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Research article

Utilization of centrate from urban wastewater plants for the production of *Scenedesmus* sp. in a raceway-simulating reactor

Ahlem Jebali [a, b], F. Gabriel Acién [b], Sami Sayadi [a], Emilio Molina-Grima [b, *]

[a] Laboratory of Environmental Bioprocesses, Sfax Centre of Biotechnology, University of Sfax, P.O. Box 1177, 3018 Sfax, Tunisia
[b] Chemical Engineering Department, University of Almería, 04120 Almería, Spain

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ABSTRACT

This work investigates the production of the native microalgae strain *Scenedesmus* sp. in semi-continuous mode at lab scale in open raceway-simulating reactors and using centrate as the culture medium. The biomass productivity and nutrient removal capacity of *Scenedesmus* sp. at different dilution rates were investigated indoors as well as its tolerance to centrate as the culture medium at different concentrations. A biomass productivity of 7.80 g/m^2 day was obtained at 200 μE/m^2 s, 5 cm culture depth, 0.30 1/day of dilution rate and 60% centrate while nitrogen and phosphorus removal rates were 1.50 g/m^2 day and 0.15 g/m^2 day, respectively. The produced biomass characterization under these conditions showed a lipid content of 12.60% d wt. along with a favorable fatty acids profile with 57.70% of total fatty acids composed of saturated and monounsaturated fatty acids. Subsequently, the effect of light intensity and culture depth on biomass productivity and nutrient uptake as well as the biochemical composition and fatty acids profile was studied using two irradiance levels (200 and 1000 μE/m^2 s) and four culture depths (5 cm, 10 cm, 15 cm and 20 cm). Under optimal conditions of 1000 μE/m^2 s, 60% centrate, 0.30 1/day dilution rate and 15 cm culture depth, a maximum biomass productivity of 22.20 g/m^2 day was obtained. Nitrogen and phosphorus removal rates of 2.00 gN/m^2 day and 0.40 gP/m^2 day, respectively, were recorded. An amount of 11.70% d wt. of lipids was determined along with a suitable fatty acids profile for biofuel production.

1. Introduction

Microalgae technology is attracting increased attention as a result of the development of renewable fuel production processes (Avagyan, 2008). At present, mass algal-based fuel commercialization is impeded due to the relatively low saponifiable lipid content of biomass feedstock as well as the techno-economic barriers and the viability concerns (Avagyan, 2017; Davis et al., 2016). These barriers are attributed to the high operation costs, due mainly to the use of fertilizers, energy and fresh water for the cultivation, which decreases the sustainability and competitiveness of microalgae–based technologies (Davis et al., 2016; Zhou et al., 2014; Zhu and Hiltunen, 2016). In fact, the current production cost range is about 5–10 €/kg of microalgae biomass (Benemann, 2013), in which the use of freshwater, fertilizers and carbon dioxide account for 23–30% of the total (Davis et al., 2016; Zhou et al., 2014). In order to effectively develop microalgae applications in low-value fields such as feed, biofertilizers and biofuels, the production cost needs to drop to 0.50 €/kg (Borowitzka and Moheimani, 2010; Chisti, 2012) and even to 0.43 €/kg in other studies (Davis et al., 2016).

Therefore, a coupled process combining waste treatment processes, particularly wastewater, to microalgae energy-rich biomass production could be an economically feasible option -microalgae cultivation in wastewater provides bioremediation while reducing the costs of algal feedstock production as well as effluent treatment (Avagyan, 2017, 2008). Indeed, wastewater algae-based treatment offers a promising route for an environmentally friendly technology (Avagyan, 2017).

Numerous studies have demonstrated that wastewater could fulfill the microalgae growth requirements for macro and micronutrients (Avagyan, 2017; Osundeko and Pittman, 2014). Microalgae can use a wide range of effluents (Avagyan, 2017). Centrate is one of the richest urban primary nutrient streams of nitrogen and phosphorus as well as of micronutrients; thus, it has been reported as potentially supporting microalgae growth (Morales-Amaral

* Corresponding author.
E-mail address: emolina@ual.es (E. Molina-Grima).

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et al., 2015a, b; Osundeko and Pittman, 2014; Zhou et al., 2012a). However, at high centrate concentrations, the nitrogen content might be toxic for microalgae, particularly in the form of ammonium, at concentrations above 100 mg/L (Collos and Harrison, 2014; Ji et al., 2014; Morales-Amaral et al., 2015a); not to mention many other compounds such as phenols, organic pollutants, pesticides and heavy metals, which at high concentrations might also inhibit growth (Avagyan, 2017; Dong et al., 2014; Muñoz et al., 2006; Muñoz and Guieysse, 2006). However, *Chlorella* has high commercial productivity in such wastewater with their water dilution to the following contents: above total N (3 g/L), NH_4 (1 g/L), chemical oxygen demand (12 gO_2/l), and biological oxygen demand (9gO_2/l) (Avagyan, 2013, 2011).

One of the key criteria for wastewater-based algae production is the selection of a robust microalgae strain that can fully adapt to such a medium, and also provide high productivity and efficient nutrient removal. The genera *Chlorella* and *Scenedesmus* are the microalgal strains most frequently used in wastewater bioremediation (Tan et al., 2015; Min et al., 2011; Batista et al., 2015; Posadas et al., 2015). Additionally, selecting an appropriate strain that presents high robustness under the various uncontrollable environmental outdoor conditions (i.e. temperature, diurnal cycles and invasive microorganisms) is a crucial step in researching economically viable, microalgal-based centrate remediation. It has been previously demonstrated the robustness of the strain used in this study, which was cultivated in untreated primary urban wastewater, highly concentrated in nutrients, yet it achieved a maximum biomass productivity of 0.9 g/L day at 0.6 1/day under a light intensity of 800 μE/m^2 s (Jebali et al., 2015).

Another key criterion is the selection of an appropriate reactor design for algae cultivation that ensures biomass production at a competitive cost for low-value products such as biofuel. Closed photobioreactors present high operation costs while open reactors, such as raceway reactors offer various advantages such as flexibility, low power consumption, simple construction and ease of operation (Acién Fernández et al., 2013; Chiaramonti et al., 2013; Rawat et al., 2013).

Nevertheless, there are problematic aspects to microalgal centrate treatment in open reactors that were poorly studied such the need for further research on the operational and environmental parameters effect.

Very few studies have researched in semi-continuously-fed cultures, the simultaneous influence of the dilution rate and centrate concentration as well as light intensity and culture depth effects on the performance of the microalgae strain in terms of productivity and nutrients removal capacity using centrate as the culture medium (Chinnasamy et al., 2010; Sepúlveda et al., 2015).

In the present study, the objective is to assess the production of microalgae using wastewater, and also contributing to recover nutrients from these effluents. To this end, indoors cultures of native *Scenedesmus* sp. were conducted in controlled conditions simulating those in real outdoor raceway reactors, and using mixtures of centrate and tap water as culture medium. Experiments were carried out in semi-continuous mode for the investigation of the simultaneous effect of dilution rate (0.04–0.40 1/day) and centrate concentration (15, 30, 45 and 60% of centrate) in order to determine the optimal combination of these two parameters for the strain's performance. It is worth noting that dilution rate is the inverse of the hydraulic retention time, the latter is the usual operational parameter used in wastewater treatment plants. Additionally, the light intensity at two levels (200 and 1000 μE/m^2 s) and four culture depths (5, 10, 15, 20 cm) were also tested at the same culture conditions. Biomass productivity, nutrients removal, as well as the biochemical composition and the saponifiable fatty acid content and profile of the produced microalgal biomass, were evaluated at steady state.

Addressing these issues is essential prior to large-scale outdoor commercial use of microalgae for centrate bioremediation and biomass production.

2. Materials and methods

2.1. Microorganism and culture media

The freshwater isolate *Scenedesmus* sp. was used for the study. This microalgae strain was selected in a previous work out of several microalgal strains potentially suitable for wastewater treatment, in which it was the top performer (Jebali et al., 2015). Culture inoculum was prepared under continuous illumination and aeration using MDM medium (Ichimura and Itoh, 1977). For the experiments, centrate from a real urban wastewater treatment plant of the city of Almería (Spain) was used as to prepare the culture medium. The centrate was obtained after separating the solids from the digestate liquid fraction resulting from the anaerobic digestion of activated sludge produced from the urban wastewater treatment. The centrate was stored in a cold room (4 °C) and then used in the subsequent experiments. Different experimental culture media were prepared, namely 15, 30, 45 and 60% v/v, by mixing tap water with the centrate. The prepared medium was sterilized to remove contaminants and to investigate the performance and nutrient removal capacity of this strain as well as its tolerance to the centrate as the culture medium. Table 1 gives the average composition of the different collected centrate batches used.

2.2. Experimental culture conditions

Experiments were run indoors using four open reactors with circular bases and opaque PVC plastic walls (diameter: 25 cm; height: 28 cm; working volume: 10 L; a volume to surface ratio V/S: 0.2 m^3/m^2). The reactors were designed to mimic the culture conditions in real raceways ponds. To that end, agitation was ensured using magnetic agitators (IKA RH basic 2, Germany) with no air used for mixing. The pH was kept within the optimum range, 7.80–8.00, by on-demand CO_2 injection at a flow rate of 0.01v/v/min. The temperature was kept constant at 25 °C by controlling the room temperature. A set of 21 W daylight fluorescent tubes (Simon Brico Daylight T5), located horizontally above the reactors, was used to artificially illuminate the vessels, simulating the circadian cycle. The light intensity was measured by a 4π quantum scalar irradiance sensor QSL-100 (Biospherical Instrument, San Diego, CA, USA).

To evaluate the performance of *Scenedesmus* sp., experiments were firstly conducted in batch mode for 6 days in the four reactors simultaneously with the four centrate dilutions in parallel, at a 5 cm culture depth and a 200 μE m^{-2} s^{-1} irradiance level, starting with an equal cell density of 1.46 10^7 cell/mL. After this, the cultures were operated in semi-continuous mode by daily diluting the cultures at different dilution rates, from 0.04 to 0.40 1/day. The second set of experiments were performed at two light intensity levels, with a maximum 200 and 1000 μE/m^2 s^{-1} to investigate the effect of light intensity on biomass productivity and nutrient removal efficiency. The other culture conditions stayed the same.

Finally, the influence of different culture depths, namely 5, 10, 15 and 20 cm, on biomass productivity and nutrient removal using the optimized culture conditions already determined (an irradiance intensity of 1000 μE m^{-2} s^{-1}, 60% centrate dilution and a dilution rate of 0.30 1/day), was investigated.

All the experiments were operated in semi-continuous mode until steady state was achieved. Once the steady state biomass

Table 1
Composition of the centrate obtained from a real wastewater treatment plant and used to prepare culture medium by mixing with freshwater at different proportions. Values represented in each experiment are the average of four centrate batches.

Nutrient	Centrate used for dilution rate selection experiment	Centrate used for light intensity and centrate percentage optimization experiment	Centrate used for culture depth selection experiment
	Concentration, mg/L		
$N\text{-}NH_4^+$	310.90 ± 40.73	318.74 ± 2.75	264.00 ± 9.24
$P\text{-}PO_4^{3-}$	119.64 ± 82.08	27.70 ± 0.01	20.40 ± 0.45
$N\text{-}NO_3^-$	13.22 ± 4.12	27.14 ± 13.46	5.46 ± 1.78

concentration for each experiment was achieved, the culture was maintained operating in these conditions for a period of time during which the total harvested culture is twice equal to the culture volume.

2.3. Determination of biomass concentration and fluorescence measurements

Samples were taken daily to measure microalgae biomass by optical density at 750 nm using a spectrophotometer (ATI UNICAM UV/Vis Spectrometer UV2, Cambridge, UK). The dry weight biomass concentration was determined by filtrating 100 mL of culture through a pre-dried 1 μm filter (Macherey-Nagel GmbH &Co.KG, Germany) and drying it in an oven at 80 °C for 24 h. In addition, chlorophyll fluorescence, as Fv/Fm, was measured daily using a fluorometer (AquaPen AP 100, Photon Systems Instruments, Drasov, The Czech Republic) in order to check the cells' physiological status. Dissolved oxygen and temperature were measured daily (Hanna Instruments, HI98193, Romania). All the analyses were carried out in duplicate and the mean value was reported.

2.4. Analytical procedure

Nutrients analyses were performed at the reactors' inlet and outlet. A 50 mL culture sample was collected and filtrated using 1 μm filters (Macherey-Nagel GmbH &Co.KG, Germany); the filtrate was used for nutrient analysis. The phosphate was measured by visible spectrophotometry through the phospho-vanado-molybdate complex (Phosphate Standard for IC: 38364). The nitrate was quantified by measuring optical density at 220 nm and 275 nm (Nitrate Standard for IC: 74246). The ammonium was measured according to the Nessler method (Ammonium standard for IC: 59755).

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 biochemical composition. Protein content was quantified according to the modified Lowry method proposed by Herbert et al. (1971). 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). Ash was determined by incinerating a 100 mg sample in an oven at 450 °C for 48 h. The carbohydrate content was estimated by subtracting the sum of the other fraction percentages (ash, lipids and proteins) from 100. All of the analyses were carried out in duplicate and the mean value was reported.

2.5. Mass transfer and mixing time

The determination of the mass transfer coefficient (K_{La}) and the mixing time was carried out to characterize the reactors. The K_{La} experiment was conducted as described by Mendoza et al. (2013b). The variation in dissolved oxygen (DO) overtime was assumed to be a function of the mass transfer coefficient (K_{La}) and the driving force ($[O_2^*]$ - $[O_2]$) according to equation (1):

$$\frac{d[O_2]}{dt} = K_{La} \cdot \left(\left[O_2^* \right] - [O_2] \right) \quad (1)$$

where K_{La} is the mass transfer coefficient (s^{-1}), $[O_2]$ is the DO concentration (mg l^{-1}), $[O_2^*]$ is the equilibrium DO concentration (mg l^{-1}) and t is time (s). The integration of this equation between time zero and time t, the mass transfer coefficient, can be obtained by (Eq. (2)) (Chisti, 1999):

$$K_{La} = \frac{\ln\left(\left(\frac{[O_2^*] - [O_2]_{t=0}}{[O_2^*] - [O_2]} \right) \right)}{t} \quad (2)$$

For mixing time determination, the experiment was conducted by adding pulses of saturated sodium hydroxide under the same culture conditions; the variation in pH was then recorded. Mixing time was defined as the time required for variations in pH to reach a final stable value. The software acquisition data used was StudioData.

3. Results

To evaluate the performance and robustness of the native microalgae strain *Scenedesmus* sp. to centrate, different tap water and centrate mixtures were prepared and different dilution rates were applied (from 0.041/day to 0.40 1/day) using a culture depth of 5 cm. Based on the literature reviews (Morales-Amaral et al., 2015a, b; Sepúlveda et al., 2015), a range of centrate concentrations (15%, 30%, 45% and 60% centrate v/v) were investigated. Table 1 shows the average composition of the centrate used for the experiments. The phosphorus concentration varied between 20.40 and 119.60 mg/L. The $N\text{-}NH_4^+$ content was in the 264.00–318.74 mg/L range. Variations in nutrient concentration of the centrate were related to the operational conditions of the urban wastewater treatment plant, and real centrate was used in this study to know the existence of these variations in order to confirm the reliability of whatever system using this type of effluents.

The characteristics of the centrate indicate that it could adequately provide most of the essential nutrients for algal growth, as previously reported by several authors (Morales-Amaral et al., 2015a, b; Osundeko and Pittman, 2014; Zhou et al., 2012a). In fact, using centrate as the sole nutrient source for microalgae biomass production offers dual returns: (1) to substitute the use of non-sustainable and expensive fertilizers, which are considered a burden in a mass algal production system and (2) to treat this type of residue and produce microalgae biomass that could be used for a range of low cost applications. At present, centrate is recycled in wastewater treatment plant by sending it to the head of the process for depuration; this makes the whole process more costly and energy consuming.

Additionally, the gas-liquid mass transfer capacity and the fluid dynamic within the reactor used were characterized. The mass transfer and mixing time determined were 4.72 h^{-1} and 13 s, respectively. Microscopic observations of the different cultures tested were performed during the experiment and showed no contamination by other microalgae strains.

3.1. Optimization of the dilution rate and centrate concentration

Fig. 1 depicts the biomass concentration, productivity and fluorescence of chlorophylls (Fv/Fm) variation during the experiment as a function of the dilution rate for the various centrate percentages used. The results demonstrated a typical variation in the dilution rate for light-limited cultures, in which the biomass concentration decreases as the dilution rate increases. The biomass concentration increased with the centrate concentration (Fig. 1A). The biomass productivities, in terms of volumetric and areal productivity, were in the 0.04–0.16 g/L day and 2.10–7.80 g/m^2 day range, respectively (Fig. 1B). It is worth noting that ash content was ranging from 3 to 12% d wt.

The highest biomass productivity obtained under the conditions of 200 μE/m^2 s and 5 cm culture depth (7.80 g/m^2 day) was obtained at 0.30 day^{-1} and for a 60% centrate dilution. Thus, this dilution rate was selected for further experiments.

The physiological status of the cells was checked daily during the experiment and the data at steady state are displayed in Fig. 1C. The results show that the Fv/Fm remained almost constant for the first three dilution rates and centrate levels; except at 0.40 day^{-1}, where a decrease was noticed for all the centrate dilutions used. It is worth noting that at 60% centrate, the Fv/Fm was up to the 0.65–0.69 range, which illustrates this strain's considerable tolerance to such a high centrate concentration compared to some strains in other studies.

The biochemical composition of the biomass was evaluated at steady state and the data are shown in Fig. 2. The results revealed that the carbohydrate content presented the main biomass fraction at the different dilution rates and centrate levels tested (Fig. 2A). The carbohydrate content varied from 49.00 to 59.80% (DW) with no significant influence from the dilution rate or centrate concentration being noticed. Regarding protein content, values varied with no direct relationship being observed to the dilution rate (Fig. 2B).

As regards lipid content, a decrease was noticed in the lipid fraction as the dilution rate increased for 15% and 30%; whereas for 45 and 60%, this tendency was not observed (Fig. 2C). The analysis of fatty acid content and the lipid fraction profile for the different dilution rates and centrate concentrations tested was conducted at steady state. The results are summarized in Table 2. For the same centrate concentration, the fatty acid content decreased as the dilution rate increased, except at 0.40 1/day. For the different dilution rates and centrate levels, the fatty acid profile was mainly composed of short-chain fatty acids; namely, C16:0, C18:3n3, C 18:0, C18:1n9, C16:3n4 and C16:3n4. There was a predominance of C16:0 where the total fatty acid values ranged from 20.50 to 14.50%, while a considerable amount of C 18:0 (7.20–11.20% of total fatty acids) was also observed. Furthermore, a significant amount of C18:2n6 at 0.04, 0.14 and 0.40 1/day was determined although this fatty acid was not detected at 0.30 1/day.

In the present study, nutrient removal analyses were conducted at steady state. Fig. S1 (supplementary materials) represents the nitrogen removal efficiency with the centrate percentage tested and the dilution rate applied. The variations of N-NH_4^+ at the inlet and outlet streams are presented in Fig. S1 in supplementary material. The highest N-NH_4^+ depuration (97.00%) was obtained at 0.04 1/day and 45% centrate whereas the lowest (25.80%) was at 0.40 1/day and 15% centrate.

For a fair comparison of the strains and reactor systems used, the recommendable parameter to study carefully in nutrient analysis data is the removal rate capacity determined in continuous mode since depuration is simply a function of the initial nutrient concentration supplied. Indeed, it is often claimed that working in continuous mode offers tighter control and a more accurate idea concerning the optimal combination of operational parameters to be employed prior to actual commercial microalgae production.

Fig. 3A displays the nitrogen removal rates as a function of the dilution rate and the different centrate mixtures tested. The results demonstrate that, for all the assays, the removal rates were lower, the lower the centrate dilution tested, and increased when the applied dilution rate increased—this correlates positively to the biomass productivity data since maximal productivities were achieved at high centrate percentages (45 and 60%) and dilution rates (0.30–0.40 1/day). The highest removal capacity was obtained at 0.40 1/day and 60% centrate while the lowest was at 0.04 1/day and

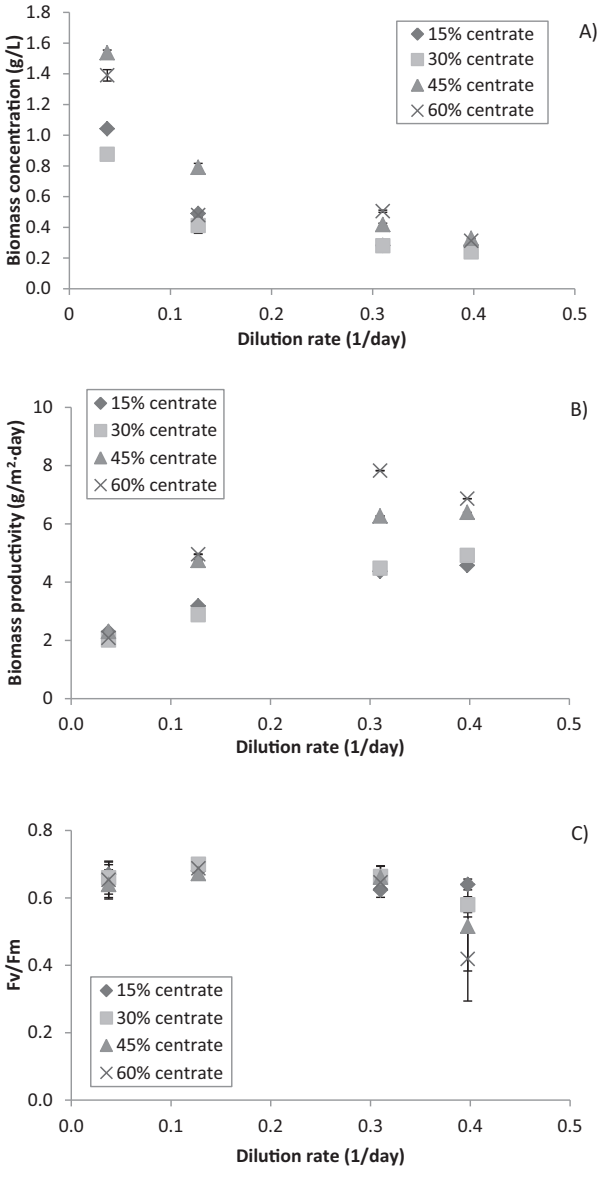

Fig. 1. Variations in biomass concentrations (A), productivities (B) and photosynthetic activities Fv/Fm (C), at steady state for the different centrate concentrations used as a function of the dilution rate. Maximum impinging irradiance 200 $\mu Em^{-2}s^{-1}$. Data values are means (±SE) of duplicate samples.

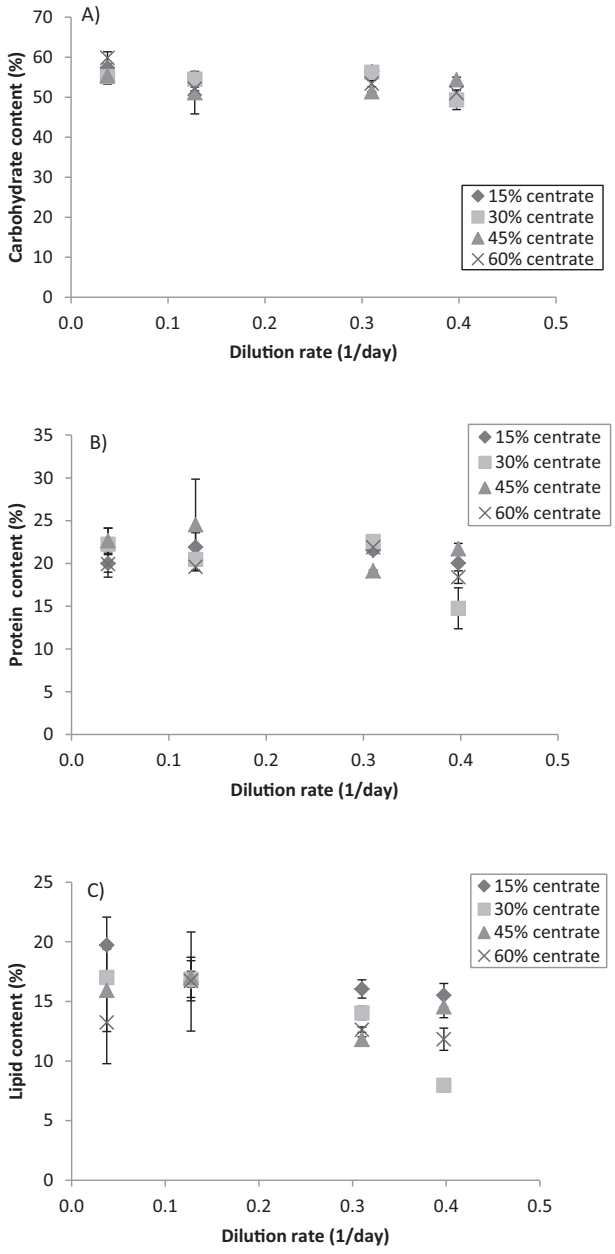

Fig. 2. Biochemical composition of *Scenedesmus* sp. at steady state as a function of different centrate concentrations and dilution rates: (A) lipid content, (B) protein content and (C) carbohydrate content. Maximum impinging irradiance, 200 μE $m^{-2}s^{-1}$.

15% centrate.

Phosphorus is another primary nutrient that plays a significant role in microalgae cell growth and metabolism. With respect to phosphorus elimination, analyses at steady state were performed. Variations in P-PO_4^{3-} concentrations at the inlet and outlet streams are shown in Fig. S2 (supplementary materials). The results show a variable trend as well as low phosphorus depuration from the different centrate mixtures used, in which no direct relationship was observed between phosphorus depuration, centrate concentration and dilution rate. Additionally, the P-PO_4^{3-} outlet concentrations were always high in all the assays, and nearly equal to those at the inlet, except at low dilution rates where the elimination efficiency was higher (relatively)-probably due to the high retention time. In fact, the highest phosphorus depuration percentage (58.05%) was reached at 0.04 1/day and 30% centrate.

The data regarding nitrogen removal rates for the centrate percentage tested and the dilution rate applied are depicted in Fig. 3B. Similar to the nitrogen results, the removal capacity increased as the dilution rate increased, and high values were obtained at high centrate percentages. The highest removal rate was recorded for 30 and 60% centrate at 0.40 1/day.

3.2. Optimization of light intensity and culture depth

The centrate compositions used in these two sets of experiments are listed in Table 1.

3.2.1. Optimization of light intensity

The effect of light intensity on biomass productivity and photosynthetic efficiency for the various experiments performed in semi-continuous mode at 0.30 day^{-1}, and using different centrate concentrations, is illustrated in Fig. 4. Results revealed that, for each light intensity level tested, the biomass productivity obviously improved as the centrate dilution increased. The highest biomass productivity (15.90 g/m^2 day corresponding to 0.30 g/L day) was determined under a light intensity of 1000 μE/m^2 s, a 5 cm culture depth and a centrate dilution of 60%; this is more than twice the productivity (7.80 g/m^2 day) obtained under the same conditions with alight intensity of 200 μE/m^2 s.

The photosynthetic activity measured as Fv/Fm data is depicted in Fig. 4B. The values did not show any stress on the cultures at the different centrate concentrations applied. At 60% centrate dilution, the ammonium concentration reached 230.00 mg/L; nevertheless, no stress was observed caused by the different media used and the strain fully adapted to it while the centrate sufficiently supported microalgae growth.

The harvested biomass was also characterized at steady state and the results are shown in Fig. 5. The protein content was lower, the higher the light intensity that was applied; this was the case for all the centrate concentrations tested (Fig. 5B). In contrast, the carbohydrate contents followed an opposite trend, increasing as the light intensity increased (Fig. 5C).

Similarly, the effect of light on lipid content was noticeable, in that the total lipid content was promoted by a decrease of light intensity for all the centrate dilutions tested (Fig. 5A). The highest lipid amount was recorded at a 15% centrate dilution and 200 μE/m^2 s (16.0%). At a 60% centrate dilution and 1000 μE/m^2 s, it was 10.5% with a lipid productivity of 33.30 mg/L day (or 1.66 g/m^2 day); these correspond to the conditions for highest biomass productivity.

Regarding the fatty acid content, its data and profile are given in Table 3. The content ranged from 2.90 to 4.90% d wt with light having no clear effect, whereas the fatty acid profile showed a slightly higher saturated and unsaturated fatty acid content under low light compared to high light, that showed a slight increase content in polyunsaturated fatty acids (C16:3-C18:3). However, a favorable fatty acid profile for C16-C18 (51.80% of total fatty acids) was observed at a 60% centrate dilution and 1000 μE/m^2 s - the optimal conditions for biomass productivity.

The influence of light irradiance on ammonium depuration and nitrogen removal rates at the optimal dilution rate (0.30 day^{-1}) was determined. The results show that ammonium depuration was higher, the higher the irradiance (except at the 30% centrate level), which correlates with the biomass productivity data (Fig. S3A, supplementary materials). At low irradiance, the nitrogen removal rates increased as centrate concentration increased but a further increase in centrate concentration from 45 to 60% did not promote a nitrogen removal rate increase (Fig. 6A). At high irradiance, the nitrogen removal rates increased consistently with the ascending centrate concentration to reach a high value of 2 g/m^2 day

Table 2
Summary of fatty acid (F.A) profiles and contents of *Scenedesmus* sp. at steady state, cultivated at different centrate concentrations in the medium (15, 30, 45 and 60%) and at different dilution rates (0.04–0.40 1/day). Data values are means (±SE) of duplicate samples.

F.A	0.04 d^{-1}				0.14 d^{-1}				0.30 d^{-1}				0.40 d^{-1}			
	15%	30%	45%	60%	15%	30%	45%	60%	15%	30%	45%	60%	15%	30%	45%	60%
16:0	17.40	15.80	15.60	15.00	17.70	16.00	15.40	15.50	20.50	18.70	17.30	19.00	17.00	15.90	15.30	14.50
16:1n7	3.10	4.30	4.50	4.30	3.90	3.80	3.80	3.50	4.00	5.90	4.60	5.50	3.90	3.10	3.70	7.50
16:2n4	2.70	3.00	2.90	2.50	3.00	2.50	3.40	2.20	10.60	13.90	12.30	11.40	2.60	4.20	4.80	2.40
16:3n4	2.10	1.20	1.50	2.30	10.40	14.80	13.30	16.20	10.60	7.70	8.10	9.00	2.00	1.50	1.80	1.90
16:4n1	8.70	11.30	10.70	10.50	–	–	–	–	–	–	–	–	15.00	11.40	11.90	9.50
18:0	11.10	9.80	8.40	9.00	10.80	8.40	9.00	7.20	9.30	7.90	9.20	9.30	8.40	10.60	11.30	9.80
18:1n9	1.00	1.10	1.20	1.30	0.60	0.70	0.80	1.00	20.60	22.40	24.50	23.80	1.40	1.90	1.30	1.90
18:1n7	14.80	12.30	12.70	10.50	11.60	9.70	12.10	14.50	–	–	–	–	7.40	7.50	10.60	8.40
18:2n6	20.20	19.10	20.20	21.60	16.00	17.30	15.20	19.50	–	–	–	–	14.20	29.60	18.00	24.00
18:3n3	2.60	0.90	1.60	1.60	1.80	1.70	1.60	2.00	4.20	2.90	2.60	3.30	2.50	2.00	1.70	1.40
FA, %d wt	5.70 ± 0.08	4.30 ± 0.05	4.40 ± 0.04	4.40 ± 0.12	4.90 ± 0.02	3.60 ± 0.23	3.60 ± 0.16	3.20 ± 0.10	4.40 ± 0.00	3.10 ± 0.09	3.30 ± 0.00	3.60 ± 0.13	3.00 ± 0.02	3.80 ± 0.30	4.00 ± 0.00	4.40 ± 0.10

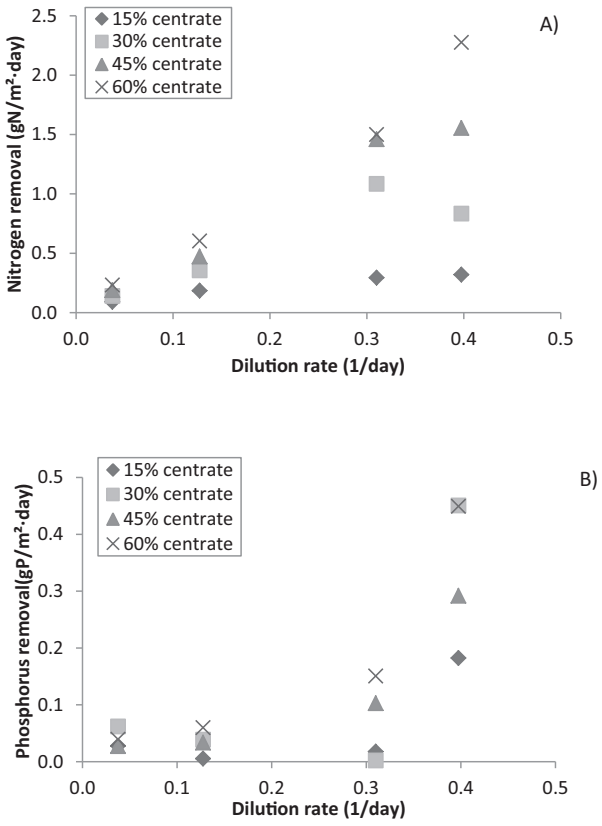

Fig. 3. Nitrogen (A) and phosphorus (B) removal rates determined at steady state for the different centrate concentrations used as a function of the dilution rate. Culture performance in semi-continuous mode. Maximum impinging irradiance, 200 μE $m^{-2}s^{-1}$.

corresponding to 39.90 mg N/L day (for 60% centrate and 1000 μE/m^2 s irradiance).

Regarding phosphorus removal, the analysis data performed at steady state are presented in Fig. 6B. The phosphorus removal efficiencies varied with media composition and light intensity - at the upper irradiance level, these were greater, the higher the centrate concentration (Fig. S3B, supplementary materials). The highest P-PO_4^{3-} removal efficiency was only (52.50%) at 1000 μE/m^2 s for an initial P-PO_4^{3-} concentration of 17.10 mg/L (60% centrate). At the other centrate concentrations, both at high and low light, depuration results were mostly less than 36%. Unexpectedly, the highest phosphorus removal rate was almost the same at 200 and 1000 μE/m^2 s at 0.15 and 0.14 g/m^2 day, respectively - at the lower light intensity, this increased as the centrate concentration increased (Fig. 6B).

3.2.2. Optimization of culture depth

To further evaluate the potential of the native strain for cultivation in an open raceway, another critical criterion that should be considered, culture depth, was investigated in semi-continuous mode at 0.30 day^{-1}.

Fig. 4C presents the variation in biomass productivity as a function of the culture depth tested. The maximum biomass productivity (22.20 g/m^2 day) was attained at 15 cm.

The photosynthetic activity measurements of the various cultures (Fig. 4D) were constant and similar for the different experiments, indicating that no growth inhibition occurred despite the high centrate percentage (60%) used.

The effect of culture depth on the biochemical composition of

the harvested biomass at steady state was determined and the results are reported in Fig. 5D. Regarding the protein content, one can observe that the highest amount (26.40% d wt) was obtained at a 10 cm culture depth while the carbohydrate contents decreased as the culture depth increased - down to 30.30% d wt at a 20 cm culture depth. As regards the lipid content, this increased as the culture depth increased (except for a slight decrease observed at 15 cm)- the maximum amount reached was 19.30% d wt. The fatty acid content and profile were examined and the data are presented in Table 4. The profile showed the dominance of C16:0, C16:2n4 and C18:1n9. The algal methyl esters were mainly saturated and monounsaturated fatty acids constituting more than 50% of the total fatty acids for the different culture depths studied -as this parameter increased, the total fatty acids increased - reaching 60.40% at 15 cm, the best culture depth for biomass productivity.

As far as the nitrogen and phosphorus removal was concerned, analyses of these nutrients inlet and outlet concentrations at steady state were performed. The variation in ammonium depuration as a function of culture depth is illustrated in Fig. S4A in supplementary materials. The results show that ammonium elimination decreased as the culture depth increased; the highest value obtained was at 5 cm (97.60%).

Regarding phosphorus, the results are provided in Fig. S4B in supplementary materials. Phosphorus elimination was not correlated to ammonium elimination; to the contrary, it increased as the culture depth increased, and the highest depuration (80%) was obtained at the 15 cm culture depth (Fig. S4B, supplementary materials). The phosphorus removal rates ranged from 0.10 to 0.40 gP/m^2 day, increasing as the culture depth increased (Fig. 6D).

4. Discussion

To determine the appropriate dilution rate to be used for ensuring both the highest microalgae productivity and efficient nutrient removal, as well as to study the tolerance of the *Scenedesmus* sp. strain to the centrate concentration, different combinations of these two parameters -the dilution rate (0.04–0.40 1/day) and the centrate percentage (15–60%) were applied. The experiments were performed in newly designed open reactors simulating the cultivation conditions in raceways, at a maximal impinging irradiance of 200 $\mu E\, m^{-2}\, s^{-1}$ during the circadian cycle and with 5 cm of culture depth.

The mixing time for these reactors was 13 s, which showed vertical mixing similar to that previously determined for raceway reactors (Mendoza et al., 2013a). Moreover, this mixing time is much lower than characteristics time of growth (days) or dilution (h), thus ensuring perfect mixing conditions from the biomass and culture medium composition point of view. Regarding the mass transfer coefficient, the value determined for the raceway-simulating reactor was 4.72 h^{-1}, which is in the value range reported for a real-scale open raceway reactor (Mendoza et al., 2013b). This value can limit the oxygen desorption if high oxygen production rates, and consequently high biomass productivities, were achieved into the culture system. Thus, the dissolved oxygen concentration can increase up to 13 mg/L (163 %Sat.) at biomass productivities up to 0.1 g/L·day, and up to 18 mg/L (225 %Sat.) at biomass productivities up to 0.2 g/L·day. Because the highest biomass productivity measured at 5 cm culture depth and 200 $\mu E/m^2$ s, was 0.16 g/L·day the maximum dissolved oxygen concentration achievable was 16 mg/L (200 %Sat.), thus confirming the potential existence of problems related to dissolved oxygen accumulation excess.

4.1. Effect of dilution rate and centrate percentage

Productivities ranging from 2.10 to 7.80 g/m^2 day were obtained (Fig. 1). These productivities are lower than those found in some studies, but they are within the range of values reported elsewhere (Table 5) The highest biomass productivity here recorded (7.80 g/m^2 day) at 200 $\mu E/m^2$ s and 5 cm culture depth is higher than reported in previous works (Table 5).

On one hand, this type of reactor suffers from certain limitations such as high dissolved oxygen accumulation (Acién Fernández et al., 2013). Since there was no air bubbling to remove the dissolved oxygen produced in this reactor system, there may have been an inhibitory effect on photosynthesis (Mendoza et al., 2013b; Molina et al., 2001) and consequently on productivity.

On the other hand, these low productivities could also be largely attributed to the low light impinging intensity (200 $\mu E/m^2$ s) utilized in the experiments rather than to an inhibiting effect due to using centrate as the culture medium; given that the results showed biomass productivity increasing along with an increase in centrate percentage in the medium, or in relation to the dilution rate imposed.

No growth inhibition was observed at high centrate percentage as demonstrated from chlorophyll fluorescence results (Fig. 1C). For instance, Osundeko and Pittman (2014) observed a detrimental effect at 40% centrate on the growth of *Parachlorella hussii* and *Chlorella letuoviridis* strains at lab scale in semi-continuous mode, whilst growth inhibition was recorded for the other strains studied. Similarly, Morales-Amaral et al. (2015b), who cultivated *Pseudokirchneriella subcapitata* and *Muriellopsis* sp. in semi-continuous mode at 0.3 1/day, reported that chlorophyll fluorescence of *P. subcapitata* went down from 0.6 to 0.49 when using 60% centrate.

The biochemical composition of the produced biomass was characterized (Fig. 2). The sugar content under the tested conditions was higher than the 21.9–42.6% reported by Batista et al. (2015), and comparable to that found by Jebali et al. (2015). The protein amounts obtained in this study were lower than those found in other works (Sepúlveda et al., 2015) and comparable to those obtained by Gómez et al. (2013) using domestic wastewater. Regarding lipid content, it is interesting to note that: (1) the highest lipid content was achieved at the lowest dilution rate tested (0.04 1/day); and (2) that no lipid content enhancement was observed as the centrate concentration increased (Fig. 2C), indicating that the medium used was not sufficiently stressful to trigger lipid accumulation, as demonstrated in many studies conducted on cultures under stress conditions (Xin et al., 2010). Comparable lipid contents were obtained by Batista et al. (2015) and Arbib et al. (2014) but higher amounts were determined in other previous studies. For example, a lipid content in the 20–42% range was reported from the cultivation of *C. vulgaris* in a semi-continuously-operated column aeration photobioreactor using artificial wastewater (Feng et al., 2011); while Ji et al. (2013) reported a lipid range of 22–32% cultivating different strains in batch mode using tertiary-treated municipal wastewater, with or without CO_2; and Sydney et al. (2011) obtained a lipid amount of 36.14% from *Botryoccocus braunii* LEM 14 produced in secondary-treated effluent after 14 days batch mode cultivation. However, as claimed by the authors, these results were relevant because cultures were grown under nutrient-deprived conditions. It is important to note that while lipid accumulation drastically increases under nutrient-limited conditions, biomass productivity increases under nutrient-sufficient conditions. Several studies have concluded that biomass productivity should be the focus of microalgae cultivation, rather than lipid content, to achieve viable biofuel production (Pittman et al., 2011; Sutherland et al., 2015).

Analyses of fatty acids were performed (Table 2). Similarly, these

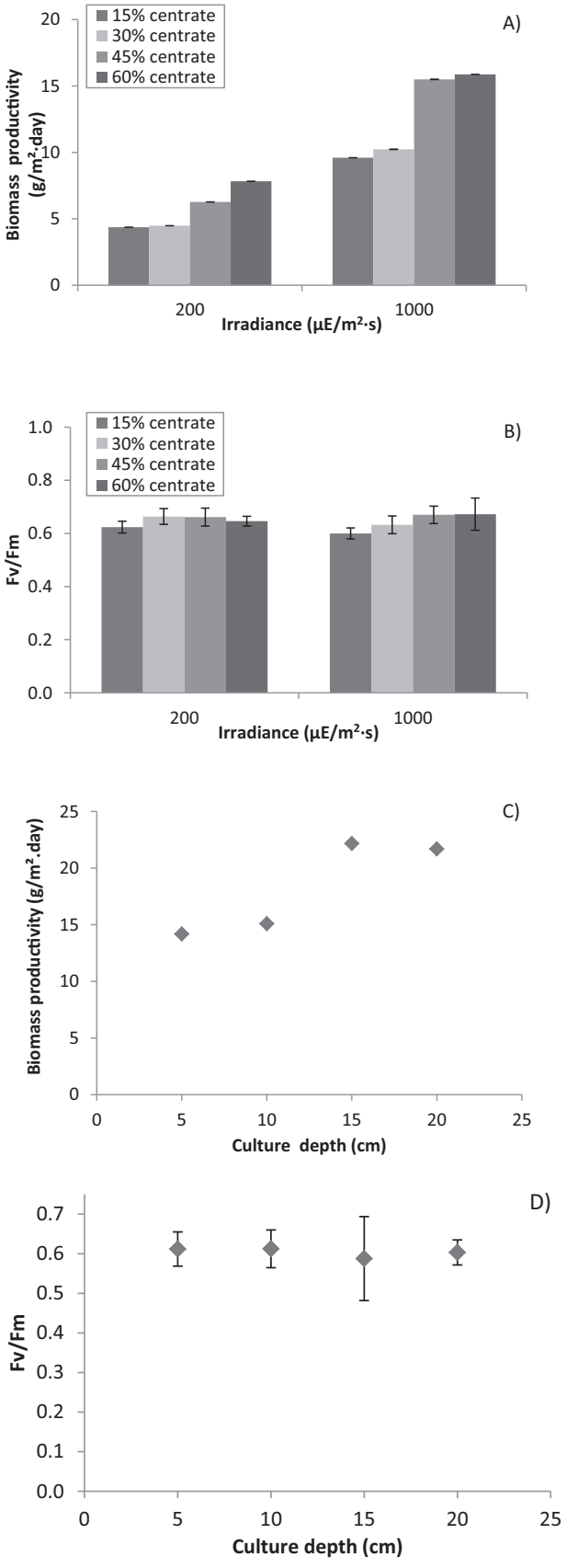

Fig. 4. Semi-continuously-fed culture of *Scenedesmus* sp. at 0.30 1/day. Effect of light intensity using different centrate concentrations (A and B) and effect of culture depth (C and D) on nitrogen and phosphorus removal rates, respectively. Maximum impinging irradiances, 200 and 1000 µE $m^{-2}s^{-1}$.

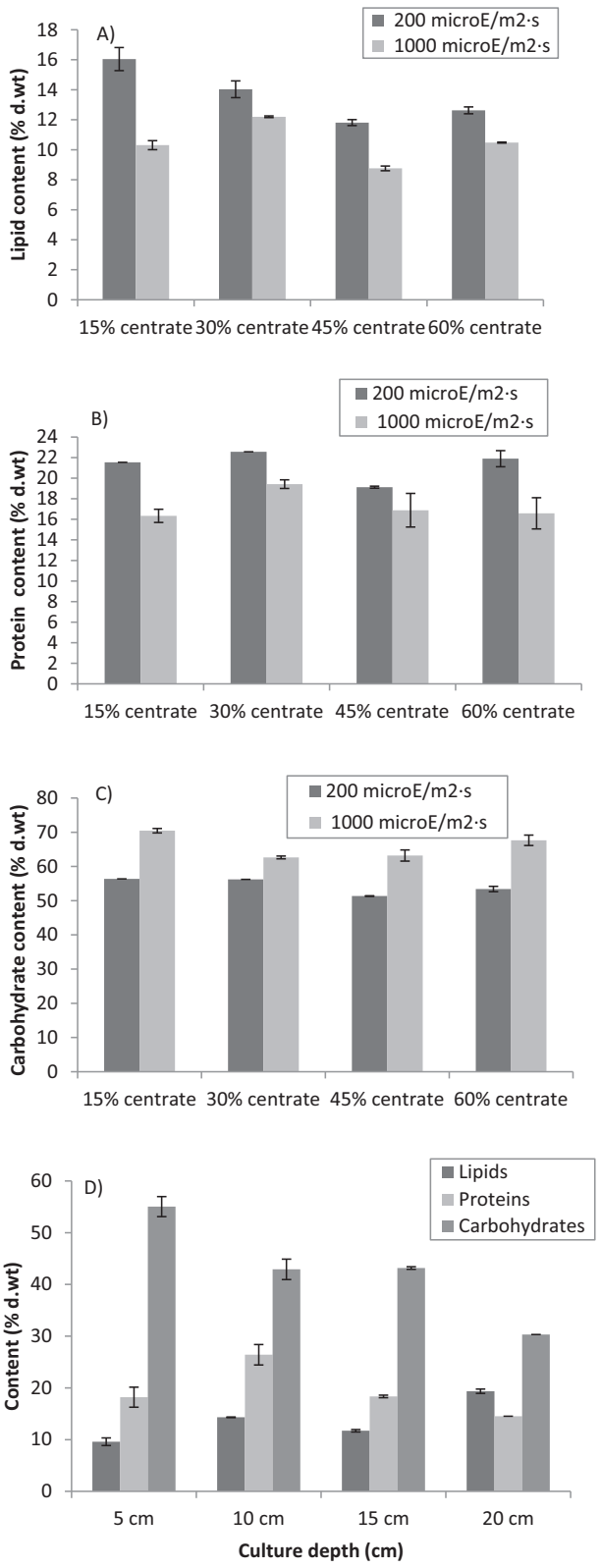

Fig. 5. Variations in the biochemical composition (A) Lipid content, (B) Protein content and (C) carbohydrate content (Maximum impinging irradiances, 200 and 1000 µE $m^{-2}s^{-1}$) as a function of light intensity using different centrate concentrations and of culture depth (C) (Maximum impinging irradiance, 1000 µE $m^{-2}s^{-1}$). Culture of *Scenedesmus* sp. was in semi-continuous mode at 0.30 1/day.

Table 3
Fatty acid profile variation as a function of the centrate percentage in the medium. Experiments were performed in semi-continuous mode (0.30 1/day) at two light intensity levels (200 and 1000 μE/m^2 s). Data values are means (±SE) of duplicate samples.

F.A.	200 μE/m^2 s				1000 μE/m^2 s			
	15%	30%	45%	60%	15%	30%	45%	60%
16:0	20.50	18.70	17.30	19.00	23.30	19.70	21.30	22.90
16:1n7	4.00	5.90	4.60	5.50	–	–	–	–
16:2n4	10.60	13.90	12.30	11.40	15.50	15.60	9.20	9.60
16:3n4	10.60	7.70	8.10	9.00	10.90	8.60	18.90	19.40
16:4n1	–	–	–	–	–	–	–	–
18:0	9.30	7.90	9.20	9.30	11.20	11.80	11.90	10.60
18:1n9	20.60	22.40	24.50	23.80	17.90	18.90	18.90	18.30
18:1n7	–	–	–	–	–	–	–	–
18:2n6	–	–	–	–	–	–	–	–
18:3n3	4.20	2.90	2.60	3.30	5.90	3.60	3.90	3.70
FA, %d wt	4.40 ± 0.00	3.10 ± 0.09	3.30 ± 0.00	3.60 ± 0.13	3.10 ± 0.08	2.90 ± 0.02	4.90 ± 0.31	4.40 ± 0.07

fatty acid classes have been determined in previous studies, at different amounts depending on the various wastewater compositions as well as the culture conditions (Jebali et al., 2015; Ji et al., 2013; Sydney et al., 2011). At the dilution rate (0.30 1/day) and centrate percentage (60%) ensuring optimal biomass productivity, the lipid content was 12.60% whilst the saturated and monosaturated fatty acid content accounted for 57.70% of total fatty acids.

The mechanisms for macro and micro-contaminant elimination in microalgae-based wastewater systems are mainly physiochemical/abiotic processes such as sorption, photodegradation, precipitation and volatilization and biotic processes including algal up-take and biodegradation (Matamoros et al., 2015; Min et al., 2014; Tan et al., 2015). The data of ammonium removal efficiency obtained are in the range of those reported in the literature (Morales-Amaral et al., 2015a, b; Zhou et al., 2012a, 2012b) and higher than those recounted in some other studies (Table 5). For instance, at 0.30 1/day and at an irradiance of 200 μE/m^2 s, the nitrogen removal efficiencies here reported were 68% and 51% for 151.10 and for 217.50 mg/L initial ammonium concentration, respectively (Fig. S1 A). This was higher than the values of around 25% and 40% at 300 and 500 μE/m^2 s, respectively, determined by Sepúlveda et al. (2015), who investigated the cultivation of *N. gaditana* at 0.25 1/day with an initial ammonium concentration of 191.5 mg/L. Regarding nitrogen removal rates (Fig. 3A), the values determined here are comparable to those previously reported in the literature (Table 5).

According to many studies, the phosphorus-removing mechanisms are either assimilation by microalgae or precipitation due to high pH (Ruiz-Martinez et al., 2012; Zhou et al., 2012b). Low phosphorus removal rates were determined (Fig. 3B). During this study, the pH was controlled at 8.00 -thus, chemical precipitation was unlikely and the phosphorus removal was presumed to be due to microalgal metabolic assimilation. Therefore, this low removal rate might indicate that phosphorus was provided in excess with respect to the nitrogen supply. Comparative low phosphorus elimination values and similar behavior was observed in previous studies. Morales-Amaral et al. (2015a) who conducted outdoor raceway cultivation of *Scenedesmus*. sp using centrate as the culture medium (10% phosphorus removal efficiency) and Posadas et al. (2014), who investigated nutrient removal from domestic water using an open algal-bacterial biofilm pond (49 and 27% phosphorus removal efficiencies were determined at hydraulic retention times of 7 and 5 days, equivalent to 0.14 1/day and 0.20 1/day of dilution rates, respectively).

The highest phosphorus removal rates reported here at 30 and 60% centrate at 0.40 1/day are similar to those previously determined (Table 5).

4.2. Light and culture depth effects

To investigate the effect of light and culture depth on biomass productivity and nutrient removal efficiency, four experiments were conducted using four different centrate and tap water mixtures (15, 30, 45 and 60%) in semi-continuous mode at the dilution rate previously selected 0.30 1/day in four lab-scale open raceway-simulating reactors, testing two different light intensity levels of a maximum 200 and 1000 μE/m^2 s. The culture depth experiments were carried out at 5, 10, 15 and 20 cm under the previously optimized conditions: dilution rate 0.30 1/day, 60% centrate and 1000 μE/m^2 s.

4.2.1. Impact of light

The biomass productivity reported here at high light intensity of 1000 μE/m^2 s (15.90 g/m^2 day) was higher than those determined in several other studies (Table 5) (Fig. 4A). On the other hand, the productivity achieved in this study fell within the range of productivities reported when using wastewater in raceways elsewhere (Table 5).

Additionally, the native *Scenedesmus* sp. strain expressed higher biomass productivity for all the centrate concentrations tested when the light intensity increased from 200 to 1000 μE/m^2 s. In fact, the photosynthesis rate is a function of the irradiance applied to the culture, linearly increasing with irradiance until saturating the photosynthetic reaction centers (Formighieri et al., 2012).

According to fluorescence chlorophyll results, no stress was observed because of high centrate percentage used (Fig. 4B). The growth inhibiting concentration of ammonium is species dependent. For instance, *Muriellopsis* sp. was capable of growing in an ammonium concentration up to 160 mg/L whereas *P. subcapitata* had reduced capability at ammonium inlet concentrations above 100 mg/L (Morales-Amaral et al., 2015b). In another study, Ji et al. (2014) claimed that *Desmodesmus* sp. EJ9-6 was inhibited by an ammonia concentration of 100 mg/L. Therefore, selecting the appropriate ammonium-tolerant strain is a crucial step in improving the stability of the microalgal-based centrate remediation process.

The biochemical composition of the produced biomass showed that lipid content increased as light intensity decreased (Fig. 5). This behavior is expected for microalgae culture where the biomass composition is known to be greatly dependent on light supply, temperature and medium composition (Molina-Grima et al., 1999; Tzovenis et al., 2003). Thus, when the growth rate was high, as in experiments at 0.30 1/day (Fig. 1C) and at 1000 μE m^{-2} s^{-1} (Fig. 4A), the total lipid content was low while the percentage of structural lipids in biomass (mainly composed of polyunsaturated fatty acids) with respect to neutral lipids was high. This may explain the

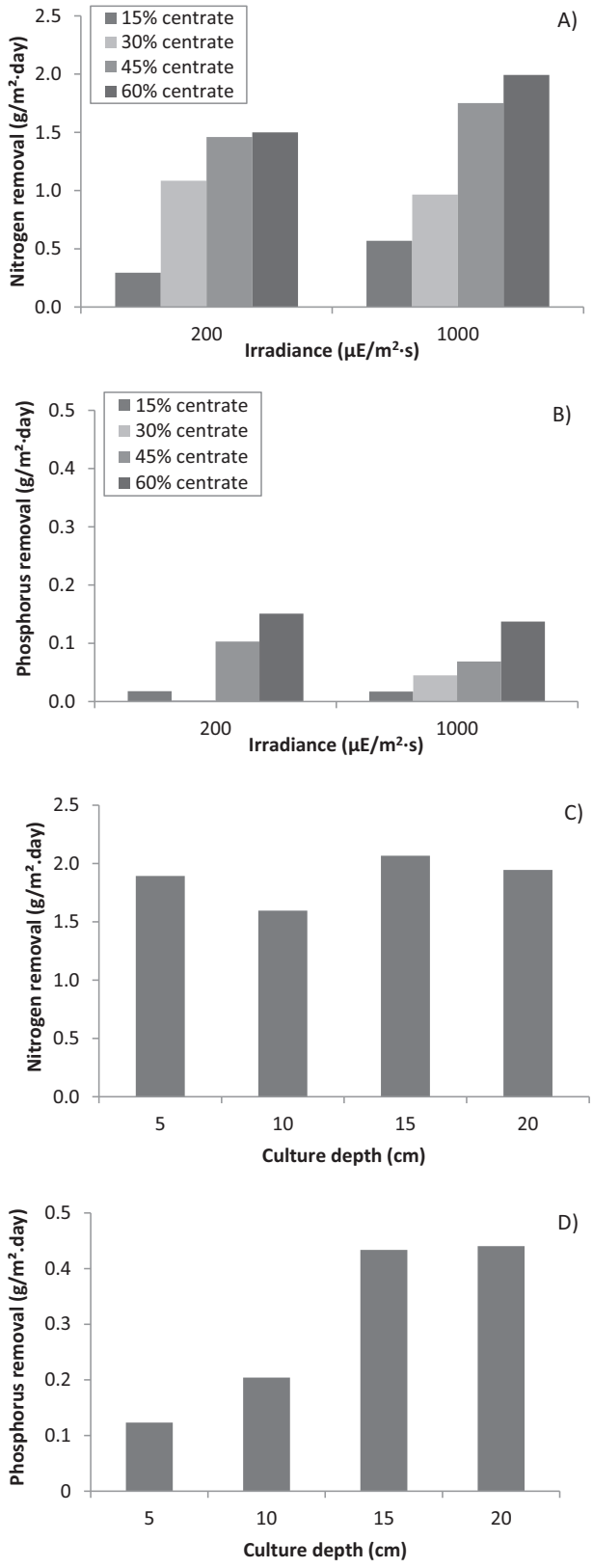

Fig. 6. Semi-continuously-fed culture of *Scenedesmus* sp. at 0.30 1/day. Effect of light intensity and centrate concentration on biomass productivity (A) and Fv/Fm (B) (Maximum impinging irradiances, 200 and 1000 µE $m^{-2}s^{-1}$). Effect of culture depth on biomass productivity (C) and Fv/Fm (D) (Maximum impinging irradiance, 1000 µE $m^{-2}s^{-1}$). Data values are means (±SE) of duplicate samples.

Table 4
Fatty-acid profile variation as a function of culture depth. Experiments were performed in semi-continuous mode (0.30 1/day) in 60% centrate. Data values are means (±SE) of duplicate samples.

F.A.	5 cm	10 cm	15 cm	20 cm
16:0	19.70	18.70	18.40	18.00
16:1n7	2.90	4.90	6.20	4.10
16:2n4	14.40	21.30	14.80	14.30
16:3n4	11.00	7.50	7.20	8.50
16:4n1	–	–	–	–
18:0	11.40	7.00	9.20	9.20
18:1n9	23.10	22.90	26.50	34.00
18:1n7	–	–	–	–
18:2n6	–	–	–	–
18:3n3	3.80	2.50	2.40	2.90
FA, %d wt	2.90 ± 0.15	2.20 ± 0.03	2.90 ± 0.17	3.00 ± 0.14

increase in the content of polyunsaturated fatty acids (16:3n4 and 18:3n3) (Tables 2 and 3) in these experiments. In contrast, under stressful conditions, as the production of all cell components and the cell growth are greatly retarded, the production of energy-rich lipids remains high resulting in their accumulation. (Molina-Grima et al., 1999; Lam and Lee, 2012). Lipid content range determined in this study fell within th values previously reported in the literature for wastewater-grown microalgae (Chinnasamy et al., 2010; Pittman et al., 2011).

Regarding nutrients removal, the higher was the light intensity, higher is the nitrogen removal (Fig. 6A). In fact, Lee and Lee (2001) stated that ATP and NADPH, both nitrogen containing compounds, are actively synthesized when microalgae undergo photosynthesis or when the cells are illuminated.

The optimal nitrogen removal rate (2 g/m^2 day) was higher or comparable to previously reported values in the literature (Table 5).

Regarding phosphorus, low removal efficiency was observed. Likewise, low phosphorus depuration of less than 30% was observed at an initial P-PO_4^{3-} concentration higher than 7.70 mg/L (Aslan and Kapdan, 2006) whereas the uptake capability of *C. kessleri* was only 8–20% under the light/dark cycle for a P-PO_4^{3-} concentration of 10 mg/L (Lee and Lee, 2001).

Phosphorus removal rates were ranging from 0.02 to 0.15 g/m^2 day (Fig. 6B). A similar range of phosphorus removal rates values is reported in the literature (Table 5).

Considering these results, one can conclude that for the native strain *Scenedesmus* sp., biomass productivity as well as nutrient uptake and removal rates were enhanced under the optimized conditions of high light intensity (1000 µE/m^2 s) and 60% centrate dilution. A biomass productivity of 15.90 g/m^2 day was obtainable, along with a nitrogen and phosphorus removal capacity of 2.00 g/m^2 day and 0.14 g/m^2 day, respectively. The native strain showed good tolerance to high ammonium concentrations up to 230.00 mg/L and it could be efficiently cultivated in semi-continuous mode for sustainable and stable biomass production and for a nutrient removal system using centrate as the culture medium. According to the International Energy Agency review (2011), high levels of sustained biomass productivity in open ponds fall within the 15–40 g m^{-2} d^{-1} range.

4.2.2. Impact of culture depth

The maximum productivity reported here at 15 cm culture depth, 1000 µE/m^2 s, 0.30 1/day and 60% centrate (22.20 g/m^2 day) is comparable to that obtained in the literature and higher than the maximum productivity reported in other studies (Fig. 4C) (Table 5).

Regarding biomass biochemical composition, lipid content was higher, the higher was the culture depth (Fig. 5D). This trend might be explained by the fact that at a greater depth, such as 20 cm, the

Table 5
Comparison of productivity, nitrogen and phosphorus removal rates in microalgae-based treatment system of wastewater.

Microalgae strain	Reactor type	Wastewater	Conditions	Productivity	N and P removal rate	Reference
Scenedesmus sp.	Raceway simulating reactor	60% Centrate	200 μmol $m^{-2}s^{-1}$ 5 cm culture depth	7.80 g/m^2 d	N: 30 mg/L d P:3 mg/L d	This study
Scenedesmus sp.	Raceway simulating reactor	60% Centrate	1000 μmol $m^{-2}s^{-1}$ 5 cm culture depth	15.90 g/m^2 d	N: 39.86 mg/L d P: 2.75 mg/L d	This study
Scenedesmus sp.	Raceway simulating reactor	60% Centrate	1000 μmol $m^{-2}s^{-1}$ 15 cm culture depth	22.2 g/m^2 d	N: 13.80 mg/L d P: 2.90 mg/L d	This study
Chlorella sp.	Multi-layer photobioreactor	20% swine manure wastewater	560.57 −476.37 μmol $m^{-2}s^{-1}$	14.59 TVSS g/m^2 d	NH_3-N: 2.65 g/m^2 d TN: 3.19 g/m^2 d P: 0.067 g/m^2 d	(Min et al., 2014)
Nannochloropsis gaditana	Raceway pond	30% of centrate	Outdoor at 0.2 d^{-1} 11 cm culture depth	0.1 g/L day	N: 14 mg/L d P: 1.4 mg/L d	(Ledda et al., 2015)
Chlorella sp.	Pilot scale photobioreactor	centrate	semi-continuous mode; 25 μmol $m^{-2}s^{-1}$ 10.2 cm culture depth	8.7–17.7 VSS g/m^2 d	N: 0.9–2.1 g/m^2 d P: 2.7–6.9 g/m^2 d	Min et al., 2011
Desmodesmus sp. EJ 9-6	flask	10% Anaerobic digestion wastewater	120 μmol $m^{-2}s^{-1}$	0.029 g/L d	N: 4.542 mg/L d P: 0.29 mg/L d	Ji et al., 2014
Scenedesmus sp.	Pilot scale raceway	centrate	Outdoor at 0.3 d^{-1} 11 cm culture depth	0.2 g/L day or 24 g/m^2 day	N: 38 mg/L d P: 3.9 mg/L d	Morales-Amaral, 2015a
C. pyrenoidosa	Rectangular photobioreactor	Diluted anaerobically -digested activated sludge	42.58–98.67 μmol $m^{-2}s^{-1}$	0.291 g/m^3 d	N: 36.8 mg/L d P: 6.1 mg/L d	Tan et al., 2015
P. subcapitata	Bubble columns	centrate	800 μmol $m^{-2}s^{-1}$	1.02 g/L d	N: 27 mg/L d P: 2.7 mg/L d	Morales-Amaral, 2015b
Chlorella vulgaris	flask	Synthetic wastewater	55.34 μmol $m^{-2}s^{-1}$	–	N: 10.5 mg/L d P: 2 mg/L d	Aslan and Kapdan, 2006
Algal consortium	raceway	carpet industrial effluent	batch mode at 18 cm batch mode at 20 cm batch mode at 30 cm	10.36 g/m^2 day 7.79 g/m^2 day 6.43 g/m^2 day	–	Chinnasamy et al. (2010)
Algal consortium	Pilot scale raceway	anaerobically digested liquid manure effluent	Outdoor; semi-continuous mode	6.83 g/m^2 day	–	Chen et al. (2012)
Chlorella vulgaris	flasks	Primary domestic effluent+25 mM glycerol	174 μmol $m^{-2}s^{-1}$	0.107 g/L day	N: 3.52 mg/L d P: 0.72 mg/L d	Cabanelas et al., 2013a
Algal consortium	photobioreactor	anaerobic membrane bioreactor effluent	Max 209 μmol $m^{-2}s^{-1}$ 0.5 d^{-1}	0.234 g/L day	N: 19.5 mg/L d P: 3.7 mg/L d	Ruiz-Martinez et al. (2012)
Chlorella vulgaris SAG211-12	Borosilicate bioreactor	centrate	150 μmol $m^{-2}s^{-1}$	0.195 g/L day	N: 9.80 mg/L d P: 1.14 mg/L d	Cabanelas et al., 2013b
Auxenochlorella protothecoides UMN280	25 L BIOCOIL reactor	Concentrated municipal wastewater	0.33 d^{-1}	1.51 g/L day	–	Zhou et al., 2012a
Nannochloropsis gaditana	bubble-column photobioreactors	centrate	500 μmol $m^{-2}s^{-1}$ 0.3 d^{-1}	0.4 g/L day	N: 35 mg/L d P: 5.70 mg/L d	Sepúlveda et al., 2015
Scenedesmus sp.	Pilot scale raceway	secondary domestic wastewater	Using flue gas Using pure CO2	13 g/m^2 d 17 g/m^2 d	N: 41-29 mg/g TSS^{-1} d^{-1} P: 5-3 mg/g TSS^{-1} d^{-1}	Posadas et al. (2015)

cultures are stressed by light limitation, which triggers lipid accumulation. Under optimal biomass productivity conditions, the lipid productivity was 17.30 mg/L day, which is within the range of values reported in the literature for wastewater-based cultures (Cabanelas et al., 2013b; Osundeko and Pittman, 2014). In the light of the fatty acid profile results determined from the different parameters tested (centrate concentration, dilution rate, light intensity and culture depth), a high monosaturated fatty acid content is observed. This would positively affect the properties of the fuel produced in terms of ignition quality, viscosity, combustion heat and oxidative stability (Rashid et al., 2008).

Consequently, the fatty acid profile of the native *Scenedesmus* sp. Microalgae strain is of great interest for use in biofuel production.

As for nutrients removal, the maximum nitrogen removal obtained was at 5 cm (97.60%). It should be noted that the highest nitrification was also observed at this culture depth (data not shown). However, it is worth noting that besides microalgae uptake, the ammonia stripping had probably contributed to ammonium removal. The expected contribution of this phenomena was low because of the low mass transfer coefficient previously determined (4.72 1/h), and the low driving force for ammonia desorption due to the controlled pH at 8.0, ammonia desorption being more relevant at pH upper than 9.

Nitrogen removal rate ranged from 1.60 to 2.00 gN/m^2 day (Fig. 6C) where the maximum (2.00 gN/m^2 day or 13.80 mgN/L day) was seen at the 15 cm culture depth; this is in accordance with the biomass productivity. The result is higher than previously reported (Table 5). Regarding phosphorus, the removal rate recorded at 15 cm was 0.40 g P/m^2 day, which is higher than the values obtained by Cabanelas et al. (2013a) and Ji et al. (2014) (Fig. 6D) (Table 5).

In this study, key parameters (dilution rate, centrate concentration and culture depth) were investigated. However, it is important to note that these different input parameters are not all independent variables, but affect each other. Additionally, these parameters variation affects also the light availability in the cultures and the overall performance of the system and accordingly influences the biomass productivity and nutrients removal capacity.

Consequently, a 15 cm culture depth seemed to be the most

appropriate depth to apply in a raceway pond using the native strain *Scenedesmus* sp. achieving a maximum biomass productivity of 22.20 g/m^2 day and nitrogen and phosphorus removal rates of 2.00 gN/m^2 day and 0.40 gP/m^2 day, respectively.

5. Conclusion

Despite the satisfactory performance of the strain, high outlet nutrient concentrations were determined in all the assays (except those at 0.04 1/day using 15, 45 and 60% centrate); these exceed the requirements of European Union Directive 98/15/CE for effluent disposal from urban wastewater treatment, which only permit 10 mg/L for total nitrogen and 1 mg/L for P-PO_4^{3-}.Therefore, regarding the effluent outlet from microalgae treatment in the present study (with its current nutrient composition), this should be either recycled in the wastewater treatment plant or used as the culture medium for microalgae growth in a second stage cultivation under nutrient-limited conditions for lipid synthesis induction. Nevertheless, the optimization of these critical parameters (dilution rate, centrate concentration and culture depth) at lab scale allows determining the optimal cultivation conditions before considering scaling up. This would be a step forward from experimental concepts to practical realities for coupling wastewater treatment with microalgae biotechnology that showed potential for economic gains. In fact, besides removing nutrients from centrate, microalgal biomass could be useful for low value products application and consequently a source of revenues. For instance, bio-oil could be obtained from the whole microalgae biomass via hydrothermal liquefaction process and preferably from biomass presenting a lipid fraction composed of saturated and mono-saturated fatty acids (Biller and Ross, 2011; Yamaberi et al., 1998). Process residues could be valorized as feed/co-substrate for anaerobic digestion for bio-methane production. Such a clean combining activities approach would offset the costs of both wastewater treatment plant operations and microalgae biomass production. The native *Scenedesmus* sp. microalgae strain was cultivated in semi-continuously-fed open raceway-simulating reactors using different mixtures of tap water and centrate. The strain showed a high tolerance to elevated centrate concentrations where a biomass productivity of 7.80 g/m^2 day was obtained at 5 cm culture depth, 0.30 1/day, 60% centrate and 200 μE/m^2 s. Under the same conditions, nitrogen and phosphorus removal rates of 1.50 g/m^2 day and 0.15 g/m^2 day were determined, respectively, along with a lipid content of 12.60%.

After optimization of the irradiance and culture depth, a maximum biomass productivity of 22.20 g/m^2 day was achieved under optimal culture conditions of a 1000 μE/m^2 s light intensity, 60% centrate, 0.30 1/day and 15 cm culture depth. The maximum nitrogen and phosphorus removal rates were 2.00 gN/m^2 day and 0.40 gP/m^2 day, respectively. A lipid content of 11.70% d wt. was determined under optimal conditions along with a suitable fatty acid profile for biofuel production.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.01.043.

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