Oliveira GA, Carissimi E, Monje-Ramirez I, Velasquez-Orta SB, Rodrigues RT, Ledesma MTO.

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Bioresource Technology 2018, 259, 334-342.

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DOI link to article:

<https://doi.org/10.1016/j.biortech.2018.03.072>

Date deposited:

12/04/2018

Embargo release date:

17 March 2019

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Comparison between coagulation-flocculation and ozone-flotation for *Scenedesmus* microalgal **biomolecule** recovery and nutrient removal from wastewater

Commented [SVO1]: Es biomolecule o macronutrient?

Gislayne Alves Oliveira^a, Elvis Carissimi^a, Ignacio Monje-Ramírez^b, Sharon B. Velasquez-Orta^c, Rafael Teixeira Rodrigues^d, *María Teresa Orta Ledesma^b

^a Departamento de Pós Graduação em Engenharia Civil, Centro de Tecnologias, Universidade Federal de Santa Maria, Camobi 97105-900, Santa Maria, RS, Brazil

^b Instituto de Ingeniería, Coordinación de Ingeniería Ambiental, Universidad Nacional Autónoma de México, Ciudad Universitaria, CP. 04510. CDMX, México.

^c School of Chemical Engineering and Advanced Materials, Newcastle University, Newcastle Upon Tyne NE1 7RU, England, UK

^d Departamento de Engenharia de Minas, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 91501-970, Porto Alegre, RS, Brazil

Abstract

The removal of nutrients by *Scenedesmus* sp. in a high-rate algal pond, and subsequent algal separation by coagulation-flocculation or flotation with ozone to recover biomolecules, were evaluated. Cultivation of *Scenedesmus* sp. in wastewater resulted in complete NH₃-H removal, plus 93% total nitrogen and 61% orthophosphate removals. Ozone-flotation obtained better water quality results than coagulation-flocculation for most parameters (NH₃-N, NTK, nitrate and nitrite) except orthophosphate. Ozone-flotation, also produced the highest recovery of lipids, carbohydrates and proteins which were $0.32 \pm$

*Corresponding author. Tel.: +52(55)56233600 Ext.: 3672; fax: +52(55)56162164.
E-mail address: tol@pumas.iingen.unam.mx

0.03, 0.33 ± 0.025 and 0.58 ± 0.014 mg/mg of biomass, respectively. In contrast, there was a low lipid extraction of 0.21 mg of lipids/mg of biomass and 0.12 - 0.23 mg of protein/mg of biomass in the coagulation-flocculation process. In terms of biomolecule recovery and water quality, ozone showed better results than coagulation-flocculation.

Keywords: *Scenedesmus* sp., coagulation-flocculation, ozone-flotation, wastewater, high rate algal pond

1. Introduction

Microalgae has stood out as a renewable raw material for biofuel generation. Many of the resources required for algal mass cultivation are present in wastewater (Ni et al., 2018); simultaneously microalgae can help remove nutrients (N, P) and metals from residual effluents (Christenson and Sims, 2011; Gupta et al., 2016). Carbon dioxide (CO₂) sequestration by microalgae can help offset air pollution caused by processes (Zhu et al., 2017). CO₂ is the main driver of biomass and lipid generation in algal production and so is an effective way to mitigate emissions whilst also producing green energy (Eloka-Eboka and Inambao, 2017).

One of the promising genera are freshwater microalgae *Scenedesmus*, which are widely available (Das et al., 2016) and a potential alternative as feedstock for biofuel, due to their high growth rates and desirable intrinsic metabolites (Miranda et al., 2012). *Scenedesmus* have been effective in treating municipal and other industrial wastewater (Mandal and Mallick, 2009; Shen et al., 2015), efficiently fix CO₂ (consumption rate 1420.6 mg.L⁻¹.d⁻¹) and show good lipid/carbohydrate production (Ho et al., 2012) for

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biofuel generation. Growing algae in wastewater offers numerous economic and environmental merits, providing one of the most sustainable ways of producing biodiesel derived from microalgae (Komolafe et al., 2014).

However, an important aspect in microalgal harvesting is finding an efficient, economical and sustainable separation method for **splitting algal biomass from the high proportion (~98%w) of liquid medium used for cultivation**. The cost of biomass harvesting can be up to 20–30% of total biofuels production costs (Rawat et al., 2011) or approximately 50% of the energy consumption in the total biodiesel production process (Seo et al., 2016). But, most harvesting methods are not cost effective enough (Sharma et al., 2013). Disrupting algal cell walls to liberate their constituents (lipids and carbohydrates among others) and making them available for the biofuel production steps is also a challenging task for large-scale biofuel production from microalgae (Christenson and Sims, 2011).

According to Japar et al. (2017), coagulation-flocculation is one of the most suitable techniques to harvest microalgae due to its high harvesting efficiencies, moderate operational/logistical costs, lack of negative impacts on the environment, and short harvesting time. Coagulation-flocculation for harvesting microalgae is applied by decreasing or neutralizing the surface charges of negatively charged cells through the application of chemical agents, resulting in the aggregation of cells that form flakes and allow removal by a solid-liquid separation unit. Numerous chemical coagulants or flocculants have been tested for microalgal flocculation (Rakesh et al., 2014). Metal salts (aluminum sulfate and ferric chloride) are generally preferred because they lead to improved harvesting efficiency (Ummalyma et al., 2016). **Aluminum salts are usually more**

effective than iron salts (Papazi et al., 2010) mostly because it is economically more viable, making them useful for the recovery of *Scenedesmus* with efficiencies of 99% (Reyes and Labra, 2016). However, the potential presence of metals in the biomass can become problematic for downstream processes (Vandamme et al., 2013). Alternative solutions to this problem include the use of plant-based bioflocculation, microbial flocculation, bioflocculation by microalgal-fungal association or autoflocculation (Ummalyma et al., 2017), using flocculants such as chitosan (Yunos et al., 2017), cationic polymers (Polydiallyldimethylammonium chloride), Superfloc (Gerchman et al., 2017) among others.

In contrast, separation using ozone-flotation has the advantage of not using chemical or biochemical agents but two physiochemical processes in one: the separation of particles by the emission of bubbles (flotation), and the oxidation of organic compounds due to the high oxidation potential of ozone. Different doses of ozone have been tested, showing an effect dependent on the liquid culture medium, morphology, microalgae structure and concentration. Betzer et al. (1980) observed for the first time, the separation of microalgae by ozone-induced flotation, with O₃ doses of 15 to 50 mg/L, which obtained a complete separation of the microalgae without the addition of coagulants. Nguyen et al. (2013) reported that pre-oxidation of microalgae by ozone can induce cell lysis and, therefore, the release of intracellular organic matter. Moreover, it is an effective cell rupture method for biodiesel production with high lipid extraction and more saturated hydrocarbon products (Huang et al., 2014), and reduces the cell's surface charge (Cheng et al., 2011).

Previous studies, evaluate other types of harvesting methods using microalgal biomass grown in culture medium while in this work wastewater is used for microalgal growth. Additionally, there is little information on the recovery of macronutrient

components such as lipids, proteins and carbohydrates for the selected harvesting methods.

The importance and efficiency of *Scenedesmus* sp. cultivation in the treatment of wastewater for nutrient removal was evaluated. After cultivation, two techniques of harvesting were compared, coagulation-flocculation which is a very efficient and proven technique for harvesting microalgae (Das et al., 2016; Reyes and Labra, 2016; Vandamme et al., 2013) and ozone-flotation, which is currently being developed (Cheng et al., 2011; Komolafe et al., 2014; Valeriano González et al., 2016; Velasquez-Orta et al., 2014; Orta Ledesma de Velásquez et al., 2017). The evaluation for both processes was performed by determining nutrient removal at different water processing stages: 1) after treatment by *Scenedesmus*, and 2) after recovering the biomass. In addition to ozone-flotation, the mass transfer of ozone and coagulation-flocculation with a dose of aluminum sulfate were evaluated. The objective of this study was to evaluate the removal of nutrients from wastewater through a mixed microalgae culture dominated by *Scenedesmus* sp via comparison the two aforementioned methods for microalgae harvesting. The conventional harvesting method (coagulation - flocculation) and the new ozone-flotation method were compared taking into account the recovery of microalgae biomass as SST and specific biomolecules of energy interest (lipids, carbohydrates and proteins). Additionally, the removal of nitrogen and phosphorus was determined as part of an integral wastewater treatment process.

2. Materials and methods

2.1 Cultivation of *Scenedesmus* sp. in wastewater

Biomass used was obtained from the cultivation of *Scenedesmus* sp. isolated from "Lago Nabor Carrillo" located in Texcoco, Mexico, grown in raw wastewater from a Ciudad Universitaria wastewater treatment plant. Microalgae were fed with residual water filtered to remove solids, prior to being transferred into a lab-scale High Rate Algal Pond (HRAP). The microalgae were produced in an HRAP with a capacity of 30 liters (height: 15 cm, width: 115 cm and diameter: 24 cm), cultivated in a ratio of 14 liters of wastewater to 14 liters of microalgae culture, from November 2016 to May 2017. They were kept under lighted conditions (photoperiod 24:0 light:darkness) and controlled agitation (21.6 cm/s).

Biomass concentration (as total suspended solids, TSS), was used to monitor microalgae growth and was determined, indirectly, by gravimetry using standard methods (APHA, 2005). The growth curve was determined from day zero, immediately after mixing wastewater and the culture, up to 16 days. Biomass concentration (TSS_r) and evaporation rate (EV) were determined according to equations 1 and 2, respectively:

$$TSS_r = (TSS * V_i) / V_d \quad (1)$$

$$EV = (V_i - V_f) / T \quad (2)$$

Where V_i is the initial volume in the reactor (in L), V_d represents the volume measured on a given day (in L); V_f represents the final volume in the reactor (in L) and T is the time the culture was maintained (in days). In all harvesting analyses, microalgal growth was monitored for 14 days. After this time coagulation-flocculation and ozone-flotation tests were carried out. The percentage of microalgal biomass harvested was calculated by subtracting the TSS_r content in the initial sample from the TSS_r remaining.

2.2 Harvesting of *Scenedesmus* sp.

2.2.1 Coagulation-flocculation

Coagulation-flocculation tests were carried out in batches, using a Phipps & Bird and model 7790-900B laboratory bench jar test, which holds six samples in cups, under the same conditions of stirrer speed and time. The following conditions were applied: (1) stirring at 120 rpm for a period of 1 min for coagulation, (2) stirring at 25 rpm for 12 min of flocculation (Reyes and Labra, 2016), and (3) a settling time of 30 min; these were used for all experiments.

2.2.2 Ozone-flotation

Ozone-flotation experiments were conducted using 1L of a freshly homogenized culture of *Scenedesmus* sp. Batch flotation tests were carried out using a skimmer-type reactor, coupled to a Labo 76 ozone generator (Emery Trailigaz, USA) with a production capacity of 19 g O₃/h. The biomass was harvested through a collector, which was located at the top of the reactor. Ozone was injected at the bottom of the reactor (using a glass diffuser: 10-15 µm pore size) at different flow rates (0.2, 0.6 or 1 L/min) and different concentrations (800 or 1200 mg/L biomass) for 20 minutes, to apply a dose of 0.14 mg O₃/mg of dry biomass which had been previously reported by Valeriano-Gonzalez, et al. 2016. The ozone concentration in the gas phase used for each test was determined using the Iodometric Method (Birdsall & Jenkins, 1952).

2.2.3 Experimental design

For the experiments, a design with two factors was proposed, with variables coded to represent the ozone flux, the aluminum sulfate dose and the biomass concentration. The design involved carrying out 16 experiments, each with four replicates. The combinations of levels were done using the statistical software Minitab 17, to obtain the best relation of the factors to the variable tested.

In the ozone-flotation tests, a 3x2 factorial was constructed, for evaluating the ozone flow rate (0.2, 0.6 and 1 L/min) and biomass concentrations (800 and 1200 mg SST/L). For coagulation-flocculation, a 5x2 factorial was constructed using doses of $\text{Al}_2(\text{SO}_4)_3$ (0.5, 1.0, 1.5, 2 and 2.5 g/L) and biomass concentrations (800 and 1200 mg SST/L). The influences of these operational variables were determined for the following responses: cell biomass recovery, amount of lipids, protein and carbohydrates extracted and water quality (ammonia nitrogen, total Kjeldahl nitrogen, orthophosphate, nitrate and nitrite).

2.3 Water quality

Water quality was determined on day zero and monitored during the 14 days of microalgal growth while evaluating nutrient removal and the efficiency of the cultivation of *Scenedesmus* sp. as a treatment technique for wastewater. After the treatment of wastewater with microalgae cultivation, the influence of the coagulation-flocculation and ozone-flotation treatments on water quality was also determined; to verify efficiency for the treatments the water quality was measured at the end of microalgae cultivation and after treatments. The parameters determined in water quality were total Kjeldahl nitrogen (TKN) and ammonia nitrogen (APHA, 2005), orthophosphate, nitrate and nitrite (HACH 3900 spectrophotometer).

2.4 Biomolecules

Total lipid, proteins and carbohydrates were extracted from the harvested microalgae biomass in coagulation-flocculation and ozone-flotation treatments. For total lipids, the sulpho-phospho-vanillin reaction by colorimetric method (Barnes and Blackstock, 1973) was used. Total proteins were quantified by the Biuret colorimetric method (Gornall et al., 1948); and total carbohydrates were determined by the colorimetric method phenol-sulfuric acid (Dubois et al., 1956). The initial lipid concentration ranged from 0.03 to 0.075 ± 0.02 mg lipid/mg of biomass and carbohydrates from 0.06 to 0.44 ± 0.16 mg carbohydrate/mg of biomass.

2.5 Statistical analysis

Statistical analysis was performed using R software version 3.2.2 with an analysis of variance (ANOVA) at the 5% significance level, to analyze and compare the experimental data. The null hypothesis was that there was no difference between different treatments. If $p \leq 0.05$, the null hypothesis was rejected, meaning that the difference was significant. If there was a significant difference between the treatments, the multiple comparisons test by the Tukey test was performed. If $p \geq 0.05$, the null hypothesis was accepted, that is, there was no significant difference between treatments.

3. Results and discussion

3.1 Growth and wastewater treatment by *Scenedesmus* sp.

Fig. 1 shows the results of the growth of *Scenedesmus* sp. and the consumption of ammonia nitrogen and orthophosphate over 16 days. The maximum biomass concentration of 1200 mg/L was obtained during the 14 days with an evaporation rate of 0.8 L/day.

As observed in Fig. 1, the growth trend of microalgae was related to nutrient consumption. A high consumption of ammonia nitrogen and orthophosphate occurred until day 12, while over that same period the exponential phase of microalgae growth occurred. A strong correlation between biomass and ammonia nitrogen consumption ($R^2 = -0.90$) and biomass with orthophosphate consumption ($R^2 = -0.97$) was obtained. For both, the correlation was inverse, which means that biomass accumulation was directly related to nutrient consumption.

Because of the scarcity of nutrients (day 12), the *Scenedesmus* sp. cells entered a stationary phase, and from the 14th day of cultivation, into a phase of decline and death. It is advantageous to consider the stationary phase for harvesting microalgal biomass, due to a lower metabolic activity and cell mobility, presenting higher intercellular interactions, as the zeta potential decreases (Barros et al., 2015).

Table 1 shows the efficiency of nutrient removal from residual water by *Scenedesmus* sp, achieving complete removal of ammonia nitrogen, 93% of total nitrogen removal and 61% orthophosphate removal. The higher nitrogen removal compared to phosphorus, was a consequence to the fact that the wastewater used in the study has a nitrogen deficiency (N/P: 2.3). It is known that the N/P ratio for an optimal growth of the genus *Scenedesmus* without limitations is around 30 (Rhee, 1978). According to these results, it can be concluded that the strain of *Scenedesmus* sp. can be an alternative for wastewater treatment, thanks to their ability to absorb nutrients, using them as a food

source for growth. In contrast, the algal treatment resulted in an increase of nitrite and nitrate levels in the final effluent after treatment and may be a consequence of favorable conditions for nitrification. The same was observed by Gupta et al. (2016), where the species *Scenedesmus obliquus* removed the N-NH₃, but resulted in an increase in the levels of nitrite and nitrate. The author related this as a consequence of favorable conditions for both, ammonia oxidation and nitrification. Lorenzen et al. (1998) also observed nitrification in the presence of microalgae and attributed it to photosynthetic oxygen.

Results from this work showed that nutrient costs can be eliminated, since no nutrients have been added for *Scenedesmus* growth besides the nutrients already present in the wastewater. At the same time, this can also help reduce the problems caused by the release of wastewater into water bodies without proper treatment.

In some studies such as that carried out by Gupta et al. (2016) the species *Scenedesmus obliquus* obtained a removal of 99 ± 3 % for nitrogen and 98 ± 4 % of orthophosphate, while Sacristán de Alva et al. (2013), using pretreated wastewater for the cultivation of *Scenedesmus acutus*, achieved 66% removal of phosphorus and 94% for organic nitrogen (94%). In another study, the cultivation of *Scenedesmus* sp. showed a maximum biomass concentration (0.90 g/L) and maximum removal of ammonia nitrogen (86%) and phosphorus (97%) (da Fontoura et al., 2017). *Scenedesmus* sp. have also been shown to effectively metabolize both nitrogen and phosphorus without suppressed assimilation of any nutrients (Kim et al., 2015).

3.2 Biomass and biomolecule recovery

The use of microalgae strains for biofuel production being economically viable depends on several factors, and according to Ho et al. (2012) it is necessary that microalgae achieve the highest productivity of lipid and carbohydrate content. Achieving microalgae species with a high growth rate, lipid content and easy harvesting plus extraction may help overcome one of the major biological challenges for the production of biofuels (Greenwell et al., 2009).

Within the variables tested, the most significant effect for biomass harvesting and proteins extracted presented an ANOVA p-value of 0.004. In the case of lipids, the initial concentration of algae and aluminum sulfate dose were the most significant factors (ANOVA p-value of 0.00002 and 0.003, respectively). For carbohydrates, both the aluminum sulfate dose and the initial concentration of algae had significant effects (p-value of 0.00) with no interaction between them (p-value of 0.13).

According to Fig. 2A, biomass harvesting values higher than 85% with a maximum recovery of 98% were obtained. For biomass harvesting, the best $\text{Al}_2(\text{SO}_4)_3$ dosage was 1 to 2.5 g/L for a microalgae concentration of 800 mg SST/L (1.3 – 3.1 mg aluminum sulfate/mg of biomass) obtaining 93-98% removal that did not statistically differ. Additionally for the biomass of 1200 mg SST/L, dosages showed no significant difference in all tested range (0.5 to 2.5 g/L of aluminum sulfate). The results of this study compared with those published by other authors are shown in Table 2. Similar results were presented by Reyes and Labra (2016) who obtained a removal efficiency of 98% using *Scenedesmus* sp. with a concentration of 1.5 g/L of $\text{Al}_2(\text{SO}_4)_3$. With the same species, Chen et al. (2013) obtained a removal of 98% after 10 min of settling with 0.3 g/L of $\text{Al}_2(\text{SO}_4)_3$.

For lipids, carbohydrates and proteins (Fig. 2B, 2C and 2D) an influence from the higher aluminum sulfate dose in the initial biomass concentration of 800 mg/L was observed. The extractions of lipids and carbohydrates (Fig. 2B and 2C) were higher for the biomass concentration of 800 mg/L, with an extraction of 0.21mg lipid/mg of biomass and 0.38 mg carbohydrates/mg of biomass. While for the proteins (Fig. 2D) the release values were 0.12 - 0.23 mg protein/mg of biomass. As shown in Fig. 2, for carbohydrates, lipids and proteins, the dose of sulfate influences the cells, that is, when the dose of coagulant applied increases, extraction increases to a certain extent. Due to the focus of the work, the concentration of metals in the biomolecules was not evaluated, however, it is recommended that further studies evaluate the influence of aluminum sulfate on biomolecules.

The best efficiency with the lowest evaluated biomass (800 mg/L) may be due to the greater contact between the coagulant and the cells, as observed by Papazi et al. (2010) in which the addition of $\text{Al}_2(\text{SO}_4)_3$ resulted in cells lysis of about 10% for the total number of cells, demonstrating a lower efficiency when using a higher concentration (1200 mg/L), even almost null the difference between the biomass harvested but with a great difference when evaluated the recovery of biomolecules.

For ozone-flotation (Figure 3), even with a low recovery of biomass (62%), there was high extraction of lipids (0.15 - 0.3 mg of lipids/mg of biomass), carbohydrates (0.25-0.3 mg of carbohydrate/mg of biomass) and proteins (0.14 – 0.5 mg of protein/mg of biomass), using a dose of ozone of 0.14 mg O_3 / mg of biomass, 3- 22 times lower compared to aluminum sulfate. This is due to the ozone actions on the cells of the microalgae by reducing the surface charge and oxidizing the cells, which favors the collection of biomass and the extraction of biomolecules.

In previous studies, Velasquez-Orta et al. (2014) found a biomass recovery of 79% and a lipid yield of 0.12 mg of lipids/mg of biomass, using higher doses of ozone (0.23 mg O₃/mg of biomass) and a lower concentration of microalgae (420 mg SST/L). Valeriano-González et al. (2016), obtained a recovery of 75% of biomass, with a lipid yield of 0.16 mg lipids/mg of biomass, with the same dose of ozone used in this study (0.14 mg O₃/mg of biomass), but a lower concentration of microalgae (400 mg/L). The differences in biomass recovery obtained in this work, with respect to previous reports, are mainly due to the type of microalgae used and the required dose of ozone. In this study, a microalgal aggregation (dominated by *Scenedesmus* sp.) grown in a wastewater in a high-rate reactor was used with an evaporation rate of 0.8 L/day. The latter causes contaminants in the culture to almost duplicate in concentration which compete for the ozone used to harvest microalgae, resulting in a less efficient process. In future studies, it is advisable to adjust the dose of ozone used to compensate for secondary ozone demands.

Biomass concentration and O₃ flow were significantly affecting algal recovery (p-value <0.00) for the evaluated response parameters (harvested biomass, lipids, carbohydrates, protein and ozone mass transfer). As shown in Fig. 3A, the maximum biomass recovery was 62% with flow greater than 0.85 L/min and high biomass concentration (1200 mg/L), predominating the physical effect of the ozone flow. Not only does the recovery require a higher dose of ozone as reported by Valeriano González et al. (2016), but also a higher flow of ozone to separate the biomass. The extraction of lipids and carbohydrates was obtained with lower concentrations of biomass (Fig. 3B and 3C) with extraction higher than 0.30 mg lipid/mg of biomass and 0.30 mg carbohydrate/mg of biomass. For proteins, the best results were obtained with a gas flow less than 0.5 L/min

(Fig. 3D) with values > 0.5 mg protein/mg of biomass, due to the higher concentration of ozone in contact with the biomass. As mentioned above, under these conditions, there is greater availability of ozone due to lower algal demand; this is consistent with what was obtained by Valeriano González et al. (2016), who reported greater recovery of proteins with higher concentrations of ozone.

According to the Tukey analysis, when a biomass concentration of 800 mg/L was used, no differences were found between the flows, but for the concentration of 1200 mg/L, the best flows with statistical difference were 0.6 and 1 L/min. Low ozone flow (0.2 L/min) was not efficient for the biomass harvest, probably due to the lower inlet bubble concentration and higher biomass concentration, which makes it difficult to float the microalgae. In order to achieve greater than 90% mass transfer of O_3 (Fig. 3E), for the range of biomass concentrations studied at 800-1200 mg/L, flows between 0.2 and 0.68 L/min were required. The largest mass transfer of ozone (95%) was achieved with a biomass of 1200 mg/L and a flow of 0.6 L/min, which did not differ statistically from the biomass of 800 mg/L for a flow of 0.2 and 0.6 L/min with transfers of 93% and 92%, respectively.

For lipid and carbohydrate extraction, the best biomass concentration was 800 mg/L, with an O_3 flow of 0.6 L/min for the lipids, obtaining an extraction of 0.32 mg lipids/mg of biomass; for carbohydrates, the best O_3 flows were 0.6 and 1 L/min, with an efficiency of 0.33 and 0.302 mg carbohydrates/mg of biomass. Keris-Sen and Gurol (2017) reported that the application of ozone leads to cell disruption in microalgae. With a lower concentration of microalgae (800 mg/L) a higher concentration of available ozone permeates the

membrane and results in a greater recovery of lipids, showing the best results compared to coagulation-flocculation.

Higher lipid extraction is observed in relation to the greater mass transfer due to the action of ozone in microalgae cells. According to Li et al. (2013) the intracellular space of the cells is occupied by lipid bodies, which is distributed in all tissues, especially in cell membranes and fat cells. Carbohydrates are similar to lipids and need treatment (O_3) to break the cells, but extraction is greater with higher concentrations of biomass. Though cells contain a large portion of stored carbohydrates, cell walls also hold carbohydrates (Kim, 2015) that are easier to extract, and their extraction was not as dependent on the transfer of O_3 as the lipids were. For proteins the largest extractions were with the highest O_3 transfers, as the proteins are present in the cell wall and inside the cell (Sierra et al., 2017); the O_3 breaks the cells, but with less transfer this action is diminished and consequently, the extraction of proteins decreases.

3.3 Nutrient removal efficiency by coagulation-flocculation and ozone-flotation

In this study, different dosages of aluminum sulfate were used to evaluate coagulation-flocculation and varying O_3 gas flows were used to evaluate ozone-flotation. Both of these were also used to evaluate two biomass concentrations (800 and 1200 mg/L) in terms of nutrient removal from residual water after treatment with microalgal culture.

The experiments found high removal for nitrate and orthophosphate (> 60%) using a biomass concentration of 800 mg/L, reaching 99% of nitrate remnants with the highest dosage (2.5 g/L) of $Al_2(SO_4)_3$ (Table 3). However, note that the efficiency for NH_3 -N, TKN and nitrite was not satisfactory (<33%), probably due to the low concentration of these in

the culture since the majority was consumed by the microalgae for its growth. As already discussed, the treatment with microalgal culture in wastewater decreases or completely removes $\text{NH}_3\text{-N}$ and TKN from the water, and can complement the coagulation-flocculation technique. It can also be observed (Table 3) that the lowest nutrient removal efficiencies were with the biomass concentration of 1200 mg/L, which in contrast to the concentration of 800 mg/L, did not obtain significant removal results. This was perhaps due to a lower interaction of sulfate with the medium due to high concentration of biomass. Table 5 shows that the biomass had a greater influence (p-value <0.05) than the dose of coagulant applied; for all statistical analyzes the best efficiency in the removal of nutrients was for the biomass of 800 mg/L with a 2.5 g/L dose of $\text{Al}_2(\text{SO}_4)_3$. This may be due to the higher interaction between aluminum sulfate and nutrients when a lower concentration of biomass is applied, which decreases their interaction at higher concentrations.

In the work of Teh et al. (2014), applying coagulation-flocculation in wastewater without the presence of microalgae obtained a greater removal of TKN (51%) and 42% total phosphorus with 0.3 g/L of $\text{Al}_2(\text{SO}_4)_3$, and for Ismail et al. (2012), using the same coagulant at a dose of 60 mg/L obtained 63% removal of PO_4^{3-} . This difference in results may be due to the initial concentration of nutrients and the absence of microalgae, which increases the nutrient-coagulant interaction.

Table 4 shows that the highest removal efficiency after treatment by ozone-flotation for nutrients (100%) was obtained for $\text{NH}_3\text{-N}$ and TKN. The maximum nitrate removal efficiency was 89% for an O_3 flux of 0.2 L/min and an initial biomass of 800 mg/L, and nitrite removal was greater than 86% for an O_3 flux of 0.6 L/min with the same biomass concentration. TKN, orthophosphate and nitrite had a significant difference (p-value <0.05)

in relation to the O₃ flux, but only nitrate and nitrite had a significant difference in relation to biomass concentration.

According to the Tukey analysis, a difference between the averages for O₃ flow (for total nitrogen, orthophosphate and nitrite) was higher than 0.6 L/min. For biomass, a difference between the averages was only obtained for nitrate, which was 800 mg/L as the ideal concentration of biomass. When applying high concentrations of biomass (1200 mg/L), the results are unsatisfactory for coagulation-flocculation (Table 3). Increasing viability and decreasing treatment costs for biofuel production, energy is better with an increased concentration of microalgae. In this case, ozone-flotation technique was more efficient when using a high concentration of biomass (Table 4).

4. Conclusions

The use of *Scenedesmus* sp. for wastewater treatment proved to be efficient and advantageous, besides being an excellent producer of biomolecules. In general, the methods tested were different; coagulation-flocculation had an efficiency between 85-98% for biomass harvesting with a dose of 0.5-2.5 g/L of Al₂(SO₄)₃ yielding 0.4 – 3.1 mg of Al₂(SO₄)₃/mg of biomass. Ozone-flotation recovery achieved 62% with a dose of ozone applied at 0.16 g O₃/L yielding 0.14 mg O₃/mg of biomass. With the application of Al₂(SO₄)₃, the microalgae yield was 3 to 22 times greater than O₃ doses alone. However, the deciding factors in the appropriate technology to use depend on the proposed objective regarding the evaluated parameters.

Acknowledgements

The authors acknowledge MSc. Isaura Yáñez-Noguez for her laboratory support, Eng. Erick Vielma for his valuable help in the laboratory, MEng. Maria Teresa Valeriano for her support with ozone-flotation, MEng. Regina González Balderas for her help with the lipid and carbohydrate techniques, MSc. Andrea Hernandez Garcia for her help with statistics. MEng. Gislayne Alves Oliveira appreciates the financial support received through by the Exceed-Swindon Project. This research project was supported by the Fondo Sectorial CONACYT, project 220704. The authors are also grateful to William LaBarre for reviewing the English.

References

- APHA-AWA-WEF, 2005. Standard Methods for the Examination of Waters and Wastewater, 21 ed. APHA, Washington, DC.
- Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanilun method for “total” lipids. J. Exp. Mar. Bio. Ecol. 12, 103–118. doi:10.1016/0022-0981(73)90040-3
- Barros, A.I., Goncalves, A.L., Simoes, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: A review. Renew. Sustain. Energy Rev. 41, 1489–1500. doi:10.1016/j.rser.2014.09.037
- Betzer, N., Argaman, Y., Kott, Y., 1980. Effluent treatment and algae recovery by ozone-induced flotation. Water Res. 14, 1003–1009. doi:10.1016/0043-1354(80)90144-X
- Chen, L., Wang, C., Wang, W., Wei, J., 2013. Optimal conditions of different flocculation methods for harvesting *Scenedesmus* sp. Cultivated in an open-pond system.

- Bioresour. Technol. 133, 9–15. doi:10.1016/j.biortech.2013.01.071
- Cheng, Y.L., Juang, Y.C., Liao, G.Y., Tsai, P.W., Ho, S.H., Yeh, K.L., Chen, C.Y., Chang, J.S., Liu, J.C., Chen, W.M., Lee, D.J., 2011. Harvesting of *Scenedesmus obliquus* FSP-3 using dispersed ozone flotation. *Bioresour. Technol.* 102, 82–87. doi:10.1016/j.biortech.2010.04.083
- Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol. Adv.* 29, 686–702. doi:10.1016/j.biotechadv.2011.05.015
- da Fontoura, J.T., Rolim, G.S., Farenzena, M., Gutterres, M., 2017. Influence of light intensity and tannery wastewater concentration on biomass production and nutrient removal by microalgae *Scenedesmus* sp. *Process Saf. Environ. Prot.* 111, 355–362. doi:10.1016/j.psep.2017.07.024
- Das, P., Thaher, M.I., Abdul Hakim, M.A.Q.M., Al-Jabri, H.M.S.J., Alghasal, G.S.H.S., 2016. Microalgae harvesting by pH adjusted coagulation-flocculation, recycling of the coagulant and the growth media. *Bioresour. Technol.* 216, 824–829. doi:10.1016/j.biortech.2016.06.014
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.* 28, 350–356. doi:10.1021/ac60111a017
- Eloka-Eboka, A.C., Inambao, F.L., 2017. Effects of CO₂ sequestration on lipid and biomass productivity in microalgal biomass production. *Appl. Energy* 195, 1100–1111. doi:10.1016/j.apenergy.2017.03.071
- Gerchman, Y., Vasker, B., Tavasi, M., Mishael, Y., Kinel-Tahan, Y., Yehoshua, Y., 2017.

- Effective harvesting of microalgae: Comparison of different polymeric flocculants. *Bioresour. Technol.* 228, 141–146. doi:10.1016/j.biortech.2016.12.040
- Gerde, J.A., Yao, L., Lio, J., Wen, Z., Wang, T., 2014. Microalgae flocculation: Impact of flocculant type, algae species and cell concentration. *Algal Res.* 3, 30–35. doi:10.1016/j.algal.2013.11.015
- Gornall, A.G., Bardawill, Charles J., David, M.M., 1948. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177, 751–766.
- Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J., 2009. Placing microalgae on the biofuels priority list: a review of the technological challenges. *J. R. Soc. Interface* 7, 703–726. doi:10.1098/rsif.2009.0322
- Gupta, S.K., Ansari, F.A., Shriwastav, A., Sahoo, N.K., Rawat, I., Bux, F., 2016. Dual role of *Chlorella sorokiniana* and *Scenedesmus obliquus* for comprehensive wastewater treatment and biomass production for bio-fuels. *J. Clean. Prod.* 115, 255–264. doi:10.1016/j.jclepro.2015.12.040
- Ho, S.H., Chen, C.Y., Chang, J.S., 2012. Effect of light intensity and nitrogen starvation on CO₂ fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresour. Technol.* 113, 244–252. doi:10.1016/j.biortech.2011.11.133
- Huang, Y., Hong, A., Zhang, D., Li, L., 2014. Comparison of cell rupturing by ozonation and ultrasonication for algal lipid extraction from *Chlorella vulgaris*. *Environ. Technol. (United Kingdom)* 35, 931–937. doi:10.1080/09593330.2013.856954
- Ismail, I.M., Fawzy, A.S., Abdel-Monem, N.M., Mahmoud, M.H., El-Halwany, M.A., 2012. Combined coagulation flocculation pre treatment unit for municipal wastewater.

- J. Adv. Res. 3, 331–336. doi:10.1016/j.jare.2011.10.004
- Japar, A.S., Takriff, M.S., Yasin, N.H.M., 2017. Harvesting microalgal biomass and lipid extraction for potential biofuel production: A review. J. Environ. Chem. Eng. 5, 555–563. doi:10.1016/j.jece.2016.12.016
- Keris-Sen, U.D., Gurol, M.D., 2017. Using ozone for microalgal cell disruption to improve enzymatic saccharification of cellular carbohydrates. Biomass and Bioenergy 105, 59–65. doi:10.1016/j.biombioe.2017.06.023
- Kim, G.Y., Yun, Y.M., Shin, H.S., Kim, H.S., Han, J.I., 2015. Scenedesmus-based treatment of nitrogen and phosphorus from effluent of anaerobic digester and bio-oil production. Bioresour. Technol. 196, 235–240. doi:10.1016/j.biortech.2015.07.091
- Kim, S.K., 2015. Handbook of Marine Microalgae: Biotechnology Advances, Handbook of Marine Microalgae: Biotechnology Advances. doi:10.1016/C2013-0-19117-9
- Komolafe, O., Velasquez Orta, S.B., Monje-Ramirez, I., Noguez, I.Y., Harvey, A.P., Orta Ledesma, M.T., 2014. Biodiesel production from indigenous microalgae grown in wastewater. Bioresour. Technol. 154, 297–304. doi:10.1016/j.biortech.2013.12.048
- Li, X., Pribyl, P., Bisová, K., Kawano, S., Cepák, V., Zachleder, V., Cízková, M., Brányiková, I., Vítová, M., 2013. The microalga *Parachlorella kessleri*--A novel highly efficient lipid producer. Biotechnol. Bioeng. 110, 97–107. doi:10.1002/bit.24595
- Lorenzen, J., Larsen, L.H., Kjaer, T., Revsbech, N., 1998. Biosensor Determination of the Microscale Distribution of Nitrate , Nitrate Assimilation , Nitrification , and Denitrification in a Diatom-Inhabited Freshwater Sediment Biosensor Determination of the Microscale Distribution of Nitrate , Nitrate Assimilati. Appl Env. Microb 64,

3264–3269.

- Mandal, S., Mallick, N., 2009. Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.* 84, 281–291. doi:10.1007/s00253-009-1935-6
- Miranda, J.R., Passarinho, P.C., Gouveia, L., 2012. Bioethanol production from *Scenedesmus obliquus* sugars: The influence of photobioreactors and culture conditions on biomass production. *Appl. Microbiol. Biotechnol.* 96, 555–564. doi:10.1007/s00253-012-4338-z
- Nguyen, T.L., Lee, D.J., Chang, J.S., Liu, J.C., 2013. Effects of ozone and peroxone on algal separation via dispersed air flotation. *Colloids Surfaces B Biointerfaces* 105, 246–250. doi:10.1016/j.colsurfb.2012.12.017
- Ni, Z.-Y., Li, J.-Y., Xiong, Z.-Z., Cheng, L.-H., Xu, X.-H., 2018. Role of granular activated carbon in the microalgal cultivation from bacteria contamination. *Bioresour. Technol.* 247, 36–43. doi:10.1016/j.biortech.2017.07.079
- Orta Ledesma de Velásquez, M.T., Monje-Ramírez, I., Velasquez-Orta, S., Rodríguez-Muñiz, V., Yáñez-Noguez, I., 2017. Ozone for Microalgae Biomass Harvesting from Wastewater. *Ozone Sci. Eng.* 39, 264–272. doi:10.1080/01919512.2017.1322488
- Papazi, A., Makridis, P., Divanach, P., 2010. Harvesting *Chlorella minutissima* using cell coagulants. *J. Appl. Phycol.* 22, 349–355. doi:10.1007/s10811-009-9465-2
- Rakesh, S., Saxena, S., Dhar, D.W., Prasanna, R., Saxena, A.K., 2014. Comparative evaluation of inorganic and organic amendments for their flocculation efficiency of selected microalgae. *J. Appl. Phycol.* 26, 399–406. doi:10.1007/s10811-013-0114-4
- Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2011. Dual role of microalgae:

- Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Appl. Energy* 88, 3411–3424.
doi:10.1016/j.apenergy.2010.11.025
- Reyes, J.F., Labra, C., 2016. Biomass harvesting and concentration of microalgae *scenedesmus* sp. cultivated in a pilot photobioreactor. *Biomass and Bioenergy* 87, 78–83. doi:10.1016/j.biombioe.2016.02.014
- Rhee, G.-Y., 1978. Effects of N : P atomic ratios and nitrate limitation on algal growth , cell composition , and nitrate uptake ' 23.
- Sacristán de Alva, M., Luna-Pabello, V.M., Cadena, E., Ortiz, E., 2013. Green microalga *Scenedesmus acutus* grown on municipal wastewater to couple nutrient removal with lipid accumulation for biodiesel production. *Bioresour. Technol.* 146, 744–748.
doi:10.1016/j.biortech.2013.07.061
- Seo, Y.H., Sung, M., Oh, Y.K., Han, J.I., 2016. Lipid extraction from microalgae cell using persulfate-based oxidation. *Bioresour. Technol.* 200, 1073–1075.
doi:10.1016/j.biortech.2015.10.106
- Sharma, K.K., Garg, S., Li, Y., Malekizadeh, A., Schenk, P.M., 2013. Critical analysis of current Microalgae dewatering techniques. *Biofuels* 4, 397–407. doi:10.4155/bfs.13.25
- Shen, Q.H., Jiang, J.W., Chen, L.P., Cheng, L.H., Xu, X.H., Chen, H.L., 2015. Effect of carbon source on biomass growth and nutrients removal of *Scenedesmus obliquus* for wastewater advanced treatment and lipid production. *Bioresour. Technol.* 190, 257–263. doi:10.1016/j.biortech.2015.04.053
- Sierra, L.S., Dixon, C.K., Wilken, L.R., 2017. Enzymatic cell disruption of the microalgae *Chlamydomonas reinhardtii* for lipid and protein extraction. *Algal Res.* 25, 149–159.

doi:10.1016/j.algal.2017.04.004

- Teh, C.Y., Wu, T.Y., Juan, J.C., 2014. Potential use of rice starch in coagulation-flocculation process of agro-industrial wastewater: Treatment performance and flocs characterization. *Ecol. Eng.* 71, 509–519. doi:10.1016/j.ecoleng.2014.07.005
- Ummalyma, S.B., Gnansounou, E., Sukumaran, R.K., Sindhu, R., Pandey, A., Sahoo, D., 2017. Bioflocculation : An alternative strategy for harvesting of microalgae – An overview. *Bioresour. Technol.* 242, 227–235. doi:10.1016/j.biortech.2017.02.097
- Ummalyma, S.B., Mathew, A.K., Pandey, A., Sukumaran, R.K., 2016. Harvesting of microalgal biomass: Efficient method for flocculation through pH modulation. *Bioresour. Technol.* 213, 216–221. doi:10.1016/j.biortech.2016.03.114
- Valeriano González, M.T., Monje-Ramírez, I., Orta Ledesma, M.T., Gracia Fadrique, J., Velásquez-Orta, S.B., 2016. Harvesting microalgae using ozoflotation releases surfactant proteins, facilitates biomass recovery and lipid extraction. *Biomass and Bioenergy* 95, 109–115. doi:10.1016/j.biombioe.2016.09.020
- Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends Biotechnol.* 31, 233–239. doi:10.1016/j.tibtech.2012.12.005
- Velasquez-Orta, S.B., Garcia-Estrada, R., Monje-Ramirez, I., Harvey, A., Orta Ledesma, M.T., 2014. Microalgae harvesting using ozoflotation: Effect on lipid and FAME recoveries. *Biomass and Bioenergy* 70, 356–363. doi:10.1016/j.biombioe.2014.08.022
- Yunos, F.H.M., Nasir, M., Mohd, N., Jusoh, W., Khatoon, H., Shiung, S., Jusoh, A., 2017. Harvesting of microalgae (*Chlorella* sp .) from aquaculture bio flocs using an environmental-friendly chitosan-based bio-coagulant. *Int. Biodeterior. Biodegradation*

124, 243–249. doi:10.1016/j.ibiod.2017.07.016

Zhu, B., Chen, G., Cao, X., Wei, D., 2017. Molecular characterization of CO₂ sequestration and assimilation in microalgae and its biotechnological applications. *Bioresour. Technol.* doi:10.1016/j.biortech.2017.05.199

FIGURE CAPTIONS

Fig. 1. Growth in wastewater and consumption of ammonia nitrogen and orthophosphates of *Scenedesmus* sp. from a Raceway reactor.

Fig. 2. Effect of Al₂(SO₄)₃ dose and biomass of coagulation-flocculation: A. recovery of biomass, B. recovery of lipid per biomass, and C. recovery of carbohydrates per biomass.

Fig. 3. Ozone dose of 0.14 mg O₃/mg biomass: recovery of biomass (A), recovery of lipid per biomass (B) and recovery of carbohydrate per biomass (C) versus ozone flow and biomass of ozone-flotation, respectively.

Table 1

Characteristics of influent and effluent with cultivation and growth of *Scenedesmus* sp.

Parameters	Inlet concentration	Outlet concentration	Removal (%)
Ammonia nitrogen (mg NH ₃ -N/L)	69.30 ± 3.50	0 ± 0.0	100
Total Kjeldahl nitrogen (mg/L)	79.80 ± 0.00	5.6 ± 0.0	93
Orthophosphate (mg/L)	28.69 ± 1.81	11.25 ± 0.11	61
Nitrate (mg/L)	1.75 ± 0.05	48.14 ± 0.62	-
Nitrite (mg/L)	0.007 ± 0.001	0.548 ± 0.001	-

-not detected.

Table 2

Coagulation-flocculation conditions used by different studies for the harvesting of microalgae biomass.

Reference	Culture medium	Microalgae	Microalgae concentration (mg/L)	Al ₂ (SO ₄) ₃ (g/L)	Biomass recovery (%)	Yield (mg biomass/ mg Al ₂ (SO ₄) ₃)
Chen et al. (2013)	Bold's	<i>Scenedesmus</i> sp.	540	0.3	98	1.8
Gerde et al. (2014)	Fertilizers	<i>Scenedesmus</i> sp.	1000	0.3	90	3.0
Reyes and Labra (2016)	Z8	<i>S. spinosus</i>	400	1.5	98	0.3
This study	Wastewater	<i>Scenedesmus</i> sp.	1200	0.5	95	2.3

Table 3Removal of nutrients by coagulation-flocculation after pre-treatment with *Scenedesmus* sp.

Parameters		NH ₃ -N		Total nitrogen		Orthophosphate		Nitrate		Nitrite	
Dose of Al ₂ (SO ₄) ₃ (g/L)	Microalgae concentration (mg/L)	Remnant concentration* (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)
0.5	800	4.2	0	7	20	10.7	60	3.5	53	0.4	6
1			17		20		75		65		3
1.5			0		30		89		67		1
2			33		20		87		75		4
2.5			0		30		76		99		5
0.5	1200	-	-	-	-	7.8	0	7.7	5	0.5	0.9
1			-		-		0.6		5		1.0
1.5			-		-		0.8		3		0.2
2			-		-		0.9		2		0.5
2.5			-		-		0.7		1		0.3

*measured after treatment with microalgae; -not detected.

Table 4

Removal of nutrients by ozone-flotation after pre-treatment with *Scenedesmus* sp.

Parameters		NH ₃ -N		Total nitrogen		Orthophosphate		Nitrate		Nitrite	
O ₃ Flow (L/min)	Microalgal concentration (mg/L)	Remnant concentration* (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)	Remnant concentration (mg/L)	Removal (%)
0.2	800	4.2	100	4.2	100	4.2	2	4.2	89	4.2	97
0.6			100		100		6		88		86
1			100		50		3		88		96
0.2	1200	-	-	-	-	16	2	48.8	79	0.5	99
0.6			-		-		11		83		97
1			-		-		5		77		99

*measured after treatment with microalgae; - not detected.

Table 5

Experimental significance of tested variables in coagulation-flocculation and ozone-flotation. The confidence level used was 95%.

	p-value				
	NH ₃ -N	NTK	Orthophosphate	Nitrate	Nitrite
Coagulation-Flocculation					
A:Dose of Al ₂ (SO ₄) ₃	0.072	0.112	0.133	7.50E-09	0.980
B:Biomass	0.027		0.00	0.00	0.279
AB	0.072		0.174	1.38E-10	0.988
Ozone-Flotation					
A: O ₃ Flow	0.387	0.00	0.027	0.401	0.004
B: Biomass	0.331		0.268	1.20E-04	0.002
AB	0.387		0.697	0.313	0.024

Figure 1

Figure 2

Figure 3