Contemporary Engineering Sciences, Vol. 11, 2018, no. 6, 283 - 291
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https://doi.org/10.12988/ces.2018.8118

Effect of Flocculation Technology on Lipids, Carbohydrates and Proteins and Extraction from *Chlorella vulgaris*

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Abstract

Nowadays, *Chlorella vulgaris* has emerged as an attractive source of metabolites such as lipids, proteins and carbohydrates, which are widely applied to obtain high value products. This work is focused on evaluating the effect of flocculation on metabolites recovery from *Chlorella vulgaris* microalgae biomass. Carbohydrates were extracted through acid and alkaline hydrolysis in order to identify the efficiency of these treatments for cell wall disruption. Protein quantification was carried out under alkaline conditions and lipids were recovered by solvent extraction with hexane. It was found that carbohydrate recovery was higher using centrifuged biomass (40.55%) than flocculated biomass (24.31%) by acid treatment, while alkaline treatment exhibited yield of 20.37% and 13.19%, respectively. In addition, efficiency of 53.75% and 32.27% in protein recovery was obtained from both centrifuged and flocculated biomass. The highest lipid extraction yield (54.2%) was achieved using acid hydrolysis and hexane solvent route when centrifuged biomass was used as metabolite source. These results suggested that flocculation affect significantly the efficiency of metabolites extraction.

Keywords: Microalgae, carbohydrates, proteins, lipids, flocculation

1 Introduction

Microalgae is recognized as novel green sources of multiple components such as proteins, carbohydrates, lipids and pigments [1]. These microorganisms exhibit the ability to grow under unsuitable conditions for other crops, needing merely some nutrients and sunlight [2]. In addition they have the advantages of photosynthetic efficiency, biomass productivity and oil content [3]. They have been extensively studied for producing biomass as a source of valuable chemicals for food, pharmaceutical, cosmetic and energy industries [4]. Lipid, protein and carbohydrate content in microalgae can vary widely according to culture conditions and organism's particular metabolic mechanism [5]. The efficient utilization of these metabolites is entirely dependent upon high lipid productivity and high carbohydrate and protein content in the microalgal biomass [6]. Among a wide variety of microalgae, *C. vulgaris* has been selected as biomass source due to its resistance against invaders, high lipid content that can reach up to 58% under stress conditions and contains highly valuable compounds, such as astaxanthin, β-carotene and some nutritional polyunsaturated fatty acids (PUFA) [7].

To extract lipid from this biomass, pretreatment and subsequent lipid collection by solvent are performed [5]. Several cell-wall disruption methods are widely used on microalgae such as: physical, chemical and enzymatic hydrolysis to recover carbohydrate and proteins [3]. The aim of this work is to evaluate the effect of flocculation on metabolites extraction by acid hydrolysis, alkaline hydrolysis and solvent extraction with hexane in order to compare recovery yield when centrifuged and flocculated biomasses are used.

2 Materials and Methods

Chlorella vulgaris samples were grown in in Bold Basal medium, microalgae was kept growing during 15 days in 2.5 L rectangular glass reactor. The reactor was coupled to a bubble aeration system for air injection and light-dark cycle 12:12 h. pH-measurement was performed daily in order to control this parameter in 7-8 value. Microalgal biomass was concentrated by adding 1.23 mL of aluminum chloride solution (40 g/L) to 100 mL of culture medium. To carry out flocculation, pH was adjusted to 7. The remaining biomass was centrifuged at 3400 rpm during 15 minutes. Finally, both flocculated and concentrated by centrifugation biomass were subjected to drying process at 105 °C during 17 hours.

Acid hydrolysis pretreatment was used for cellular wall disruption according to the procedure described by González-Delgado et al.[5]. Biomass (5 g) was added to 50 mL HCl (0.5 M). The mixture was continuously stirred at 500 rpm during 2 hours and neutralized by KOH (1 M). Then, separation and filtration of liquor and

hydrolyzed biomass was carried out. For Alkaline hydrolysis Biomass (5 g) was mixed with 30 mL of NaOH (3.76 M) and heated during 20 minutes at 55ºC [8]. Finally, liquor and hydrolyzed biomass was separated by filtration.

For quantification of carbohydrates, liquor obtained in acid hydrolysis was subjected to phenol-sulfuric acid colorimetric method[9] for determining carbohydrates content. Hydrolyzed biomass (1 mL) was mixed with 0.5 ml of 5% phenol and 2.5 ml of 95% sulfuric acid. Absorbance measurement was carried out at 485 nm (wavelength) after revealing mixture coloration. Methodology described by Lowry et al.[10] was used to determine protein content in the extract. The sample (1 mL) was mixed with Lowry solution (1.4 mL) and homogenized during 5 minutes. Then, 0.2 mL of Folin-water solution was added.

After revealing coloration, absorbance measurements were performed at 750 nm using Spectroquant Pharo 300 spectrophotometer (Merck). Protein content was determined by calibration curve, previously standardized from the analytical grade albumin reagent. After monosaccharides production was subjected to lipid extraction by Soxhlet using 20 mL of hexane as solvent. The lipid extract was separated from the solvent in a roto-evaporator, followed by weighing to determine the recovery of lipids according to Equation 1. Experiments for metabolites extraction using centrifuged and flocculated biomass were carried out in triplicate. Statistical analysis of the results were performed by T test (n=3), in which average of data is considered significantly different when P value is less or equal to 0.05.

$$\% \text{ extraction } = \frac{\text{oil weight}}{\text{biomass weight}} \times 100 \quad (1)$$

3 Results and Discussion

3.1. Carbohydrate extraction with Acid treatment (HCl)

The results of carbohydrates extraction from *Chlorella vulgaris* through acid hydrolysis are presented in Figure 1. As is shown, 40.55% of the carbohydrate content in centrifuged biomass and 25.31% in flocculated biomass was recovered under acid extraction conditions.

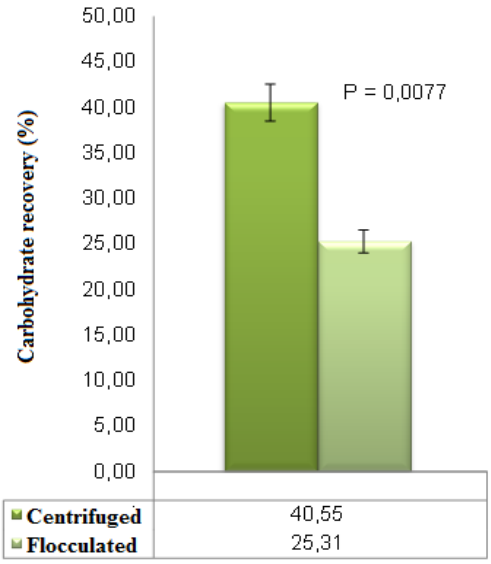

Figure 1. Carbohydrates recovery by acid hydrolysis using centrifuged and flocculated biomass

The analysis of experimental data by T test indicated that the difference between data averages is significant, because P value is less than 0.05. These results show that flocculation affects the performance of acid hydrolysis in the extraction of carbohydrates from microalgae biomass.

3.2. Carbohydrate extraction via Alkaline treatment (NaOH)

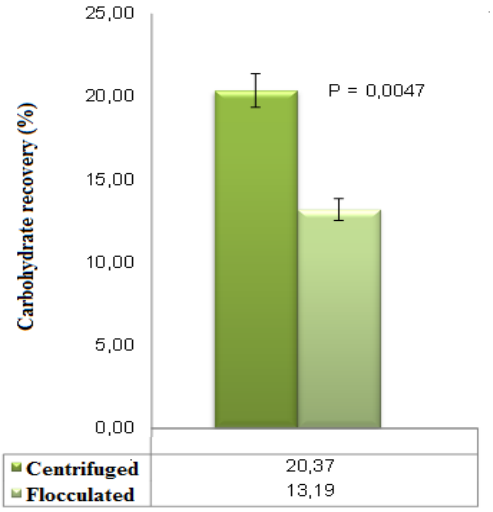

Figure 2. Carbohydrates recovery by alkaline hydrolysis using centrifuged and flocculated biomass

Figure 2 shows carbohydrate recovery from *Chorella vulgaris* using alkaline hydrolysis. The results indicated a carbohydrate extraction yield of 20.37% and 13.19% for centrifuged and flocculated biomass, respectively. It is observed that P value is less than 0.05 indicating the negative effect of flocculation on hydrolysis reaction with NaOH. Alkaline treatment exhibit lower carbohydrate recovery in comparison with acid hydrolysis. Robert et al. [11] claimed that the yield of extraction can decrease up to 23.17%, for *T. pseudonana* flocculated, using an alkaline treatment (1 M NaOH, at 100 °C during 10 minutes).

3.3. Protein extraction

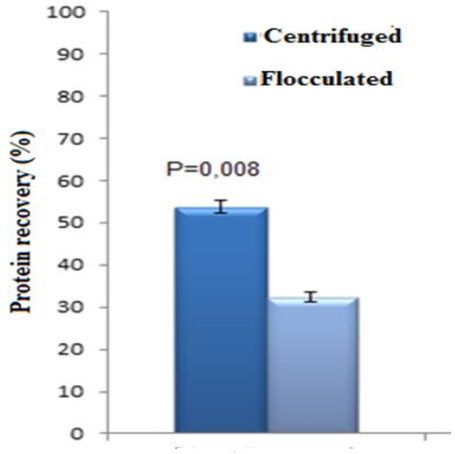

Figure 3. Protein recovery from centrifuged and flocculated biomass

Figure 3 shows protein extraction from *Chlorella vulgaris* biomass (flocculated and centrifuged). It was observed an efficiency of 53.75% in protein recovery with centrifuged biomass and 32.27% with flocculated biomass. These results indicated that the use of flocculant in culture medium has negative effect on protein recovery, which is confirmed by P value less than 0.05. Some authors confirm the inhibitory effect of flocculants in protein extraction by alkaline hydrolysis (1M NaOH at 100 °C). According to Robert et al. [11], process efficiency can decrease by almost 30% when ferric chloride is used to concentrate *T. pseudonana*. On the other hand, Prasertsan et al. [12] claim to be able to recover up to 52.9% of protein content in *Chlorella sp*, using aluminum potassium sulfate in culture medium. Regarding acid hydrolysis (HCl), this procedure can degrade amino acids present in biomass, hence protein extraction was not quantified using acid treatment.

3.4. Lipid extraction after acid treatment (HCl)

The results of lipid extraction from *Chlorella vulgaris* when acid hydrolysis is used for cellular wall disruption are presented in Figure 4. It is observed a lipid recovery of 54.2% and 51% for centrifuged and flocculated biomass, respectively. Other studies report yields up to 60% in lipid extraction from microalgae *Nannochloropsis salina* following acid route (5% H_2SO_4) and using hexane as extractive agent [13].

Hence, it is recommended to evaluate the effect of flocculant on biomass production under different culture conditions and consider other routes of hydrolysis during pretreatment.

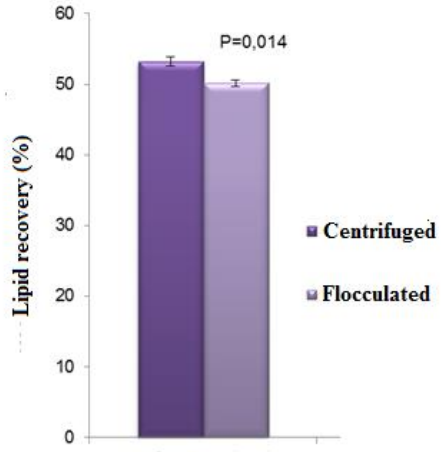

Figure 4. Lipid recovery from centrifuged and flocculated biomass (acid treatment)

3.5. Lipid extraction after alkaline treatment (NaOH)

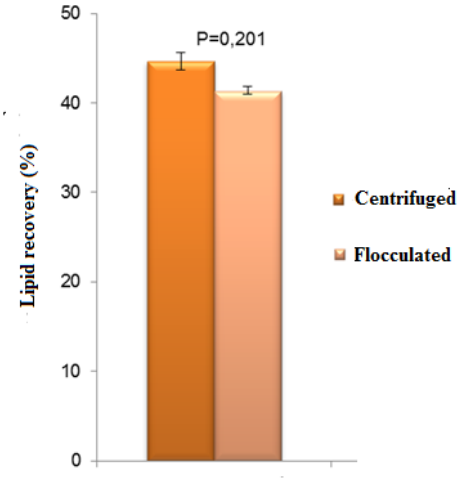

Figure 5. Lipid recovery from centrifuged and flocculated biomass (alkaline treatment)

As is shown in Figure 5, lipid extraction yield from centrifuged biomass (44.67%) is higher than flocculated biomass (40.89%). Statistical analysis exhibited P>0.05, which suggested that flocculant in culture medium did not affect the performance of this process. In addition, extraction performance also depends on solvent selectivity and nature. Hexane is a non-polar substance, this characteristic allows it to exhibit a greater affinity towards non-polar and amphipathic lipids. According to Bellou & Aggelis [14] *C. vulgaris* accumulates up to 92% of non-polar lipids.

Nevertheless, cultivation conditions (light, availability of nutrients, carbon sources, among others) can modify the metabolic activity of cells and accumulate certain amount of metabolites [15].

4 Conclusions

This work attempted to evaluate the effect of flocculation on carbohydrate, lipid and protein extraction from *Chlorella vulgaris* biomass. The highest metabolites recovery were obtained when centrifuged biomass was used instead of flocculated biomass, which was confirmed by statistical analysis and suggested that flocculant in culture medium affects significantly the performance of these extraction. Acid hydrolysis exhibited carbohydrate recovery of 40.55% and 25.31% using centrifuged and flocculated biomass, respectively, higher than thus obtained by alkaline hydrolysis. To extract proteins, it was found an efficiency of 53.75% with centrifuged biomass and 32.27% with flocculated biomass. The highest lipid recovery (54.2%) was achieved using centrifuged biomass and acid hydrolysis-solvent extraction procedure.

Acknowledgements. Authors thank to Universidad Francisco de Paula Santander and University of Cartagena for supporting this research.

References

[1] R. Zhang, J. Chen, X. Zhang, Extraction of intracellular protein from Chlorella pyrenoidosa using a combination of ethanol soaking, enzyme digest, ultrasonication and homogenization techniques, *Bioresource Technology*, **247** (2018), 267–272. https://doi.org/10.1016/j.biortech.2017.09.087

[2] E. Blanco-Carvajal, E. Sánchez-Galvis, Á. González-Delgado, Janet Bibiana Garcia Martinez, Andres Fernando Barajas-Solano, Cultivation of Chlorella vulgaris in Aquaculture Wastewater for Protein Production, *Contemporary Engineering Sciences*, **11** (2018), 93–100. https://doi.org/10.12988/ces.2018.712203

[3] Á. González-Delgado, J. García-Martínez, Y.Y. Peralta-Ruíz, Evaluation of Two Pre-Treatments for Improving Lipid Extraction from Microalgae Navicula sp., *Contemporary Engineering Sciences*, **10** (2017), 851–859. https://doi.org/10.12988/ces.2017.78792

[4] L.K. Torres-carvajal, Á.D. González-Delgado, A.F Barajas-Solano, Jhon Hermogenes Suarez-Gelvez, Nestor Andres Urbina-Suarez, Astaxanthin Production from Haematococcus pluvialis : Effects of Light Wavelength and Salinity, *Contemporary Engineering Sciences*, **10** (2017), 1739–1746. https://doi.org/10.12988/ces.2017.711196

[5] Á. González-Delgado, J. García-Martínez, Y.Y. Peralta-Ruíz, Cell Disruption and Lipid Extraction from Microalgae Amphiprora sp . Using Acid Hydrolysis-Solvent Extraction Route, *Contemporary Engineering Sciences*, **10** (2017), 841–849. https://doi.org/10.12988/ces.2017.78791

[6] Y. Heoa, H. Leea, C. Lee, Juwon Kang, Joon-Woo Ahn, Young Min Lee, Kyu-Young Kang, Yoon-E Choi, Jae-Jin Kim, An integrative process for obtaining lipids and glucose from Chlorella vulgaris biomass with a single treatment of cell disruption, *Algal Research*, **27** (2017), 286–294. https://doi.org/10.1016/j.algal.2017.09.022

[7] E. Saraylooa, S. Simseka, Y. Unlud, Gul Cevahir, Can Erkey, Ibrahim Halil Kavakli, Enhancement of the lipid productivity and fatty acid methyl ester profile of Chlorella vulgaris by two rounds of mutagenesis, *Bioresource Technology*, **250** (2018), 764–769. https://doi.org/10.1016/j.biortech.2017.11.105

[8] M. Chang, D. Li, W. Wang, Dongchu Chen, Yuyuan Zhang, Huawen Hu, Xiufang Ye, Comparison of sodium hydroxide and calcium hydroxide pretreatments on the enzymatic hydrolysis and lignin recovery of sugarcane bagasse, *Bioresource Technology*, **244** (2017), 1055–1058. https://doi.org/10.1016/j.biortech.2017.08.101

[9] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric Method for Determination of Sugars and Related Substances, *Analytical Chemistry*, **28** (1956), 350–356. https://doi.org/10.1021/ac60111a017

[10] O.H. Lowry, N.J. Rosebrough, A.L. Farr and RJ. Randall, Protein measurement with the Folin phenol reagent, *The Journal of Biolical Chemistry*, **193** (1951), 265–275.

[11] R. Robert, R-M. Knuckey, D-M-F. Frampton, M-R. Brown, Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds, *Aquacultural Engineering*, **35** (2006), 300–313. https://doi.org/10.1016/j.aquaeng.2006.04.001

[12] Y.I. Mandik, B. Cheirsilp, P. Boonsawang, P. Prasertsan, Optimization of flocculation efficiency of lipid-rich marine Chlorella sp. biomass and evaluation of its composition in different cultivation modes, *Bioresource Technology*, **182** (2015), 89–97. https://doi.org/10.1016/j.biortech.2015.01.125

[13] M.R. Talukder, P. Das, J.C. Wu, Microalgae (Nannochloropsis salina) biomass to lactic acid and lipid, *Biochemical Engineering Journal*, **68** (2012), 109–113. https://doi.org/10.1016/j.bej.2012.07.001

[14] S. Bellou, G. Aggelis, Biochemical activities in Chlorella sp. And Nannochloropsis salina during lipid and sugar synthesis in a lab-acale open pond simulating reactor, *Journal of Biotechnology*, **164** (2013), 318–329. https://doi.org/10.1016/j.jbiotec.2013.01.010

[15] S.P. Cuellar-Bermudez, I. Aguilar-Hernandez, D.L. Cardenas-Chavez, N. Ornelas-Soto, M. A. Romero-Ogawa and Roberto Parra-Saldivar, Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins, *Microbial Biotechnology*, **8** (2014), 190–209. https://doi.org/10.1111/1751-7915.12167

Received: February 8, 2018; Published: March 3, 2018