Optimization and scale-up of an LED-illuminated microalgal photobioreactor for wastewater treatment


ABSTRACT

The use of light-emitting diode (LED)-illuminated photobioreactors with microalgae has been extensively studied for wastewater treatment. Most studies have used isolated microalgae species; however, this practice does not match the reality of conditions in wastewater treatment plants. Operational conditions that promote greater growth of algal biomass and that remove pollutants most effectively are disputed in the literature. In this context, LED-illuminated photobioreactors with microalgae were evaluated using multivariate analysis in order to optimize removal of pollutants (nitrogen, phosphorus, and carbonaceous organic matter). Three variables were evaluated: operating time, LED wavelength, and luminous flux intensity. A microalgae consortium was used in the photobioreactor. In addition to the LED-illuminated photobioreactors, control photobioreactors illuminated by sunlight were also operated. Using the results obtained in the optimization, a scaled-up reactor approximately 8.5 times larger in volume was operated to evaluate if the behavior would be maintained. The best operational conditions for the removal of pollutants were observed in LED-illuminated photobioreactors operated under a light intensity of 700 μmol·m⁻²·s⁻¹ for 15 days. Under these conditions, it was possible to remove 89.97% of carbonaceous organic matter, 86.50% of nitrogen, and 30.64% of phosphorus. The scaled-up photobioreactor operated with similar performance.

INTRODUCTION

There is a growing demand for technologies capable of treating wastewater that have low energy consumption, can mitigate greenhouse gas emissions and can potentially generate value-added products (Hwang et al. 2016). The use of microalgae consortia (consortia of microalgae and microalgae-bacteria) satisfies this demand; they have the capacity to treat wastewater with high rates of pollutant removal, and they create possibilities for biomass and bioproducts generation (Olguin 2012; Unnithan et al. 2014; Gonçalves et al. 2017).

The use of microalgae in wastewater treatment is widely discussed in the literature. Microalgae treatment systems emerged in 1950 and since then several studies have shown the efficiency of microalgae for removing pollutants (Gonçalves et al. 2017). Wastewater treatment systems with microalgae have their efficiency impaired under conditions of low light intensity and at certain wavelengths (Wang et al. 2007). In open-air systems, unstable light intensity can inhibit the growth of microalgae as a result of insufficient light intensity during rainy and cloudy days or excessive light during sunny days. Thus, the use of artificial light sources to grow microalgae is considered an alternative solution (Pilon et al. 2011). Among the choices of artificial lighting sources suitable for photobioreactors, light-emitting diodes (LEDs) are favored for several reasons, and their use is the subject of much recent research. They are more energy efficient than traditional lighting and allow better performance and operational control (Pattison et al. 2018). The disadvantage of using LEDs is the initial cost, including the purchase of lamps, which is still higher than the cost of other lighting sources such as fluorescent lamps (Johnson et al. 2018). However, it is expected that LEDs will soon have
costs comparable to traditional lighting (Pessoa & Ghisi 2014).

Several studies have evaluated the removal of contaminants through the use of LED-illuminated microalgal photobioreactors. Researchers such as Wang et al. (2007), Yan et al. (2015), and Das et al. (2011) have obtained different responses regarding the best wavelength, light intensity, and operation time for biomass growth and removal of pollutants using LED-illuminated photobioreactors. Most of the studies have been carried out at the laboratory scale using photobioreactors with a volume of less than 0.5 L, and there is concern that scaling up could alter the performance of these photobioreactors, mainly due to the nonhomogeneous distribution of light inside the photobioreactor as a consequence of the auto-shading generated by the cells, which can compromise the performance of the system (Camacho et al. 2014). Therefore, further studies are needed since there is dissent in the literature on the optimal operating conditions and scale-up in photobioreactors is complex.

Therefore, the objective of this study is to optimize the removal of organic matter, nitrogen, and phosphorus in wastewater using LED-illuminated photobioreactors populated with a microalgal consortium. The research also evaluates the response of the photobioreactor to an attempt to scale up under optimized conditions.

**METHODS**

**Photobioreactor optimization**

Small-scale photobioreactors were used to optimize operational conditions, including wavelength, luminous flux intensity, and operating time. They were operated at a working volume of 2.8 L, each with an internal diameter of 0.15 m and height of 0.25 m (Figure 1(a)). A thermostat (brand: Roxin, model: HT-1900) was installed inside each photobioreactor to keep the temperature constant at 24 °C. A black cloth was used to prevent interference from external lights, cover around the sides. The cloth did not prevent gas exchange, because the liquid surface was open.

A submerged aquarium pump (brand: Salor Better, model: SB1000C) was installed inside each photobioreactor for slight agitation and to homogenize the exposure of the biomass to the available light. The aquarium pump was programmed by a plug-in timer (brand: Fox Lux, model: FX TBD) to operate for 3 min and then turn off, repeating this cycle 20 times a day, during the entire operation of the photobioreactor. Cycling the pump on and off was necessary in order to avoid heating the photobioreactors.

**Photobioreactor scaling**

Photobioreactors intended for testing whether optimized conditions would scale up were constructed. They were mounted in a black polyethylene box and operated at a working volume of 24 L; they had rectangular geometry (0.41 m × 0.34 m). Submersible aquarium pumps and thermostats were also installed in these photobioreactors. The pumps ran continuously. One of the photobioreactors was illuminated with a luminous plate (0.41 m × 0.33 m) composed of eight RGB LEDs (brand: Maxtel, model: R100RGB, China) (Figure 1(b)). This was the configuration resulting in the best performance achieved during the optimization step.

**Substrate composition**

Wastewater used in this study was synthetic (OEDC 1996). The composition was as follows: tryptone (160 mg·L⁻¹),...
meat extract (650 mg·L⁻¹), urea (50 mg·L⁻¹), monopotassium phosphate (28 mg·L⁻¹), sodium chloride (7 mg·L⁻¹), calcium dihydrate (4 mg·L⁻¹), magnesium sulfate heptahydrate (2 mg·L⁻¹), and heart and brain infusion broth containing a culture of Escherichia coli (10¹² MNP/100 mL). This composition resulted in: 818 mg·L⁻¹ of chemical oxygen demand, 135 mg·L⁻¹ of total Kjeldahl nitrogen and 31 mg·L⁻¹ of phosphorus.

**Inoculum**

The inoculum was produced from 3 L of a sample (which contained a consortium of microalgal species) from an artificial lake located in the Botanic Garden of the Institute of Exact and Biological Sciences of the Federal University of Ouro Preto. The lake was chosen because it is an environment rich in nutrients, which favors the growth of microalgae. The lake water sample was mixed with 20 L of synthetic wastewater (described above).

A 24-L photobioreactor for inoculum cultivation was set up as described above. The illumination was provided by means of a light plate (0.41 m × 0.33 m) composed of white LED strips (brand: IP4, model: 3528 IP20 3M) with a luminous flux of 160 μmol·m⁻²·s⁻¹. The photoperiod of the photobioreactor was set to 24 h light, 0 h dark.

**Analytical methods**

The following variables were analyzed in accordance with standard procedures: filtered chemical oxygen demand (COD₅, 5220 D (APHA 2012)); total Kjeldahl nitrogen (TKN₅, 4500 D (APHA 2012)); filtered total Kjeldahl nitrogen (TKN₅, 4500 D (APHA 2012)); ammoniacal nitrogen (NH₄-N, 4500 C (APHA 2012)); nitrite (Hach 8507); nitrate (Hach 8871); volatile suspended solids (VSS, 2540 B (APHA 2012)); soluble phosphorus (4500-P D (APHA 2012)), and chlorophyll a (NUSH 1981). For the filtered analyses, the samples were vacuum filtered through a nitrocellulose membrane with porosity of 0.45 μm. The samples were collected on the surface and in triplicate (i.e. three samples were collected in the same reactor).

Measurements of pH, dissolved oxygen (DO), and temperature were collected using multiparameter probes (brand: Hach, model: HQ40D with probes LCD101 and pHCl101). For consistency, temperature was always measured with the thermometer on the pH electrode. The light flux in the photobioreactors was measured on the surface of the liquid with a photo-radiometer capable of measuring photosynthetically active radiation (PAR) (brand: Delta Ohm, model: HD21012.1).

**Optimization procedures – statistical approach**

Two different multivariate statistical analysis were conducted in this study. First, in order to determine the conditions that are most successful for removing nitrogen, phosphorus, and organic matter, we implemented the screening step using a 2² full factorial design (two factors at two levels) with central point, with four repeats at center point. A screening step was performed for three wavelengths – which we refer to as white, blue, and red – and for which we used white, blue, and red LEDs, respectively. These wavelengths were selected based on studies that identified them as having a better response from the microalgae in terms of how well they remove pollutants (Wang et al. 2007; Yan et al. 2013; Choi & Lee 2015; Kang et al. 2018).

For each of the wavelengths (white, red, and blue) two variables were evaluated: the intensity of the luminous flux (X₁) and the length of time the photobioreactors operated (X₂). The levels of the luminous flux (X₁) studied in the screening step were 500, 1,250, and 2,000 μmol·m⁻²·s⁻¹ (Table 1); these values were defined based on studies by Wang et al. (2007), Yan et al. (2013), and Silva et al. (2017). For the time variable (X₂), the durations studied were 4, 8, and 12 days; the selection of these intervals was based on the studies of Wang et al. (2007) and Yan et al. (2013).

Then, based on the results of the screening step, the response surface step was conducted. In this step, we implemented the central composite design (CCD) technique using more refined levels for the variables X₁ and X₂, with five repeats at the center point. The luminous flux levels investigated in this part of the study were 96.45, 200, 450, 700, and 803.55 μmol·m⁻²·s⁻¹, while the durations studied for the time variable were 7.76, 9, 12, 15, and 16.24 days (Table 1). The time and luminous flux levels investigated in the response surface step were based on the results obtained on the screening step.

The experimental matrix for the screening step and response surface was generated from the electronic spreadsheets of Teófilo & Ferreira (2006) (Table 1). Analysis of variance (ANOVA) was used to analyze the results (α = 0.05). The errors associated with each effect were estimated by the t test.

The photobioreactors were filled with 10% inoculum and 90% wastewater. The operation was carried out in batch mode. The photobioreactors operated under 24 h of...
light, 0 h of dark. The analyses of CODf, phosphorus, and TKNf were performed at the beginning and end of photobioreactor operation.

**Scale-up procedures**

A photobioreactor (FLED) ∼8.5 times larger than those used in the previous stage was operated with the same conditions that presented the best global efficiency in the treatment of wastewater during the optimization stage. We found 15 days to be the optimal time; however, we chose to prolong the operation of the photobioreactor to evaluate what happens after the period considered optimal.

Physical and chemical analyses were performed every three days, except for analyses of pH, DO, and CODf, which were performed daily. The samples were collected on the surface and in triplicate (i.e., three samples were collected in the same reactor). Just as in the optimization phase, the photobioreactors were filled with 10% inoculum and 90% wastewater. The operation was carried out in batch mode, with a photo-period of 24 h of light and zero hours dark.

**Control experiments**

Control photobioreactors (Fsol) were illuminated with sunlight and exposed to ambient conditions. The objective of using the control photobioreactors was to allow us to assess the efficiency of removing organic matter, nitrogen, and phosphorus in the artificial light system (FLED) as compared with a sunlit system. In the optimization step, the controls were assembled and operated in the same manner as were the LED-illuminated photobioreactors. For each wavelength evaluated in the 22 complete factorial design and the CCD, controls were also evaluated (Table 1). In the scale-up step, this meant that a photobioreactor of the same size was operated but it was exposed to ambient conditions. The average of sunlight was 1,100 μmol·m⁻²·s⁻¹ during the period of experiments. There were no controls without microalgae. This decision was made due to the ability of microalgae to remove pollutants from wastewater is already widely discussed in the literature.

Table 1 | Experimental conditions used in the screening step and for determination of the response surface for photobioreactor efficacy

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<th>Screening step (2²)</th>
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Note: The prefixes identify photobioreactors illuminated with the white (B), red (V), and blue (A) LEDs; controls (C); and experiments performed on the response surface (SR). Values in parentheses are the encoded values for the CCD and 2² factorial matrix analyses.
RESULTS AND DISCUSSION

Optimization of photobioreactors

Photobioreactors illuminated with blue LEDs were more efficient for the removal of pollutants (carbonaceous organic matter, nitrogen, and phosphorus) (Table 2). We verified that the variable time and intensity of the luminous flux with the blue LED were significant for the three responses studied. Additionally, it was observed that for nitrogen (TKNf) the interaction between the two studied variables has a significant effect on the use of the blue LED. When an effect is found to be significant, this indicates that the variable has an influence on the studied system.

Removal of organic matter (as measured by CODf) was highest in photobioreactor A7 (89.02%), which was illuminated by blue light with a luminous flux of 500 μmol·m⁻²·s⁻¹ for 12 days. For the removal of nitrogen, among the LED-illuminated photobioreactors the best TKNf removal rates were obtained in photobioreactors A7 (73.26%) and A8 (78.55%). Like photobioreactor A7, A8 was also illuminated by blue light, but with a luminous flux of 2,000 μmol·m⁻²·S⁻¹ for 12 days. For phosphorus, the best removal rate was found in photobioreactor A1 (40.32%), which had a luminous flux of 500 μmol·m⁻²·s⁻¹ and four days of operation. The low efficiency of phosphorus removal by photobioreactors may have occurred due to the stoichiometric ratio between nitrogen and phosphorus, which was 4:1. According to Yan et al. (2015) the optimal N:P rate is 5:1, and small differences can influence the removal of phosphorus.

From the results obtained in the screening step, we observed that for the removal of nitrogen, phosphorus, and organic matter, the blue LED-illuminated photobioreactors presented the best results (photobioreactors A1, A7, and A8) and that the differences between them were less than 17%. These results and the fact that the energy consumption of the blue LED operating at an intensity of 500 μmol·m⁻²·s⁻¹ is 50% lower than that of the same LED operating at 2,000 μmol·m⁻²·s⁻¹ determined the parameters for the next part of the study. For the response surface experiments, we investigated various luminous flux levels and times starting from the conditions used for the A7 photobioreactor band (blue light with a flux of 500 μmol·m⁻²·s⁻¹ for 12 days).

Applying the response surface methodology (Table 1) indicated that the best conditions for the removal of organic matter and nitrogen occurred under the experimental conditions of the SR4 photobioreactor (700 μmol·m⁻²·s⁻¹ light flux for 15 days). This configuration was able to remove 86.55% of TKNf, 89.97% of organic matter, and 30.64% of phosphorus (Table 2).

The optimization procedures approach allowed us to obtain a significant mathematical model for the removal of nitrogen, with $p = 0.0009$ for the model and $p = 0.3248$ for the lack of fit. For the organic matter removal, $p = 0.0058$ was obtained for the model and $p = 0.0076$ for the lack of fit. For the phosphorus removal, $p = 0.0362$ was obtained for the model and $p = 0.4166$ for the lack of fit. The coefficients of determination ($r^2$) were 0.92 for nitrogen, 0.88 for organic matter, and 0.76 for phosphorus. For the removal of organic matter, although the mathematical model was not significant, the multivariate optimization approach used provided a greater removal of organic matter than in the screening step. For the removal of phosphorus, although the model has been adjusted, the errors are not associated with the pure error. Therefore, more experiments are needed so that the model represents the system better. Using the multivariate analysis approach, it was possible to optimize the removal of organic matter and nitrogen in photobioreactors. The models were obtained by multiple linear regression.

Control experiments

Results with the control photobioreactors showed that sunlight is effective for the removal of organic matter, nitrogen, and phosphorus. However, efficiency varies due to the weather. The largest nitrogen removals were found in the control photobioreactors. However, C6 operated for the same length of time (12 days) presented the lowest rate of nitrogen removal (71.34%) in comparison to the trials with the highest rates of removal, in photobioreactors C3 and C9 (89.75% and 84.62%, respectively). While the experiment on photobioreactors C3 and C9 was being carried out, no precipitation was recorded, which favored the microalgae activity. By contrast, during the operation of C6, precipitation did occur, which decreased the microalgae’s pollution removal efficiency. This result shows that sunlight is effective for nitrogen removal. However, weather conditions such as precipitation and either excessive or insufficient illumination can influence the efficiency of the system. The same behavior was noted for the removal of phosphorus and organic matter. This shows that although sunlight is a freely available energy source, its availability can significantly influence the efficiency of photobioreactors.
available resource, its use does not guarantee constancy in the efficiency of photobioreactors. Therefore, the use of artificial lighting is an alternative that can ensure efficiency and reliability in photobioreactors.

### Photobioreactor scaling

From the results obtained with the optimization procedures, a photobioreactor with optimized conditions (SR4) was
operated with these parameters: blue LED, a luminous flux of 700 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), and 15 days of operation.

Dissolved oxygen (DO) at the beginning of the experiment was 7.03 mg L\(^{-1} \), as the wastewater was made with tap water. Twenty-four hours after the start of the experiment, DO values fell close to zero (Figure 2(a)) in both the LED-illuminated photobioreactor and the sunlight-illuminated control (F\(_{\text{LED}}\) and F\(_{\text{sol}}\), respectively). The drop in DO might have occurred due to degradation of organic matter by aerobic bacteria. Starting on the second day, the DO in the F\(_{\text{LED}}\) increased again, which might have resulted from the growth of microalgae through photosynthesis. The same DO reduction behavior was observed in the experiments of Silva et al. (2017), but in an illuminated photobioreactor with low luminous flux (160 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). DO values only began to increase on the sixth day of operation.

The pH values in the two photobioreactors (F\(_{\text{LED}}\) and F\(_{\text{sol}}\)) also increased during the experiment (Figure 2(b)). In the F\(_{\text{LED}}\), the highest pH value (10.66) was obtained, which occurred on the 18th day of operation. The DO started to increase on the third day. On the ninth day of operation, it had a maximum concentration of 15.78 mg L\(^{-1} \). After that time, it oscillated. In F\(_{\text{sol}}\), the highest pH value (10.57) was obtained on the tenth day of operation. On the seventh day of operation, F\(_{\text{sol}}\) presented a DO concentration of 17.47 mg L\(^{-1} \), which was the highest of the two photobioreactors. The increase in pH during the experiments made the medium more basic due to the consumption of carbon dioxide (CO\(_2\)) by the microalgae, which is its source of carbon. Similar behavior was observed in studies by Yan et al. (2015) and Silva et al. (2017).

In the two photobioreactors (F\(_{\text{LED}}\) and F\(_{\text{sol}}\)) the chlorophyll \( a \) concentration increased markedly between the beginning of the experiment and the third day (Figure 2(c)). In F\(_{\text{LED}}\), starting from day six, chlorophyll \( a \) was maintained at an approximately constant value. After analyzing the data for chlorophyll \( a \) and VSS, we conclude that the biomass in F\(_{\text{LED}}\) exhibited the typical growth cycle of microorganisms, with a lag phase, an exponential growth phase, and a plateau. Eventually, we would expect the microorganism population to decline. However, we did not observe a decline phase within the 18 days of the experiment. We also observed that, even though VSS and chlorophyll \( a \) are both considered to be proxy variables to estimate biomass, their results in these experiments were not similar. This is most likely because VSS is associated also with microbial biomass and not just with photosynthetic organisms. Thus, even at times when chlorophyll \( a \) decreases, VSS continues to increase, possibly indicating the presence of other microorganisms in addition to photosynthesizers.

Removal of the organic matter was efficient for both photobioreactors (F\(_{\text{LED}}\) and F\(_{\text{sol}}\)). The best result was produced by F\(_{\text{LED}}\), with 91% removal, while F\(_{\text{sol}}\) presented 89% removal. On the third day of operation of the photobioreactors, a decrease of the organic matter concentration was observed; after that time, the concentration showed small oscillations (Figure 2(d)). The results obtained for the removal of organic matter were like those found in other experiments, even with the difference in wavelength. Yan et al. (2013) was able to remove 42.23% with the use of blue LEDs and 76.46% with a red LED. Their study used a luminous flux of 2,000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), which implies a greater energy expenditure. In addition, the volume of the photobioreactor used in the study was 0.4 L.

Although the percentages removed were comparable, the time taken to remove organic matter in this study was lower than that reported in the work of Yan et al. (2012) and Xu et al. (2013). This may be related to the fact that in the studies cited the synthetic wastewater used was prepared in a sterile manner. Another possibility is that the inoculum used in this work contained a consortium of microalgae and other microorganisms, and in the studies of Yan et al. (2013) and Xu et al. (2013) was a pure culture.

In the two photobioreactors (F\(_{\text{sol}}\) and F\(_{\text{LED}}\)), the organic nitrogen at the beginning of the experiment had a concentration of 150 mg L\(^{-1} \), which was converted to ammoniacal nitrogen (which is the preferred form for assimilation by microalgae). The concentration of ammoniacal nitrogen increased between time zero and the third day of the experiment, after which it decreased to below the limit of detection for the method used in this research (Figure 2(g) and 2(h)). In the F\(_{\text{LED}}\), starting on the ninth day the presence of ammoniacal nitrogen was not detected. In the F\(_{\text{sol}}\) it was undetectable only starting on the fifteenth day. F\(_{\text{LED}}\) showed a 96.34% removal of TKN\(_{f}\). Starting from the ninth day, in the F\(_{\text{LED}}\) TKN\(_{f}\) maintained its almost constant concentration, with values remaining below 10 mg L\(^{-1} \) (Figure 2(g)). The two photobioreactors showed themselves to be efficient in the removal of nitrogen, which can be explained by the processes of volatilization and biological assimilation (Reed 1985). Removal values were similar to those in the study by Yan et al. (2013) that used C. vulgaris but obtained the best results in light intensity of 200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) with red LED (78.56%). The superior results for nitrogen removal in this study compared to the studies of Yan et al. (2013), Xu et al. (2013), and Yan et al. (2012) – even with the use of a smaller luminous flux – may
be due to the slow stirring system we used. This did not allow the formation of a layer of microalgae on the surface that would prevent the passage of light into the deeper layers of the photobioreactors.

Soluble phosphorus in the FLED showed a reduction of 43.54% between the beginning of the experiment and the third day and remained practically constant until the ninth day. After this time, there was an increase of soluble
phosphorus in the photobioreactor, and at the end of the experiment, the photobioreactor had a concentration of 20 mg·L\(^{-1}\), which represented 35% removal (Figure 2(e)). This was an improvement in the F\(_{LED}\) result compared to the response surface result (SR4) that removed 30.64%. In the F\(_{sol}\), a reduction was observed during the first three days of the experiment, and after some oscillations of the concentration, at the end of the experiment it was found to have removed 68.55%. Further removal of soluble phosphorus in F\(_{sol}\) may have occurred due to the production of precipitates that caused the photobioreactor to overflow and its volume to dilute.

Removal of phosphorus was below the values found in other studies. Xu \(\text{et al.}\) (2015) in 10 days obtained 55% removal of phosphorus with blue light at an intensity of 2,000 \(\mu\text{mol·m}^{-2}·\text{s}^{-1}\) and 73.93% with a red light at the same luminous flux level. Yan \(\text{et al.}\) (2015) in 10 days reached 41% removal with blue light at an intensity of 2,000 \(\mu\text{mol·m}^{-2}·\text{s}^{-1}\), and 73.93% with red color with the same intensity. In addition to the low efficiency of phosphorus removal by the photobioreactors, it was observed that in B7 an increase of soluble phosphorus occurred in the photobioreactors, so further studies are needed to understand the behavior in this system. The differences between the results of the removal obtained in the studies may be related to the ratio between nitrogen and phosphorus. Yan \(\text{et al.}\) (2015) state that the optimal N:P ratio for removal of phosphorus is 5:1, but Choi & Lee (2015) state that this ratio varies according to species. It is known that the photobioreactors contained different genera (Chlorella, Pseudanabaena, Euglenophyta, Bacillaciophytas), so the N:P ratio, despite being 5:1 at the beginning of the experiments, may not have favored removal by these species.

Scaling up the volume did not decrease the efficiency of the photobioreactor. With the F\(_{LED}\) photobioreactor it was possible to obtain better removal results for the removal of TKN\(_{f}\) (96.34%) and COD\(_{f}\) (91%) compared to SR4 (Figure 3). Removal of the pollutants was possibly maintained because the intensity of the light flux was maintained after the scale increase (Molina \(\text{et al.}\) 1999). With the increased scale, the geometry of the photobioreactor was modified, but we maintained the slow stirring and the overall configuration, which had illumination coming from above the surface of the liquid. Molina \(\text{et al.}\) (1999) and Camacho \(\text{et al.}\) (2011) when doing a similar scale-up, maintained the geometry of their photobioreactor, and according to the authors this is what guarantees success. The photobioreactors evaluated here, although their geometry changed, maintained their performance.

**CONCLUSIONS**

For removal of filtered total Kjeldahl nitrogen and organic matter, the blue LED showed the best response with a light intensity of 700 \(\mu\text{mol·m}^{-2}·\text{s}^{-1}\) and a 15-day operating time. Further studies are needed to achieve better removal of phosphorus. After increasing the size of the photobioreactor, it was possible to remove 96.34% of filtered nitrogen, 91% of organic matter and 35% of phosphorus. This shows that after the increase in the scale of the photobioreactor, pollutant removal efficiency was maintained.

![Figure 3](http://iwaponline.com/wst/article-pdf/80/12/2352/672902/wst080122352.pdf) | The best pollutant removal rates obtained with the LED-illuminated photobioreactors for each experimental step, according to the screening step (\(2^2\) full factorial design statistical analysis), response surface (central composite design (CCD) statistical analysis) and the scale-up experiment. The error bars refer to the sampling in triplicate.
when compared to the results of the optimization process. LED-illuminated photobioreactors with microalgae are a promising technology for wastewater treatment applications.

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