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Harvesting microalgae using ozoflotation releases surfactant proteins, facilitates biomass recovery and lipid extraction

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Abstract

In this paper, a mixed microalgal consortium (including cyanobacteria) was separated from treated wastewater by ozoflotation to evaluate the method's effect on protein excretion, lipids extraction, and cell recovery. Microalgae suspensions obtained from an artificial lake, Lake Nabor Carrillo, were subjected to different ozoflotation conditions (0.028-0.136 mg O₃/mg biomass). During ozoflotation, proteins released exhibited surfactant activity in the water disrupting the surface tension of water to values of 42.73 mN/m. A critical micelle concentration (CMC) of at least 550 ± 17 mg protein /L was needed to produce foaming and biomass separation of the aqueous medium. The C-Phycocyanin beta chain was identified as one of the main proteins released during the ozoflotation process. Biomass recovery was found to be directly proportional to the ozone dose; best results (75% as TSS)

were obtained at the highest dose used (0.14 mg O₃/mg biomass). In contrast, the best lipid recovery (16% mg lipid/mg biomass) was achieved when using low ozone doses (0.047 mg O₃/mg biomass).

Keywords: Ozoflotation, Microalgae biomass, Protein, Lipids, Surface tension

1. Introduction

Microalgae biomass harvesting is a critical step in the production of third-generation biofuels. For example, it has been reported that harvesting contributes between 20-30% of the total cost of biodiesel production [1]. Conventional harvesting techniques include centrifugation, coagulation-flocculation, filtration, sedimentation and dissolved air flotation. However, these methods have several disadvantages such as the addition of chemicals to biomass harvested, low efficiency, high water content, high retention time, high energy consumption and scaling problems [2, 3].

Ozoflotation is an alternative method of harvesting microalgae, which was first reported in 1980s [4]. Since then, there has been increased interest in using ozone to harvest microalgal biomass [5, 6, 7, 8]. Ozone doses tested in studies range between 0.005 to 0.5 mg O₃/mg biomass depending on the bulk liquid and microalgae source, type, and concentration. Betzer et al. [4] showed that ozoflotation could achieve 98% removal of microalgae, as well as solids and total coliforms, from oxidation pond effluents. Cheng et al. [7, 8], reported that the harvesting of *Chlorella vulgaris* and *Scenedesmus obliquus* using dispersed flotation was not successful when using air alone and required the use of ozone doses. Additionally, the authors reported the effect of ozone on changing the zeta potential through the release of proteins and polysaccharides from microalgae. Velásquez-Orta et al.

[5] found that ozone concentration and ozonation time were the main variables affecting microalgae yield when harvesting native mixed microalgae from wastewater.

Ozoflotation is a physicochemical process that combines the physics of flotation with the chemical oxidative properties of ozone. The oxidative cell damage triggered by ozone is responsible for algal cell lysis and the release of biopolymers like proteins, lipids, carbohydrates, and DNA [9]. Another phenomenon that occurs during microalgae ozoflotation is foaming, which promotes recovery of the biomass from the culture broth. Cheng et al. [7, 8], suggested that the proteins released by cell lysis act as surfactants. These proteins cause the surface of the bubble to become increasingly hydrophilic, which makes bubble-cell collisions more effective, thus forming a layer of foam on the surface where microalgae cells concentrate. Surfactant proteins can decrease the surface tension and contribute to the stability of emulsions or the presence of foam by increasing their viscosity and strength. In fact, protein foamability has been correlated to a decrease in surface tension [10]. However, to date, the surfactant capacity of proteins has not been quantified nor characterized in microalgae recovery by ozoflotation.

Both, Cheng et al. [7] and Velásquez-Orta et al. [5] found that ozoflotation improved lipids recovery after extraction and modified the fatty acid methyl ester (FAME) profile. Cheng et al. [8] found that, the fraction of fatty acid C16:0 in *Chlorella vulgaris* cells increased to more than 55% of total lipids. Velasquez-Orta, et al. [5] observed that the amount of extracted lipids increased to 50% (with respect to centrifugation) when microalgae was harvested by ozoflotation; however, an excess of ozone (>0.24 mg O_3 /mg dried biomass) had the opposite effect. Komolafe, et al. [6] reported that the use of ozoflotation as a method of harvesting microalgae increases the degree of saturation in the FAME extracted.

Sadowska, et al. [11] also reported that exposure of vegetable oils to ozone had decreased the degree of unsaturation of the oils. This indicates that determining the ideal ozone condition should not only consider an increase in the quantity of lipids extracted, but also the release of proteins; all while assuring the quality of the harvested biomass (e.g. lipid FAME profile). It is noteworthy that ozoflotation as harvesting method has some drawbacks such as high-energy consumption; Nava et al. [12] reported that ozoflotation has an energy cost of 9.1 kWh/m³. The aim of this work is to identify, for the first time, proteins released during ozoflotation and evaluate their surfactant capacity as well as to establish a relationship between surfactant protein released, biomass, and lipids recovery.

Methodology

2.1 Microalgae samples

A native consortium of microalgae from the “Lago Nabor Carrillo” (an artificial lake fed with treated wastewater located in Texcoco, Mexico) was used in this study. During April 2014, samples of the native consortium were taken from the lake each week, immediately stored at 4°C and processed the following day. The concentration of biomass as total suspended solids (TSS), was determined gravimetrically using standard methods [13]. Results showed an average TSS concentration of 400 ± 20 mg/L. Microorganisms were identified and counted using a microscope (Leitzlaborlux S, Germany). It was found that cyanobacteria (i.e., *Arthrospira* sp. *Spirulina* sp. *Oscillatoria* sp.) and green algae (i.e., *Desmodesmus* sp. *Scenedesmus* sp.) were the most abundant phyla.

2.2 Harvesting by ozoflotation

Ozoflotation experiments were conducted using 950 ml of fresh homogenized suspensions of mixed microalgae. Batch flotation tests used a specially designed 1.3 L glass column reactor (height: 66 cm; inner diameter: 4.9 cm). For ozoflotation, lab-scale experiments used a Labo 76 ozone generator (Emery Trailigaz, USA) with a production capacity of 19 g O₃/h. Ozone injected at a flow rate of 0.2 L/min using a glass diffuser (10-15 µm pore-sizes) located at the bottom of the column, Figure 1. The ozone concentration in the gas phase was determined using the Iodometric Method [14].

In the harvesting process, two types of samples were taken: the first sample, “concentrated protein”, contained the cell biomass and proteins dissolved in the bulk liquid recovered from the top of the reactor; the second sample, “reactor protein”, contained the proteins that remained in the column bulk liquid. At the end of each experiment, total protein concentration and surface tension were measured for both samples.

The percentage of microalgal biomass harvested was calculated by subtracting the TSS content in the initial sample from the TSS remaining in the ozoflotation reactor.

Figure 1. Experimental setup for testing the ozoflotation with microalgae.

2.3 Experimental design

Biomass and lipid recovery were evaluated using the response surface method with a two factorial central composite rotational design (CCRD). The design involved carrying out 13 experiments, each with three replicates. These included five central points and an axial point, with a selected α of 1.68. It resulted in a total of nine tests, involving ozone concentrations in the gas phase and ozonation times between 3-7 mgO₃/L and 15-35 min, respectively. The influence of these two operating variables were determined for the following responses: 1) amount of proteins released, 2) cell biomass recovery, and 3) amount of lipids extracted. Minitab 17 statistical software was used to perform an analysis of variance.

2.4 Surface tension of proteins

Surface tension was measured in samples of proteins obtained by separating the microalgae from its natural environment (treated wastewater) and using the pre-washed biomass to eliminate possible interference of the constituents present in treated wastewater (eg. detergents). The washing consisted of removing water by centrifugation and re-suspending the pellet in distilled water; the procedure was repeated three times. Finally, 40L of microalgae suspension were prepared at a concentration of 400 mg TSS/L in distilled water for ozoflotation tests. Surface tension tests were conducted for all protein samples obtained.

Samples from the reactor and concentrated protein were filtered using a 0.22µm Millipore membrane (GVWP02500) and total protein was quantified using the Biuret method (Merck 1103070500), according to the manufacturer's specifications.

The surface tension was determined according to Pardo-Cervantes et al. [15]. Samples were allowed to rest at least 2 h at room temperature (20±2 °C) in a Petri dish to allow proper equilibrium. The Ring method was chosen to evaluate surface tension. Equipment was assembled using a balance (Adventurer, Ohaus. Pine Brook, NJ) with a hook underneath to hang the DuNouy platinum ring (CSC Scientific Co. Inc., USA) having a 5.992 cm mean circumference and an $R/r=53.6$. Samples were placed on a platform with a speed-controlled elevator (Orbisphere Labs., Neuchatel/Geneva, Switzerland) which pushed samples until a distance between the sample's surface and the ring was reached. The platform descended and the maximum weight pulled by the ring was registered. Surface tension was determined using equation 1.

$$\sigma = \frac{Mg}{4\pi R} * F \quad \text{Eq. 1}$$

Where M is the maximum weight supported by the ring, g is the local gravity (977.94 cm/s², UNAM), R is the radius of the ring and F is a correction factor that adjusts the size of the ring (R^3/V , where V is the maximum volume supported by the ring), obtained from tables [16].

In order to determine the concentration of protein necessary to lower surface tension as well as the ozone dose needed for microalgae separation, the critical micelle concentration (CMC) was determined. CMC is the minimum concentration that triggers micelle formation [17] and was calculated using equation developed by Viades-Trejo and Gracia-Fadrique [18].

2.5 Identification of protein released by ozoflotation

Total protein released into aqueous medium was identified after ozoflotation using three different doses of ozone (27.8 mgO₃/L, 43.3 mgO₃/L and 54.4 mgO₃/L). To validate that proteins identified were released by the breakage of cells due to ozone, the values were compared with microalgae proteins extracted by a conventional alkaline method [19]. In the alkaline method, a solution of 50 mL of NaOH/water at pH 12 was added to 1 g of dry microalgal biomass. The sample was heated at 40°C with stirring for one hour. Extracted proteins were recovered from the aqueous phase by centrifugation at 5000 g for 10 min.

Proteins were isolated using the technique of polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) at 12%. Twenty-five microliters of total proteins obtained from the concentrated samples were injected to an electrophoresis gel, along with a marker of molecular weight ranging from 15 to 250 KDa (Dual-color, BioRad). Electrophoresis was performed at 45V for 24h at room temperature in a running buffer SDS-PAGE, on a 600 SE Vertical Unit (GE-Healthcare). The SDS-PAGE was stained with Coomassie blue.

Next, the SDS-PAGE bands of interest were excised with a sterile scalpel and were treated with gel trypsin digestion. Gel digestion was performed with modified porcine trypsin 30 µL in a solution containing 20 ng/µL. Samples were incubated at 37°C for 18 h.

The identification of proteins was performed using tandem mass spectrometry (LC/MS/MS); a nano-LC-ESI-MS/MS integrated system (Mass Spectrometer/flight time, SYNAPT G2 HD, Waters Corporation) equipped with a NanoLockSpray ion source; and a nanoACQUITY-UPLC (Waters Corporation).

Results were processed through ProteinLynx Global software (PLGS) (Waters Corporation) and compared with the database UNIPROT. Peptides were considered a match to the PLGS scores at a 96% confidence level or higher.

2.6 Lipid extraction of microalgae biomass

Microalgae separated by ozoflotation were centrifuged at 15,000×g at 20°C for 10 min using a Beckman Coulter centrifuge (AVANTI-J26S XPI). Biomass was oven dried at 50°C. Lipid extraction was performed using a homogeneous solution of chloroform-methanol ratio of 2:1 (v/v) [6]. The volume of the chloroform and methanol solution was 45 times the mass of the solution. Therefore, for 1g of algae biomass, the volumes of chloroform and methanol were 30mL and 15ml, respectively. The solution was left overnight in the fridge at a temperature of 4°C. After overnight extraction, samples were vacuum filtered using a Whatman glass microfiber filter paper. The filtrates were poured into separating funnels and a weak salt solution of potassium chloride (KCl, 0.88 vol%) was added at 25% of the starting weight. The solutions were well mixed and allowed to separate into two layers. Lipid layers were carefully removed into pre-weighed conical flasks and left to dry in a fume cabinet until constant weight. The mass of lipids in microalgae species was finally obtained by deducting the vessel weight from the final constant weight.

3. Results and discussion

3.1 Protein release during ozoflotation

During microalgae ozoflotation, an intense foaming due to the release of proteins was observed; this was confirmed by quantifying the total protein content. As was previously

reported, this foaming contributed to the separation and concentration of microalgae [8, 7]. In this study, the percentage of recovered biomass and protein concentration in the “concentrate protein” was observed to increase proportionally according to the ozone dose (Figure 2). A direct relationship was obtained between the protein concentration and the recovery of biomass. A maximum concentration of 2.5 g/L of “concentrated protein” was obtained from the microalgal biomass at the end of the ozoflotation (Figure 2). There were also 351 mg/L of “reactor protein” after the ozoflotation process, a value 7 times lower than the maximum “concentrated protein” yield.

Figure 2. Relationship between the recovery of biomass and protein concentration during ozoflotation of the “concentrated protein”. Standard deviations are indicated by error bars.

3.2 Surfactant effect of proteins in the ozoflotation.

Proteins are amphiphilic molecules that reduce surface tension and promote foaming [17], allowing microalgae separation from the aqueous medium. In this paper, the surface tension was measured in samples of proteins obtained by ozoflotation of microalgae from its

natural environment (treated wastewater) and from pre-washed microalgae, to eliminate possible interference of the constituents present like detergents. As can be seen in Figure 3, the surface tension in all protein samples decreased with an increasing ozone dose, which could be associated to the higher protein content in the samples (see Figure 2). For both samples (washed and unwashed microalgae), a similar behavior is observed; the analysis of variance indicated no significant difference between them (p-value = 4.13). This means that changes in the surface tension during ozoflotation were primarily attributable to the proteins released by ozone, and not to the chemicals present in wastewater.

Figure 3. Effect of the ozone dose in the reduction of surface tension by the effect of the protein released from microalgae. Standard deviations are indicated by error bars.

On the other hand, Figure 4 shows the effect of protein concentration on the reduction of surface tension, from 65.71 mN/m (initial value) to values of 42.73 mN/m and 50.04 mN/m, in samples of proteins, obtained from ozoflotation of unwashed and washed microalgae, respectively. Foegeding et al. [20] reported that proteins in solution reduce the

surface tension of distilled water from 72 mN/m to approximately 45 mN/m. Chronakis et al. [21] also showed a decrease of surface tension to 37.5 mN/m, using microalgae proteins obtained by alkaline extraction.

Figure 4. Reduction of the surface tension according to the concentration of protein released during ozoflotation. Standard deviations are indicated by error bars.

Results indicate that a CMC of 550 ± 17 mg/L of microalgae protein is required to reduce the surface tension of water to 42 mN/m, which is achieved with an ozone dose of 0.025 mg O_3 /mg biomass. Higher ozone doses produced a higher concentration of surfactant with no significant effect in surface tension; however, an increased ozoflotation time was necessary to complete biomass harvesting. It is reported that increased protein concentrations generate more stable and dense foam [10].

3.3 Identification of proteins released by ozoflotation

233 Results of SDS-PAGE gel of the different protein samples are shown in Figure 5. In the
234 alkaline extract (A), two bands between molecular weights of 15 and 10 kDa, were
235 detected. Conversely, multiples bands of proteins, depending on the ozone dose used on
236 microalgae ozoflotation were observed. When we use low ozone dose (B), an important
237 number of proteins, with molecular weight between 10 - 100 kDa, were identified; seven
238 proteins are the more relevant. The effect of ozoflotation is observed as an intensifying
239 band in the three samples around the 20 and 37 kDa which shows higher accumulation with
240 an increasing ozone dose (Figure 5B, C, and D). This was possibly due to higher cell
241 damage and protein concentration release. On the other hand, the bands around 75 kDa
242 disappear with excess ozone dose. We suggest this effect is a result of possible per-
243 oxidation of the proteins.

244

245 Figure 5. SDS-PAGE gel of samples of microalgae protein: Dual Color, Bio-Rad molecular
 246 weight marker, kDa (MWM); alkaline extract, without ozone (A); Ozoflotation: 0.0695 mg
 247 O₃/mg biomass (B), 0.1082 mg O₃/mg biomass (C), and 0.136 mg O₃/mg biomass (D).

248 The protein identified when using alkaline extraction (A) was RuBisCo (ribulose 1,5
 249 biphosphate carboxylase-oxygenase (MW: 56kDa). This protein is contained in the
 250 carboxysomes of the cyanobacterias of genus *Cyanothece* sp. [22]. Other proteins like
 251 RuBisCo were identified in the alkaline extract such as Allophycocyanin beta chain (MW:
 252 17 kDa) and the C-phycocyanin alpha chain (MW: 17.3 kDa), from *Arthrospira platensis*
 253 cyanobacteria.

The main protein obtained after ozoflotation (B, C, and D) was identified as the C-Phycocyanin beta chain with a molecular weight of 18 kDa, a biliprotein (pigment) normally found in the cell wall of cyanobacteria [23]. These results suggest that ozone acts on the cell wall, which contains the C-phycocyanin beta chain. It follows that, the ozoflotation process specifically promotes the release of surfactant proteins located in the cell wall of microalgae.

3.4 Biomass and lipid recovery by ozoflotation

Previous studies by Velásquez-Orta et al. [5] found a maximum recovery of biomass (79%) and lipids (12%) when using doses of 0.23 mg O₃/mg biomass. In addition, these studies revealed that a dose of >0.24 mg O₃/mg biomass decreased lipid recovery.

In this paper, ozoflotation was studied using low ozone doses (0.023 - 0.123 mg O₃/mg of dried biomass) using the critical micellar concentration (CMC) of the surfactant protein as a starting point. Table 1 shows the effect of tested variables (ozone concentration and ozonation time) in the recovery of biomass (TSS%) and lipids (%).

Table 1. Experimental significance of tested variables in ozoflotation. Variables tested were ozone concentration and ozonation time; responses were biomass and lipid recoveries. The confidence level used was 95%.

	Biomass recovery (TSS %)		Lipid recovery (%)	
	F-Ratio	p-value	F-Ratio	P-Value
A: Ozone concentration	79.91	0.0000	0.41	0.5432
B: Ozonation time	52.09	0.0002	64.88	0.0001
AA	1.16	0.3176	16.50	0.0048

AB	11.40	0.0118	5.31	0.05471
BB	0.04	0.8411	16.36	0.0049

272

273 With regards to the recovery of biomass, three effects were found to be significant in the
274 following order: ozone concentration (A), with a p-value of 0.000; ozonation time (B), with
275 a p-value of 0.002; and finally AB interaction, with a p-value of 0.01. Similarly, ozonation
276 time (B) was found to have a significant effect on the recovery of lipids (p-value <0.05).
277 According to Pareto charts (results not shown), the variable B negatively affected lipid
278 recovery.

279 Figure 6 shows the model response surface, indicating that the maximum recovery of
280 biomass (75%) was obtained with an ozone concentration of 7.53 mgO₃/L and ozonation
281 time of 37.6 min, equivalent to a dose of 0.142 mgO₃/mg biomass. On the other hand, the
282 highest lipid recovery (16%) was achieved with a low dose of ozone (0.047 mgO₃/ mg
283 biomass). The results of biomass harvesting and recovery of lipids were similar to those
284 reported by Velasquez-Orta et al. [5], but using 1.6 to 4.9 times less ozone doses. In
285 previous studies, Cunha et al. [24] observed that high ozone doses produce lipid oxidation,
286 for this reason, low doses of ozone were chosen to permit membrane lysis for surfactant
287 protein release (biomass recovery) and lipid recovery while avoiding lipid oxidation.

Figure 6. Recovery of biomass (A) and lipid (B) versus time and ozone concentration of ozoflotation respectively. Contours show estimates of response surface for the recovery of biomass and lipid.

As seen in Figure 7, a higher recovery of microalgal biomass (Figure 7A) did not result to an increase in lipid recovery (Figure 7B). In fact, lipid recovery decreased with an increasing dose of ozone (≥ 0.058 mg O_3 / mg biomass). The same behavior was reported in Velasquez-Orta et al. [5], but with higher ozone doses (≥ 0.25 mg O_3 /mg biomass). Figure 7C shows that, in this case, the harvested biomass had lower lipid content when recovered at higher doses of ozone. Lipid reduction can be attributed to a possible lipid peroxidation. It has been reported that the major target for lipid peroxidation occurs in unsaturated fatty acids. Lipid peroxidation was previously observed with prolonged ozone exposure times (20-30 minutes) [24, 25].

Figure 7. Effect of the ozone dose to microalgae: A. Recovery of biomass, B. Recovery of lipids, C. Recovery of lipid per biomass, and D. Recovery efficiency.

Taking into account the efficiency of the process (Figure 7D), in both cases the best results were obtained with low doses of ozone (0.028 mg O₃/mg biomass). These results were consistent with those reported in the literature for microalgae biomass recovery [8], [7] and [4]. However, the link between harvested biomass and lipid recovery by ozoflotation is still incipient and must be further studied. These results indicate that even at low ozone dose lipid recovery is affected (Figure 7C). It seems that high exposure to ozone, either by the

use of high concentrations in the gas phase or by prolonged ozonation time, negatively affects the quantity of lipids extracted from the microalgal biomass harvested. Therefore, further research is needed to achieve a good biomass recovery with high lipid content.

Conclusions

The surfactant effect of protein released during the ozoflotation of microalgae was found to decrease the surface tension of the system (42 mN/m) thereby promoting foaming and biomass recovery. Beta C-Phycocyanin was the main protein identified to be released during the ozoflotation process. The critical micelle concentration (CMC) of 550±17 mg/L was achieved using ozone dose of 0.025mg O₃/mg biomass. The highest percentages of biomass recovery were achieved at ozone doses of 0.142 mg O₃/mg biomass. However, a high exposure of biomass to the oxidizing effect of ozone was found to impact lipid recovery. The best recovery efficiencies of microalgal biomass and lipids were respectively: 8 mg biomass/mg O₃ and 1.25 mg lipids/mg O₃.

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Bibliography

[1] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Biodiesel from microalgae: A critical
evaluation from laboratory to large scale production, *Fuel*. 103 (2013) 444-467.

[2] A. Barros, A. Goncalves, M. Simoes, J. Pires, Harvesting techniques applied to
microalgae: A review, *Renewable and Sustainable Energy Reviews*. 41 (2015) 1489-1500.

[3] M. Gerardo, S. Van Den Hende, H. Vervaeren, T. Coward, S. Skill, Harvesting of
microalgae within a biorefinery approach: A review of the developments and case studies
from pilot-plants, *Algal Research*. 11 (2015) 248-262.

[4] N. Betzer, Y. Argaman, Y. Kott, Effluent treatment and algae recovery by ozone-
induced flotation, *Water Research*. 14 (1980) 1003-1009.

[5] S.B.Velásquez-Orta, R. García-Estrada, I. Monje-Ramirez, A. Harvey, M.T. Orta
Ledesma, Microalgae harvesting using ozoflotation: Effect on lipid and FAME recoveries,
Biomass and Bioenergy. 70 (2014) 356-363.

[6] O. Komolafe, S.B. Velasquez-Orta, I. Monje-Ramirez, I. Yañez Noguez, A. P. Harvey,
M.T. Orta-Ledesma, Biodiesel production from indigenous microalgae grown in
wastewater, *Bioresource Technology*. 154 (2014) 297-304.

350 [7] Y.L. Cheng, Y.C. Juang, G.Y. Liao, P.W. Tsai, S.H. Ho, K.L. Yeh, C.Y. Chen, J.S.
 351 Chang, J.C.Liu, W.M. Chen, D.J. Lee, Harvesting of *Scenedesmus obliquus* FSP-3 using
 352 dispersed ozone flotation, *Bioresource Technology*. 102 (2011) 82-87.

353 [8] Y.L. Cheng, Y.C. Juang, G.Y. Liao, S.H. Ho, K.L. Yeh, C.Y. Chen, J.S. Chang,
 354 J.C.Liu, D.J. Lee, Dispersed ozone flotation of *Chlorella vulgaris*, *Bioresource Technology*.
 355 101 (2010) 9092-9096.

356 [9] B. Langlais, D. A. Reckhow, D. R. Brink, *Ozone in water treatment: application and*
 357 *engineering*. Lewis, USA, 1991.

358 [10] J. F. Zayas, *Functionality of proteins in food. Foaming properties of proteins*. Springer
 359 Verlag.(1996).

360 [11] J. Sadowska, B. Johansson, E. Johannessen, R. Friman, L. Broniarz-Press, J.
 361 Rosenholm, Characterization of ozonated vegetable oils by spectroscopic and
 362 chromatographic methods, *Chemistry and Physics of Lipids*. 151 (2008) 85-91.

363 [12] M. Orta-Ledesma, I. Nava Bravo, S.B. Velásquez-Orta, I. Monje-Ramírez, I. Yáñez-
 364 Noguez, A.P. Harvey. (2014), Microalgae in Nabor Carrillo Lake suitable for Bioenergy
 365 production. IWA Water-Energy-Climate Conference 21-23 May. Mexico City. IWA -
 366 International Water Association, ANEAS - Asociacion Nacional de Empresas de Agua y
 367 Saneamiento de México.

368 [13] APHA-AWWA-WPCF. *Standard methods for the examination of water and*
 369 *wastewater*. Diaz Santos. Madrid, Spain. (1992).

370 [14] C. Birdsall, A. Jenkins, E. Spadinger, Iodometric determination of ozone, *Analytical*
 371 *Chemistry*. 24 (1952) 662-664.

372 [15] A. Pardo-Cervantes, J. Viades-Trejo, J. Gracia-Fadrique, Activity coefficients at
 373 infinite dilution and Critical micelle concentrations of Poly(oxy-1,2-ethanedyl), α -
 374 Tridecyl- ω -hydroxy ethers (C₁₃Ei=6,10,18) in aqueous media by the volmer surface equation
 375 of state and group contributions, Journal of chemical and engineering data. 56 (2011) 2812-
 376 2817.

377 [16] W. D. Harkins, H. F Jordan, A method for the determination of surface and interfacial
 378 tension from the maximum pull on a ring, Journal American Chemical Society. 52 (1930)
 379 1751-1772.

380 [17] C.N. Mulligan, Enviromental applications for biosurfactants, Enviromental Pollution.
 381 133 (2005) 183-198.

382 [18] J. Viades-Trejo, J. Gracia-Fadrique, A new surface equation of state: Hydrophobic-
 383 hydrophilic contributions to the activity coefficient, Fluid Phase Equilibria. 264 (2008) 12-
 384 17.

385 [19] C. Safi, M. Charton, A. Ursu, C. Laroche, B. Zebib, P. Pontalier, C. Vaca-Garcia,
 386 Release of hydro-soluble microalgal proteins using mechanical and chemical treatments,
 387 Algal Research. 3 (2014) 55-60.

388 [20] E. A. Foegeding, P. Luck, J. Davis, Factors determining the physical properties of
 389 protein foams, Food Hydrocolloids. 20 (2006) 284-292.

390 [21] I. S. Chronakis, A. N. Galatanu, T. Nylander, B. Lindman, The behaviour of protein
 391 preparations from blue-green algae (*Spirulina platensis* strain Pacifica) at the air/water
 392 interface, Colloids and Surface A: Physicochemical and Engineering Aspects. 173 (2000)
 393 181-192.

- 394 [22] J. V. Moroney, A. Somanchi, How do algae concentrate CO₂ to increase the efficiency
395 of photosynthetic carbon fixation?, *Plant Physiology*. 119 (1999) 9-16.
- 396 [23] R. Chiklahan, N. Chirasuwan, V. Loha, S. Tia, B. Bunnang, Separation and
397 purification of phycocyanin from *Spirulina* sp. using a membrane process, *Bioresource*
398 *Technology*. 102 (2011) 7159-7164.
- 399 [24] H.N. Cunha, F.L.A.J. Silva, J. Melo, E.H.S. Cavalcanti, T.M. Fonseca, E.U.K.
400 Melcher, A.M.N. Lima, H.M. Laborde, H. Neff, The interaction of ozone with bio-fuel,
401 revealed by electrical conduction and infrared spectroscopy, *Fuel Processing Technology*.
402 92 (2011) 2160-2168.
- 403 [25] M.N. Vaultier, Y. Jolivet, Ozone sensing and early signaling in plants: An outline from
404 the cloud, *Environmental and Experimental Botany*. 114 (2015) 144-152.