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

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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 $\mu mol \cdot m^{-2}s^{-1}$ 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.

Key words | light-emitting diode, microalgae, multivariate optimization, removal of pollutants, scale-up

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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

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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.* (2013), 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.* 2011). 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 microalgae 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}),

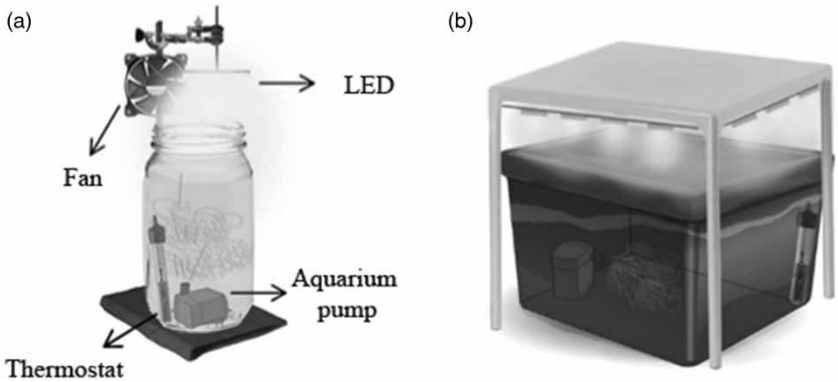

Figure 1 | Photobioreactors. (a) Photobioreactor used in screening step and response surface analysis. (b) Photobioreactor used in scale-up experiments.

meat extract (650 mg·L^{-1}), urea (30 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* (10^{12} 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 (COD_f, 5220 D (APHA 2012)); total Kjeldahl nitrogen (TKN, 4500 D (APHA 2012)); filtered total Kjeldahl nitrogen (TKN_f, 4500 D (APHA 2012)); ammoniacal nitrogen (NH_4-N, 4500 C (APHA 2012)); nitrite (Hach 8507); nitrate (Hach 8171); 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 LDO101 and pHC101). 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 2^2 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 (X_1) and the length of time the photobioreactors operated (X_2). The levels of the luminous flux (X_1) 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 (X_2), 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 X_1 and X_2, 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 ($\alpha =$ 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

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

Screening step (2^2)			Determination of response surface (CCD)		
Assay (Photobioreactors)	**X_1 (Luminous flux, $\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**X_2 (Time, in days)**	**Assay (Photobioreactors)**	**X_1 (Luminous flux, $\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**X_2 (Time, in days)**
B1, V1, A1	500 (−1)	4 (−1)	SR1	200 (−1)	9 (−1)
B2, V2, A2	2,000 (1)	4 (−1)	SR2	700 (1)	9 (−1)
B3, V3, A3	1,250 (0)	8 (0)	SR3	200 (−1)	15 (1)
B4, V4, A4	1,250 (0)	8 (0)	SR4	700 (1)	15 (1)
B5, V5, A5	1,250 (0)	8 (0)	SR5	96.45 (−1.41)	12 (0)
B6, V6, A6	1,250 (0)	8 (0)	SR6	803.55 (1.41)	12 (0)
B7, V7, A7	500 (−1)	12 (1)	SR7	450 (0)	7.76 (−1.41)
B8, V8, A8	2,000 (1)	12 (1)	SR8	450 (0)	16.24 (1.41)
C1, C4, C7	Sunlight	4	SR 9	450 (0)	12 (0)
C2, C5, C8	Sunlight	8	SR10	450 (0)	12 (0)
C3, C6, C9	Sunlight	12	SR11	450 (0)	12 (0)
			SR12	450 (0)	12 (0)
			SR13	450 (0)	12 (0)
			C10	Sunlight	7.76
			C11	Sunlight	9
			C12	Sunlight	12
			C13	Sunlight	15
			C14	Sunlight	16.24

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 2^2 factorial matrix analyses.

light, 0 h of dark. The analyses of COD_f, phosphorus, and TKN_f were performed at the beginning and end of photobioreactor operation.

Scale-up procedures

A photobioreactor (F_{LED}) ~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 COD_f, 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 photoperiod of 24 h of light and zero hours dark.

Control experiments

Control photobioreactors (F_{sol}) 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 (F_{LED}) 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 2^2 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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.

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 (TKN_f) 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 COD_f) was highest in photobioreactor A7 (89.02%), which was illuminated by blue light with a luminous flux of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 days. For the removal of nitrogen, among the LED-illuminated photobioreactors the best TKN_f 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 $\mu mol \cdot m^{-2} \cdot S^{-1}$ for 12 days. For phosphorus, the best removal rate was found in photobioreactor A1 (40.32%), which had a luminous flux of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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.* (2013) 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is 50% lower than that of the same LED operating at 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light flux for 15 days). This configuration was able to remove 86.55% of TKN_f, 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, $p = 0.0038$ 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

Table 2 | Results of the screening experiments and the experiments to characterize the response surface

Screening step (2^2)				Response surface (CCD)			
	Removal of pollutants (%)				Removal of pollutants (%)		
Photobioreactors	COD	N	P	Photobioreactors	COD	N	P
B1 (500/4)	−7.06	0.33	6.45	SR1 (200/9)	51.65	45.89	19.35
B2 (2000/4)	22.96	23.21	11.29	SR2 (700/9)	62.47	58.40	25.81
B3 (1250/8)	51.60	34.11	28.23	SR3 (200/15)	64.90	70.37	27.42
B4 (1250/8)	57.36	32.84	25.81	SR4 (700/15)	89.97	86.55	25.00
B5 (1250/8)	63.95	30.58	26.61	SR5 (96.45/12)	66.27	57.26	17.74
B6 (1250/8)	66.16	47.37	20.97	SR6 (803.55/12)	76.77	73.32	27.42
B7 (500/12)	72.80	50.27	−8.06	SR7 (450/7.76)	12.15	43.95	28.23
B8 (2000/12)	69.06	55.24	30.65	SR8 (450/16.24)	75.85	68.23	30.65
C1 (sunlight/4)	47.60	45.57	5.65	SR9 (450/12)	85.42	72.20	25.00
C2 (sunlight/8)	76.77	60.23	4.03	SR10 (450/12)	82.10	66.86	29.03
C3 (sunlight/12)	87.95	89.75	−10.48	SR11 (450/12)	76.90	59.98	28.23
V1 (500/4)	15.84	47.50	8.06	SR12 (450/12)	80.75	69.46	25.00
V2 (2000/4)	47.25	26.76	13.71	SR13 (450/12)	83.09	64.44	29.84
V3 (1250/8)	77.68	60.75	24.19	C10 (sunlight/7.76)	62.47	57.41	51.61
V4 (1250/8)	69.67	52.80	25.00	C11 (sunlight/9)	77.94	59.22	41.13
V5 (1250/8)	71.46	62.12	22.58	C12 (sunlight/12)	79.09	72.38	45.97
V6 (1250/8)	75.71	58.88	21.77	C13 (sunlight/15)	83.39	54.37	37.90
V7 (500/12)	83.13	70.12	28.23	C14 (sunlight/16.24)	82.27	56.18	36.29
V8 (2000/12)	85.27	71.03	28.23				
C4 (sunlight/4)	42.15	45.84	27.42				
C5 (sunlight/8)	68.80	64.62	25.00				
C6 (sunlight/12)	84.01	71.34	27.42				
A1 (500/4)	42.04	36.98	34.00				
A2 (2000/4)	52.66	36.29	33.87				
A3(1250/8)	69.97	68.77	33.87				
A4 (1250/8)	65.09	64.35	30.65				
A5 (1250/8)	72.14	64.55	29.03				
A6 (1250/8)	68.00	64.12	29.03				
A7 (500/12)	89.02	73.26	32.26				
A8 (2000/12)	84.11	78.55	27.42				
C7 (sunlight/4)	81.75	56.95	48.39				
C8 (sunlight/8)	75.84	69.91	50.00				
C9 (sunlight/12)	79.09	84.62	48.39				

Note: The values in parentheses indicate the light flux ($\mu mol \cdot m^{-2} \cdot s^{-1}$) and the time (days) for each photobioreactor. The prefixes 'B', 'V', 'A', 'C', and 'SR' identify experiments performed with white (B), red (V), and blue (A) LEDs; controls (C); and experiments performed with blue light on the response surface (SR). The negative values indicate an increase in the pollutant concentration.

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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 15 days of operation.

Dissolved oxygen (DO) at the beginning of the experiment was 7.03 $mg \cdot L^{-1}$, as the wastewater was made with tap water. Twenty-four hours after the start of the experiment, DO values fell to close to zero (Figure 2(a)) in both the LED-illuminated photobioreactor and the sunlight-illuminated control (F_{LED} and F_{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_{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 $\mu mol \cdot m^{-2} \cdot s^{-1}$), DO values only began to increase on the sixth day of operation.

The pH values in the two photobioreactors (F_{LED} and F_{sol}) also increased during the experiment (Figure 2(b)). In the F_{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 $mg \cdot L^{-1}$. After that time, it oscillated. In F_{sol}, the highest pH value (10.57) was obtained on the tenth day of operation. On the seventh day of operation, F_{sol} presented a DO concentration of 17.47 $mg \cdot L^{-1}$, 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.* (2013) and Silva *et al.* (2017).

In the two photobioreactors (F_{LED} and F_{sol}) the chlorophyll *a* concentration increased markedly between the beginning of the experiment and the third day (Figure 2(c)). In F_{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_{LED} exhibited the typical growth cycle of microorganisms, with a lag phase, an exponential growth phrase, 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_{LED} and F_{sol}). The best result was produced by F_{LED}, with 91% removal, while F_{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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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_{sol} and F_{LED}), the organic nitrogen at the beginning of the experiment had a concentration of 150 $mg \cdot L^{-1}$, 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_{LED}, starting on the ninth day the presence of ammoniacal nitrogen was not detected. In the F_{sol}, it was undetectable only starting on the fifteenth day. F_{LED} showed a 96.34% removal of TKN_f. Starting from the ninth day, in the F_{LED} TKN_f maintained its almost constant concentration, with values remaining below 10 $mg \cdot L^{-1}$ (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 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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

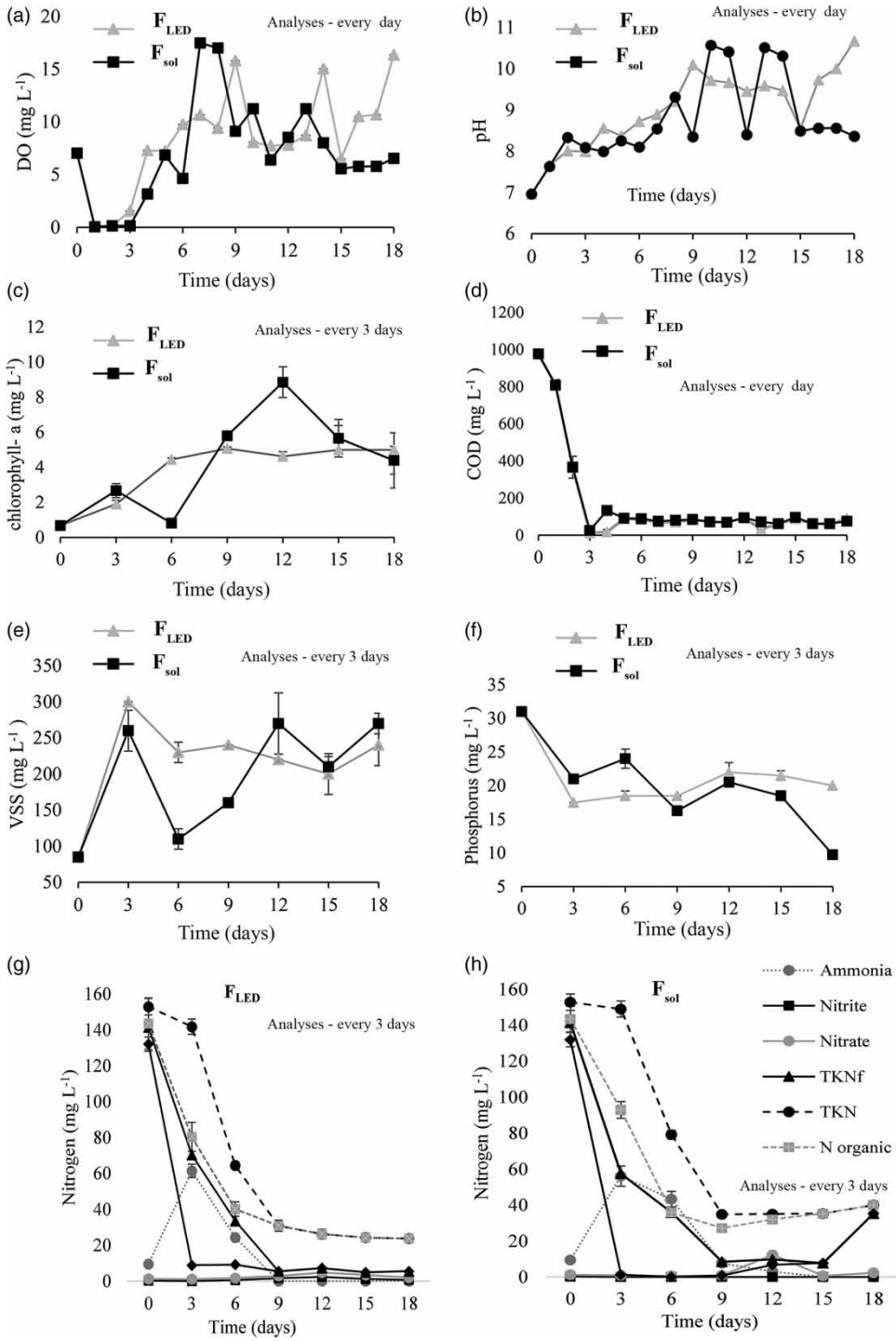

Figure 2 | Photobioreactor scaling. Comparison between F_{LED} (photobioreactor illuminated with LEDs) and F_{sol} (control photobioreactor) for the variables: (a) DO (dissolved oxygen); (b) pH; (c) chlorophyll *a*; (d) COD (chemical oxygen demand); (e) VSS (volatile suspended solids); (f) phosphorus; (g) nitrogen, F_{LED} series; and (h) nitrogen, F_{sol} series. The error bars refer to the sampling in triplicate.

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 F_{LED} 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 *et al.* (2013) in 10 days obtained 55% removal of phosphorus with blue light at an intensity of 2,000 μmol·m^{-2}·s^{-1} and 73.93% with a red light at the same luminous flux level. Yan *et al.* (2013) in 10 days reached 41% removal with blue light at an intensity of 2,000 μmol·m^{-2}·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 *et al.* (2013) 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 *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 *et al.* (1999) and Camacho *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 μmol·m^{-2}·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

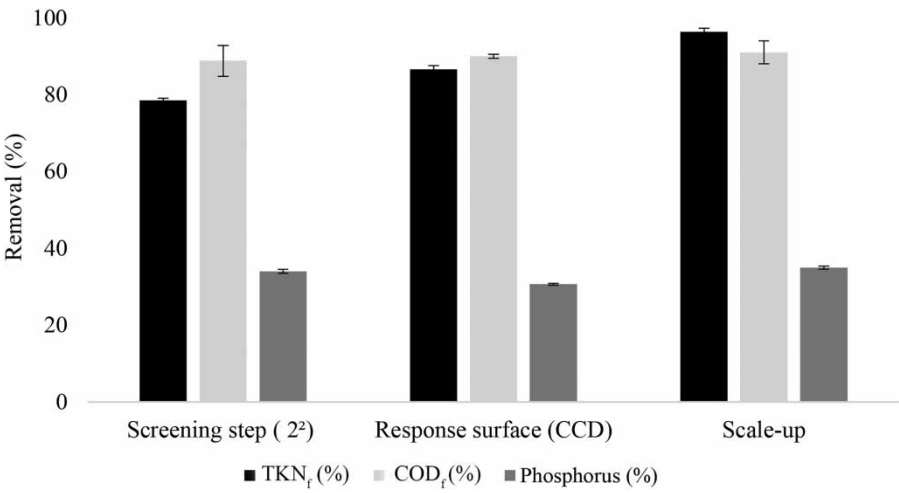

Figure 3 | The best pollutant removal rates obtained with the LED-illuminated photobioreactors for each experimental step, according to the screening step (2^2 full factorial design statistical analysis), response surface (central composite design (CCD) statistical analysis) and the scale-up experiment. 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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