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

P. Sanchis-Perucho, F. Duran, R. Barat, M. Pachés and D. Aguado

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 anaerobic membrane bioreactor pilot plant treating real wastewater. Four experimental periods were carried out at different light intensities (36 and 52 μmol s⁻¹ m⁻²) and phosphorus 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 species when light intensity was at the lower level tested, and was competitively displaced by a mixed culture of Scenedesmus and Monoraphidium when light was increased. When phosphorus concentration in the photobioreactor was raised up to 15 mgP L⁻¹, a growth of cyanobacterium became the dominant species in the culture. The highest nutrient removal efficiency (around 58.4 ± 15.8% and 96.1 ± 16.5% of nitrogen and phosphorus, respectively) was achieved at 52 μmol s⁻¹ m⁻² of light intensity and 6.02 mgP L⁻¹ of phosphorus concentration, reaching about 674 ± 86 mg L⁻¹ of volatile suspended solids. The results obtained reveal how the light intensity supplied and the phosphorus concentration available are relevant operational factors that determine the microalgae species 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.

Key words | 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 CO₂ as carbon source, contributing to a reduction in greenhouse gas emissions; and (iii) the generation of a valuable biomass which may be used to produce biogas (Collet & Hélias 2014), manufacture biofuels (Chisti 2007) or improve the energetic balance by direct combustion of algae biomass (Sturm & Lamer 2011).

In addition, anaerobic membrane bioreactors (AnMBR) 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 improving the energetic balance through a generation of biomethane and a lower production of sludge (Giménez et al. 2011; Bornare et al. 2013). However, this technology is not able to remove inorganic nutrients efficiently. Therefore, the discharge of effluent into aquatic environments could cause important eutrophication problems.

Coupling the AnMBR technology with microalgae cultivation can benefit from all the previously 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 AnMBR technology (Ruiz-Martinez et al. 2012). The main challenge still persists which 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 (NRE) (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, when there is 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 species 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 that can survive in the culture. Thus, at the other extreme, when there is 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 previously mentioned studies have focused on the competition of species in batch conditions. The studies that focused on the effect of this competition in a continuous culture are very scarce (e.g., Pisman 2002), feeding the culture with synthetic water, without the inherent variability associated with 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, it 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, NRE 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 laboratory-scale photobioreactor (LabPBR) was obtained from the effluent of the submerged AnMBR pilot plant located in Carraixet WWTP (Valencia, Spain) and owned by the CALAGUA research team. This pilot plant is fed with the effluent of the pre-treatments 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 (Giménez et al. 2011; Robles et al. 2015).

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>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.29 ± 0.10</td>
</tr>
<tr>
<td>COD (mg COD L⁻¹)</td>
<td>58.6 ± 10.2</td>
</tr>
<tr>
<td>BOD₅ (mg BOD L⁻¹)</td>
<td>26 ± 9</td>
</tr>
<tr>
<td>VFA (mg COD L⁻¹)</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Alk (mg CaCO₃ L⁻¹)</td>
<td>817.24 ± 22.56</td>
</tr>
<tr>
<td>NO₃-N (mg N L⁻¹)</td>
<td>0.37 ± 0.18</td>
</tr>
<tr>
<td>NO₂-N (mg N L⁻¹)</td>
<td>1.42 ± 0.67</td>
</tr>
</tbody>
</table>

COD: chemical oxygen demand; BOD: biochemical oxygen demand; VFA: volatile fatty acids.
Laboratory-scale photobioreactor operation

The LabPBR consisted of a cylindrical clear tank with 19 cm of internal diameter (9 L working volume) (see Figure 1(a)), 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 CO₂ (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 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. It was then fed with the nutrient-loaded effluent from AnMBR system, and operated in a semi-continuous mode, without retention of biomass. A peristaltic pump controlled by a PC was used to feed every 3 hours (8 feed cycles a day) a flow of 280 mL in order to keep constant the sludge retention time (SRT) in 4 days, maintaining the total volume with an overflow at the top of the reactor. Moreover, allythiourea was fed to inhibit nitrification bacteria growth, thus assuring that nitrogen removal was due to the microalgae activity.

The illumination was provided by eight uppercase LED strips (Efector LED, SMD5050 60LED/M 5M RGB IP65) fixed in an external cylinder (31 cm internal diameter) around the LabPBR (see Figure 1(b)). Lighting was supplied 24 hours a day, and two photo sensors (Sensor PAR Apogee SQ-222) were disposed under and inside the LabPBR in order to measure light intensity supplied to the culture (see Figure 1(a)). PAR sensor 1 was used to determine and control the light intensity supplied to the photobioreactor while PAR sensor 2 was used to determine 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. Photograph 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 phosphorus 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 CO₂ to the LabPBR, only light, ammonium and phosphate concentrations were considered as limitations to the microalgae growth.

Analytical methods

Nutrient recovery by microalgae was assessed three times a week by recording nitrogen and phosphate concentration in both the influent and the soluble fraction collected from the LabPBR purge. This soluble fraction was obtained by membrane filtration with 0.45 mm pore size filters of polycarbonate glass fibre.

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

The nitrogen and phosphorus content of the dry biomass were measured in triplicate once every 15 days. For this determination an acid-digestion of the dry biomass was performed.

Solids, phosphorus 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 (Smartchem200 de AMS/Alliance Instruments). Nitrogen biomass content was determined via spectrophotometric method using commercial kit (MERCK, 100613) (Spectroquant® Pharo 300 MERCK).

Microbiological method

To assess the microalgae community evolution, a cell count was performed twice a week. 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 dehydrated through successive washes with ethanol (50%, 80%, 90% and 99%). Cell counts were accomplished by the 100× oil immersion lens of an epifluorescence microscopy on a Leica DM2500. In the cell counts, a minimum of 300 cells were counted, assuring that at least 100 cells of the most abundant genera with an error of less than 15% were counted (Pachés et al. 2012). All the measurements were obtained in triplicate.

Calculations

NRE was calculated for influent and effluent on a daily balance basis.

In the nitrogen balance, only NH₄ was considered to be available for biomass growth. This assumption was made based on the concentration of the other soluble species (NO₃ and NO₂), which were negligible during all experimental periods (below 2.20 mg N L⁻¹). Likewise, nitrification was not considered because allylthiourea was used to inhibit the nitrifying bacteria growth. Nitrogen gas loss (N₂ or NH₃) was not considered because the pH was kept around 7.5 (at this pH value, the predominant form of ammonia nitrogen is by far NH₄).

In the phosphorus balance, phosphorus 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, NRE was calculated as follows:

\[ NRE (%) = \left(1 - \frac{E}{T}\right) \times 100 \]  

(1)

where \(I\) and \(E\) are the ammonium or phosphate concentration in the influent and effluent, respectively (mg L⁻¹).

Table 2 | Operational conditions of the LabPBR during each experimental period

<table>
<thead>
<tr>
<th>Period</th>
<th>Duration (d)</th>
<th>Light intensity (μmol s⁻¹ m⁻²)</th>
<th>Ammonium (mg NH₄–N L⁻¹)</th>
<th>Phosphorus (mg PO₄⁻–P L⁻¹)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>50</td>
<td>36.3 ± 5.3</td>
<td>60.62 ± 2.81</td>
<td>6.05 ± 0.73</td>
<td>27.4 ± 0.8</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>54</td>
<td>52.2 ± 4.8</td>
<td>59.31 ± 6.64</td>
<td>5.95 ± 1.22</td>
<td>27.7 ± 1.0</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>9</td>
<td>52.1 ± 0.9</td>
<td>59.32 ± 0.10</td>
<td>15.23 ± 0.03</td>
<td>28.1 ± 0.4</td>
</tr>
<tr>
<td>Exp. 4</td>
<td>16</td>
<td>52.2 ± 1.7</td>
<td>66.06 ± 4.02</td>
<td>7.73 ± 0.23</td>
<td>27.5 ± 0.8</td>
</tr>
</tbody>
</table>
Likewise, intracellular nutrients concentration (INC) were calculated as follows:

$$INC\,\% = \frac{T - E}{VSS} \cdot 100$$  \hspace{1cm} (2)

where $T$ is the total nitrogen or phosphorus 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.

$$\frac{N}{P_E} = \frac{I_{NHA} - E_{NHA}}{I_{PO4} - E_{PO4}}$$  \hspace{1cm} (3)

$$\frac{N}{P_I} = \frac{T_{NHA} - E_{NHA}}{T_{PO4} - E_{PO4}}$$  \hspace{1cm} (4)

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

$$NNU = \frac{I - E}{CC}$$  \hspace{1cm} (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 assessing the growth, NRE and species competition under two different light intensities ($36.3 \pm 5.3$ and $52.2 \pm 6.1 \, \mu mol \, s^{-1} \, m^{-2}$), maintaining all other working conditions constant. Figure 2 shows the time profile evolution of nutrient removal, light intensity, VSS and relative microalgae species abundance obtained along the experimental period.

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

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

The increase in the VSS could indicate that in Exp. 1 the most important biomass growth limiting factor was light. When no other factor is limiting the microalgae growth, enhanced light intensity speeds up the microalgae metabolism as long as it stays under the optimum value (Martín & Marzal 1999).

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 competitiveness of $Scenedesmus$ and $Monoraphidium$ genres in the first experimental period could be explained by the low light intensity supplied. Although a given microalgae species can proliferate whenever the actual value of $I_{out}$ is above their critical light intensity (Huisman & Weissing 1994; Weissing & Huisman 1994), in competition, the species with the lowest $I_{critic}$ displaces 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 below the critical light intensities of all others species (Huisman & Weissing 1994; Weissing & Huisman 1994).

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

This relation is commonly estimated by the Lambert–Beer equation:

$$I_{out} = I_0 \cdot \exp\left(-k_e \cdot c_b \cdot z\right)$$  \hspace{1cm} (6)

where $I_0$ is the intensity measured at the surface of the water column ($\mu$mol s$^{-1}$ m$^{-2}$), $k_e$ is the light attenuation coefficient (m$^{-1}$ 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 to the results obtained in a cylindrical reactor illuminated from its perimeter. The relation between VSS and $I_{out}$ in a cylindrical reactor can therefore be adjusted by the equation proposed by Molina Grima et al. (1997):

$$I_{out} = \frac{I_0}{k_e \cdot c_b \cdot L_{eq}} \left(1 - \exp\left(-k_e \cdot c_b \cdot L_{eq}\right)\right)$$  \hspace{1cm} (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 VSS concentration and the fitting provided by both the Lambert–Beer and Molina Grima et al. equations. As can be seen in this figure, the Molina Grima et al. equation provides a much better fit.

Moreover, when the Molina Grima equation is used, the \( k_e \) coefficient 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 centre of the photobioreactor.

Usually, \( k_e \) depends mostly on the genre and conditions of the algal culture because the light that is supplied to the photobioreactor is mostly absorbed by microalgae. However, the thickness, geometry and material of the photobioreactor must be taken into account because it represents an additional resistance to the light passage.

In this study, the \( k_e \) was deduced from the Molina Grima et al. equation, achieving a value of 0.0859 m² gTSS⁻¹. This value has been calculated assuming a VSS/TSS relation of 86%, which has been deduced from the values obtained in this study.

The \( k_e \) obtained is similar to that reported by other authors (Molina Grima et al. 1994; Ruiz-Martínez et al. 2016), especially by that reported by Ruiz-Martínez et al. (2016), whom assumed a value of 0.0758 m² gTSS⁻¹ operating a flat-plate photobioreactor in outdoor conditions at similar TSS concentrations. Consequently, it can be deduced that the effects of photobioreactor resistance to the light passage on the \( k_e \) coefficient can often be considered negligible, in accordance with Molina Grima et al. (1994) who reported that \( k_e \) depends mainly on the algal light absorption.

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

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

**Effect of nutrients concentration**

Another important operational factor with strong influence on the competition between microalgae species is the nutrient concentration (Yang et al. 2016). In Exp. 1, NRE was low enough (see Figure 2(a)) to maintain a nutrient concentration in the reactor high enough to not limit the microalgae growth. However, in Exp. 2, the NRE increased reaching high values, especially for the phosphorus concentration (up to 96% of P removal). Therefore, nutrient concentration was low enough to be considered a growth limiting factor.

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 because, according to Wu et al. (2014), Chlorella vulgaris can live at the lower nitrogen concentration, but is very difficult to survive in the absence of phosphorus. Therefore, phosphorus is the limiting factor for its growth.

The N/P elimination ratio that 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 Figure 4). The intracellular nitrogen and phosphorus content achieved (see Table 3) seems to indicate that Chlorella needs higher phosphorus concentration for its growth than Scenedesmus and Monoraphidium. Consequently, this result suggests that Chlorella has more
dependence on phosphorus 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 phosphorus concentration are able to change the microalgae dynamics in the LabPBR. Phosphorus concentration was raised to 15 mg L\(^{-1}\) in Exp. 3 and were reduced to normal concentrations in Exp. 4 (see Table 2). The increase in phosphorus concentration feed led to an important cyanobacterium growth (until a 72.6% of relative abundance), which displayed a dramatic decrease until it disappeared when the phosphorus concentration was lowered to the typical concentration level recorded in the AnMBR effluent (see Figure 2). This phenomenon can be explained in the same way as that of the *Chlorella* competitive displacement by the low phosphorus concentration, which presents the cyanobacterium with an even higher phosphorus dependence. This result is in accordance with the N/P

Table 3 | Intracellular nutrient content obtained by each consortium of microalgae

<table>
<thead>
<tr>
<th>Day</th>
<th>Scenedesmus</th>
<th>Chlorella</th>
<th>Monoraphidium</th>
<th>Cyanobacterium</th>
<th>%N</th>
<th>%P</th>
<th>N/P ratio</th>
<th>VSS (mg L(^{-1}))</th>
<th>(I_\text{in} (\mu\text{mol s}^{-1} \text{m}^{-2}))</th>
<th>(I_\text{out} (\mu\text{mol s}^{-1} \text{m}^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>24</td>
<td>75</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1.70</td>
<td>–</td>
<td>236</td>
<td>36</td>
<td>9.93</td>
</tr>
<tr>
<td>29</td>
<td>27</td>
<td>70</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1.83</td>
<td>–</td>
<td>270</td>
<td>36</td>
<td>11.21</td>
</tr>
<tr>
<td>34</td>
<td>17</td>
<td>82</td>
<td>1</td>
<td>–</td>
<td>9.99</td>
<td>1.53</td>
<td>6.52</td>
<td>344</td>
<td>36</td>
<td>9.00</td>
</tr>
<tr>
<td>42</td>
<td>29</td>
<td>59</td>
<td>13</td>
<td>–</td>
<td>11.02</td>
<td>1.80</td>
<td>6.12</td>
<td>268</td>
<td>52</td>
<td>10.22</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
<td>–</td>
<td>60</td>
<td>–</td>
<td>9.40</td>
<td>1.27</td>
<td>7.41</td>
<td>623</td>
<td>52</td>
<td>7.94</td>
</tr>
<tr>
<td>92</td>
<td>28</td>
<td>–</td>
<td>72</td>
<td>–</td>
<td>1.31</td>
<td>580</td>
<td>52</td>
<td>268</td>
<td>52</td>
<td>5.07</td>
</tr>
<tr>
<td>97</td>
<td>32</td>
<td>–</td>
<td>68</td>
<td>–</td>
<td>5.79</td>
<td>0.76</td>
<td>7.61</td>
<td>790</td>
<td>52</td>
<td>3.94</td>
</tr>
<tr>
<td>108</td>
<td>55</td>
<td>2</td>
<td>–</td>
<td>43</td>
<td>2.93</td>
<td>0.70</td>
<td>4.21</td>
<td>1,600</td>
<td>52</td>
<td>1.22</td>
</tr>
<tr>
<td>120</td>
<td>96</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>5.93</td>
<td>–</td>
<td>–</td>
<td>810</td>
<td>52</td>
<td>7.31</td>
</tr>
</tbody>
</table>

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.

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by guest
elimination and intracellular contents ratios obtained when cyanobacterium was the predominant microalgae in the culture (see Figure 4 and Table 3), which achieved the lowest N/P elimination and intracellular content ratios among all genres that predominated in each experimental period when cyanobacterium was present in the PBR.

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 moderately related to 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) throughout the experimental period when this genus was present in the PBR.

This result suggests that the biomass growth itself could produce changes in the medium that influence microalgae competition because light availability and nutrient concentration will decrease growth until microalgae culture reaches the equilibrium (i.e., the pseudo-steady state). Although the ideal conditions for one species were achieved in a photobioreactor, the culture evolves until reaching its own equilibrium. Further research is being carried to provide additional data confirming this interesting finding.

Moreover, in regard to the effluent quality produced, it can be seen in Figure 2 that in the second period (52 μmol s⁻¹ m⁻² of light supplied), the microalgae culture was able to remove nearly all phosphorus concentration. Nevertheless, it could not remove enough nitrogen to meet legal discharge limits, reaching an ammonium concentration in the effluent about 25 mg NH₄-N L⁻¹. Thus, because microalgae stop activity in the absence of any required nutrients (Hoff & Snell 2001), the microalgae cultivated in this study would not be able to treat AnMBR effluent properly, and would require an additional process to reduce ammonium in the effluent. However, AnMBR effluent treatments by microalgae have been studied by many different authors (i.e. Ruiz-Martínez et al. 2016; Viruela et al. 2016), who have reported promising results with outdoor pilot plants, which shows the potential of the microalgae as a feasible tertiary treatment for urban wastewater.

Biomass composition and nutrient uptake efficiency

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

Figure 5 shows a scatter plot of the intracellular nutrient content, Iₜₐₓ and PBR available phosphorus versus VSS. As can be seen in this figure, the nutrient content decreases with a VSS increase.

According to different authors, a reduction in the culture available light causes an increase in the biomass phosphorus content (Hessen et al. 2002; Powell et al. 2008; Ruiz et al. 2014). This phenomenon has been interpreted as a reduction in ATP accumulation when sufficient light energy is available. Hessen et al. (2002) also reported that high light intensity caused reductions in the biomass nitrogen content. Consequently, it can be assumed that by supplying low light intensity to the culture, the nitrogen and phosphorus 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 phosphorus concentration began to be low enough to be considered a growth limiting factor, reaching values under 0.2 mg P L⁻¹. It can be assumed that the decrease in the biomass phosphorus content is due to the competition among microalgae species for the scarce available phosphorus concentration. In addition, nitrogen content in the microalgae biomass decreased with the low available phosphorus concentration, although the available nitrogen concentration was high enough to not limit the biomass growth (data not shown). This could be explained by the fact that microalgae require both nutrients from the environment, stopping their activity in the absence of either (Hoff & Snell 2001). According to Marcilhac et al. (2014), when phosphorus 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).
Figure 6 shows the normalized nutrient uptake as a function of microalgae population. The normalization took 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 reaching a minimum of uptake efficiency. This fact can be explained in the same way as the intracellular nutrient content diminution, which is the lower nutrient uptakes achieved due to the low availability of phosphorus in the PBR. Also, the available light that is able to reach the microalgae culture decreases due to the auto-shadow effect, thereby also reducing the nutrient uptake. Consequently, although the total nutrient removal can increase due to the biomass growth, the NRE per biomass unity in low biomass concentrations is significantly higher than that displayed at high biomass concentrations.

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

The results obtained suggest that light and phosphorus 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 phosphorus concentration, nutrient removal and competition among microalgae species in a continuous fed photobioreactor has been studied. In the experimental period, *Chlorella* 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 photobioreactor, with a nutrients removal efficiency of 35.6 ± 9.9% and 71.8 ± 13.3% of nitrogen and phosphorus, 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* and *Monoraphidium* which increased the VSS until 674 ± 86 mg L⁻¹ and reached a nutrients remove efficiency around 58.4 ± 15.8% and 96.1 ± 16.5% of nitrogen and phosphorus, respectively. The results obtained suggests that *Chlorella* shows a lower I_critic that *Scenedesmus* and *Monoraphidium* as previous studies have reported.

**Table 4** Intracellular nutrient content reported by different authors

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

*Figure 6* | Nutrient uptake normalized by the cell count versus the population density.
Nutrient removal ratio analysis reflects that Chlorella presents higher dependence for phosphorus concentration than Scenedesmus and Monoraphidium, showing lower N/P remove ratios. Moreover, when phosphorus concentration was raised from 6 to 15 mgP L\(^{-1}\), the culture was dominated by cyanobacterium, decreasing in abundance until it disappeared when phosphorus was reduced to 6 mgP L\(^{-1}\) again. These results clearly indicate that phosphorus 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 phosphorus concentration (4.21 ± 0.05, 6.32 ± 0.28 and 7.51 ± 0.14 for a consortium of cyanobacterium and Scenedesmus, and cultures mostly dominated by Chlorella and Monoraphidium, respectively).

This study highlights the importance of light and nutrient concentration in the competence among microalgae, and shows the dramatic impact that changes in these two variables can have in the microalgae species that can survive in a culture and, consequently, in the nutrient remove efficiency. It must also be highlighted that these changes can be induced by the microalgae themselves because during their growth, microalgae shift the available nutrient concentration and/or the available light.

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