al., 2011), the urgent need to improve the treatment approach is recognized. Microalgae-based processes offer a promising option for an eco-friendly wastewater bioremediation process; however, this needs to go hand-in-hand with the production of a valuable co-product, which generates revenue to offset costs (Park et al., 2011). Microalgae can grow in poor quality waters such as wastewater while, at the same time, assimilate inorganic and organic nutrients for growth, thus simultaneously treating the water and producing high-potential biomass (Jebali et al., 2018, 2015; Qi et al., 2018; Zhu et al., 2017; Arbib et al., 2014; Rawat et al., 2011). Such a combined process could counterbalance the operational costs related, firstly, to conventional wastewater treatment system costs due to their high energy consumption up to 0.5 kWh/m^3 (personal communication from Aqualia), and secondly, to mass microalgae production, which currently uses expensive, non-sustainable artificial fertilizers in the culture medium (Zhu and Hiltunen, 2016). This coupling approach could help to increase the feasibility of using microalgae in low-value products. In fact, various studies have pointed out that mass microalgae commercialization in this field is impeded by the upstream and downstream processing costs (Zhu et al., 2017; Park et al., 2013; Chisti, 2012; Park et al., 2011), making the whole process non cost-effective. For instance, pure CO_2 supply can account for 30% of the microalgae production cost (Acién Fernández et al., 2013); this, however, could be reduced by using flue gas instead (Chiu et al., 2011). Additionally, life cycle analysis research conducted by Yang et al. (2011) concluded that up to 90% of the freshwater could be saved by using wastewater for microalgae cultivation; while 94% and 100% of nitrogen and micronutrient requirements, respectively, could be provided using wastewater. Microalgal production integrated with wastewater treatment and CO_2 mitigation is still not commercialized, although the relevant costs can be reduced. The resulting microalgae biomass could be applied as a feedstock to produce biofertilizers, biohydrogen, biodiesel, bio-oil, bio-materials and other biochemical derivatives (Rinna et al., 2017; Cabanelas et al., 2013b; Park et al., 2013; Rawat et al., 2011).

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Centrate is obtained from the digested sludge dewatering process after the anaerobic digestion of secondary treated urban wastewater. It is considered one of the richest urban wastewater nutrient streams resulting in higher biomass productivity (Li et al., 2011; Wang et al., 2010). Numerous studies have reported the robustness and tolerance of several microalgae strains to centrate as the culture medium, most notably those of the genera *Scenedesmus* and *Chlorella*. However, most of these studies were conducted at laboratory scale (Li et al., 2011, Sepúlveda et al., 2015; Zhou et al., 2012b); few confirmed the results obtained at the large scale and in semi-continuous mode (Min et al., 2011; Morales-Amaral et al., 2015a); nor did they investigate one of the key operational parameters, namely, the appropriate culture depth to use.

The aim of this work is to investigate the feasibility of producing microalgae using centrate as the culture medium and flue gas for CO_2 supply. For this, the native strain *Scenedesmus* sp. was selected as it previously showed robustness and tolerance to this wastewater. Experiments were carried out under real outdoor conditions in three pilot scale raceway reactors to assess the reliability of the process. In this respect, key operational parameters, namely different dilution rates ($0.2\,d^{-1}$ and $0.3\,d^{-1}$), centrate percentages (30% and 45%) and culture depths (5, 10 and 15 cm) were studied to achieve maximum process performance. Thus, the effect of these parameters on biomass productivity, nutrient removal capacity, lipid content and the fatty acids profile was evaluated. In addition, the biomass optical properties were studied to assess the cultures' light-use efficiency.

2. Materials and methods

2.1. Microorganism and culture media

The freshwater isolate *Scenedesmus* sp. was used in this study after previously being selected as a potentially suitable strain for wastewater treatment (Jebali et al., 2015). The strain inoculum was maintained at 25 °C under continuous aeration and illumination of $80\,\mu E\,m^{-2}\,s^{-1}$ using MDM medium (Ichimura and Itoh, 1977). Centrate from the urban wastewater treatment plant in Almería (Spain) was used as the culture medium for the experiments. Experimental culture media were prepared by mixing tap water with centrate at different percentages: 30% and 45% v/v. Centrate is a wastewater stream that has a high nutrient concentration; it is obtained from the digested sludge dewatering process following the anaerobic digestion of treated urban wastewater. The composition of the centrate used is given in Table 1. The medium was prepared daily by using a greenhouse irrigation system (Nutritec 9000, Ritec, Riegos y Tecnología S.L., Spain). It was firstly subjected to ozonization and then passed through 200 and 25 μm filters.

2.2. Reactors and operational conditions

Experiments were performed outdoors in three pilot-scale raceway reactors, $7.2\,m^2$ in area each. The reactors consisted of polyethylene algal ponds, channels 5.0 m in length and 0.6 m wide connected by 180° bends. Culture circulation is ensured by a paddlewheel, 0.6 m in diameter, rotating at 0.3 m/s and regulated by an electric motor (Ebarba, Barcelona, Spain); the speed was regulated by a frequency inverter (Yaskawa AC Drive V1000, Yaskawa Electric Europe GmbH, Germany). The pH was maintained in the 7.8–8.0 range by on-demand CO_2 injection coming from flue gas (with an average CO_2 concentration of 10%); this was obtained from a diesel heating boiler. The flue gas at the boiler outlet was cooled by passing through a passive stainless steel serpentine; it then went to the compressor for storage. The air and flue gas were sparged at the bottom of the reactor sump through a diffuser. Air was continuously provided to the systems at 0.03 v/v/min (FR4L72BVBN flow meters, Key Instruments, USA) to ensure dissolved oxygen removal. The parameters for temperature, pH and dissolved oxygen concentration were measured by probes (5342 pH electrode and 5120 OD electrode, Crison Instruments S.A., Spain) connected to MM44 transmitter units (Crison Instruments, Spain) which were controlled online. The impinging irradiance was measured by a thermoelectric pyranometer connected to an AC-420 adapter (LP-02, Geónica S.A., Spain). The data for all of these parameters were logged using DaqFactory 5.0 software (Azeotech Inc., USA). The medium inlet flow rate was regulated by flow meters (FCIV0201D, 10100, FIP, Italy).

The raceway reactors were operated at 3 different culture depths (0.05, 0.10 and 0.15 m, corresponding to culture volumes of 360, 720 and 1080 L, respectively) and two different centrate concentrations (30% and 45%) and dilution rates $0.2\,d^{-1}$ and $0.3\,d^{-1}$. Experiments were firstly performed in batch mode, where the raceways were inoculated with 10% of inoculum at the exponential phase of cell density of $1.2\cdot10^7$ cell mL^{-1}, cultivated in an outdoor 100 L bubble-column photobioreactor. Subsequently, the cultures were shifted to semi-continuous mode by the daily addition of fresh medium during daylight periods (10 a.m–2 p.m) and the same culture volume was harvested in order to achieve dilution rates of $0.2\,d^{-1}$ and $0.3\,d^{-1}$. This was performed until steady state was achieved; at this point, a constant biomass concentration was attained. Experiments were conducted during, May, June and July in Almería (36°48′N, 2°43′W), Spain.

Table 1
Composition of the centrate obtained from a real urban wastewater treatment plant used for preparing the different culture media.

Parameter	mg/L	Parameter	mg/L
TSS	0.12 ± 0.08	Boron	0.6
$N\text{-}NH_4^+$	628.05 ± 60.66	Calcium	27
$N\text{-}NO_3^-$	7.17 ± 2.64	Magnesium	22
$P\text{-}PO_4^{3-}$	64.03 ± 26.23	Sulfate	31
TOC	182.67 ± 69.58	Potassium	241
TIC	563.10 ± 131.18	Iron	0.51
Carbonate	20	Copper	< 0.20
Bicarbonate	1523	Manganese	< 0.10
Sodium	234	Zinc	0.15

pH = 7.53 ± 0.19; Conductivity = 5.80 ± 0.96 ms/cm.

2.3. Biomass concentration and fluorescence measurement

The optical density of the three cultures at 750 nm was measured daily using a spectrophotometer (DR/4000 UV/Vis Spectrophotometer, HACH, USA). The dry weight biomass concentration was determined by filtering 100 mL of each culture through a 1 μm pre-dried filter (Macherey-Nagel GmbH & Co.KG, Germany) and drying it in an oven at 80 °C until constant weight was obtained.

Additionally, the maximum photosynthetic efficiency of photosystem II, calculated as the Fv/Fm ratio, was measured daily using a fluorometer (AquaPen AP 100, Photon Systems Instruments, Drasov, The Czech Republic) to check the physiological status of the cell. The quantum yield, the extinction coefficient and the average light intensity were calculated as detailed by Molina Grima et al. (1997). The extinction coefficient (Ka) was calculated by dividing the average absorption in the photosynthetically-active radiation range, PAR (400–700 nm), by the biomass concentration (Cb) and the cuvette light path (p) (Eq. (1)):

$$Ka = \frac{Abs}{C_b \times p} \tag{1}$$

The average irradiance was calculated as follows (Eq. (2)):

$$I_{av} = \frac{I_0}{K_a \cdot C_b \cdot p}(1-\exp(-K_a \cdot C_b \cdot p)) \tag{2}$$

where I_0 was the irradiance in the absence of cells.

Quantum yield is the amount of microalgal biomass produced by a unit of radiation (moles of photons) absorbed by the culture. This can

be calculated as follows (Eqs. (3) and (4)):

$$F_{vol} = I_{av} \cdot K_a \cdot C_b \quad (3)$$

$$\Psi_E = \frac{P_b}{F_{vol}} \quad (4)$$

where P_b is the volumetric biomass productivity and F_{vol} stands for the photon flux absorbed by a volume unit; it is obtained by multiplying the average irradiance (I_{av}), on a culture volume basis, by the extinction coefficient (K_a) and the biomass concentration (C_b).

The photosynthetic efficiency (PE) value was obtained by calculating the ratio of energy stored in the produced biomass to the energy impinging on the reactor surface. The biomass combustion heat (ΔH) was calculated considering the specific caloric value of the lipids (38.9 kJ g^{-1}), proteins (24 kJ g^{-1}) and carbohydrates (16.6 kJ g^{-1}), and by knowing the biochemical composition of the biomass. The ratio of the reactor's volume to surface (V/S) and the PAR to the global ratio of light, which was 2 E MJ^{-1}, were used for the photosynthetic efficiency calculation as follows (Eq. (5)):

$$PE = \frac{P_b \cdot \Delta H \cdot V}{I_0 \cdot S} \quad (5)$$

2.4. Analytical procedure

The nutrient analyses at the reactor inlet and outlet were carried out using colorimetric methods (Ministry of Agriculture, 1982). The phosphate was measured by visible spectrophotometry through the phospho-vanado-molybdate complex. The nitrate was determined by measuring the optical density at 220 nm and 275 nm. The ammonium was quantified according to the Nessler method. Nutrient depuration was calculated as follows (Eq. (6)):

$$\text{Nutrient depuration} = \frac{[\text{Nutrient}]_{inlet} - [\text{Nutrient}]_{outlet}}{[\text{Nutrient}]_{inlet}} \times 100 \quad (6)$$

At steady state, biomass was harvested by centrifugation at 7500 rpm for 5 min (SIGMA 4–15 Sartorius, Goettingen, Germany), washed twice with distilled water, freeze dried and used to determine the lipid content. Total lipids were determined as described by Kochert (1978). The fatty acids content and profile were obtained by direct transesterification and gas chromatography (Agilent Technologies 6890 N Series Gas Chromatograph, Santa Clara, CA, USA) as described by Rodríguez-Ruiz et al. (1998).

All the samples were withdrawn from cultures at steady state. All of the analyses were carried out in duplicate and the mean value was reported. Standard deviation (± SD) was calculated using excel tools.

3. Results and discussion

3.1. Culture growth

To determine the feasibility of the outdoor production of the native microalga *Scenedemus* sp. in raceway ponds using centrate as the sole nutrient source and flue gas for the CO_2 supply, experiments were performed with the reactors operating at a dilution rate of 0.2 d^{-1} and 0.3 d^{-1}, using centrate diluted with freshwater at different percentages (30% and 45%) and operating at three different culture depths (5, 10 and 15 cm).

The centrate contains nutrients such as nitrogen and phosphorus in addition to other trace elements that could efficiently support microalgae growth. The centrate exhibited the highest ammonium concentration among the urban wastewater streams (Morales-Amaral et al., 2015b; Osundeko and Pittman, 2014; Zhou et al., 2012a). Numerous studies reported that N-NH_4^+ is the preferred form for microalgae because its assimilation process does not require the redox reaction, which means a lower energy requirement (Cai et al., 2013). Indeed, it was

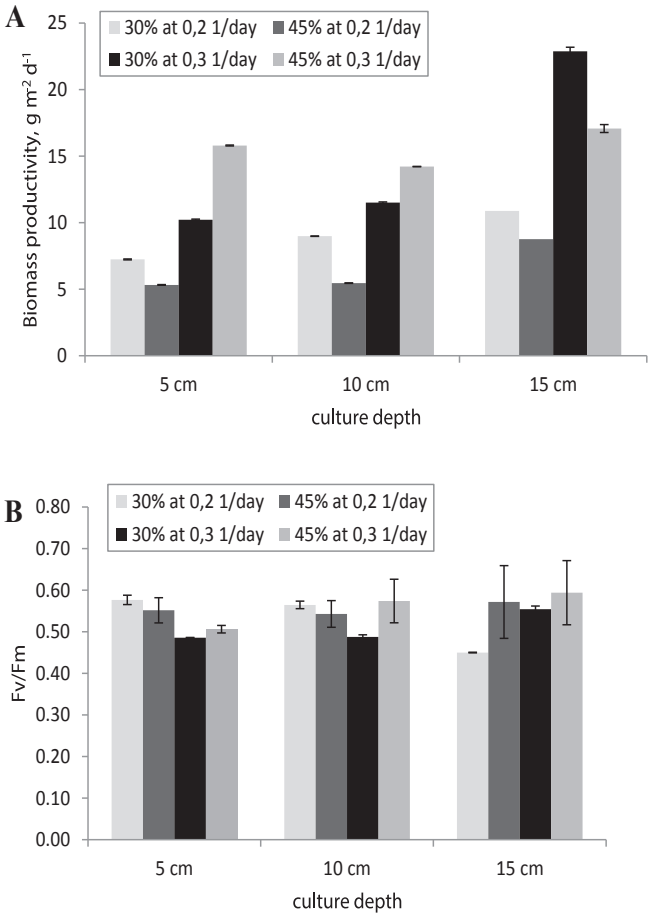

Fig. 1. Variation in biomass productivity (A) and chlorophyll fluorescence (B) of *Scenedesmus* sp. culture as a function of the centrate percentage and culture depth. Experiments were conducted in semi-continuous mode at 0.2 d^{-1} and 0.3 d^{-1}.

stated that assimilated inorganic nitrogen forms are reduced to ammonium before being incorporated into amino acids within intracellular fluid (Cai et al., 2013). Furthermore, another study showed that algae start consuming nitrate when ammonium is depleted from the medium (Maestrini et al., 1986).

From May to July, the cultures were subjected to an average impinging irradiance range of 1094.5 μE m^{-2} s^{-1} (i.e. 238 w/m^2), equivalent to 470 μE m^{-2} s^{-1} (PAR), while the CO_2 supply was ensured by injecting flue gas. The temperature of the pond and the dissolved oxygen over the course of the experiment ranged from 13.6 to 26.0 °C and 131.0% sat. to 502.4% sat., respectively (note that 100% sat correspond to the solubility of O_2 in water when it is bubbled with air).

The biomass productivity results under these conditions, as a function of the centrate dilution and the dilution rate for the different culture depths tested, are presented in Fig. 1A. For raceways operating at 5 and 10 cm culture depths, a further increase in the centrate percentage from 30% to 45% led to an increase in biomass productivity. An additional experiment was conducted at 5 cm and 60% centrate, which demonstrated that above this centrate percentage, the biomass productivity declined at this depth. On the other hand, for the raceway operated at 15 cm, 45% centrate led to culture inhibition and consequently to a decrease in biomass productivity. This could be more likely related to the increase of ammonium concentration in the medium that entailed culture growth inhibition. In fact, high ammonium concentrations are widely known to negatively affect microalgae growth. For instance, Morales-Amaral et al., (2015b) reported inhibition due to excessive ammonium at a concentration in the order of 200 mg/L for

Muriellopsis sp. and *Pseudokirchneriella subcapitata* cultures operating indoors in semi-continuous mode at 0.3 d^{-1}; He et al. (2013), on the other hand, claimed that *C. vulgaris* growth declined markedly at ammonium concentrations higher than 143 mg/L.

The maximum biomass productivity was 22.9 g m^{-2} d^{-1} at the 15 cm culture depth, at 0.3 d^{-1} and 30% centrate. This was far greater than the 4.9 g m^{-2} d^{-1} reported for the cultivation of a microalgal consortium in a raceway pond of 950 L using wastewater predominantly made up of carpet mill effluent mixed by municipal wastewater (Chinnasamy et al., 2010b), the 8.04–10.36 g m^{-2} d^{-1} biomass productivity range determined by the cultivation of a microalgae consortium in batch mode using untreated carpet industry effluent in raceway pond at a 18 cm culture depth of 500 L volume (Chinnasamy et al., 2010a). However, it was comparable to the 24 g m^{-2} d^{-1} obtained from the semi-continuous cultivation of *Scenedesmus* sp. in 12 cm deep pilot-scale raceway reactors using 30% centrate (Morales-Amaral et al., 2015a).

The optimum centrate percentage determined outdoors (30%) is lower than that determined at the laboratory scale (60%) in our previous study (Jebali et al., 2018). This could be explained by the fact that, compared to indoor experiments, outdoor culture conditions are subject to various uncontrollable parameters such as temperature and diurnal light fluctuations over the course of a single day, which might add to the stress on microalgae growth in addition to the high ammonium content in the centrate. Moreover, in this study, the ammonium content reported in the centrate (up to 628 mg/L) was too high whereas in the previous study, it was far lower, in the 264–318.7 mg/L range (Jebali et al., 2018).

In this work, the effect of two dilution rates (0.2 d^{-1} and 0.3 d^{-1}) on strain performance was investigated (Fig. 1A). For all of the centrate concentrations tested and the different culture depths studied, one can observe that the higher the dilution rate, the higher the biomass productivity. This would be explained by the fact that the culture optimal growth conditions were attained at 0.3 d^{-1} dilution rate. Thus, an adequate adjustment of the optimal dilution rate to be applied is very critical to maximize biomass productivity. Indeed, various studies have concluded that continuous culture systems allow the biomass productivity to be maximized by operating the optimal process parameter combination, which is closer to that for optimal microalgae production (McGinn et al., 2012; Osundeko and Pittman, 2014). It is worth mentioning that the optimum dilution rate, determined here at 0.3 d^{-1}, was in accordance with the results obtained in our previous study with *Scenedesmus* sp. using indoor open raceway-simulating reactors (Jebali et al., 2018).

The optimum culture depth determined in this outdoor study is in agreement with our previous results at the laboratory scale using raceway-simulating reactors, where 15 cm was the recommended culture depth to be used (Jebali et al., 2018). It should be pointed out that the culture depth is an important operational feature that affects CO_2 loss in the raceway pond (Rawat et al., 2011) as well as the light availability inside the reactor; and consequently the effective light use by microalgae to maximize photosynthetic activity (Sutherland et al., 2014). In fact, light plays a determining role in microalgae productivity through photosynthesis, when inorganic nutrients are converted to organic molecules and, ultimately, to biomass, which is a form of stored chemical energy. In dense cultures, light intensity decreases exponentially from the illuminated surface to the bottom of the reactor due to light attenuation and mutual shading (Acién Fernández et al., 2013; Sutherland et al., 2015, 2014), where cells move from one position to another receiving modulated light (Acién Fernández et al., 2013). Thus, it has been claimed that culture depth, along with biomass concentration and the turbulence regime, determine the light availability to the cell and the degree of light attenuation (Sutherland et al., 2014).

The measurement of the maximum quantum yield of photosynthesis, which reflects the maximal photochemical efficiency of photosystem II (Fv/Fm) (Fig. 1B) revealed the culture's tolerance to centrate as the culture medium as well as the culture's adaptation to outdoor conditions. It is worth noting that bacteria and other *Chlorophyceae* microalgae strains were observed by microscopic observations; however, the strain with which the reactors were inoculated was predominant.

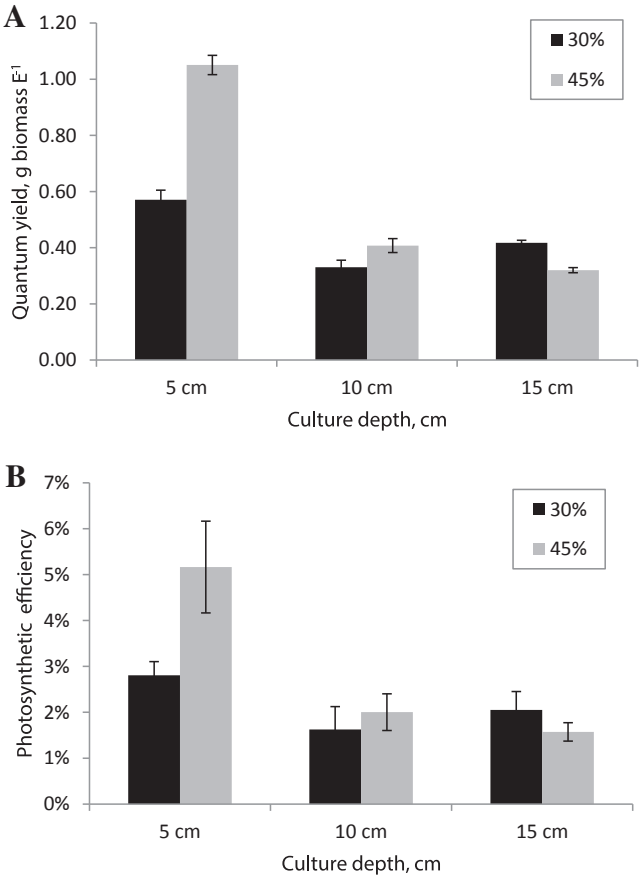

Fig. 2. Effect of centrate percentage in the culture medium and culture depth (5, 10 and 15 cm) on quantum yield (A) and photosynthetic efficiency (B) in semi-continuously fed *Scenedesmus* sp. culture at 0.3 d^{-1}.

The results obtained here prove that centrate can be an effective nutrient source option mitigating the otherwise high operational costs for culture mediums reported in large-scale microalgae cultivation. Additionally, our results showed that the cultures were tolerant to flue gas even though it contains a mixture of gases, some of which are potentially toxic to microalgae (e.g. NO_x and SO_x). This suggests that microalgae offer a promising bio-based alternative for CO_2 sequestration, one of the main greenhouse gases (GHGs) emitted into the atmosphere (Chiu et al., 2011).

The results for the optical properties of the culture are presented in Fig. 2. For the quantum yield, this was maximal up to 1.0 g E^{-1} using 45% centrate and a 5 cm culture depth; it reduced to 0.3 g E^{-1} at 45% centrate and a 15 cm culture depth indicating low light-use efficiency under these conditions (Fig. 2A). The marine strain *N. gaditana* reached a maximal value of 1.3 g E^{-1} using centrate as the culture medium under laboratory conditions (artificial light) (Sepúlveda et al., 2015). The photosynthetic efficiency (Fig. 2B) was greater at low culture depths: efficiency of up to 5.2% was recorded at a 5 cm culture depth and 45% centrate. Morales-Amaral et al. (2015a) reported a maximal photosynthetic efficiency of 9% from semi-continuously fed culture performed in thin-layer reactor of 2 cm culture depth. Furthermore, the results showed maximum light availability, 262.6 μE m^{-2} s^{-1}, at a culture depth of 15 cm and 45% centrate; this decreased to 125.5 μE m^{-2} s^{-1} at a 15 cm culture depth and 30% centrate due to the high biomass concentration under these optimal growth conditions.

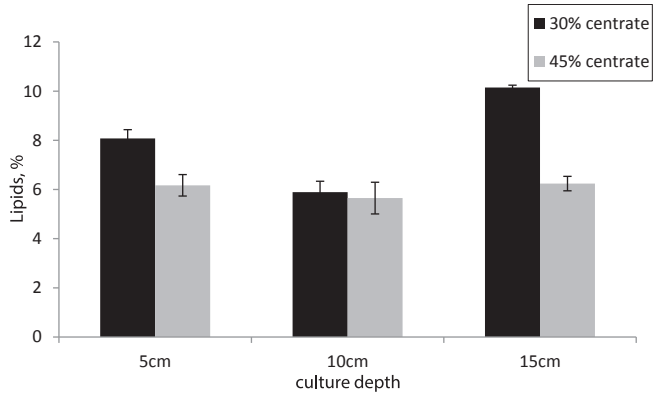

Fig. 3. Variation in the lipid content of semi-continuously cultivated *Scenedesmus* sp. at a 0.3 d^{-1} dilution rate as a function of the culture depth and centrate percentage.

Similarly, Sepúlveda et al. (2015) observed light availability decrease within the optimal centrate percentage range for *N. gaditana* growth using centrate. The extinction coefficient was maximal (0.13 $m^2 g^{-1}$) at 30% centrate and a 15 cm culture depth but reduced to 0.06 $m^2 g^{-1}$ when using 45% centrate and a 5 cm culture depth, meaning that more light had to be available under these conditions. These results fell within the range of values obtained by Morales-Amaral et al. (2015a).

3.2. Lipid content and fatty acids profile

With respect to the biomass produced, the lipid content and fatty acids profile at 0.3 d^{-1} were analyzed. Fig. 3 shows the variation in lipid content with the centrate percentage in the medium and the culture depth. The results revealed that no particular tendency was seen with regard to the centrate percentage and culture depth. The highest amount of lipids, 10.1%, which corresponded to a lipid productivity of 2.3 g $m^{-2} d^{-1}$, was determined in the 15 cm-deep pond using 30% centrate. The relatively low lipid contents obtained (Fig. 3) revealed that the centrate as culture medium was not stressful enough to trigger lipid production by microalgae which confirm the recorded data from chlorophyll fluorescence (Fv/Fm) that showed an adaptation and tolerance of the strain to this kind of wastewater. This was within lipid productivity range (8.3–15.8 mg L d^{-1}) determined by Rinna et al. (2017) who cultivated *Botryococcus braunii* strains in artificial medium, pre-treated and treated domestic wastewater and within the 8.1% range determined from *Scenedesmus obliquus* cultivation using urban wastewater in an outdoor tubular vertical photobioreactor (Batista et al., 2015). It was claimed that high lipid percentages were not usually observed in open pond microalgae cultivation; in particular, under wastewater conditions (Pittman et al., 2011), and that one should focus on high microalgae biomass productivity leading to significant total lipid productivity, which is preferable for a large-scale production system (Pittman et al., 2011).

The fatty acids profile is given in Table 2. The contents ranged from 2.3% d.wt. to 4.5% d.wt. with no particular trend observable as a function of the depth whereas it did decrease as the centrate percentage increased in the medium. The short-chain fatty acids C16:0, C18:0, C18:1n7 and C18:3n3 predominated – with a maximum 20.0% (of total fatty acids) obtained at a 15 cm pond depth and 30% centrate; 17.6% (of total fatty acids) at a 15 cm pond depth and 45% centrate; 21.5% (of total fatty acids) at a 5 cm pond depth and 60% centrate and 31.4% (of total fatty acids) at a 5 cm pond depth and 45% centrate, respectively. These fatty acid classes were similarly reported elsewhere but in different percentages, probably due to the culture conditions and differences in wastewater composition (Jebali et al., 2015; Ji et al., 2013; Sydney et al., 2011).The content of the monosaturated fatty acids C16:1 and C18:1 ranged from 34.0% to 18.0% (of total fatty acids). It has been widely reported that these monosaturated fatty acids are favorable for high-quality biofuel production in terms of their cetane number, oxidative stability and cold flow characteristics (Biller and Ross, 2011; Hoekman et al., 2012).

Table 2
Variation in the fatty acids profile of the *Scenedesmus* sp. culture in semi-continuous mode at 0.3 d^{-1} as a function of the centrate percentage in the medium and the culture depth.

F.A	15 cm pond depth		10 cm pond depth		5 cm pond depth	
	30%	45%	30%	45%	30%	45%
16:0	20.04	17.30	17.39	18.32	18.58	16.80
16:1n7	3.65	6.50	3.87	6.32	2.44	4.19
16:2n4	4.56	2.94	3.78	4.38	3.17	–
16:3n4	8.56	–	–	–	4.83	7.70
16:4n1	3.49	8.33	6.24	6.65	9.01	8.20
18:0	5.75	17.60	5.09	3.71	4.57	2.71
18:1n9	3.66	3.66	–	–	4.34	13.17
18:1n7	13.46	10.78	16.10	17.51	16.78	–
18:3n3	24.02	19.54	30.72	25.57	20.55	–
FA, %d.wt	2.59	3.05	3.24	2.36	4.43	4.28

3.3. Nutrient removal capacity of Scenedesmus sp.

Nutrient elimination processes in wastewater treatment systems can be biotic or abiotic mechanisms (Qi et al., 2018; Matamoros et al., 2015; Min et al., 2014). In this work, nitrogen and phosphorus in the inlet and outlet at steady state were analyzed in order to evaluate nutrient removal under the different cultivation conditions tested. Fig. 4A shows the variation in nitrate at the inlet and outlet, respectively, for 30% and 45% centrate as a function of the culture depth at 0.3 d^{-1} dilution rate. The results indicate that a nitrification phenomenon possibly took place in some of the assays, where the final nitrate concentration increased compared to the initial concentration. The highest nitrate outlet rate (9.1 mg/L) was observed at 0.3 d^{-1} at a 5 cm culture depth and with 30% centrate. This nitrification is due to the transformation of ammonium to nitrite by *Nitrosomonas* bacteria, and then to nitrate by *Nitrobacter* bacteria (Ruiz-Martinez et al., 2012). This phenomenon would imply that microalgae growth and consequently nitrogen up-take was lower than the growth of existing bacteria in the culture.

Fig. 4B shows the influence of the centrate percentage and the culture depth on the ammonium outlet concentration at 0.3 d^{-1}. The results revealed that the higher the inlet ammonium loading, the greater the outlet ammonium concentration. The culture depth had an effect on the final ammonium concentration, increasing as the culture depth increased. In addition, one can observe that, when using 45% centrate, the removal efficiency was enhanced, where the highest (94%) was determined at a 5 cm culture depth, higher than 77.8% obtained from the co-culture of microalgae and bacteria using fermentation wastewater as culture medium (Qi et al., 2018). In fact, it was previously stated that these parameters – pond depth and hydraulic retention time (note that dilution rate is the inverse of the hydraulic retention time) – are key operational features to adjust the culture density and improve light availability in the pond while avoid self-shading (Park et al., 2011). It is worth pointing out that ammonium volatilization possibly contributed to nitrogen removal for open outdoor cultivation though the control of pH and temperature. The data showed that no relevant values were recorded.

Regarding nitrogen removal rate, it can be observed that, in line with the biomass productivity results, the removal rate increased as the culture depth increased (Fig. 4C). Moreover, the higher the centrate percentage in the medium, the greater was the removal rate – the maximum obtained (5.2 g N $m^{-2} d^{-1}$ or 34.7 mg N $L^{-1} d^{-1}$) was with a 15 cm pond depth at 0.3 d^{-1} and 45% centrate.

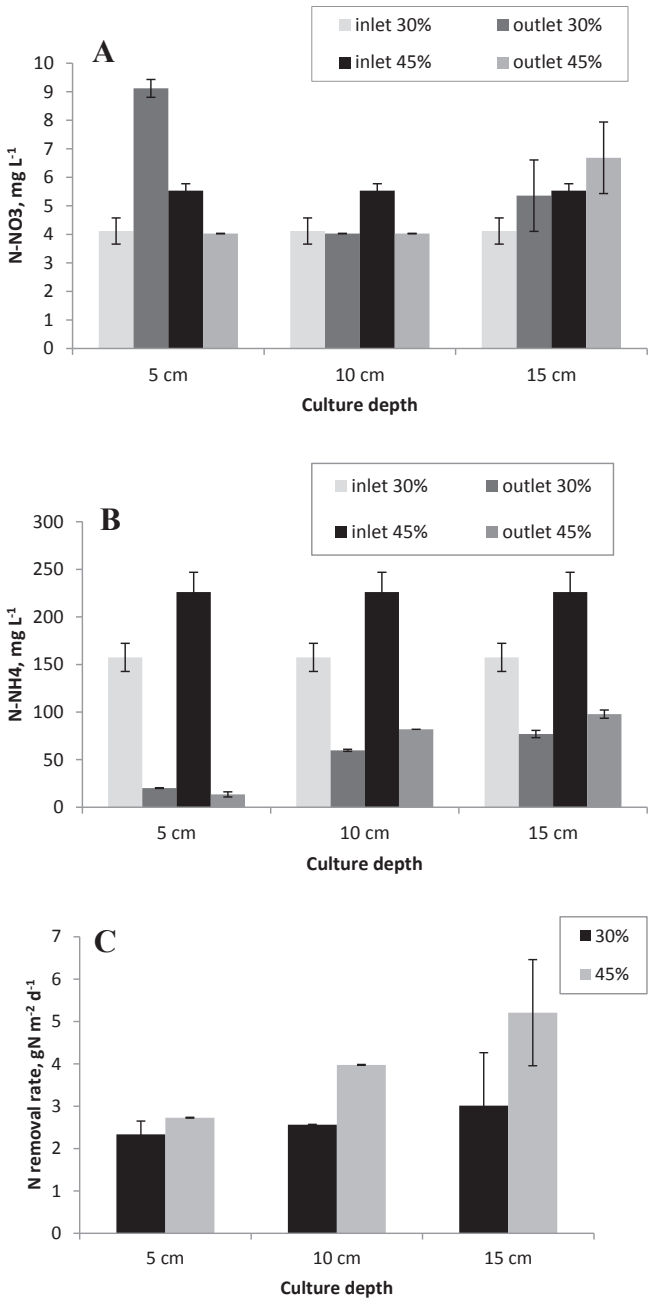

Fig. 4. Variation in nitrate (A), ammonium (B) inlet and outlet concentrations at 30% and 45% centrate and nitrogen removal rate (C) for *Scenedesmus* sp. culture as a function of the culture depth. Experiments were conducted in semi-continuous mode at 0.3 d^{-1}.

With respect to phosphorus, Fig. 5 shows the variation in the phosphorus inlet and outlet with regard to the centrate percentage and the culture depth at 0.3 d^{-1}. Similarly, the final phosphorus concentration increased as the centrate percentage and the culture depth increased. The highest phosphorus removal efficiency (90.6%) was obtained at 0.3 d^{-1} at a 5 cm culture depth and 30% centrate, higher than 45.6% determined by Qi et al. (2018) from microalgae and bacteria co-culture using fermentation wastewater.

Regarding phosphorus removal rates (Fig. 5C), the results showed that no specific pattern was obvious in correlation with the centrate percentage, whereas it increased inversely with culture depth. The maximum phosphorus removal rate, 2.3 g P m^{-2} d^{-1}, was obtained using 30% centrate at 0.3 d^{-1} with a 5 cm pond depth.

Nonetheless, the outlet concentrations for nitrogen and phosphorus

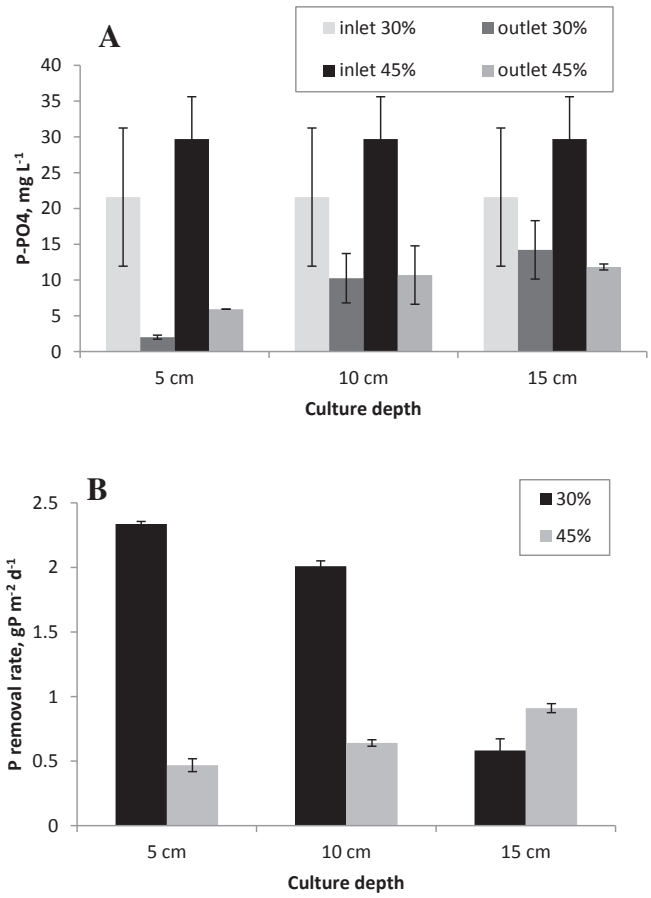

Fig. 5. Variation in phosphorus inlet and outlet at 30% and 45% centrate (A) and phosphorus removal rate (B) for *Scenedesmus* sp. culture as a function of the culture depth. Experiments were conducted in semi-continuous mode at 0.3 d^{-1}.

reported here were higher than the standards permitted by European UnionDirective 98/15/CE, which regulates water release into the environment (10 mg N/L and 1 mg P/L).

Under optimal conditions for biomass productivity (30% centrate, 0.3 d^{-1} of dilution rate and a 15 cm culture depth), up to 3.0 g N m^{-2} d^{-1} and 0.6 g P m^{-2} d^{-1} were removed. These values are within the 3.2 g N m^{-2} d^{-1} and 0.41 g P m^{-2} d^{-1} range reported from the cultivation of a microalgal-bacterial consortium using synthetic wastewater as the culture medium (Boelee et al., 2014), higher than the 1.0 g N m^{-2} d^{-1} and 0.13 g P m^{-2} d^{-1} obtained using real municipal wastewater for a microalgal consortium culture (Boelee et al., 2011) and the 2.1 g Total Kjeldhal Nitrogen m^{-2} d^{-1} determined from *Chlorella* sp. culture in a pilot-scale photobioreactor using a 1/4 harvesting rate and utilizing centrate as the culture medium (Min et al., 2011).

In the light of the results obtained here, one can conclude that, although the 5 cm shallow pond provides higher values in terms of volumetric productivity and nutrient removal rates because of the short light path, deeper ponds are more commercially viable offering high areal productivity and nutrient uptake capacity. It has also been claimed that shallow ponds might exhibit greater temperature fluctuations compared to deep ponds (Sutherland et al., 2015) whereas very deep ponds might experience light limited problems. For instance, Sutherland et al. (2014) reported that a higher treated wastewater volume and areal productivity were achieved using a 400 mm culture depth than when using a 200 mm-deep pond; whereas Silva Benavides et al. (2013) reported that limited solar radiation decreased the biomass productivity. As such, a compromise between culture depth and light

availability in the pond should be considered in open raceway reactors in order to make this technology economically viable.

To sum up, the results presented here demonstrate that using centrate as the culture medium seems to be an economically feasible and viable option for reducing microalgae production costs and, consequently, ensuring process sustainability. Besides, this would significantly contribute, on the one hand, to wastewater treatment in conventional wastewater treatment plants by offering a cost-effective and eco-friendly bioremediation approach and, on the other hand, to the agricultural sector by recycling nutrients for soil amendments. Thus, a microalgae-based system provides an integrated process for nutrient recovery via wastewater treatment as well as a potentially viable source of biomass for low-value product application such as biofertilizers, feed and biofuels.

4. Conclusion

The native *Scenedesmus* sp. seems to be a good candidate for outdoor production using centrate. The optimal culture conditions were 30% centrate, 0.3 d^{-1} and 15 cm culture depth. A maximum biomass productivity of 22.9 g m^{-2} d^{-1} was obtained; corresponding to an estimated 80 tonnes ha^{-1} $year^{-1}$, which is far greater than the productivity of oleaginous vegetal plants. The process could remove up to 10.8 tonnes N ha^{-1} $year^{-1}$ and 2.2 tonnes P ha^{-1} $year^{-1}$. Furthermore, 10.1% of lipids were determined corresponding to an estimated 8.3 tonnes ha^{-1} $year^{-1}$, along with a favorable fatty acids profile for biofuel production.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.biortech.2018.04.057.

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