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

R. M. González-Balderas, S. B. Velásquez-Orta, I. Valdez-Vazquez and M. T. Orta Ledesma

**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 O3/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 O3/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

**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 O3/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 |
|-----|------------------------|
| CHO | Carbohydrates          |
| MW  | Microwave              |
| Ortho-PO4³⁻ | Orthophosphate |
| P   | Phosphorus             |
| TP  | Total phosphorus       |

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doi: 10.2166/wst.2020.404
INTRODUCTION

Microalgae contain multiple marketable products. Despite the high economic value of some of the biocomponents and products accumulated in microalgal 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. 2013), struvite precipitation or incineration (Egle et al. 2016) are chemically, energetically, and operationally intensive, and not ecologically sustainable. Enhancing the utilization of microalgal-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 microalgal biomass disintegration and increases the surface area for a subsequent biodegradability process (González-Fernández & 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 (González-Fernández & 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 O3/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-PO4 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 opolensis (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 NH₄⁻N, 67 ± 2 mg/L ortho-PO₄³⁻, 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 microalgal cultures were composed of 90% Desmodesmus sp., 5% Cyanobacteria sp., and 5% Mychonastes sp. During microalgal biomass cultivation, total suspended solids (TSS), ammonia concentrations (NH₄-H), total phosphorus (TP), ortho-PO₄³⁻, nitrate (NO₃), and COD were measured. TSS and NH₄-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-PO₄³⁻ by the amino acid method, NO₃ by the cadmium reduction method, and COD by the dichromate test. TP, ortho-PO₄³⁻, NO₃, 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 5 ± 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 O₃/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 O₃/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 microalgal 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-PO₄³⁻, and TP released into the aqueous phase were quantified separately. For this purpose, microalgal 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-PO₄³⁻ by the amino acid method. At least three replicates of each analysis were performed.

After ozone pretreatment CHO, ortho-PO₄³⁻, 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-PO₄³⁻ by the amino acid method. At least three replicates were performed.

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 O₃/L, the longest contact time of 35 min and pH of 11, which depicts an ozone consumption of 420 mg O₃/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 microalga 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 microalgal 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 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 & Munoz 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 microalgal 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.

Proteins and lipids extraction after ultrasound pretreatment

Proteins were recovered from the aqueous phase after ultrasound pretreatment. For that, the microalgal 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, microalgal 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 & Munoz 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 microalgal 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.
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 O₃/100 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 ± 4.6%) was achieved at the experimental parameters of ozone concentration of 27 mg O₃/L, contact time of 35 min and pH of 11, Figure 3(a). For ortho-PO₄³⁻ recovery only pH showed a significant effect on the released ortho-PO₄³⁻, with a p-value of 0.029. The released ortho-PO₄³⁻ increased as pH increased. The maximum released ortho-PO₄³⁻ (53 ± 3.6%) was achieved at the experimental parameters of ozone concentration of 9 mg O₃/L, contact
time of 35 min and pH of 11, Figure 3(b). On the other hand, ortho-PO$_4^{3-}$/C$_0$ 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$_4^{3-}$/C$_0$, a highly soluble form that prevents precipitation (Egle et al. 2016).

P recovery was evaluated in terms of released TP and ortho-PO$_4^{3-}$/C$_0$ 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$_4^{3-}$/C$_0$ 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$_4^{3-}$/C$_0$ for microalgal 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
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$_4^{3-}$/C$_0$ 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$_4^{3-}$/C$_0$ 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, poly-
phosphates 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 for-
mation 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 pro-
vide good response for simultaneous recovery of CHO and
P. For simultaneous recovery, the maximum released CHO
(84 ± 2.4%) and P (58 ± 2%) were achieved at the highest
ozone concentration of 45 mg O₃/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 recov-
ery 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 hemicellu-
lose 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 chemi-
cal hydrolysis of microalgal 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; Tra-
vaini 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 patho-
gens). 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 stru-
vite; 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

![Desirability surface](image-url)
at alkaline pH of 11 and atmospheric conditions in comparison to typical hydrolysis pretreatment (98% H₂SO₄, 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 O₃/L, contact time of 35 min and pH of 11 for ozone pretreatment. A total of 56% TP was released as ortho-PO₄³⁻. 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.

**ACKNOWLEDGEMENTS**

The authors thank MSc Isaura Yañez-Noguez and Dr Ignacio Monje-Ramirez for their valuable help in laboratory and ozonation processes. The experimental work was carried out in the Laboratorio de Ingeniería Ambiental, Instituto de Ingeniería, UNAM.

**FUNDING**

This work was supported by the Fondo Sectorial CONACYT-SENER-Sustentabilidad Energética (project 220704) and by Consejo Nacional de Ciencia y Tecnología (CONACYT grant 385966/253075).

**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflicts, no informed consent was necessary, and no human or animal rights were applicable.
All relevant data are included in the paper or its Supplementary Information.

REFERENCES

APHA-AWWA-WPCF 2005 Métodos normalizados para el análisis de aguas potables y residuales (Standardized Methods for Drinking Water and Wastewater Analysis), 17th edn. Díaz Santos, Madrid, Spain.

Birdsall, C. M., Jenkins, A. C. & Spadinger, E. 1952 Iodometric determination of ozone. Anal. Chem. 24, 662–664.

Brown, N. & Shilton, A. 2014 Luxury uptake of phosphorus by microalgae in waste stabilisation ponds: current understating and future direction. Rev. Environ. Sci. Biotechnol. 13, 321–328.

Chandra, R., Iqbal, H. M. N., Vishal, G., Lee, H. & Nagra, S. 2019 Algal biorefinery: a sustainable approach to valorize algal-base biomass towards multiple products recovery. Bioresour. Technol. 278, 346–359.

Chen, C., Zhao, X., Yen, H., Ho, S., Cheng, D. L., Bai, F. & Chan, J. 2013 Microalgal carbohydrates for biofuels production. Biochem. Eng. J. 78, 1–10.

Cosgun, S. & Semerci, N. 2019 Combined and individual application of ozonation and microwave treatment for waste activated sludge solubilization and nutrient release. J. Environ. Manage. 241, 76–85.

Deng, Y., Zhang, T., Sharma, B. & Nie, H. 2019 Optimization and mechanism studies on cell disruption and phosphorus recovery from microalgae with magnesium modified hydrochar in assisted hydrothermal system. Sci. Total Environ. 646, 1140–1154.

Dixon, C. & Wilken, L. R. 2018 Green microalgal biomolecule separation and recovery. Bioresour. Bioprocess. 5, https://doi.org/10.1186/s40643-018-0199-3.

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.

Egle, L., Rechberger, H., Krampe, J. & Zessner, M. 2016 Phosphorus recovery from municipal wastewater: an integrated comparative technological, environmental and economic assessment of P recovery technologies. Sci. Total Environ. 571, 522–542.

Farias Silva, C. E., Meneghello, D. & Bertucco, A. 2018 A systematic study regarding hydrolysis and ethanol fermentation from microalgae biomass. Biocatal. Agric. Biotechnol. 14, 172–182.

González-Balderas, R. M., Velásquez-Orta, S. B., Valdez-Vázquez, I. & Orta Ledesma, M. T. 2020 Intensified recovery of lipids, proteins, and carbohydrates from wastewater-grown microalgae Desmodesmus sp. by using ultrasound or ozone. Ultrason. Sonochem. 62, 104852.

Gonzalez-Fernandez, C. & Muñoz, R. 2017 Microalgae-Based Biofuels and Bioproducts. Woodhead Publishing, Duxford, UK.

Harun, R. & Danquah, M. K. 2011 Enzymatic hydrolysis of microalgal biomass for bioethanol production. Chem. Eng. J. 168 (3), 1079–1084.

Huang, R., Wan, B., Hultz, M., Díaz, J. M. & Tang, Y. 2018 Phosphatase-mediated hydrolysis of linear polyphosphate. Environ. Sci. Technol. 52, 1183–1190.

Keris-Sen, U. D. & Gürul, M. D. 2017 Using ozone for microalgal cell disruption to improve enzymatic saccharification of cellular carbohydrates. Biomass Bioenergy 105, 59–65.

Mirsiga, M. & Reardon, K. F. 2015 Conversion of lipid-extracted Nannochloropsis salina biomass into fermentable sugars. Algal Res. 8, 145–152.

Misha, S. K., Su, W., I., Farooq, W., Moon, M., Shrivastav, A., Park, M. S. & Yang, J. W. 2014 Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. Bioresour. Technol. 155, 330–333.

Safi, C., Charton, M., Ursu, A., Laroché, C., Zebib, B., Pontalier, P. & Vaca-Garcia, C. 2014 Release of hydro-soluble microalgal proteins using mechanical and chemical treatments. Algal Res. 3, 55–60.

Solovchenko, A., Khizin-Goldberg, I., Selyakh, I., Semenova, L., Ismagulova, T., Luckyanov, A., Mamedov, I., Vinogradova, E., Karpova, O., Konuykho, I., Vasileva, S., Mojzes, P., Dijkema, C., Vecherskaya, M., Zyvagin, I., Nedbal, L. & Gorelova, O. 2019 Phosphorus starvation and luxury uptake in green microalgae revisited. Algal Res. 43, 101651.

Travaini, R., Martín-Juárez, J., Lorenzo-Hernando, A. & Bolado-Rodríguez, S. 2016 Ozonolysis: an advantageous pretreatment for lignocellulosic biomass revisited. Bioresour. Technol. 199, 2–12.

Uzun, H., İbanoğlu, E., Catal, H. & İbanoğlu, S. 2012 Effects of ozone on functional properties of proteins. Food Chem. 134, 647–654.

Valeriano-González, M. T., Monje-Ramírez, I., Orta-Ledesma, M. T., Gracia Fadrique, J. & Velásquez-Orta, S. B. 2016 Harvesting microalgae using ozonoflotation releases surfactant proteins, facilitates biomass recovery and lipid extraction. Biomass Bioenergy 95, 109–115.

Yin, G., Liao, P. H. & Lo, K. V. 2007 An ozone/hydrogen peroxide/microwave-enhanced advanced oxidation process for sewage sludge treatment. J. Environ. Sci. Health 42, 1177–1181.

Zabed, H. M., Akter, S., Yun, J., Zhang, G., Awad, F. N., Qi, X. & Sahu, J. N. 2019 Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew. Sustain. Energy Rev. 105, 105–128.