Microalgae population dynamics growth with AnMBR effluent: effect of light and phosphorous concentration.

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Abstract

The aim of this study was to evaluate the effect of light intensity and phosphorus concentration on biomass growth and nutrient removal in a microalgae culture and their effect on their competition. The photobioreactor was continuously fed with the effluent from an AnMBR pilot plant treating real wastewater. Four experimental periods were carried out at different light intensities (36 and 52 µmol s^{-1} m^{-2}) and phosphorous concentrations (around 6 and 15 mgP L^{-1}). Four green algae: *Scenedesmus*, *Chlorella*, *Monoraphidium* and *Chlamydomonas* and cyanobacterium were detected and quantified along whole experimental period. *Chlorella* was the dominant specie when light intensity was at the lower level tested, being competitively displaced by a mixed culture of *Scenedesmus* and *Monoraphidium* when light was increased. When phosphorous concentration in the photobioreactor was raised up to 15 mgP·L^{-1}, a growth of cyanobacterium became the dominant specie in the culture. The highest nutrient removal efficiency (around 58.4 ± 15.8 % and 96.1 ± 16.5 % of nitrogen and phosphorous respectively) was achieved at 52 µmol s^{-1} m^{-2} of light intensity and 6.02 mgP·L^{-1} of phosphorous concentration, reaching about 674 ± 86 mg L^{-1} of VSS. The results obtained reveal how the light intensity supplied and the phosphorous concentration available are relevant operational factors that determinate the microalgae specie that is able to predominate in a culture. Moreover, changes in

microalgae predominance can be induced by changes in the growth medium produced by the own predominant species.

Keywords

Anaerobic membrane bioreactor; continuous photobioreactor; microalgae competition; nutrient removal; wastewater.

INTRODUCTION

The cultivation of microalgae has recently attracted growing interest as a solution for tertiary wastewater treatment. This interest is based on several potential benefits as: i) the simultaneous removal of nitrogen and phosphorus compounds in wastewater with a lower sludge generation than in conventional treatments; ii) the use of CO_2 as carbon source, contributing to reduce greenhouse gas emissions and iii) the generation of a valuable biomass which may be used to produce biogas (Collet & Hélias, 2011), manufacture biofuels (Chisti, 2007) or improve the energetic balance by direct combustion of algae biomass (Sturm & Lamer, 2011).

In addition, Anaerobic Membrane Bioreactors (AnMBRs) technology has been presented as a treatment able to reach high removal of Total Suspended Solids (TSS) and Chemical Oxygen Demand (COD), producing a high quality effluent while improve the energetic balance through a generation of biomethane and a lower production of sludge (Bornare *et al*., 2015; Giménez *et al*., 2011). However, this technology is not able to remove inorganic nutrients efficiently. Therefore, when the effluent is discharged into aquatic environments could cause important eutrophication problems.

Coupling the AnMBR technology with microalgae cultivation can benefit from all the above mentioned advantages, thus, being an interesting technology for wastewater treatment.

Up to now, very few studies have demonstrated the feasibility of a microalgae post-treatment for the effluent produced by AnMBRs technology (Ruiz-Martinez *et al*.,

2012). The main challenge still persists and is to obtain a stable microalgae culture able to reduce nitrogen and phosphorus concentration to values below the discharge limits established in Council Directive 91/271/EEC.

Nutrient removal by microalgae is influenced by many factors: physical, such as light, nutrient concentration, pH or temperature (Richmond, 2004), as well as biological, such as competition between bacteria and microalgae or between different species of microalgae.

Light is the most relevant parameter in microalgae growth (Jonker & Faaij, 2013). It has to be supplied at the optimum intensity, duration and wavelength to reach the maximum algal growth and nutrient removal efficiency (Termini *et al*., 2011). Moreover, light can also determine which phytoplankton can proliferate in the culture. Hence, the predominant microalgae species determine nutrient removal.

At one extreme: no nutrient limitation culture, microalgae usually compete for light. Light that has not been absorbed by microalgae reaches the bottom of the water column with intensity I_{out} (Huisman *et al*., 1999). Hence, I_{out} is variable as a function of the microalgae growth. Therefore, the critical light intensity (I_{critic}) of a specie is defined as the light intensity registered at the bottom of a well-mixed water column at which this species can just survive (Passarge *et al*., 2006). In a constant and well-mixed environment, theory predicts that the species with the lowest I_{critic} will be the superior competitor for light (Huisman & Weissing, 1994). Experiments reported by Huisman *et al*. (1999) and Litchman (2003) with phytoplankton in light limited conditions support this prediction.

Nutrient concentration can also determine the phytoplankton which can survive in the culture. Thus, at the other extreme: no light limitation culture, in a constant and well-mixed environment, the species with lowest nutrient requirements will be the superior nutrient competitor (Passarge *et al*., 2006). This prediction has been upheld by numerous nutrient competition studies (e.g., Van Donk & Kilham, 1990; Ducobu *et al*., 1998; Passarge *et al*., 2006).

Nevertheless, the abovementioned studies have been focused on the competition of species in batch conditions. So the studies focused in the effect of this competitions in

a continuous culture are very scarce (e.g., Pisman, 2002), feeding the culture with synthetic water, without the inherent variability associate to the real influents. For this reason, in this work different experimental conditions are tested in order to assess the possibility to remove nutrients (meeting legal requirements) from the effluent of a pilot plant AnMBR (processing real wastewater) with microalgae. For this purpose, is essential to analyze the microalgae population dynamics using real AnMBR effluent to ensure the accomplishment of discharge limits established.

Therefore, the aim of this study is to analyze the effect of light intensity and nutrient concentration on growth, nutrient removal efficiency and species competition in an indigenous microalgae culture fed by AnMBR effluent which treated real urban wastewater.

MATERIAL AND METHODS

Inoculum

The microalgae used as inoculum in this study came from the photobioreactors pilot plants located in Carraixet WWTP (Valencia, Spain) and owned by the CALAGUA research team. This inoculum was initially composed by *Monoraphidium* and *Scenedesmus* with a relative abundance of 73 % and 27 % respectively.

Culture medium

The fresh culture medium fed into the lab-scale photobioreactor (LabPBR) was obtained from the effluent of the Submerged anaerobic membrane bioreactor pilot plant (AnMBR) located in Carraixet WWTP (Valencia, Spain) and owned by the CALAGUA research team. This pilot plant is feed with the effluent of the pre-tratments units of the Carraixet WWTP (a full-scale urban wastewater treatment plant that treats 131050 PE). Further details about AnMBR process can be found in previous studies (Robles *et al.*, 2015; Giménez *et al*., 2011).

To feed the LabPBR, the AnMBR effluent was collected in opaque glass bottles and taken to laboratory every three days. In order to prevent the proliferation of microorganisms in the collected effluent, it was kept in the dark at a temperature of 5 °C. The average main composition of AnMBR effluent is shown in Table 1.

Table 1. Average AnMBR effluent composition.

Parameter	Mean ± SD
pH	7.29 ± 0.10
COD (mg COD L^{-1})	58.6 ± 10.2
BOD_L (mg BOD L^{-1})	26 ± 9
VFA (mg COD L^{-1})	2.0 ± 0.3
Alk (mg $CaCO_3$ L^{-1})	817.24 ± 22.56
NO_2-N (mg N L^{-1})	0.37 ± 0.18
NO_3-N (mg N L^{-1})	1.42 ± 0.67

Lab-scale Photobioreactor operation

The LabPBR consisted of a cylindrical clear tank with 19 cm of internal diameter (9 L working volume) (See Fig. 1a), installed in an incubator chamber with temperature control.

In order to achieve appropriate homogenization and maintain the pH fixed at 7.5, the culture was agitated with air across four fine bubble diffusers positioned crosswise on the bottom and pure CO_2 (99.9%) was injected into the gas flow from a gas bullet.

The temperature and pH were monitored online and logged on a PC through a self-made data acquisition software, processing the signal by a multiparametric analyzer (CONSORT C832, Belgium).

During the start-up, the LabPBR was operated in batch mode for 3 days, and then, was fed with the nutrient-loaded effluent from AnMBR system, and was operated in a semi-continuous mode, without retention of biomass. A peristaltic pump controlled by a PC was used to feed every three hours (8 feed cycles a day) a flow of 280 mL in order

to keep constant the SRT in 4 days, maintaining the total volume with an overflow at the top of the reactor. Moreover, allylthiourea was feed to inhibit nitrification bacteria growth, thus assuring that nitrogen removal was due to the microalgae activity.

The illumination was provided by eight led strips (Efecto LED, SMD5050 60LED/M 5M RGB IP65) fixed in an external cylinder (31 cm internal diameter) around the LabPBR (see Fig. 1b). Lighting was supplied 24 hours a day, and two photo sensors (Sensor PAR Apogee SQ-222) were disposed under and inside of the LabPBR in order to measure light intensity supplied to the culture (see Fig. 1a). PAR sensor 1 was used to determinate and control the light intensity supplied to the photobioreactor while PAR sensor 2 was used to determinate the value of I_{out} as a function of the microalgae concentration.

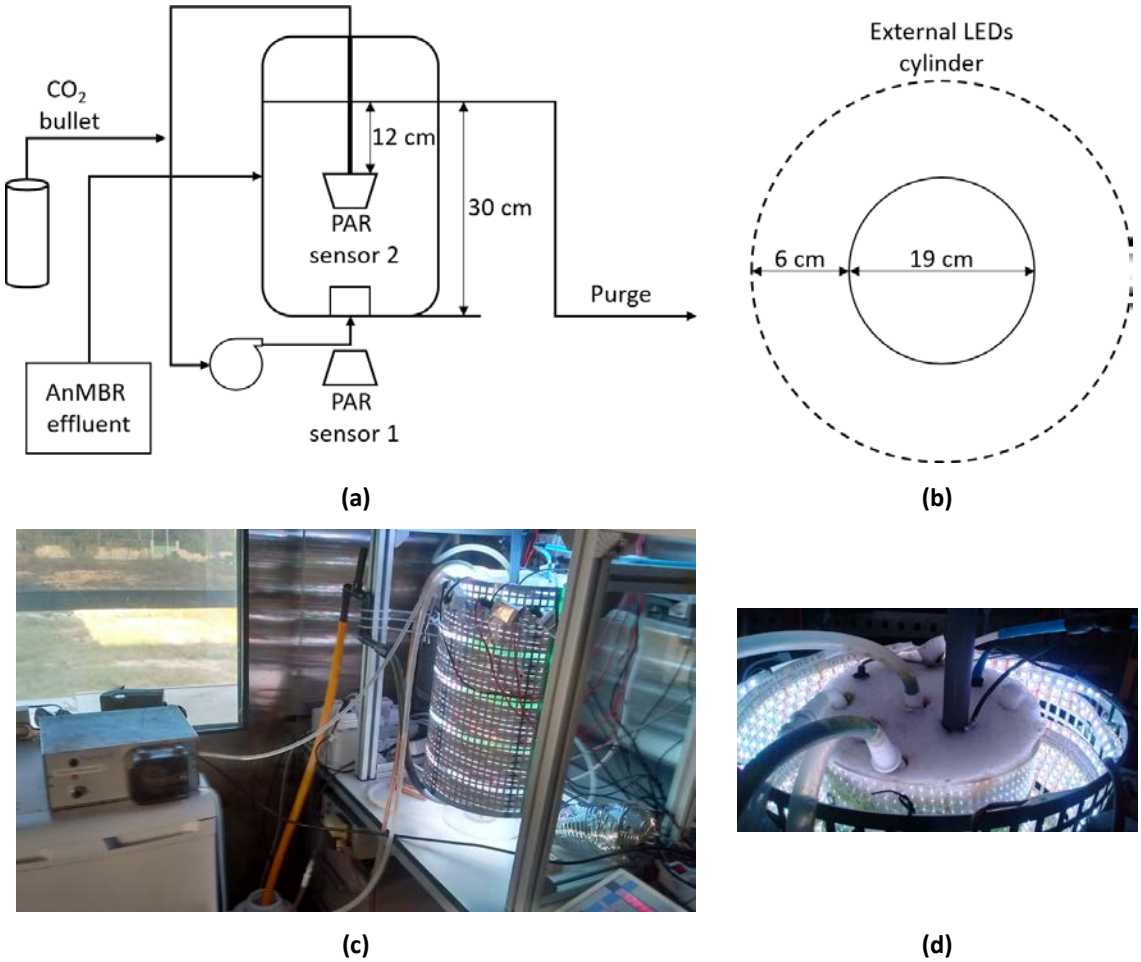


Figure 1. Experimental set-up: Scheme of the (a) laboratory scale photobioreactor and (b) Illumination system. Photo of the (c) laboratory scale photobioreactor and (d) Illumination system.

Experimental design

The study aimed at assessing the influence on the microalgae culture of two variables: light supplied on the reactor surface (at two different intensities: 36.3 and 52.2 µmol s^{-1} m^{-2}) and phosphorous concentration feed (around 6.02 and 15.23 mgP L^{-1}), keeping constant all others operational conditions. Four experimental periods were conducted. Table 2 displays the operational conditions imposed at each experimental period.

Since the concentration of nitrite and nitrate were negligible in the AnMBR effluent and the continuous addition of CO_2 to the lab-PBR, only light, ammonium and phosphate concentrations were considered as limitations to the microalgae growth.

Table 2. Operational conditions of the lab-scale photobioreactor during each experimental period.

Period	Duration (d)	Light intensity (µmol s^{-1} m^{-2})	Ammonium (mg NH_4-N L^{-1})	Phosphorus (mg PO_4^{3-}-P L^{-1})	Temperature (°C)
Exp. 1	50	36.3 ± 5.3	60.62 ± 2.81	6.05 ± 0.73	27.4 ± 0.8
Exp. 2	54	52.2 ± 4.8	59.31 ± 6.64	5.95 ± 1.22	27.7 ± 1.0
Exp. 3	9	52.1 ± 0.9	59.32 ± 0.10	15.23 ± 0.03	28.1 ± 0.4
Exp. 4	16	52.2 ± 1.7	66.06 ± 4.02	7.73 ± 0.23	27.5 ± 0.8

Analytical methods

Nutrient recovery by microalgae was assessed by recording thrice a week nitrogen and phosphate concentration in both, the influent and the soluble fraction collected from the lab-scale PBR purge. This soluble fraction was obtained by membrane filtration. There were used 0.45 mm pore size filters of polycarbonate glass fiber.

Total and volatile suspended solids (TSS and VSS) were determined thrice a week to evaluate biomass growth under each experimental period.

The nitrogen and phosphorous content of the dry biomass were measured in triplicate once every fifteen days. For this determination an acid-digestion of the dry biomass was performed.

Solids, phosphorous biomass content and all nutrients (ammonium, nitrate and phosphate) were obtained according to *Standard Methods for the Examination of Water and Wastewater* (APHA, AWWA, WEF, 2012). These methods were implemented in a multiparametric analyzer (*Smartchem*200 de *AMS/Alliance Instruments*). Nitrogen biomass content was determined via espectrofotometric method using commercial kit (MERCK, 100613) (Spectroquant® Pharo 300 MERCK).

Microbiological method

To assess the microalgae community evolution, twice a week a cell count was performed. A sample of 50 µL was filtered through 0.2 µm membranes. In order to eliminate the retained salt, the filters were washed using distilled water and then, dehydrated through successive washes with ethanol (50%, 80%, 90% and 99%). Cell counts were accomplished by the 100x oil immersion lens of an epifluorescence microscopy on a Leica DM2500. In the cell counts, a minimum of 300 cells were counted, assuring that were counted at last 100 cells of the most abundant genera with an error of less than 15% (Pachés *et al*., 2012). All the measurements were obtained in triplicate.

Calculations

Nutrient removal efficiency was calculated considering influent and effluent terms in a daily balance basis.

In the nitrogen balance, only NH_4 was considered to be available for biomass growth. This assumption was made based on the concentration of the other soluble species (NO_3 and NO_2) were negligible (below 2.20 mg N L^{-1}). Likewise, nitrification was not considered since allylthiourea was used to inhibit the nitrifying bacteria growth. Nitrogen gas loss (N_2 or NH_3) was not considered since the pH was kept always around 7.5 (at this pH value, the predominant form of ammonia nitrogen is by far NH_4).

In the phosphorous balance, phosphorous precipitation was assumed to be negligible due to the low solubility of the possible precipitating compounds (as struvite) in water at neutrality (Laliberte *et al*., 1997).

Therefore, nutrient removal efficiency (NRE) was calculated as follows:

$$NRE\ (\%) = \left(1 - \frac{E}{I}\right) \cdot 100 \quad (eq.1)$$

Where *I* and *E* are the ammonium or phosphate concentration in the influent and effluent respectively (mg L^{-1}).

Likewise, intracellular nutrients concentration (INC) were calculated as follows:

$$INC\ (\%) = \left(\frac{T - E}{VSS}\right) \cdot 100 \quad (eq.2)$$

Where *T* is the total nitrogen or phosphorous concentration in the purge.

Moreover, the N/P elimination and intracellular ratios (N/P_E and N/P_I respectively) were calculated in order to assess the different nutrients needs of each microalgae covered.

$$N/P_E = \frac{I_{NH4} - E_{NH4}}{I_{PO4} - E_{PO4}} \quad (eq.3)$$

$$N/P_I = \frac{T_{NH4} - E_{NH4}}{T_{PO4} - E_{PO4}} \quad (eq.4)$$

Finally, nutrients normalized uptake (NNU) was determined on a daily basis through the following equation:

$$NNU = \frac{I - E}{CC} \quad (ec.5)$$

Where *CC* is the archived value of cell counts.

RESULTS AND DISCUSSION

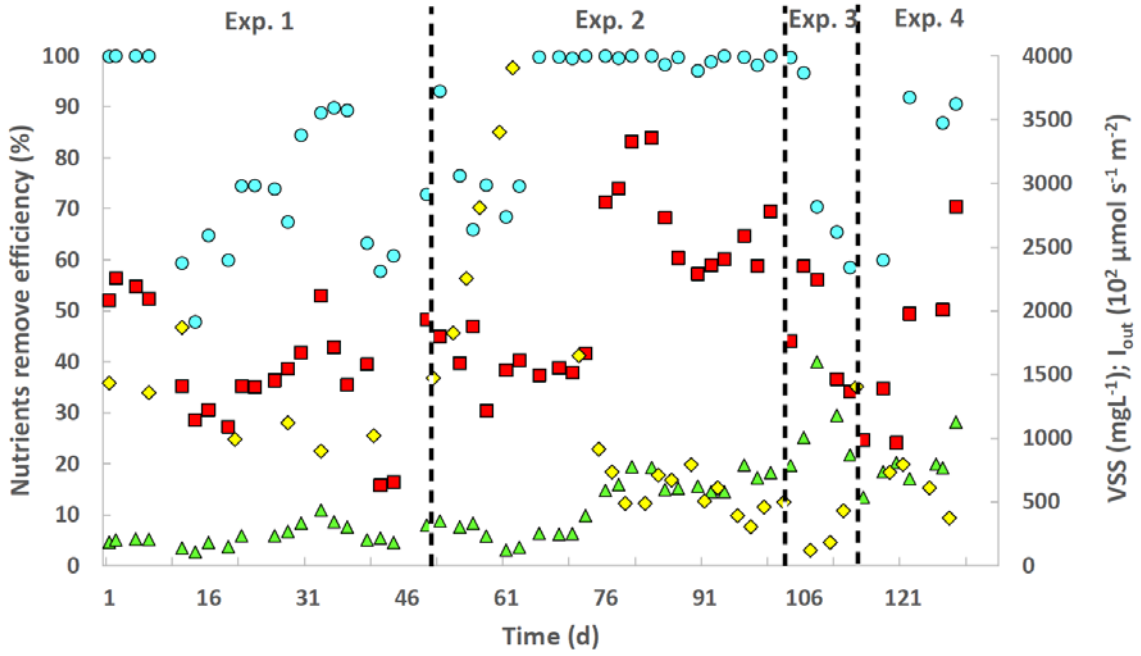
Effect of light

The first two experimental periods (Exp. 1 and Exp. 2) were aimed at assess the growth, nutrient removal efficiency and species competition under two different light intensities (36.3 ± 5.3 and 52.2 ± 6.1 µmol s^{-1} m^{-2}), maintaining constant all others working conditions. Figure 2 shows the time profile evolution of nutrient removal, light intensity, volatile suspended solids and relative microalgae species abundance obtained along the experimental period.

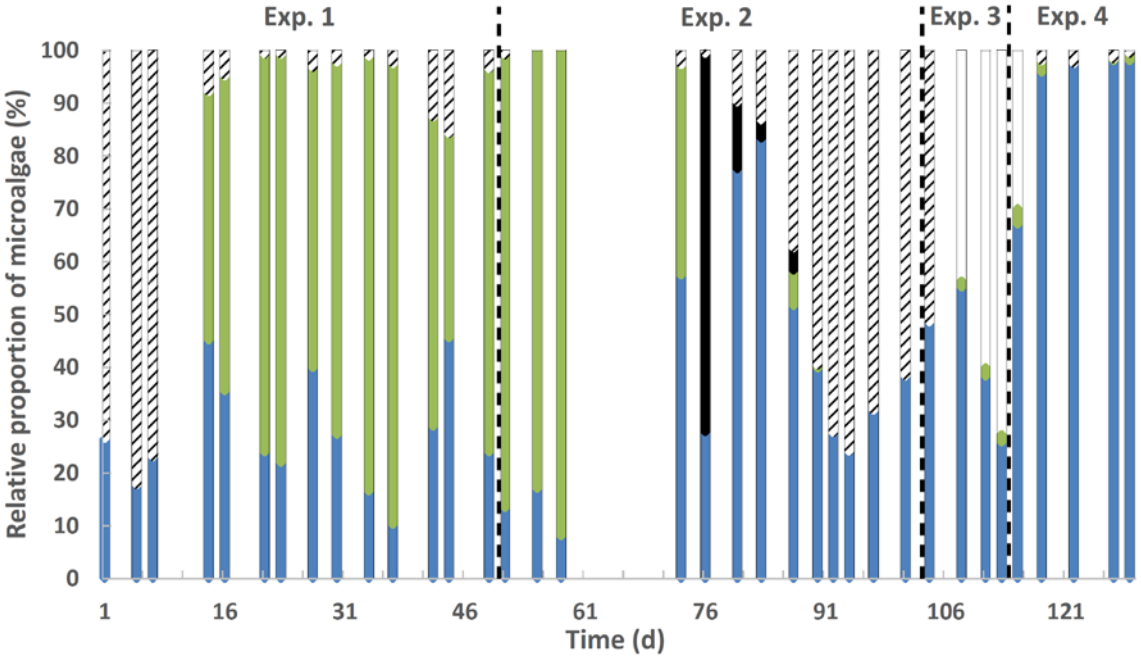
The first experimental period lasted 50 days and the volatile suspended solid concentration reached about 244 ± 83 mg L^{-1} of VSS. After a week, the genres included in the original inoculum (*Scenedesmus* and mostly *Monoraphidium*) were competitively displaced by genre *Chlorella*, becoming the predominant microalgae in the culture. As can be seen in Figure 2b this genre not reached a stable value of relative abundance, despite being the predominant genus. It can also be observed a decrease in the daily ammonium and phosphorus removal efficiency with this specie change, achieving values about 52.8 ± 5.7 % and 99.9 ± 0.1 % of nitrogen and phosphorous respectively when *Monoraphidium* was the dominant specie versus the 35.6 ± 9.9 % and 71.8 ± 13.3 % of nitrogen and phosphorous removal when *Chlorella* was dominant.

However, when light intensity was raised from 36 to 52 µmol s^{-1} m^{-2}, green algae *Chlorella* was competitively displaced by both, *Scenedesmus* and *Monoraphidium*. In this second experimental period (54 days of duration), VSS increased until 674 ± 86 mg L^{-1}. Specie abundance stability was not reached in this experimental period. During this second period, the daily ammonium and phosphorus removal efficiency increased until reaching values about 58.4 ± 15.8 % and 96.1 ± 16.5 %, respectively.

The increase in the volatile suspended solids could indicate that in Exp. 1, the most important biomass growth limiting factor was light. When no other factor is limiting the microalgae growth, enhance light intensity speed up the microalgae metabolism as long as it stays under the optimum value (Martín and Marzal, 1999).



(a)



(b)

Figure 2. Evolution of (a) nutrient removal efficiency (nitrogen ■ and phosphorous ●), volatile suspended solids (▲), I_{out} x10^2 (♦) and (b) relative abundance of microalgae (*Chlorella* ■, *Scenedesmus* ■, *Monoraphidium* ■, *Chlamydomonas* ■ and *Cyanobacterium* □) in the LabPBR during each experimental period. Vertical black dotted lines indicate an experimental period change.

Moreover, this increase in the light supplied to the reactor surface allowed *Scenedesmus* and *Monoraphidium* genres to be more competitive than *Chlorella* genre. This result suggests that *Scenedesmus* and *Monoraphidium* genres growth requires noticeably higher I_{out} than the *Chlorella* genre. This conclusion is in agreement with

that reported by Huisman *et al*. (1999), who reported that *Scenedesmus* had a much higher critical light intensity than *Chlorella,* being competitively excluded under deficient light conditions. In the same way, Passarge *et al.* (2006) reported that in two pure microalgae culture growths, *Monoraphidium* showed higher I_{critic} than *Chlorella*.

Therefore, the lesser competitivity of *Scenedesmus* and *Monoraphidium* genres in the first experimental period could be explained by the low light intensity supplied. Although a given microalgae specie can proliferate whenever the actual value of I_{out} is above their critical light intensity (Huisman and Weissing, 1994; Weissing and Huisman, 1994), in competition, the species with the lowest I_{critic} displace all others species. This observation is due to the fact that, during its growth, the species with the lowest I_{critic} is able to reduce the light penetration to the bottom of the reactor bellow the critical light intensities of all others species (Huisman and Weissing, 1994; Weissing and Huisman, 1994).

Consequently, since light usually represents the limiting factor in the cultures of photosynthetic microalgae (Cuaresma *et al*., 2011), and influences their competence (Passarge *et al.*, 2006; Huisman *et al*., 1999), it is imperative to be able to estimate the value of light reaching the center of a photobioreactor in relation with the VSS in order to supply the accurate light intensity.

Commonly this relation is estimated by the Lambert-Beer expression:

$$I_{out} = I_0 \cdot exp(-k_e \cdot c_b \cdot z) \quad (eq. 6)$$

Where I_0 is the intensity measured at the surface of the water column (µmol s^{-1} m^{-2}), k_e is the light attenuation coefficient (m^2 Kg^{-1}), c_b is the solid concentration (Kg m^{-3}) and z is the thickness of the water column (m).

Since equation 6 was developed in plane coordinates, it does not adjust well enough to the results obtained in a cylindrical reactor illuminated from all its perimeter. Then, the relation between VSS and I_{out} in a cylindrical reactor can be adjusted in a better way by the equation proposed by Molina *et al.* (1997):

$$I_{out} = \frac{I_0}{k_e \cdot c_b \cdot L_{eq}}(1 - exp(-k_e \cdot c_b \cdot L_{eq})) \quad (eq.7)$$

Where L_{eq} is the equivalent optical length of the system, which is a function of the radius and in the most usual conditions takes the value of $L_{eq} = 1.60 \cdot r$, where r is the radius of the cylinder object of study (9.5 cm in the conditions of this study).

Figure 3 shows the experimental I_{out} values measured for each volatile suspended solid concentration and the fitting provided by both, Lambert-Beer and Molina *et al.* equations. As can be seen in this Figure, the Molina *et al.* equation provided a much better fit.

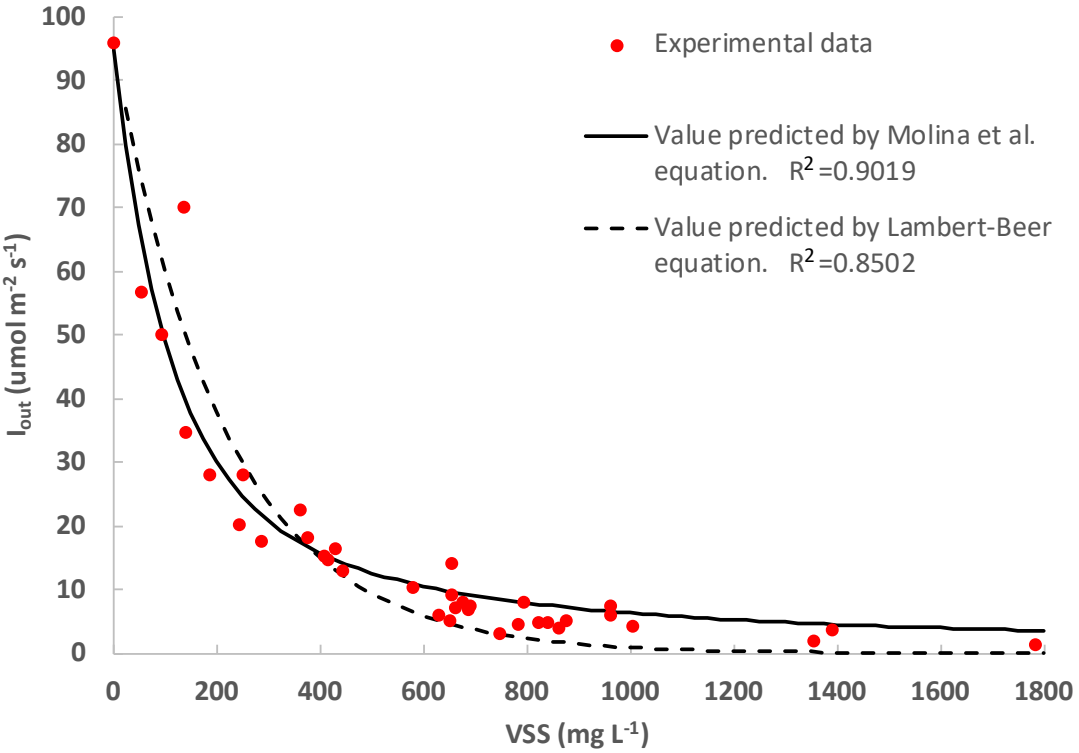


Figure 3. Fitting the experimental I_{out} values registered with an external light intensity on the surface's photobioreactor of 52 µmol s^{-1} m^{-2} using the equations of Molina *et al.* (1997) and Lambert-Beer.

Moreover, from this equation fitted it can be deduced the k_e coefficient, which can provide an interesting information by the fact that represents the efficiency with which light can be harnessed. The higher the k_e coefficient, the higher the amount of supplied light will be needed to reach the center of the photobioreactor.

298 Usually, k_e depends mostly on the genre and conditions of the algal culture, due that
299 the mainly light that is supplied to the photobioreactor is absorbed by microalgae.
300 However, the thickness, geometry and material of the photobioreactor must be taken
301 in account due that it represents an additional resistance to the light passage.

302 In this study, it has been deduced the k_e from the Molina *et al.* equation, achieving a
303 value of 0.0859 m^2 $gTSS^{-1}$. This value has been calculated assuming a VSS/TSS relation
304 of 86 %, which has been deduced from the values obtained in this study.

305 The k_e obtained is similar to that reported by other authors (Ruiz-Martínez *et al.*, 2016;
306 Molina-Grima *et al*., 1994), specially by that reported by Ruiz-Martínez *et al.* (2016),
307 whom assumed a value of 0.0758 m^2 $gTSS^{-1}$ operating a flat-plate photobioreactor in
308 outdoors conditions at similar TSS concentrations. Consequently, it can be deduced
309 that the effects of photobioreactor resistance to the light passage on the k_e coefficient
310 can be often considered negligible, being in accord with Molina-Grima *et al*. (1994)
311 whom reported that k_e depends mainly on the algal light absorption.

312 It must be highlighted that, despite microalgae genre was changed in each
313 experimental period (with inherent variability of size and shape), no significant
314 difference in the provided auto-shadow was observed. Thus, these equations could be
315 applicate to estimate the I_{out} of any microalgae culture, pure or in consortium.

316 On the other hand, during the second experimental period (Exp. 2) the value of I_{out}
317 decreased due to the increase in the VSS, reaching values lower than those registered
318 in Exp. 1 (see Fig. 2a), but no significant presence of *Chlorella* was observed.
319 Consequently, it can be concluded that, although the increase in the supplied light to
320 the reactor surface improved the *Scenedesmus* and *Monoraphidium* competition, the
321 dominance of the culture by these genres was not only due to light intensity supplied.

322

323 *Effect of nutrients concentration*

324 Another important operational factor with strong influence on the competition
325 between microalgae species is the nutrient concentration (Yang *et al*., 2016). In Exp. 1,

nutrient removal efficiency was low enough (see Fig. 2a) to maintain a nutrient concentration in the reactor high enough to not limit the microalgae growth. However, in Exp. 2, the nutrient removal efficiency increased reaching high values, especially for the phosphorus concentration (up to 96% of P-removal). Therefore, nutrient concentration was low enough to be considered also a growth limiting factor.

Then, it can be assumed that the increase in nutrient removal was produced by the increment in the external light on the photobioreactor's surface. Nevertheless, this low phosphorus concentration in the reactor could have favoured the *Scenedesmus* and *Monoraphidium* genres competition against *Chlorella* since according to Wu *et al*. (2014), *Chlorella vulgaris* can live at the lower nitrogen concentration, but it is very difficult to survive in the absence of phosphorus. Therefore, phosphorus is the limiting factor for its growth.

The N/P elimination ratio showed while *Chlorella* was the predominant genus in the culture, was notably lower than that displayed by the consortium formed by *Scenedesmus* and *Monoraphidium* (5.0 ± 0.8 and 6.8 ± 0.3 respectively, see Fig. 4). The intracellular nitrogen and phosphorous content achieved (see Table 3), seems to indicate that *Chlorella* need higher phosphorus concentration for its growth than *Scenedesmus* and *Monoraphidium*. Consequently, this result suggests that *Chlorella* has more dependence on phosphorous concentration than other green algae like *Scenedesmus* or *Monoraphidium*.

Considering the strong influence that the concentration of phosphorus has on the competence between microalgae species as evidenced in this study, Exp. 3 and Exp. 4 were planned to confirm how the variations in the phosphorous concentration are able to change the microalgae dynamics in the LabPBR. Phosphorous concentration was raised to 15 mg L^{-1} in Exp. 3 and then were reduced to normal concentrations in Exp. 4 (see Table 2). The increase in P-concentration led to an important cyanobacterium growth (until a 72.6 % of relative abundance) and decreasing until its vanishment when the phosphorous concentration was lowered to the typical concentration level recorded in the AnMBR effluent (see Fig. 2). This phenomenon can be explained in the same way that *Chlorella* competition, having cyanobacterium higher phosphorous

dependence. This result is in accordance too with the N/P elimination and intracellular contents ratios obtained when cyanobacterium was the predominant microalgae in the culture (see Fig. 4 and Table 3), achieving the lower N/P elimination and intracellular content ratios among all others genres that predominated along each experimental period.

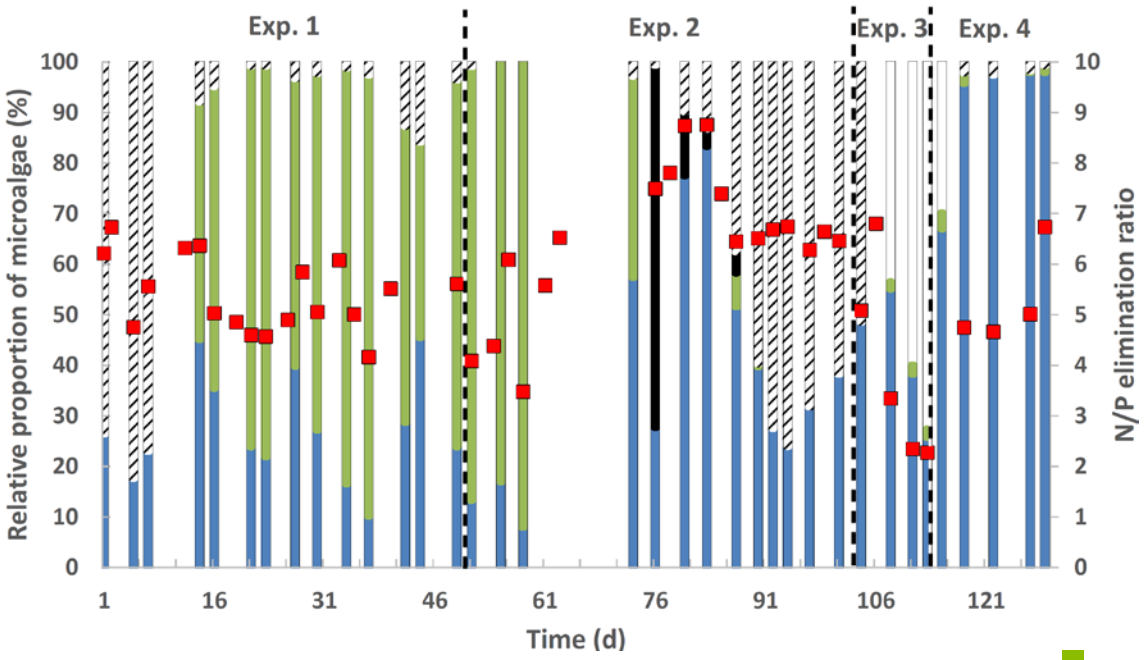


Figure 4. Evolution of N/P elimination ratio (■) and relative microalgae abundance (*Chlorella* ■, *Scenedesmus* ■, *Monoraphidium* ▨, *Chlamydomonas* ■ and *Cyanobacterium* □) in the LabPBR along the experimental period.

During the second period a rapid growth of *Chlamydomonas* genre was observed (day 76 in Figure 4), which vanished after a few days (day 87 in Figure 4). The *Chlamydomonas* growth seems to be lightly related with the available ammonium concentration in the PBR, just like *Chlorella* and cyanobacterium with the phosphate concentration, showing a significant improvement in the ammonium removal efficiency (73.5 ± 7.0 %), and registering the higher N/P elimination ratio (7.8 ± 0.9) of the whole experimental period when this genus was present in the PBR.

This result suggests that the biomass growth itself could be able to produce changes in the medium that influence microalgae competition since in their growth, light availability and nutrient concentration will decrease until microalgae culture reaches the equilibrium (i.e., the pseudo-steady state). Then, although the ideal conditions for one specie were achieved in a photobioreactor, the culture evolves until reaching its

377 own equilibrium. Further research is being carried to provide additional data
378 confirming this interesting finding.

379 Moreover, in regard to the effluent quality produced, it can be seen in Figure 2 that in
380 the second period (52 µmol s^{-1} m^{-2} of light supplied), the microalgae culture was able
381 to remove practically all phosphorous concentration. Nevertheless, it could not
382 remove enough the nitrogen concentration to meeting legal discharge limits, reaching
383 an ammonium concentration in the effluent about 25 mgNH_4-N L^{-1}. Thus, since that
384 microalgae stop their activity in the absence of any of required nutrients (Hoff & Snell,
385 2001), the microalgae cultivated in this study would not be able to treat AnMBR
386 effluent properly, requiring an additional process to reduce ammonium in the effluent.
387 However, AnMBR effluent treatment by microalgae have been studied by many
388 different authors (i.e. Ruiz-Martínez *et al*., 2016; Viruela *et al*., 2016), whom have
389 reported promising results operating with outdoors pilot-plants, showing the potential
390 of the microalgae as a feasible tertiary treatment for urbane wastewaters.

391

392 *Biomass composition and nutrient uptake efficiency*

393 Table 3 shows the nitrogen and phosphorous content in the biomass obtained by each
394 consortium of microalgae in each experimental period.

395 **Table 3**. Intracellular nutrient content obtained by each consortium of microalgae.

Day	Algae Population (%)				%N	%P	N/P ratio	VSS (mg L^{-1})	I (µmol s^{-1} m^{-2})	I_{out} (µmol s^{-1} m^{-2})
	Scenedesmus sp.	*Chlorella sp.*	*Monoraphidium sp.*	*Cyanobacterium*						
21	24	75	1	-	-	1.70	-	236	36	9.93
29	27	70	2	-	-	1.83	-	270	36	11.21
34	17	82	1	-	9.99	1.53	6.52	344	36	9.00
42	29	59	13	-	11.02	1.80	6.12	268	52	10.22
90	40	-	60	-	9.40	1.27	7.41	623	52	7.94
92	28	-	72	-	-	1.31	-	580	52	5.07
97	32	-	68	-	5.79	0.76	7.61	790	52	3.94
108	55	2	-	43	2.93	0.70	4.21	1600	52	1.22
120	96	2	2	-	5.93	-	-	810	52	7.31

Figure 5 shows a scatter plot of the intracellular nutrient content, I_{out} and PBR available phosphorus versus the Volatile Suspended Solids (VSS). As can be seen in this Figure, the nutrient content decreases with the VSS increase.

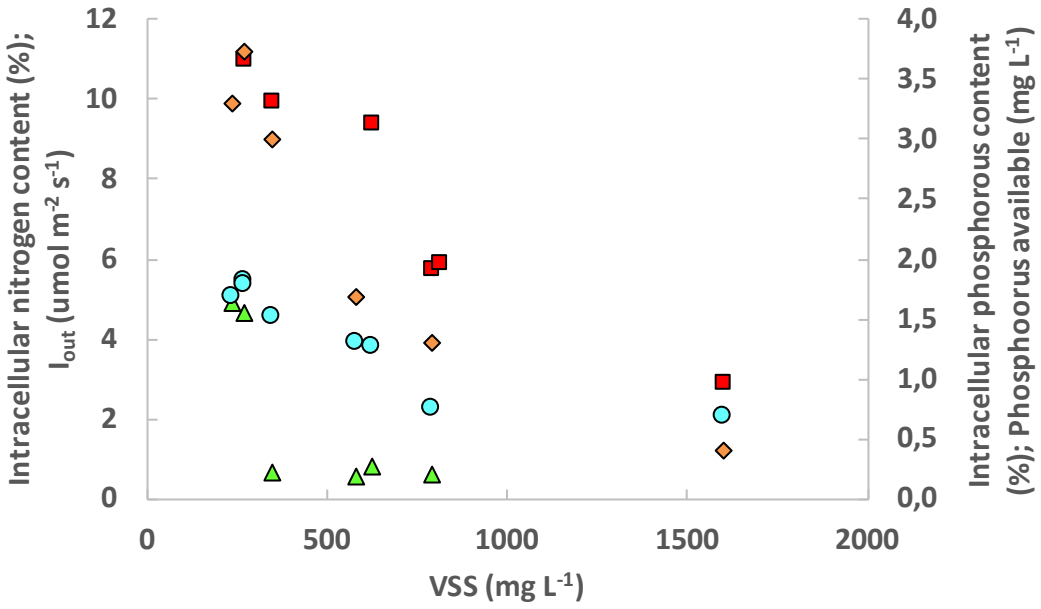


Figure 5. Intracellular nutrient content (nitrogen ■ and phosphorous ●), I_{out} (♦) and available phosphorous (▲) versus VSS concentration.

According to different authors, a reduction in the culture available light cause an increase on the biomass phosphorus content (Hessen *et al*., 2002; Powell *et al*., 2008; Ruiz *et al*., 2014). It has been construed as a reduction in ATP accumulation when is available enough light energy. Additionally, Hessen *et al*. (2002) reported that high light intensity caused reductions in the biomass nitrogen content. Consequently, it can be assumed that supplying low light intensity to the culture, the nitrogen and phosphorous biomass content must be higher than that obtained at elevated light intensities.

However, from approximately 350 mg VSS L^{-1} onwards (see Figure 5), the available phosphorous concentration began to be low enough to be considered a growth limiting factor, reaching values under 0.2 mg P L^{-1}. Then, it can be assumed that the decreasing in the biomass phosphorous content is due to the competition among microalgae species for the scarce available phosphorous concentration. In addition, nitrogen content in the microalgae biomass decreased also with the low available

phosphorous concentration although the available nitrogen concentration was high enough for not limiting the biomass growth (data no shown). This could be explained by the fact that microalgae require both nutrients from the environment, stopping their activity in the absence of any of them (Hoff & Snell, 2001). According Marcilhac *et al.* (2014), when phosphorous concentration was below 0.1 ppm, nitrogen uptake was limited.

The nitrogen and phosphorus content obtained in this study is similar to the contents reported by other authors in different species (see Table 4).

Table 4. Intracellular nutrient content reported by different authors.

Microalgae specie	% N	% P	N/P	Authors
Chlorella sorokiniana	10.10	-	-	Richardson *et al*. (1969)
Scenedesmus Obliquus	-	0.99	-	Martínez *et al*. (2000)
Consortium (*Scenedesmus* dominant)	-	3.16	-	Powell *et al.* (2008)
Consortium fed at low loading rate	6.55	0.94	6.97	Mulbry *et al*. (2008)
Consortium fed at high loading rate	5.45	0.82	6.65	Mulbry *et al*. (2008)
Nanoochlorpsis oculta	8.30	-	-	Hsueh *et al*. (2009)
Consortium	9.27	0.87	10.66	Chinnasamy *et al*. (2010)
Scenedesmus sp.	-	3.50	-	Yin-Hu *et al.* (2012)
Scenedesmus Obliquus inoculated at low biomass	4.85	0.68	7.13	Ruiz *et al.* (2014)
Scenedesmus Obliquus inoculated at high biomass	5.89	0.78	7.55	Ruiz *et al.* (2014)
Consortium at low biomass (*Chlorella sp*. dominant)	9.99	1.53	6.52	This study
Consortium at medium biomass (*Scenedesmus sp./Monoraphidium sp.*)	5.79	0.76	7.61	This study
Consortium at high biomass (*Scenedesmus sp./*cyanobacterium)	2.93	0.70	4.21	This study

Figure 6 is shows the normalized nutrient uptake as a function of microalgae population. The normalization has been carried out taking into account the number of cell counts. In this Figure, it can be observed that higher nutrient uptake was achieved at the lower biomass concentrations, decreasing with the increase in microalgae population until reach a minimum of uptake efficiency. This fact can be explained in the same way that the intracellular nutrient content diminution, being the competence

among microalgae in the culture for the scarce available phosphorous the limiting factor. Also, the available light that is able to reach the microalgae culture decrease due the auto-shadow effect, reducing the nutrient uptake too. Consequently, although the total nutrient removal can increase due to the biomass growth, the nutrient removal efficiency per biomass unity in low biomass concentrations is significantly higher than that displayed at high biomass concentrations.

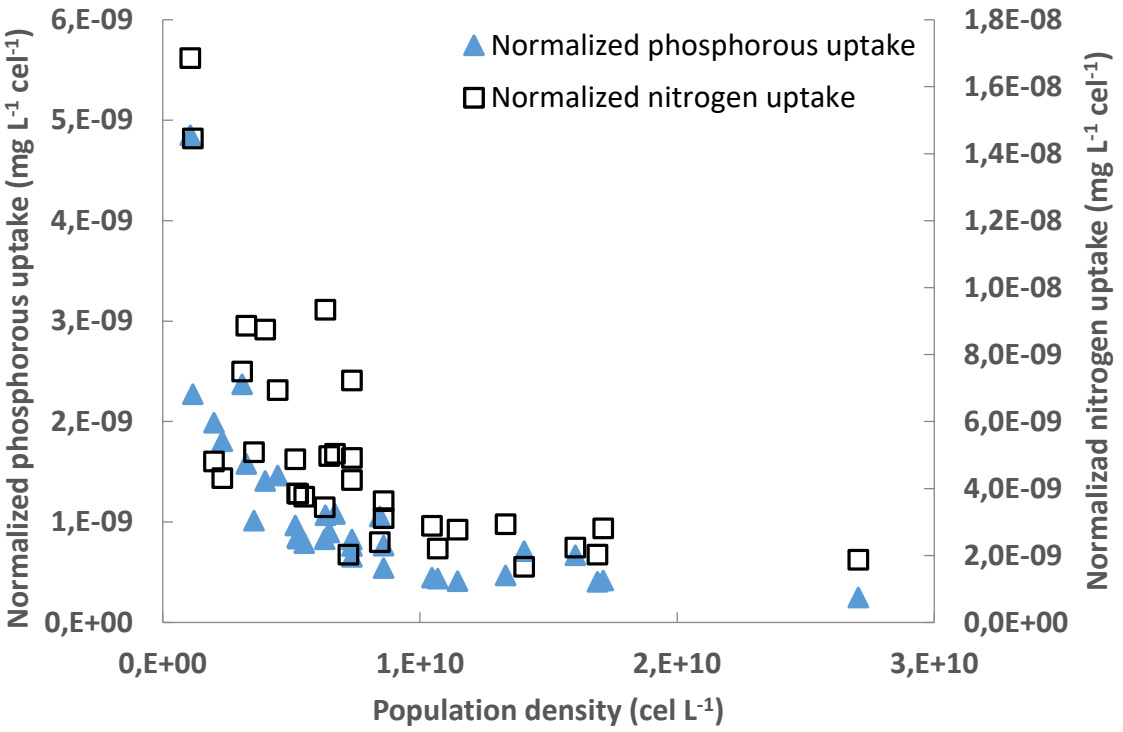


Figure 6. Nutrient uptake normalized by the cell count versus the population density.

Regarding the normalized nitrogen uptake, the experimental data acquired displays a higher dispersion than that registered by the normalized phosphorous uptake. This phenomenon can be explained by the phosphorous limitation effect too. Since the removal of nitrogen in the reactor was not only a function of light or available nitrogen concentration, but also to the available phosphate concentration.

The results obtained suggest that light and phosphorous concentrations seem to be the most relevant variables for the microalgae growth in this study, supporting the previous conclusions.

CONCLUSIONS

The light intensity supplied, the available phosphorous concentration, nutrient removal and the competition among microalgae species in a continuous fed photobioreactor has been studied. In the experimental period, *Chlorella sp.* was the dominant specie when light intensity was low (36 µmol $s^{-1}m^{-2}$), reaching about 244 ± 83 mg L^{-1} of VSS in the photobiorreactor, with a nutrients removal efficiency of 35.6 ± 9.9 % and 71.8 ± 13.3 % of nitrogen and phosphorous, respectively. Conversely, when the light intensity supplied was increased to 52 µmol $s^{-1}m^{-2}$, the culture was dominated by a consortium of *Scenedesmus sp.* and *Monoraphidium sp*. increasing the VSS until 674 ± 86 mg L^{-1} and reaching a nutrients remove efficiency around 58.4 ± 15.8 % and 96.1 ± 16.5 % of nitrogen and phosphorous respectively. The results obtained suggests that *Chlorella* sp. shows a lower I_{critic} that *Scenedesmus sp.* and *Monoraphidium* sp. species, such as previous studies reported before.

Nutrient removal ratio analysis reflects that *Chlorella sp.* presents higher dependence for phosphorous concentration than *Scenedesmus sp.* and *Monoraphidium sp.*, showing lower N/P remove ratios. Moreover, when phosphorous concentration was raised from 6 to 15 mgP L^{-1}, the culture was dominated by cyanobacterium, decreasing its abundance until vanish their relative abundance when phosphorous was reduced to 6 mgP L^{-1} again. These results clearly indicate that phosphorous concentration has an important influence in the competition among microalgae.

The analysis of the biomass intracellular nutrients was coherent with previous conclusions, achieving lower N/P ratios in the composition of microalgae which was attributed more dependence for phosphorous concentration (4.21 ± 0.05, 6.32 ± 0.28 and 7.51 ± 0.14 for a consortium of cyanobacterium and *Scenedesmus sp.* and cultures mostly dominated by *Chlorella sp.* and *Monoraphidium sp.* respectively).

This study highlights the importance of light and nutrient concentration in the competence among microalgae, showing the dramatic impact that changes in this two variables can have in the microalgae species that can survive in a culture, and consequently, in the nutrient remove efficiency. Besides, it must be highlighted that

these changes can be induced by the microalgae themselves since with their growth, shift the available nutrient concentration and/or the available light.

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