Sequential pretreatment to recover carbohydrates and phosphorus from *Desmodesmus* sp. cultivated in municipal wastewater

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ABSTRACT

This study focused on the simultaneous recovery of carbohydrates (CHO) and phosphorus (P) from *Desmodesmus* sp. biomass cultivated in municipal wastewater, through a sequential pretreatment. The pretreatment consisted first of ultrasound to trigger cell disruption followed by ozonation to recover CHO and P. For ozone pretreatment, three different parameters were considered: ozone concentration (9, 15, 21, 27, 36, and 45 mg O_3/L), contact time (15, 25 and 35 min), and pH (8 and 11). The maximum simultaneous release of 84% of CHO and 58% of P was achieved at the experimental parameters of ozone concentration of 45 mg O_3/L, contact time of 35 min, and pH of 11. Also, P was concentrated in solution by 8- to 14-fold with respect to municipal wastewater. The sequential pretreatment was conducted at alkaline pH of 11 and atmospheric conditions, which may considerably reduce energy demand and reagents, in comparison to a traditional hydrolysis pretreatment. The results found suggest that the sequential pretreatment could be feasible on a large scale.

Key words | ozonation, phosphorus recovery, polysaccharides recovery, simultaneous recovery, ultrasound

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HIGHLIGHTS

- Simultaneous carbohydrates (CHO) and phosphorus (P) recovery from microalgae.
- Ultrasound was used to trigger cell disruption followed by ozonation to recover CHO and P.
- Simultaneous release of 84% of CHO and 58% of P was achieved at an ozone concentration of 45 mg O_3/L, contact time of 35 min, and pH of 11.
- Results suggest that the sequential pretreatment could be feasible on a large scale.

ABBREVIATIONS

COD	Chemical oxygen demand	Ortho-PO_4^{3-}	Orthophosphate
CHO	Carbohydrates	P	Phosphorus
MW	Microwave	TP	Total phosphorus

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INTRODUCTION

Microalgae contain multiple marketable products. Despite the high economic value of some of the biocomponents and products accumulated in microalgae cells, their commercialization has still not reached its maximum potential (Gonzalez-Fernandez & Muñoz 2017) since existing downstream processing operations are primarily designed for the extraction and recovery of one target biocomponent (Dixon & Wilken 2018). The simultaneous recovery of microalgal biocomponents and products provides a more sustainable and economical approach (Chandra *et al.* 2019). Studying the simultaneous recovery of carbohydrates (CHO) and phosphorus (P) from microalgae biomass is meaningful as microalgae have between 2 and 60%w carbohydrate and approximately 10%w of nitrogen and P-nutrients (Chen *et al.* 2013; Deng *et al.* 2019).

Using microalgal feedstock has several advantages over lignocellulosic feedstock for recovering CHO. These include the absence of lignin and low hemicellulose content; therefore, the conversion of usable sugars from microalgal biomass can be achieved more efficiently than from lignocellulosic biomass (Chen *et al.* 2013). These advantages thus allow microalgae to be a clean, efficient, and sustainable feedstock for biofuels production (Chen *et al.* 2013). On the other hand, P is a crucial element for food production. The shortage of P reserves is a major concern in many countries; therefore, renewable secondary P resources such as microalgal biomass should be developed as soon as possible (Deng *et al.* 2019). Usually, the raw materials used for P recovery are sewage sludge and wastewater; therefore, the studies focusing on P recovery from microalgal biomass are limited (Egle *et al.* 2016).

The processes applied for CHO and P recoveries including chemical pretreatments, steam explosion (Zabed *et al.* 2019), struvite precipitation or incineration (Egle *et al.* 2016) are chemically, energetically, and operationally intensive, and not ecologically sustainable. Enhancing the utilization of microalgae-based CHO and P requires the combination of pretreatment techniques that increase the recovery efficiency and decrease the energy demand during the recovery process. In this context, ultrasound pretreatment is recognized as an efficient cell disruption technique that induces microalgae biomass disintegration and increases the surface area for a subsequent biodegradability process (Gonzalez-Fernandez & Muñoz 2017). González-Balderas *et al.* (2020) studied the effect of ultrasound pretreatment on cell disruption and biocomponents recovery from *Desmodesmus* sp. They found that ultrasound pretreatment caused high cell disruption releasing microalgae proteins (97 ± 0.4%). Ozone pretreatment, on the other hand, proved to be attractive and promising with advantages over traditional methods for CHO saccharification and P recovery. These advantages include low production of inhibitor compounds, low chemical consumption, both pretreatments can be performed under atmospheric conditions, and the generation of easily degradable subproducts (Gonzalez-Fernandez & Muñoz 2017). González-Balderas *et al.* (2020) studied the effect of ozone pretreatment on *Desmodesmus* sp. carbohydrate recovery. They reported a recovery of 85 ± 2%, under pretreatment conditions of 9 mg O_3/L of ozone concentration, 25 g/L of biomass concentration, and 5 min of contact time. Cosgun & Semerci (2019) studied the effect of ozone pretreatment on activated sludge solubilization and nutrient release. Their result showed that ozonation is an effective technology for P solubilization: the reactive P content in the sludge increased from 1.9 to 3.6 mg orthophosphate (ortho-PO_4^{3-})/g mixed liquor suspended solids (89.5% increase).

In this study, ultrasound and ozone pretreatments were evaluated in sequence to simultaneously recover CHO and P from *Desmodesmus* sp. cultivated in municipal wastewater. The sequential pretreatment studied consisted first of ultrasound to trigger cell disruption followed by ozone to recover CHO and P.

MATERIALS AND METHODS

Microalgae species

The microalgae, *Desmodesmus* sp., used in this study were native microalgae isolated from the artificial lake 'Lago Nabor Carrillo' located in Texcoco, Mexico. This lake is fed with effluent from a facultative lagoon wastewater treatment plant also located in Texcoco. *Desmodesmus* sp. were characterized considering their morphology via light microscopy observations with the aid of identification manuals. The species identified were *Desmodesmus intermedius* (Chodat) Hegewald, *Desmodesmus magnus* (Meyen) Tsarenko, *Desmodesmus communis* (Hegewald) Hegewald and *Desmodesmus opoliensis* (Richter) Hegewald.

Microalgae cultivation and composition

Desmodesmus sp. biomass was grown in 10 L sequencing batch reactors under static conditions without external

aeration, incubated at room temperature (20 ± 3 °C), using raw municipal wastewater (pH 8.5, 85 ± 16 mg/L NH_4^+-N, 67 ± 2 mg/L ortho-PO_4^{3-}, 73 ± 1 mg/L TP, and 407 ± 6 mg/L chemical oxygen demand (COD)) as a culture medium. The light was provided by fluorescent lamps under light/dark periods of 12:12 h. After 27 days of cultivation, the biomass was harvested by sedimentation. Then, the biomass was oven dried at 40 °C for 12 h and stored at 4 °C until its use. The overall microalgal population composition was determined by cell count with a Neubauer counting chamber and microscope (AX10 Lab A1, Zeizz, Jena, Germany). The microalgae cultures were composed of 90% *Desmodesmus* sp., 5% *Cyanobacteria* sp., and 5% *Mychonastes* sp. During microalgal biomass cultivation, total suspended solids (TSS), ammonia concentrations (NH_4^+-H), total phosphorus (TP), ortho-PO_4^{3-}, nitrate (NO_3), and COD were measured. TSS and NH_4^+-H concentrations were measured according to the APHA standard methods (APHA-AWWA-WPCF 2005). TP was assayed by the USEPA PhosVer 3 with acid persulfate digestion method, ortho-PO_4^{3-} by the amino acid method, NO_3 by the cadmium reduction method, and COD by the dichromate test. TP, ortho-PO_4^{3-}, NO_3, and COD assays were carried out by using a Hach 3900 spectrophotometer following the manufacturer's recommendations. The *Desmodesmus* sp. culture at the beginning of the cultivation process was composed of 180 ± 15 mg TSS/L. At least three batches of biomass cultivation were performed.

After growth, the microalgal biomass composition was analysed on dry biomass using the methods reported by Valeriano-González *et al.* (2016), Safi *et al.* (2014), and USEPA PhosVer 3 to determine lipids, proteins, and TP on a per gram basis, respectively. CHO were analysed on wet biomass using the methods reported by Mirsiaghi & Reardon (2015). The obtained results were: 102 ± 4 mg/g of lipids, 660 ± 56 mg/g of proteins, 12 ± 1 mg/g of TP and, 117 ± 6 mg/g of CHO.

Sequential pretreatment: procedure and experimental design

The design of experiments comprised a response surface methodology based on a multilevel factorial design. The design creation and subsequent statistical analysis were performed using statistical software (Statgraphics Centurion XVI, Statgraphics Technologies, The Plains, VA, USA). This approach was used to find a combination of the experimental parameters that provided a good response for releasing CHO and P simultaneously. The sequential pretreatment designed consisted of ultrasound pretreatment to induce cell disruption followed by ozonation to release CHO and P. Based on our previous study, ultrasound parameters that provided good response for cell disruption were: biomass concentration of 75 g/L, applied energy 50 kWh/kg dry biomass and ultrasonic intensity of 0.32 W/L (González-Balderas *et al.* 2020). Thus, ultrasound pretreatment was carried out in 100 mL Erlenmeyer flasks containing 25 mL of the microalgal suspension immersed in a 2.81 L ultrasound bath (2510-MT, Branson, Hampton, NH, USA) performing at 100 W and 42 kHz under controlled temperature of 3 ± 1.5 °C using ice-water.

For ozone pretreatment, 30 tests were conducted to determine the effects of ozone concentration at six levels (9, 15, 21, 27, 36, and 45 mg O_3/L), contact time at three levels (15, 25, and 35 min), and pH at two levels (8 and 11), each test was performed in triplicate. Ozonation was conducted using 250 mL Erlenmeyer flasks containing 25 mL of microalgal suspensions of 25 g/L after ultrasound pretreatment. Ozone was provided using an ozone generator (Labo76, Emery Trailigaz, Wayne, NJ, USA) with a production capacity of 19 g O_3/h. Ozone was injected at a flow rate of 0.5 L/min. The ozone concentration in the gas phase was determined using the iodometric method (Birdsall *et al.* 1952). All experiments were done using the same batch of microalgae biomass to ensure consistency.

The desirability function was applied in order to find out the combination of experimental parameters that provided good response for simultaneous recovery of CHO and P during ozone pretreatment. In this method, each set of responses obtained by applying an experimental design were transformed into dimensionless values called individual desirabilities, which were then aggregated into a single response called overall desirability. By fitting a mathematical model to the values of desirabilities in order to adequately describe its behaviour, it is possible to optimize the variables while considering all the available responses. The obtaining of this scale is essential, as it makes possible the combination of the various responses of different orders of magnitude into a single response, without running the risk of overlapping the effect of another one. Desirability values can range within a scale from 0 (undesirable response) to 1 (completely desirable response) (González-Balderas *et al.* 2020).

The analysis of variance was also performed to identify the significant operating parameters at a 95% level ($p < 0.05$). Experiments were performed in triplicate or more.

Quantification of biocomponents released into the aqueous phase

After ultrasound pretreatment, lipids, proteins, CHO, ortho-PO_4^{3-}, and TP released into the aqueous phase were quantified separately. For this purpose, microalgae suspensions were centrifuged at 2,500 *g*, 20 °C, for 15 min. Lipids were determined by the sulfo-phospho-vanillin assay (Misha *et al.* 2014), proteins by the Biuret method (Uzun *et al.* 2012), CHO by using the phenol–sulfuric acid method (Dubois *et al.* 1956), TP by the USEPA PhosVer 3 method, and ortho-PO_4^{3-} by the amino acid method. At least three replicates of each analysis were performed.

After ozone pretreatment CHO, ortho-PO_4^{3-}, and TP released into the aqueous phase were quantified separately. CHO were determined by using the phenol–sulfuric acid method (Dubois *et al.* 1956), TP by the USEPA PhosVer 3 method, and ortho-PO_4^{3-} by the amino acid method. At least three replicates were performed.

Proteins and lipids extraction after ultrasound pretreatment

Proteins were recovered from the aqueous phase after ultrasound pretreatment. For that, the microalgae suspension was centrifuged at 2,500 *g*, 20 °C, for 15 min. At least three replicates were performed.

Lipid extraction from the ultrasound pretreated biomass before ozone pretreatment was performed using the procedure reported by González-Balderas *et al.* (2020). For that, microalgae suspensions were centrifuged at 2,500 *g*, 20 °C, for 15 min. At least three replicates were performed.

RESULTS AND DISCUSSION

Cell disruption after ultrasound pretreatment

Ultrasound pretreatment disrupts cell membranes through several mechanisms. The first is through the cell wall membrane expansion and compression due to thrust and rarefaction of the ultrasound field. The cell membrane rigidity plays a significant role in this type of rupture: highly elastic cell membranes can expand and shrink significantly without breaking; however, rigid cell membranes may break during the expansion. The second mechanism for cell rupture is through shear forces generated around the cell membrane due to acoustic cavitation outside the cell walls. The energy released into the suspension when bubbles collapse may also induce cell disruption. The third mechanism is intramembrane cavitation, where space between lipid monolayers inflates and deflates due to ultrasound (Gonzalez-Fernandez & Muñoz 2017). It is difficult to predict the extent to which each of these mechanisms contributes to cell rupture; however, it may be evaluated in terms of microalgae biocomponent release. In this study, ultrasound pretreatment achieved high cell disruption since 92 ± 4% of the available proteins in the *Desmodesmus* sp. biomass were released to the aqueous phase. Cell rupture by ultrasound also extracted 68 ± 4% of total lipids. The released CHO and P after ultrasound pretreatment were evaluated to determine their contents in the residual biomass before ozonation. Ultrasound pretreatment released to the aqueous phase 15 ± 4% of CHO and 15 ± 1% of TP.

Effect of ozone pretreatment on carbohydrate release

All the experimental parameters, ozone concentration, contact time, and pH, showed a positive effect on the released CHO, with p-values <0.05. The released CHO increased linearly with ozone concentration, contact time, and pH. The maximum released CHO (84 ± 2.4%) was achieved at the highest ozone concentration of 45 mg O_3/L, the longest contact time of 35 min and pH of 11, which depicts an ozone consumption of 420 mg O_3/g biomass, Figure 1.

The increase in the released CHO as ozone concentration and contact time increased can be explained by an increase in ozone consumption. Ozone consumption is directly dependent on process parameters such as ozone concentration and contact time. It is one of the most important variables of ozone pretreatment as it is closely related to carbohydrate depolymerization (Travaini *et al.* 2016). The high ozone demand for CHO released can be explained by microalgal biomass recalcitrance. The cell wall of microalgae contains a polysaccharide matrix such as agar, alginate, hemicellulose, pectin, and glycoprotein in the external and internal layer. However, the major carbohydrate in the microalgal cell wall is cellulose which provides rigidity and recalcitrance to microalgal biomass, preventing effective biodegradability (Zabed *et al.* 2019). In addition, ultrasound pretreatment could also increase the recalcitrance of the microalgae biomass. Microbubbles created during ultrasound pretreatment produced thermolysis of water forming highly reactive free radicals such as H• and OH•. These radicals in aqueous solution react with cellulose by abstracting an H-atom from their carbon that results in random cleavage of glycosidic bonds. Also, an initial electrophilic attack followed by a hydroxylation may

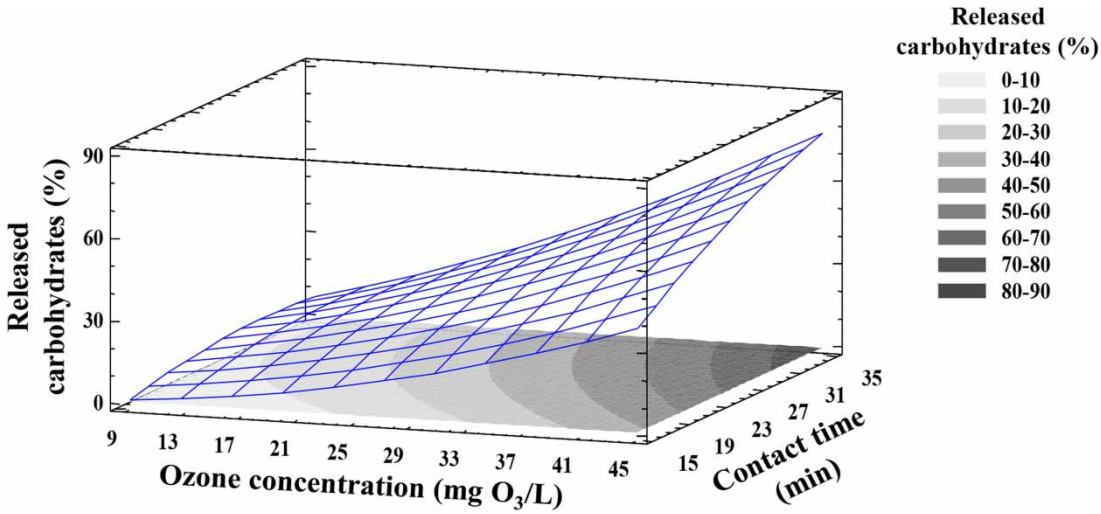

Figure 1 | Response surface of released carbohydrates as a function of contact time and ozone concentration (pH 11). Triplicate measurements ($n = 3$) are shown for each data set.

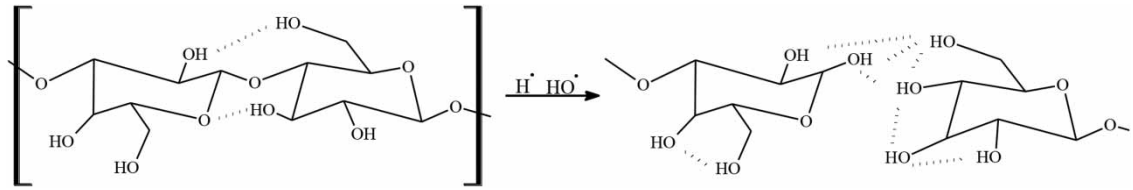

Figure 2 | Possible mechanism for the addition of hydroxyl groups in the cellulose molecules.

have occurred (Travaini *et al.* 2016). The addition of hydroxyl groups in the cellulose molecules of the microalga produces a crystalline structure due to extensive hydrogen bonding, Figure 2. This structure makes cellulose molecules stronger and more rigid, which in turn increases the ozone consumption for releasing CHO (Zabed *et al.* 2019).

Ozonation for pretreatment of a variety of biomass such as wheat straw, sugarcane bagasse, and maize stover among others consumed between 12 and 33 g O_3/100 g of dry biomass (Chen *et al.* 2013). Lignocellulosic biomasses consume four times more ozone than microalgal biomass (Travaini *et al.* 2016). Therefore, CHO-enriched species of microalgae are less expensive feedstocks for fermentative biofuels. As mentioned earlier, the released CHO increased linearly with contact time. An increase in contact time favoured the reaction between ozone or hydroxyl radicals with CHO. However, a contact time longer than 60 min must be avoided to prevent the formation of fermentation inhibitors such as carboxylic acids (Travaini *et al.* 2016). pH had a significant positive effect during ozone pretreatment since alkaline pH produces hydroxyl radicals. It has been reported that in acidic pH, ozone can react directly with an organic substrate through a slow and selective reaction. However, a fast and non-selective reaction of hydroxyl radical (OH•) with an organic substrate is favoured in an alkaline pH. Thus, the hydroxyl radicals formed in the liquid phase are decisive in the breakdown and solubilization of compounds difficult to degrade such as cellulose (Travaini *et al.* 2016).

Effect of ozone pretreatment on P release

Contact time and pH had a positive effect on the released TP, with p-values <0.05, while ozone concentration did not show a significant effect with a p-value of 0.2305, (Table S3, Supplementary Information). The released TP increased as contact time and pH increased. The maximum released TP ($88 \pm 4.6\%$) was achieved at the experimental parameters of ozone concentration of 27 mg O_3/L, contact time of 35 min and pH of 11, Figure 3(a). For ortho-PO_4^{3-} recovery only pH showed a significant effect on the released ortho-PO_4^{3-}, with a p-value of 0.029. The released ortho-PO_4^{3-} increased as pH increased. The maximum released ortho-PO_4^{3-} ($53 \pm 3.6\%$) was achieved at the experimental parameters of ozone concentration of 9 mg O_3/L, contact

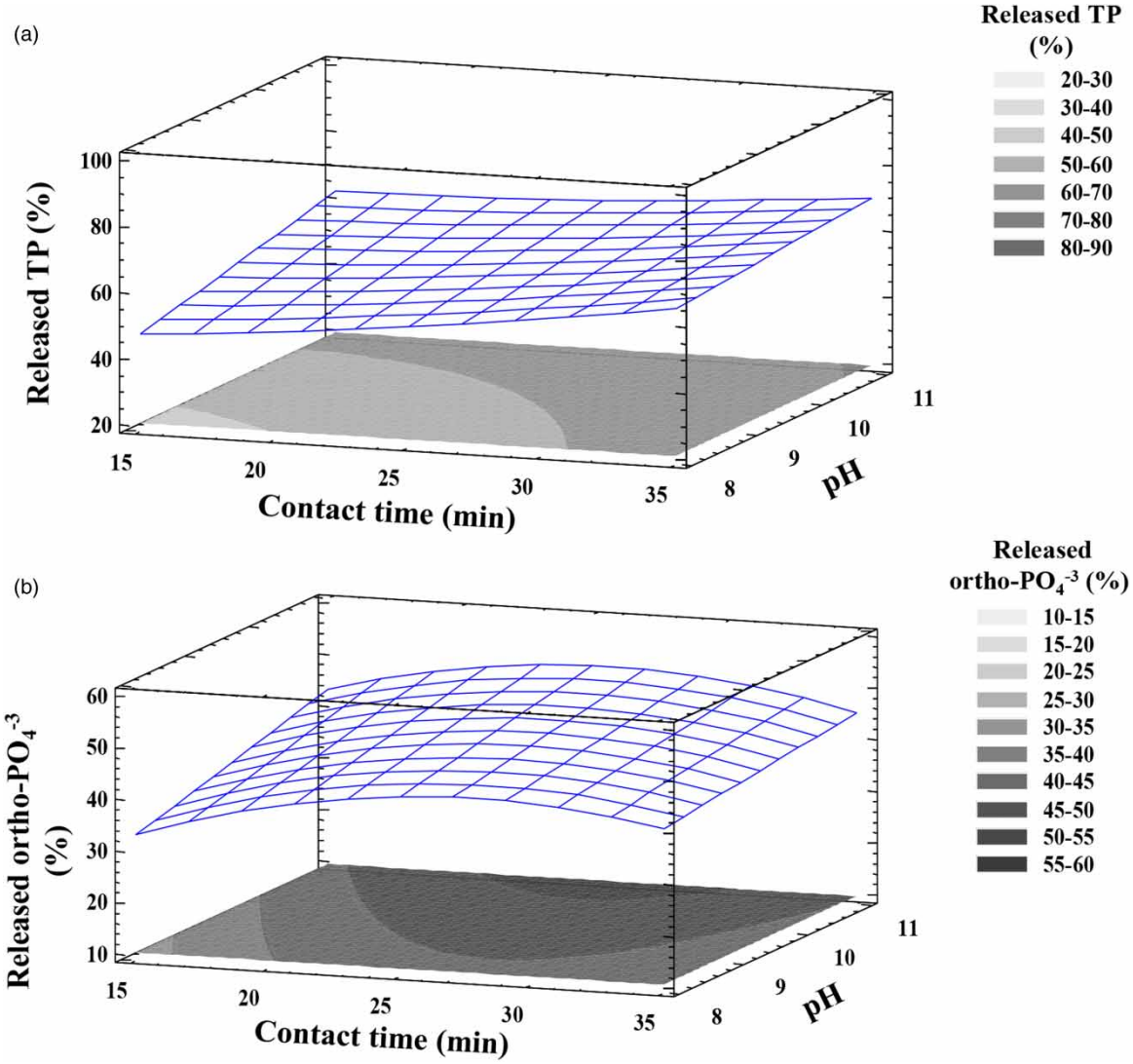

Figure 3 | Response surface of released TP and ortho-PO_4^{3-} as a function of pH and contact time (27 mg O_3/L ozone concentration). Triplicate measurements ($n = 3$) are shown for each data set.

time of 35 min and pH of 11, Figure 3(b). On the other hand, ortho-PO_4^{3-} release decreased when contact time increased at pH 8. This can be explained since some P compounds as struvite may spontaneously precipitate at a pH of 8.5. Also, the increase in contact time may induce the release of some ions such as calcium from microalgal cells. These released ions at a solution pH of 8.5 induce P precipitation. This effect did not occur at pH 11 because at this pH the P in solution is found as ortho-PO_4^{3-}, a highly soluble form that prevents precipitation (Egle *et al.* 2016).

P recovery was evaluated in terms of released TP and ortho-PO_4^{3-} to know the P forms and their availability in the aqueous phase after pretreatment. At the parameters used for maximum release of TP, 51% was ortho-PO_4^{3-} which comprised 57% of the TP released. Therefore, 43% of the TP was released as other soluble forms. P is an essential element required in the form of ortho-PO_4^{3-} for microalgae cellular constituents such as phospholipids, nucleotides and nucleic acids (Brown & Shilton 2014). However, under certain conditions microalgae can be triggered to take up much more P than is necessary for survival. This additional P uptake may be stored as polyphosphate which can then be used by the cell as an internal resource when the external P concentration is limiting for growth (Huang *et al.* 2018). While polyphosphate formation was not directly measured, and further data is needed, interesting results were found during the cultivation process as two P uptake phases were distinguished. The maximum

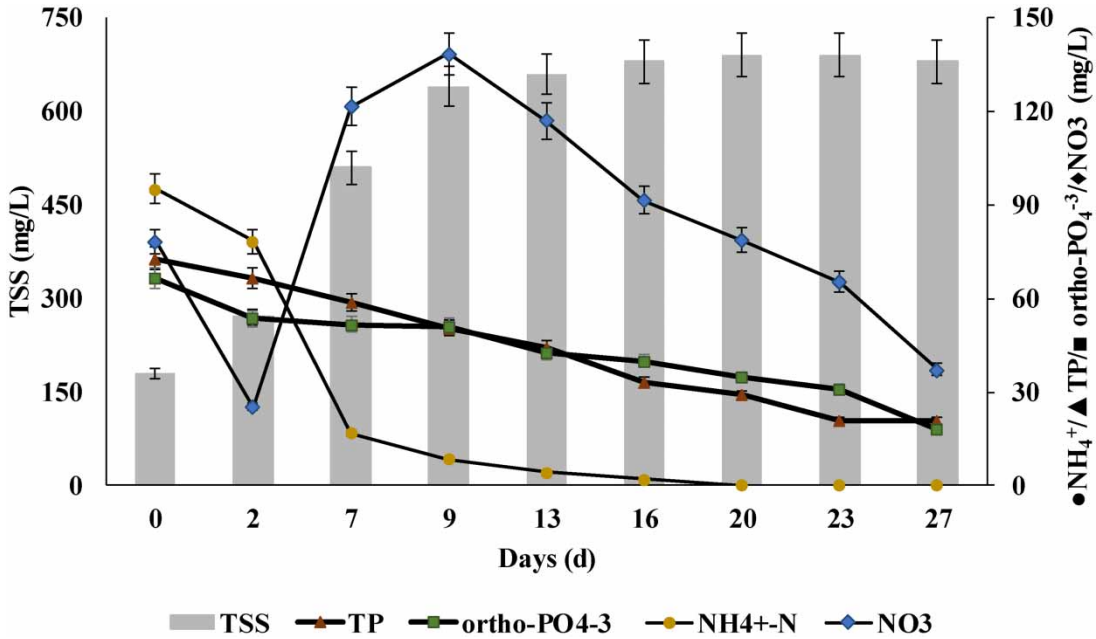

Figure 4 | Time course profiles of microalgal growth and nutrient removals (NH_4^+-N, NO_3, TP, and ortho-PO_4^{3-}). Triplicate measurements ($n = 3$) are shown for each data set.

concentration of TSS was 680 ± 7 mg/L after 27 days of cultivation, Figure 4. The microalgae exhibited exponential growth (37 mg/(L·d)) in the first 13 days of cultivation 37% of the ortho-PO_4^{3-} available in municipal wastewater were removed. After day 13 of cultivation, the biomass productivity slowed down (1.5 mg/(L·d)). This effect was because, from day 13, the ammonium concentration was less than 5 mg/L. Therefore, biomass productivity from day 13 was produced by nitrates uptake; nitrate content decreased considerably from this day. Despite the low biomass productivity, after day 13 of cultivation, 36% of the ortho-PO_4^{3-} in wastewater was removed. Therefore, it can be hypothesized that ortho-PO_4^{3-} uptake in the last 14 days of cultivation could have induced the biosynthesis of polyphosphate. The *Desmodesmus* sp. culture required 192 ± 17 mg of NH_4^+-N and 89 ± 13 mg of ortho-PO_4^{3-}/g biomass produced. Efficient nutrient removal was observed after microalgal growth: 100% of NH_4^+-N, 72 ± 2% of NO_3, and 73 ± 9% of ortho-PO_4^{3-} were removed. Similar results were found by Solovchenko *et al.* (2019), who distinguished two phases of ortho-PO_4^{3-} uptake during the polyphosphate formation in *Chlorella vulgaris* cultures. During the first phase, microalgae took up ortho-PO_4^{3-} at a high rate and then the microalgae resumed exponential growth. Afterwards, the content of polyphosphate in the microalgae started to increase again when cell division slowed down. The biosynthesis of polyphosphate during slow cell division helps to retain the ortho-PO_4^{3-} excess, which is taken up by the cell but cannot be immediately consumed (Solovchenko *et al.* 2019). Polyphosphate recovery from microalgae could be meaningful as the global phosphorus reserve is considerably declining.

Similar to carbohydrate release, ultrasound pretreatment enhanced P release due to the high cell disruption achieved. Cell disruption may have favoured the reaction between ozone or hydroxyl radicals and intracellular components containing P. TP release had a higher ozone demand than ortho-PO_4^{3-} release. The maximum released ortho-PO_4^{3-} was achieved at 9 mg O_3/L while the maximum released TP was achieved at 27 mg O_3/L, (Table S4, Supplementary Information). This result can be explained by a difference between P disposition and structure within the microalgae cell. In microalgae, ortho-PO_4^{3-} is located intracellularly in microalgal components such as adenosine triphosphate, lipids, and nucleic acid. These molecules are susceptible to strong oxidants such as ozone and hydroxyl radicals. For this reason, in addition to high cell disruption ozone is further consumed to achieve ortho-PO_4^{3-} release to the aqueous phase. On the other hand, the ozone demand for TP release may be explained by oxidation requirements for polyphosphate release. In microalgae cells, polyphosphate is found as acid-insoluble long-chains in metachromatic cytoplasmic granules (Huang *et al.* 2018). As mentioned earlier, ultrasound pretreatment improves P

release by cell disruption. However, similar to CHO, polyphosphates must be hydrolysed for their release, which increases ozone consumption. The increase of released TP over ozone concentration suggests that ozone pretreatment hydrolysed polyphosphate to shorter chains that were released to the aqueous phase, in accordance to literature (Huang *et al.* 2018). However, since polyphosphate formation in microalgae cells was not verified in this study, the effect of ozone pretreatment on polyphosphate recovery should be further investigated.

Simultaneous recovery of CHO and P

The desirability function was applied in order to find the experimental parameters for ozone pretreatment that provide good response for simultaneous recovery of CHO and P. For simultaneous recovery, the maximum released CHO ($84 \pm 2.4\%$) and P ($58 \pm 2\%$) were achieved at the highest ozone concentration of 45 mg O_3/L, longest contact time of 35 min and pH of 11; the desirability of this experiment was 0.75, Figure 5.

Comparison with other studies and large scale approach

In comparison to previous studies focused on carbohydrate recovery, the sequential pretreatment presented here achieved a similar yield of released CHO (~90%), Table 1. Moreover, the sequential pretreatment achieved higher yields of P recovery except for hydrothermal digestion pretreatment. However, the hydrothermal digestion pretreatment recovered one microalgae component, while the sequential pretreatment recovered two components simultaneously.

The sequential pretreatment proposed here for CHO recovery satisfies most of the criteria required for a large scale application such as (1) alteration of cellulose for efficient biofuels production, (2) minimum loss of hemicellulose and cellulose, (3) generation of no or fewer inhibitors, (4) no or low residues formation, and (5) consumption of little or no chemical (Zabed *et al.* 2019). In this regard, the sequential pretreatment achieved high yields of released CHO, and no residues were produced, Table 1. NaOH was used to increase both the pH of the microalgal suspension and the hydroxyl radicals concentration. Also, ozone pretreatment does not generate the common inhibitors generated during the chemical hydrolysis of microalgae biomass such as furfural and 5-hydroxymethylfurfural. These inhibitors potentially repress the fermentation process of biofuels production and also require costly downstream treatments (Chen *et al.* 2013; Travaini *et al.* 2016; Zabed *et al.* 2019). On the other hand, an ideal large scale technology for P recovery would feature P recovery yields close to 100% and destruction of potentially hazardous substances (organic micropollutants and pathogens). In this regard, the sequential pretreatment achieved a TP yield of 58%, Table 1. The P solution obtained after ozone pretreatment contained between 178 and 275 mg P/L, which were 8- and 14-fold higher than the concentration of P usually found in wastewater. These P concentrations increase considerably the feasibility of P recovery since they reduce the energy demand for P valorization as struvite; under these conditions P recovery may have a value up to €39.8/kg P recovered (Egle *et al.* 2016). Additionally, both ultrasound and ozone pretreatment were conducted

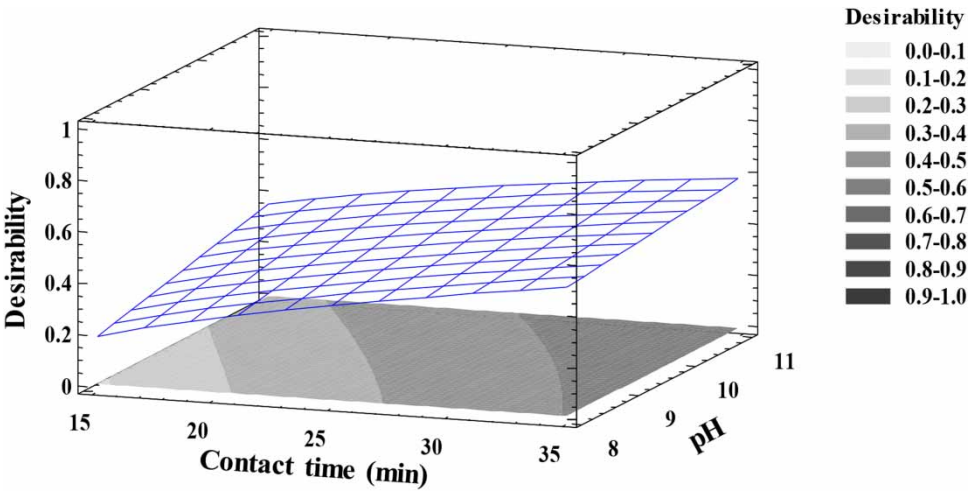

Figure 5 | Response surface for desirability function of ozone pretreatment as a function of pH and contact time. Triplicate measurements ($n = 3$) are shown for each data set.

Table 1 | Comparison of case studies on CHO and P recovery

Biocomponent recovered	Pretreatment	Species	Result*	Reference
Carbohydrates	Acid hydrolysis	*Chlorella vulgaris* and *Scenedesmus obliquus*	90% carbohydrate	Farias Silva *et al.* (2018)
	Ultrasound/ hydrolysis	*Chlorococum humicola*	64% carbohydrate	Harun & Danquah (2011)
	Ozone	Mixed microalgal	81% carbohydrate	Keris-Sen & Gurol (2007)
Phosphorus	O_3/H_2O_2/MW	Activated sludge	4 g P/kg sludge	Yin *et al.* (2007)
	Hydrothermal digestion	*Scenedesmus dimorphus*	9.7 g P/kg biomass	Deng *et al.* (2019)
Carbohydrates and phosphorus	Ultrasound and ozone	*Desmodesmus* sp.	84% carbohydrate and 58% TP 7 g P/kg biomass, 5 mg P/L wastewater	This study

*Result % (w/w) algal biomass. MW: microwave.

at alkaline pH of 11 and atmospheric conditions in comparison to typical hydrolysis pretreatment (98% H_2SO_4, 100–130 °C, and P ~1 atm), which increases the sustainability of the process since it reduces the energy demand and reagent waste for CHO and P solubilization considerably (Farias Silva *et al.* 2018).

The usefulness of the sequential pretreatment here presented for the simultaneous recovery of P and CHO was demonstrated, but the technology and identified parameters must be tested using real conditions, i.e. wastewater instead of distilled water. Wastewater may increase ultrasound intensity and ozone demand because other components, such as heavy metals, could come into solution.

CONCLUSIONS

The sequential pretreatment of ultrasound followed by ozonation achieved the simultaneous recovery of CHO and P from *Desmodesmus* sp. biomass. The maximum release of CHO and TP was 84 and 58%, respectively, at the experimental parameters of biomass concentration of 75 g/L, applied energy of 50 kWh/kg dry biomass, and ultrasonic intensity of 0.32 W/L for ultrasound pretreatment, and ozone concentration of 45 mg O_3/L, contact time of 35 min and pH of 11 for ozone pretreatment. A total of 56% TP was released as ortho-PO_4^{3-}. The sequential pretreatment concentrated the P in solution 8- to 14-fold with respect to P concentration usually found in municipal wastewater.

The sequential pretreatment met most of the criteria required for large scale recovery of CHO and P, such as minimum loss of the compound of interest, generation of no or fewer inhibitors, no or low residues, and consumption of little or no reagents. Also, both ultrasound and ozone pretreatments were conducted at alkaline pH of 11 and atmospheric conditions. This increased the sustainability of the process in comparison to typical hydrolysis pretreatment reported in literature for microalgal biomass.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts, no informed consent was necessary, and no human or animal rights were applicable.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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