Optimization of microalgae harvesting by sedimentation induced by high pH

Luan de Souza Leite and Luiz Antonio Daniel

ABSTRACT

Microalgae harvesting is a major hurdle for the production of high-value microalgal bioproducts on a large scale. Among harvesting techniques, pH-induced sedimentation stands out as an inexpensive and technically viable method. Nevertheless, there is little information available on the application of this method for microalgae cultivated in wastewater. In this context, the present study investigated the optimization of sedimentation parameters for *Chlorella sorokiniana* harvesting from wastewater. Parameter optimization was statistically determined by the response surface methodology. The optimal values included a velocity gradient of 250 s^{-1}, mixing time of 10 seconds, and pH of 12, which enabled microalgae harvesting efficiencies of more than 97.8%. These optimal parameters also showed resilience through the physico-chemical variation of the photobioreactor effluent. Furthermore, wastewater quality improved significantly after microalgae harvesting. High removal was found for turbidity (97.9–98.3%), apparent color (92.2–97.2%), total Kjeldhal nitrogen (91.0–94.4%), and total phosphorus (92.8–98.6%). Centrifugation, as the dewatering method, and its operational parameters were also evaluated. Sedimentation followed by centrifugation increased the initial microalgae concentration by about 123 times. This study shows the importance of operational optimization and the results can be used as practical guidelines for microalgae harvesting on a large scale.

Key words | *Chlorella sorokiniana*, microalgae harvesting, sedimentation, wastewater

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INTRODUCTION

Microalgae is a source of a wide range of bioproducts with commercial applications (e.g. carbohydrates, phenols, protein, lipids, fatty acids, and fine chemicals) (Patel *et al.* 2017). However, the high costs incurred by biomass production do not make microalgal bioproducts commercially viable. Major savings can be achieved by appropriately selecting the harvesting method as the biomass recovery represents 20–60% of the total production costs (Molina Grima *et al.* 2003). High energy demand is required in this production step due to the low microalgae concentration (0.5–5.0 $g{\cdot}L^{-1}$), small cell diameter (5–50 μm), and negative surface charge (Sukenik & Shelef 1984).

Different harvesting methods have been used for microalgae recovery, such as filtration, centrifugation, sedimentation, and flotation (Kadir *et al.* 2018). Sedimentation stands out as an inexpensive and technically viable method for large-scale operations as it can be used to pre-concentrate the microalgal suspension prior to the dewatering/drying method (Fasaei *et al.* 2018). As in commercial systems, centrifugation is used as a harvesting method, using sedimentation followed by centrifugation could significantly reduce the costs (Schlesinger *et al.* 2012). In this case, the sedimentation and centrifugation processes would be used as the harvesting and the dewatering methods, respectively.

Among the types of sedimentation, microalgal sedimentation induced by high pH is an effective method and requires a cheap base (e.g. sodium hydroxide) instead of coagulants (e.g. metal salts and polymers) (Vandamme *et al.* 2012). In alkaline conditions, sedimentation occurs due to the coprecipitation of inorganic salt precipitates (e.g. calcium phosphate, calcium carbonate, magnesium hydroxides, struvite, and calcite) (Lei *et al.* 2018). These precipitates interact with the negative microalgae cells by the charge neutralization mechanism and sweep flocculation, and microalgae-precipitates flocs are formed (Leite *et al.* 2020).

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Microalgae sedimentation at high pH has been widely investigated for microalgae cultivated in a culture medium (Wu *et al.* 2012; Rakesh *et al.* 2014; Ummalyma *et al.* 2016; Pérez *et al.* 2017). Nevertheless, there is little information about the application of this method for microalgae cultivated in wastewater (Mennaa *et al.* 2019). A thorough investigation is required to apply the method to the complex matrix that is wastewater, because some compounds can significantly affect the efficiency of microalgae sedimentation (Leite *et al.* 2019a).

Furthermore, the optimal operational parameters of sedimentation in alkaline conditions are unclear. The previous studies did not consider the influence of parameters on harvesting efficiency (Wu *et al.* 2012; Rakesh *et al.* 2014; Ummalyma *et al.* 2016; Pérez *et al.* 2017). In general, the experiments were conducted in beakers, which were stirred at a stated velocity (in rpm) by a mechanical agitator or magnetic stirrer. However, information given as rpm does not allow reproducibility because it is a variable according to the equipment and volume. Agitation information should be given as a velocity gradient, which reflects the mixing intensity considering the relative speed and vessel geometry (Mhaisalkar *et al.* 1986). Thus, practical guidelines are necessary to enable a scale-up of the process.

In this context, this paper investigated the optimization of sedimentation induced by high pH for *Chlorella sorokiniana* harvesting from wastewater. The main goals of this paper are: (1) to optimize the sedimentation parameters (pH, velocity gradient, and mixing time) for *Chlorella sorokiniana* harvesting; (2) to evaluate centrifugation as a dewatering method; and (3) to check the reproducibility of the optimal sedimentation parameters and the wastewater quality after the microalgae harvesting.

MATERIAL AND METHODS

Microalgae cultivation

Chlorella sorokiniana 211-8 k (Culture Collection of Algae and Protozoa, Argyll, Scotland) was used as a strain model in this study, and cultivated in a wastewater mixture previously treated by an upflow anaerobic sludge blanket (UASB) reactor. A raw wastewater mixture was prepared weekly using 400 L of wastewater from a swine farm (Brotas, Brazil) and 1,200 L of municipal wastewater from a full-scale wastewater treatment plant (São Carlos, Brazil). The raw wastewater was pumped and anaerobically treated in a UASB reactor, operating with a volume of 650 L and upflow velocity of 220 $L \cdot day^{-1}$. Then, the treated effluent was collected to feed three 50 L flat panel photobioreactors for the microalgae growth. The swine farm wastewater was used as an additional nutritional source to increase the nitrogen and phosphorus concentrations in the wastewater mixture.

Batch cultivation was performed according to the procedures described previously (Leite *et al.* 2019b). The conditions were maintained with an average light intensity of 196 $\mu mol \cdot m^{2} \cdot s^{-1}$, aeration rate of 0.6 vvm, 10 L of microalgae acclimated in wastewater as the inoculum, a photoperiod of 16:8 (day:night), and 30 °C of temperature. After 7 days, the photobioreactor effluent (PBRE) was collected, homogenized, and used for characterization and sedimentation tests.

Microalgae concentration was estimated by measuring the absorbance at 680 nm and dry weight (DW). DW was determined gravimetrically by filtering 15 mL of PBRE through a 0.45 μm glassmicrofiber membrane (Macherey-Nagel, Germany), which was then dried for 24 hours at 100 °C. Then, DW was quantified by the difference in membrane weight before and after drying. The pH and alkalinity of the PBRE samples were also characterized according to procedures described in *Standard Methods for the Examination of Water and Wastewater* (APHA 2012).

Sedimentation experiments

Microalgae harvesting at high pH was investigated in a jar test apparatus with six positions for 2.5 L jars (model 218-6LDB, Nova Ética, Brazil). In the optimization process, the following sedimentation parameters were evaluated and optimized: pH, velocity gradient (G_V) and mixing time (T_M). In general, coagulation and flocculation processes are required for the microalgae settling. Nevertheless, high sedimentation efficiency was reached using only coagulation in this study.

The tests were performed in a defined environment, where the initial pH of the PBRE (2 L, 0.5 g L^{-1}) was modified (pH 9–13) by adding 5 N NaOH (Qhemis, Brazil). The pH values tested were selected based on preliminary tests. The coagulation process was carried out in rapid mixing of the PBRE and NaOH using different associations of coagulation parameters ($G_V \times T_M$). The appropriate quantity of NaOH was added to give the required pH. After that, floc settling was allowed for 7 min. Samples of the sedimentation test effluent (STE) were taken from 7 cm below the liquid level in the jars.

Each condition was tested in triplicate and the maximum experimental error was ±5%. The microalgae concentration was estimated by absorbance at 680 nm using a UV-vis spectophotometer (model 5500, Hach, USA). Sedimentation efficiency (SE, %), i.e. the percentage of microalgae removed from the wastewater, was calculated according to Equation (1).

$$SE(\%) = \frac{Abs_i - Abs_f}{Abs_i} \times 100 \quad (1)$$

where Abs_i and Abs_f are the absorbance values at 680 nm before and after sedimentation, respectively.

Experimental procedure

The experimental procedure for the optimization coagulation parameters was divided into two steps:

(1) Sedimentation efficiency was evaluated at various pH values (pH 9–13) using pre-chosen parameters ($G_V = 500\ s^{-1}$ and $T_M = 30$ seconds). The pH values which reached the highest efficiencies were selected and tested in the following optimization step.
(2) A response surface methodology (RSM) was used to quantify the effect of coagulation parameters (G_V, T_M) on the microalgae sedimentation efficiency (SE). These parameter values were selected because they are usually applied in full-scale wastewater treatment plant facilities (Metcalf & Eddy 2003). The analysis was carried out using two factors (Table 1) and optimized using a central composite design, based on a three-factor level (−1, 0, +1) design with an alpha face centred (a = 1). A total of 13 experiments were carried out to complete the experimental design for each pH. The experimental data were analyzed by multiple regression and analysis of variance (ANOVA) using the Minitab software (version 18.1, Minitab LLC., PA, USA). Each pH graphic was plotted using different colors (pH 12 – green, pH 12.5 – blue and pH 13 – gray), because of their different SE ranges.

Table 1 | Experimental factors and their coded and actual values

		Coded values		
Variables	**Unit**	**−1**	**0**	**+1**
G_V	s^{-1}	250	500	750
T_M	s	10	20	30

To ensure the same quality, all experiments were performed in triplicate using the same PBRE with a microalgae concentration, in terms of DW, of $0.5\ g\ L^{-1}$ and a pH of 9. The high pH was used because there was no control of this variable during the microalgae cultivation and no application of CO_2.

Zeta potential measurements

Zeta potential (ZP) measurements were performed to understand the effect of each pH on the microalgae harvesting by sedimentation. The ZP at different pH values (pH 9–13) was measured using the Zetasizer Nano-ZS (Malvern, UK) at 25 °C. Samples were taken immediately after the coagulation process. All samples were measured at least eight times.

Centrifugation experiments

Firstly, the biomass harvested using the optimized conditions was carefully collected, homogenized, and placed in graduated tubes of 15 ± 0.2 mL. Centrifugation tests were performed in a laboratory centrifuge (model 5810, Eppendorf, Germany) at 25 °C. Microalgae concentration was evaluated at different values of centrifugal forces (800; 1,000; 1,200; 1,500, 1,750; 2,000; and 2,250 × g) and centrifugation times (5, 10, and 15 min) in terms of DW.

After the centrifugation test, the volume occupied by the biomass in the tubes was noted and the supernatant was discharged. Then, the centrifuged biomass was carefully transferred to a porcelain recipient for DW determination. This DW method was used due to the difficulty in filtering the concentrated biomass. The discharged supernatant was also analyzed by absorbance at 680 nm. All experiments were performed in triplicate.

Parameter reproducibility and wastewater quality

In order to check the reproducibility of the optimized parameters, sedimentation tests were performed using PBRE from three different cultivation cycles. This investigation was carried out to test PBRE with different quality and microalgae concentrations.

The PBRE and STE quality were also evaluated using physico-chemical analysis. The following parameters were determined in triplicate according to the procedures described in *Standard Methods for the Examination of Water and Wastewater* (APHA 2012): apparent color, chemical oxygen demand (COD), dissolved organic carbon

(DOC), soluble COD (sCOD), total Kjeldahl nitrogen (TKN), total phosphorus, total suspended solids (TSS), true color, and turbidity. For DOC, sCOD, and true color analysis, the samples were previously filtered through glass microfiber filters of 0.45 μm (Macherey-Nagel, Germany).

Statistical analyses

All results were expressed as a mean value ± standard deviation. The significance of the results and differences among the operational parameters tested were evaluated using a two-way ANOVA analysis and Tukey test. The Tukey test was also used to compare the PBRE and STE wastewater quality. Statistical analyses were performed with a significance level of 0.05 using Minitab software (version 18.1, Minitab LLC., PA, USA).

RESULTS AND DISCUSSION

Optimization process

The sedimentation tests were carried out at different pH values (9–13) and the efficiency was estimated by absorbance at 680 nm removal (Figure 1). Statistical analysis of the results showed a significant effect of pH variation on SE (Tukey test, $p < 0.05$). Tests performed on unmodified PBRE (pH 9) showed the lowest SE (8.6%), while the highest SE (97.0%) was obtained at pH 13.

ZP measurements were carried out immediately after the coagulation to understand the pH effect in the electrostatic interactions (Figure 1). The pH increment significantly increased the ZP values (Tukey test, $p < 0.05$). ZP values remained negative in the whole pH range (pH 9–13) from −27.9 mV at pH 9 to −9.7 mV at pH 13. This ZP variation is in agreement with a previous study using *Chlorella sorokiniana* (Leite *et al.* 2019a).

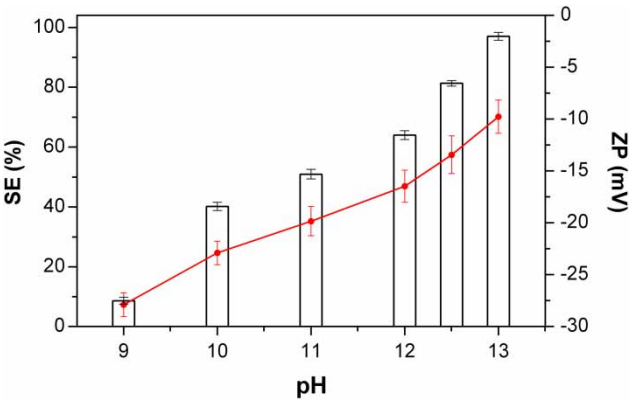

Figure 1 | Effect of pH value on sedimentation efficiency (SE) of *Chorella sorokiniana* (bars) and on zeta potential measurements (ZP, red line). Tests were carried out using $G_V = 500\ s^{-1}$ and $T_M = 10$ seconds. ZP measurements were taken immediately after the coagulation process for each condition. Please refer to the online version to see this figure in color: http://dx.doi.org/10.2166/wst.2020.106.

Sedimention at alkaline pH is often caused by the charge neutralization mechanism and sweep flocculation (considered to be the second form of charge neutralization) (Ghernaout & Ghernaout 2012). The highest sedimentation efficiency is usually found close to neutrality (ZP = 0) (Wu *et al.* 2012). Here, the highest SE (= 97.0%, pH 13) was reached in a ZP of −9.7 mV, in which it is not close to ZP neutralization. It may have been due to the presence of ions and solids in the wastewater that are not involved in the harvesting process. This fact makes it difficult to determine the predominant method based on the ZP results.

There is little information about microalgae sedimentation in alkaline conditions for microalgae cultivated in wastewater. Mennaa *et al.* (2019) studied the harvesting of indigenous microalgae by sedimentation induced by pH. The authors found efficiencies of 70.0% at pH 12 and 94.0% at pH 13, which is in agreement with the results obtained in this study. Different optimal pH values (10–12) have been reported for microalgae harvesting from a culture medium by alkaline pH (Wu *et al.* 2012; Rakesh *et al.* 2014; Ummalyma *et al.* 2016; Pérez *et al.* 2017). The variation in the results makes it clear that the sedimentation process also depends on the chemical species present in the medium (e g. component of precipitates and possible interfering compounds). Low concentration of precipitates and the presence of proteinaceous compounds in the solution can significantly reduce the microalgae sedimentation efficiency (Leite *et al.* 2019a). Considering the results obtained, the best pH values (12, 12.5 and 13) were selected to optimize the sedimentation parameters.

The SE of *Chlorella sorokiniana* using different values of velocity gradient (G_V) and mixing time (T_M) for each pH is shown in Figure 2. A high range of SEs was obtained in sedimentation tests at pH 12 (48.3–91.4%, Figure 2(a)), pH 12.5 (70.3–94.0%, Figure 2(b)), and pH 13 (89.4–98.1%, Figure 2(c)). Statistical analysis showed a significant effect of G_V and T_M on SE for the three pH values tested (Tukey test, $p < 0.05$).

A second-order polynomial equation was generated by multiple regression analysis for each pH. ANOVA analysis showed that the quadratic model was significant ($p < 0.001$) and the lack of fit was statistically insignificant ($p > 0.05$) for the experimental data. Good correlation

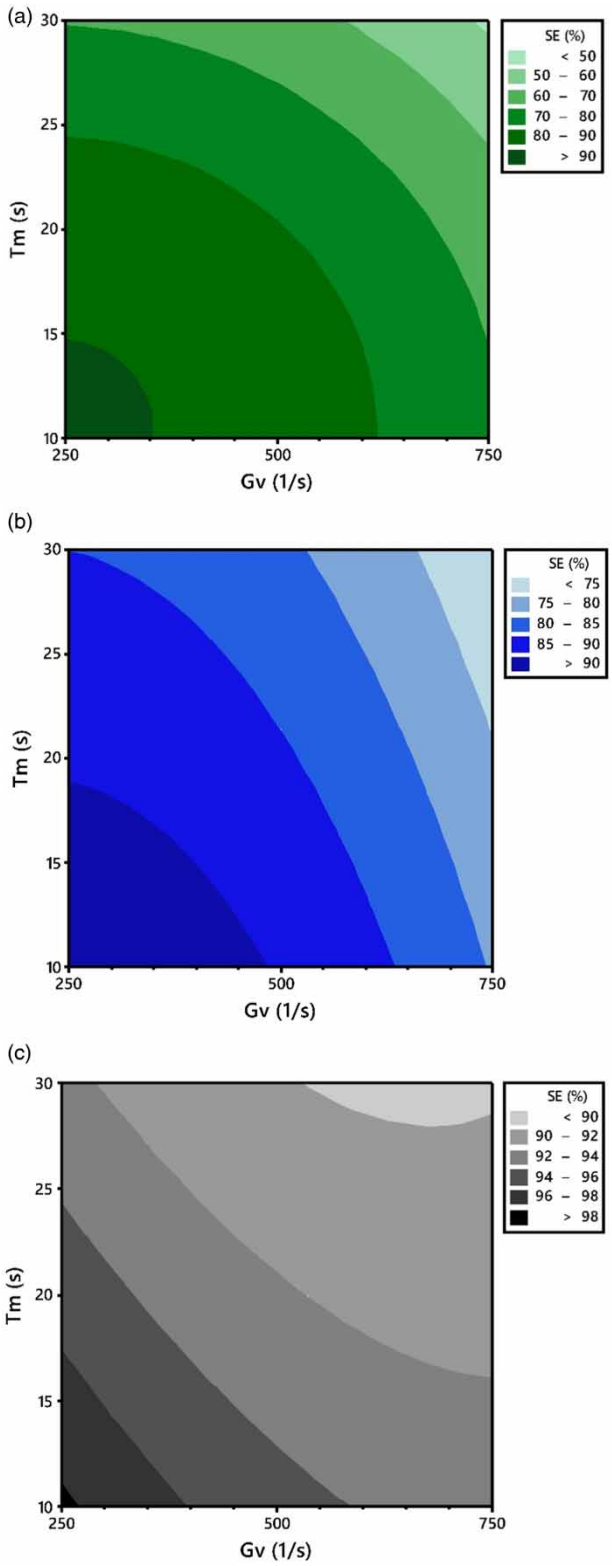

Figure 2 | Variation of sedimentation efficiency (SE) of *Chlorella sorokiniana* using different values of mixing times (T_M) and velocity gradient (G_V) at pH (a) 12, (b) 12.5, and (c) 13. Please refer to the online version to see this figure in color: http://dx.doi.org/10.2166/wst.2020.106.

between experimental ($R^2 > 0.97$) and predicted (adj. $R^2 > 0.96$) data was obtained. Each equation term was also individually analyzed and only significant terms were retained in the final equations. Therefore, the final equations for pH 12, 12.5, 13, expressed in terms of actual values, are shown in Equations (2)–(4), respectively.

$$\text{SE } (\%) = 79.92 + 0.03759 \times G_V + 1.241 \times T_M - 0.000076 \times G_V^2 - 0.05734 \times T_M^2 \quad (2)$$

$$\text{SE } (\%) = 94.01 + 0.02228 \times Gv - 0.262 \times T_M - 0.000049 \times Gv^2 \quad (3)$$

$$\text{SE } (\%) = 108.14 - 0.02901 \times G_V - 0.437 \times T_M + 0.000016 \times G_V^2 + 0.000244 \times G_V \times T_M \quad (4)$$

The optimization process through RSM was successful for the three pH values tested. The sedimentation efficiency increased significantly for the three pH values tested (Tukey test, $p < 0.05$) varying the operational parameters. For instance, the SE at pH 12 increased from 64.0% ($G_V = 500\ s^{-1}$ and $T_M = 30$ seconds) to 91.8% ($G_V = 250\ s^{-1}$ and $T_M = 10$ seconds). This fact endorses the importance of optimization studies for the operational parameters to promote effective microalgae harvesting.

Considering the results, the optimal operational values were a G_V of $250\ s^{-1}$ and a T_M of 10 seconds for the three pH values tested. The highest SEs were 91.4, 94.0 and 98.5% for pH 12, 12.5, and 13, which are close to the predicted values of 91.0, 93.9 and 98.1% obtained by Equations (2)–(4), respectively.

During the coagulation process, the interparticle collision promoted by G_V and T_M directly affects the microalgae floc diameter, and the floc size can directly affect the sedimentation efficiency, as observed in a previous study (Aktas *et al.* 2013). Based on this, the optimal values found may promote floc formation with a better size for the sedimentation process.

The difference between the highest SE obtained, using the three pH values, was significant (Tukey test, $p < 0.05$). Besides the efficiency, the choice of the optimal pH must also consider the NaOH concentration used to reach the pH tested. In the tests, the NaOH concentrations of 0.6, 2.8 and $6.4\ g\ L^{-1}$ were used to modify the pH values to pH 12, 12.5 and 13, respectively. Considering this, pH 12 was considered the optimal value because the NaOH concentration was considerably lower than the other pH

values (12.5 and 13) and also promoted efficient microalgae harvesting (SE = 91.8%).

Centrifugation as a dewatering process

After the sedimentation process at optimal conditions (pH 12, $G_V = 250\ s^{-1}$ and $T_M = 10$ seconds), the harvested biomass was centrifuged. The microalgae concentration was evaluated at different values of centrifugal forces (800; 1,000; 1,200; 1,500, 1,750; 2,000 and 2,250 × g) and centrifugation times (5, 10 and 15 min) in terms of DW (Figure 3). Statistical analysis of the results showed a significant effect of the centrifugal force and centrifugation time on DW (Tukey test, $p < 0.05$). However, centrifugal force values above 2,000 × g did not promote a significant improvement in the DW in the three centrifugation times tested (Tukey test, $p < 0.05$). Considering these findings, the highest microalgae concentration (DW = 64.5 g·L^{-1}) was obtained using a centrifugal force of 2,000 × g and a centrifugation time of 15 min.

Centrifugation as a dewatering method requires caution due to the high shear and gravitational forces that can cause damage to the microalgae cell structure during the process (Molina Grima *et al.* 2003). The cell damage is not desired because it may result in loss of bioproducts from the microalgae biomass. Heasman *et al.* (2000) studied the effect of centrifugation on the cell viability of 10 microalgae species. Most of the microalgae species exhibited very low apparent cell damage (0–3%), when submitted to a high centrifugal force of 13,000 × g. So it is expected that the cell damage is even less in this study considering the lower values of centrifugal force tested (800–2,250 × g).

Usually, absorbance is used to determine the centrifugation efficiency for microalgae harvesting (Schlesinger *et al.* 2012; Dassey & Theegala 2013). To compare the results obtained by DW, the supernatant absorbance was determined at 680 nm. However, absorbance appears not to be appropriate to evaluate the real consequences of the centrifugation process, since all centrifugation conditions tested led to efficiencies higher than 91.3% based on absorbance at 680 nm removal. Therefore, the differences between the results found that centrifugal forces and centrifugation time are not significant (Tukey test, $p > 0.05$), which is not in agreement with the microalgal concentration determined by DW (Figure 3). This happens because the initial absorbance of the biomass harvested by sedimentation is too high (3.90) and this method measures the efficiency based on the supernatant absorbance. Considering this, it is also important to measure the final volume occupied by the harvested biomass to determine exactly the centrifugation effect on the microalgal biomass concentration.

A summary of the microalgae concentration evaluation using the optimal conditions for sedimentation (pH 12, $G_V = 250\ s^{-1}$, and $T_M = 10$ seconds) and centrifugation (centrifugal force = 2,000 × g and centrifugation time = 15 min) is shown in Figure 4. Sedimentation increases the initial microalgae concentration by around 23 times whereas centrifugation increases the concentration obtained from the sedimentation test by 99 times. So the harvesting sequence studied increases the initial microalgal concentration by around 123 times, from 0.5 to 61.3 g·L^{-1}.

The results found here are in agreement with those reported in the literature for harvesting and dewatering processes. Schlesinger *et al.* (2012) studied alkaline sedimentation in a culture medium followed by centrifugation (50 × g, 10 min) and found a concentration factor of 10 in terms of number of cells. Therefore, a concentration

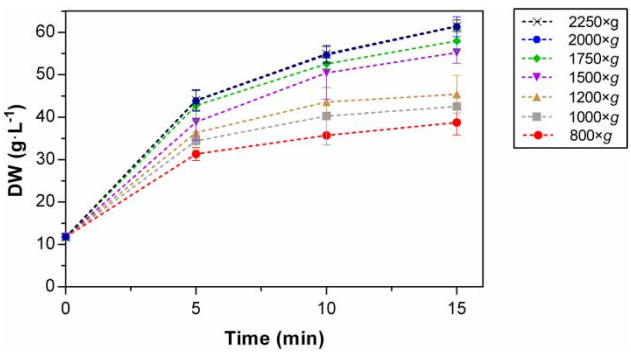

Figure 3 | Microalgae concentration evaluation, expressed as dry weight (DW), using different centrifugal forces and centrifugation times. The initial DW of 11.8 ± 0.2 g·L^{-1} was obtained in the sedimentation tests at the optimal conditions (pH 12, $G_V = 250\ s^{-1}$, and $T_M = 10$ seconds).

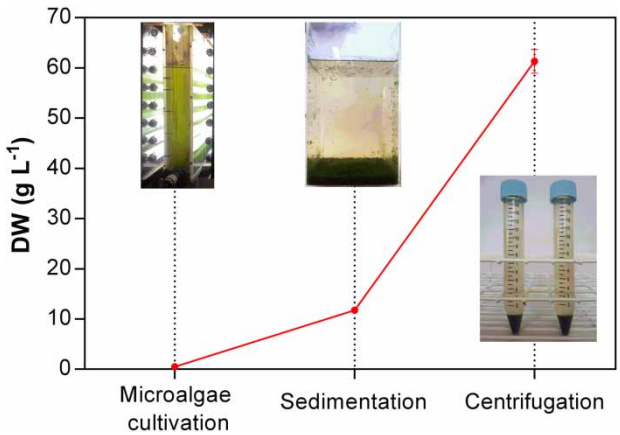

Figure 4 | Evaluation of microalgae concentration during the harvesting processes studied in this work.

factor of 4–250 is usually reported by different centrifugation types in terms of suspended solids (Molina Grima *et al.* 2003). A more comparative discussion is not possible because harvesting studies usually report only the efficiencies based on the supernatant analysis.

Sedimentation reproducibility and wastewater quality

The composition of the wastewater used for microalgae cultivation is subject to variation in each cultivation cycle, because it is mainly municipal wastewater. This variation happens because the wastewater quality depends on the weather conditions, daily patterns, and eventual industrial wastewater discharge (Chys *et al.* 2018). This fact may affect microalgae production, nutrient removal, and final wastewater composition. It is therefore vital to check the reproducibility and resilience of the microalgae sedimentation using the optimal parameters throughout the wastewater quality variations.

Considering all this information, sedimentation tests were carried out using three different photobioreactor effluents, each one over a week (Figure 5). The results show that the sedimentation parameter optimization was successful and reproducible. SEs higher than 97.8% were obtained for each pH tested (12, 12.5 and 13) in the three PBREs analyzed. Moreover, no significant differences were found between the SEs obtained in the pH tested (Tukey test, $p < 0.05$). An interesting point is that the efficiencies obtained are higher than those found during the optimization process.

The SEs found (97.8–99.1%) are higher than those reported in the literature for microalgae sedimentation at alkaline pH in a culture medium (Wu *et al.* 2012; Rakesh *et al.* 2014; Ummalyma *et al.* 2016; Pérez *et al.* 2017). It proves that very good results were achieved even working with microalgae cultivated in a complex matrix such as wastewater. Furthermore, this work shows the excellent potential of sedimentation at high pH as a harvesting method.

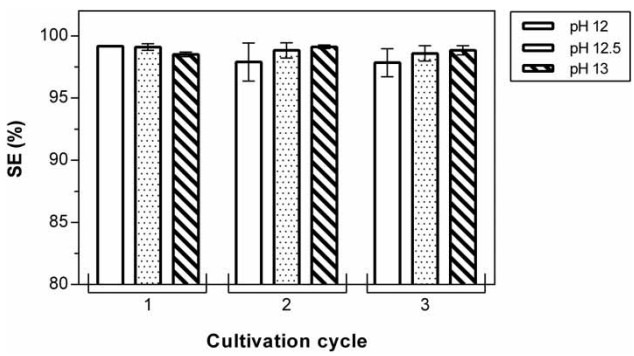

Figure 5 | Sedimentation efficiencies (SEs) of *Chlorella sorokiniana* using three different photobioreactor effluents (PBREs). The tests were carried out using the optimized parameters ($G_V = 250\ s^{-1}$ and $T_M = 10$ seconds).

The high pH required for microalgae sedimentation at optimal conditions (pH 12, $G_V = 250\ s^{-1}$, and $T_M = 10$ seconds) does not impair the results found in this study. Cell viability analysis showed minimal damage on the microalgae cell structure in the harvesting process at pH 12 (Vandamme *et al.* 2012; Ummalyma *et al.* 2016). The harvesting method also has an advantage for wastewater treatment: high pH (10–12) has a bactericidal effect on pathogenic bacteria (Starliper & Watten 2013).

Microalgae growth coupled with wastewater treatment improves the wastewater quality as well as the high-value biomass production (Shchegolkova *et al.* 2018). Microalgae absorb wastewater contaminants (carbon, nitrogen and phosphorus) during cultivation, while aerobic bacteria degrade organic matter (Foladori *et al.* 2018). For these reasons, the quality of PBRE and STE after microalgae sedimentation at the optimal parameters (pH 12, $G_V = 250\ s^{-1}$, and $T_M = 10$ seconds) was analyzed (Table 2). Analyses widely used for wastewater characterization were used, which quantify the physical (apparent color, true color, turbidity, COD, sCOD, and TSS) and chemical (DOC, TKN, and total phosphorus) parameters of the samples.

The wastewater quality improved significantly after microalgae sedimentation tests (Tukey test, $p < 0.05$). High removal was found for turbidity (97.9–98.3%), apparent color (92.2–97.2%), COD (82.7–87.4%), TSS (90.3–98.9%), TKN (91.0–94.4%), and total phosphorus (92.8–98.6%). These results were obtained because the parameters are directly associated with the microalgal biomass and wastewater solids removed during the sedimentation tests. Cassini *et al.* (2017) found similar analysis removals (85.5–95.2% of total nitrogen, 85.7–90.8% of total phosphorus, and 80.0–90.0% of COD) after microalgae sedimentation using natural and inorganic coagulants. In general, the efficiencies obtained here were higher than those obtained in our previous study using high pH followed by dissolved air flotation with the same PBRE (Leite *et al.* 2020).

The sedimentation test showed a low removal capacity of dissolved organic matter from wastewater, which was in agreement with a previous study (Katsoyiannis & Samara 2007). Removal efficiencies of 35.5–41.5%, 42.6–55.4%, and 21.4–24.8% were obtained for true color, sCOD, and DOC, respectively.

These results support the use of sedimentation at alkaline conditions for microalgae harvesting and also the efficient integration of microalgae production with

Table 2 | Quality of photobioreactor effluent (PBRE) and sedimentation test effluent (STE) in three different cultivation cycles

Cultivation cycle	Sample	Parameter								
		Turbidity (NTU)	Apparent color (Pt-Co)	True color (Pt-Co)	TSS ($mg{\cdot}L^{-1}$)	COD ($mg\ O_2{\cdot}L^{-1}$)	sCOD ($mg\ O_2{\cdot}L^{-1}$)	DOC ($mg{\cdot}L^{-1}$)	TKN ($mg\ N{\cdot}L^{-1}$)	Total phosphorus ($mg\ P{\cdot}L^{-1}$)
1	PBRE	175	4,564	258	454 ± 30	778 ± 2	136 ± 1	35.9 ± 0.2	42.8 ± 0.8	35.3 ± 1.5
	STE	3 ± 0 (98.3)	130 ± 2 (97.2)	151 ± 4 (41.5)	5 ± 1 (98.9)	98 ± 1 (87.4)	78 ± 2 (42.6)	28.2 ± 0.9 (21.4)	2.4 ± 0.4 (94.4)	0.5 ± 0.0 (98.6)
2	PBRE	196	6,740	214	409 ± 6	774 ± 2	211 ± 1	48.6 ± 0.1	37.6 ± 1.1	38.3 ± 1.2
	STE	5 ± 0 (97.4)	325 ± 8 (95.2)	138 ± 10 (35.5)	40 ± 4 (90.3)	107 ± 5 (86.2)	94 ± 13 (55.4)	40.6 ± 0.3 (21.5)	3.4 ± 0.3 (91.0)	1.7 ± 0.5 (95.6)
3	PBRE	284	10,430	296	563 ± 6	1,000 ± 3	289 ± 1	62.2 ± 0.2	99.8 ± 1.4	71.0 ± 1.6
	STE	6 ± 1 (97.9)	440 ± 5 (92.2)	183 ± 6 (38.2)	34 ± 2 (94.0)	173 ± 7 (82.7)	164 ± 13 (43.2)	46.8 ± 1.1 (24.8)	8.9 ± 0.15 (91.1)	5.1 ± 1.4 (92.8)

The sedimentation tests were carried out using the optimized parameters ($G_V = 250\ s^{-1}$, $T_M = 10$ seconds, and pH 12). Initial microalgae concentration was 0.4, 0.5 and 0.7 $g{\cdot}L^{-1}$ in PBRE 1, 2, and 3, respectively. Average removal values are shown in parentheses.

wastewater treatment. Further studies are reccomended to evaluate the cost and energy consumption of this optimized process on a large scale. Furthermore, it is recommended to check the viability of manipulating the NaOH required for the process.

CONCLUSIONS

The present study investigated the optimization of sedimentation parameters for *Chlorella sorokiniana* harvesting from wastewater. Parameter optimization was successfully determined by RSM, which found efficiencies higher than 97.8%. Optimal operational values were a velocity gradient of 250 s^{-1}, mixing time of 10 seconds, and pH of 12. Centrifugation was used as the dewatering method to obtain a final concentration of 61.3 $g{\cdot}L^{-1}$, which represents an increment of 123 times from the initial microalgae concentration. Optimal sedimentation parameters were also effective with the physico-chemical variation of the photobioreactor effluent. Furthermore, wastewater quality also improved significantly after microalgae harvesting. High removal was found for turbidity (97.9–98.3%), apparent color (92.2–97.2%), COD (82.7–87.4%), TKN (91.0–94.4%), and total phosphorus (92.8–98.6%). This study showed the importance of operational optimization and the results can be used as practical guidelines for microalgae harvesting on a large scale.

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