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Improvement of Biorefinery Efficiency for Microalgae *Nannochloropsis sp.* via Harvesting Technology Evaluation

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Abstract

Recently, microalgal biomass has attached much attention due to the wide diversity of compounds synthesized from different metabolic pathways. This work attempts to study metabolites recovery from *Nannochloropsis sp*. biomass concentrated by centrifugation and flocculation. Carbohydrates were obtained using acid and alkaline hydrolysis required for cell disruption. Protein extraction was performed after alkaline pretreatment and lipids were recovery by acid hydrolysis- Soxhlet and alkaline hydrolysis- Soxhlet extraction routes. It was found that carbohydrates were recovered by acid hydrolysis in 41 % and 35.39 % for centrifuged and flocculated biomass, respectively, values higher than thus reported using alkaline hydrolysis. For protein extraction, centrifuged biomass exhibited higher recovery yield (55.48%) than flocculated biomass (38.40%). The lipid extraction route that achieved highest yield (43.45%) was acid hydrolysis with HCl followed by Soxhlet extraction with hexane. In addition, statistical analysis by T test suggested that flocculants affect negatively biomass culture, hence, efficiency of metabolites extraction.

Keywords: Harvesting, metabolites, biomass, microalgae

1 Introduction

Microalgae are nowadays considered to be the best source of a wide range of highly valuable products, including polyunsaturated fatty acids (PUFA), carotenoids, phycobiliproteins, polysaccharides and phycotoxin, which find applications in health, pharmacology, nutrition and biotechnology[1, 2]. They are aquatic photosynthetic organisms that have higher growth rates than terrestrial plants and do not compete with food crops and agricultural lands due to its ability for growing in unsuitable conditions needing merely some nutrients and sunlight [3, 4]. The interest for these organisms lies in their potential to produce biomass for food, feed and valuable chemicals but mainly for the possibility of synthetizing biofuels from microalgae biomass[5, 6]. Generally, there are three main components in microalgae: carbohydrates, proteins, and lipids. Depending on the species of microalgae, the mass contents of these main components and other elements are different [7]. However, microalgae culture conditions can be modified to induce the production of carbohydrates, proteins or other specific compounds within the microorganism [8]. Microalgae produces high amount and high quality proteins, which are the source of essential amino acids [9]. Its carbohydrates have low lignin content and their saccharification is much easier, thus being a more promising and sustainable biomass source for bioethanol production [10]. In addition, microalgae produce lipids such as triacylglyceride (TAG) that can be converted into biodiesel but also numerous nonfuel lipids [11]. Different procedures have been applied for recovering metabolites from microalgal biomass. To extract lipid, cell-wall disruption and subsequent lipid collection by solvent are performed [12]. To disrupt cell-wall, several pretreatment methods are widely used on microalgae such as: physical, chemical and enzymatic hydrolysis[13]. In this work, acid and alkaline hydrolysis and Soxhlet extraction were applied to recover metabolites from *Nannochloropsis sp*. using centrifuged and flocculated biomass in order to evaluate the effect of flocculation on efficiency of metabolites extraction.

2 Materials and Methods

Biomass pretreatment: The microalgal samples were grown in Bold Basal medium during 15 days with light-dark cycle 12:12 h. A 2.5 L regular glass reactor was coupled to bubble aeration system for injecting air. The pH was controlled in 7-8 value by measuring daily this parameter. *Nannochloropsis sp*. biomass was mixed with 1.23 mL of 40 g/L aluminum chloride solution in order to concentrate it to 100 mL of culture medium. Flocculation was performed under pH 7 and the remaining biomass was centrifuged at 3400 rpm during 15 minutes. Then, drying was carried out to both flocculated and concentrated biomasses at 105°C during 17 h. Cellular wall disruption required to extract metabolites was achieved by acid hydrolysis pretreatment as reported by González-Delgado et al.[12]. This procedure was based on stirring 5 g of biomass with 50 mL of 0.5 M HCl at 500 rpm during

2 h and neutralizing by KOH. For alkaline hydrolysis, biomass was mixed with 30 mL of 3.76 M NaOH and heated at 55°C during 20 min [14]. It was required to separate liquor and pretreated biomass by filtration as final stage.

Carbohydrate extraction: To quantify amount of carbohydrate extracted from *Nannochloropsis sp*. biomass, phenol-sulfuric acid colorimetric method was carried out to liquor obtained in acid hydrolysis pretreatment [15]. Hence, pretreated biomass was mixed with 0.5 mL of 5% phenol and 2.5 mL of 95% sulfuric acid. After exhibiting mixture coloration, absorbance measurement was recorded at 485 nm.

Protein extraction: Regarding protein quantification, methodology described by Lowry et al. [16] was implemented by mixing 1 mL of sample with 1.4 mL of Lowry solution. Then, 0.2 mL of Folin-water solution was added to experiment changes in solution coloration and perform absorbance measurement at 750 nm. Calibration curve used to determine protein content was previously standardized from analytical grade albumin reagent.

Lipid extraction: For determining the amount of lipid in biomass, Soxhlet method was implemented using 20 mL of hexane as solvent. Then, a roto-evaporator was used to separate liquid extract and solvent and after weighing the sample, recovery of lipids was calculated by Equation 1. All experiments for extracting metabolites using both centrifuged and flocculated biomass were carried out in triplicate. In addition, statistical analysis was applied by T test (n=3).

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

3 Results and Discussion

3.1. Carbohydrate extraction with Acid treatment (HCl)

Figure 1 shows the results for quantification of carbohydrates extracted from *Nannochloropsis sp*. when acid treatment was used. It was found a carbohydrate recovery of 41% and 35.39% in centrifuged and flocculated biomass, respectively, which suggested that flocculation affect significantly carbohydrate extraction.

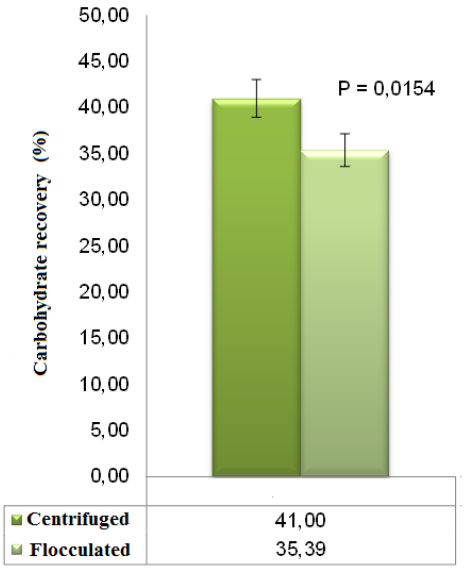

Figure 1. Carbohydrates recovery by acid hydrolysis using centrifuged and flocculated biomass

The negative effect of flocculation on carbohydrate extraction was confirmed by the analysis of experimental data by T test, which reported a P value less than 0.05, hence, the difference between data averages is significant.

3.2. Carbohydrate extraction via Alkaline treatment (NaOH)

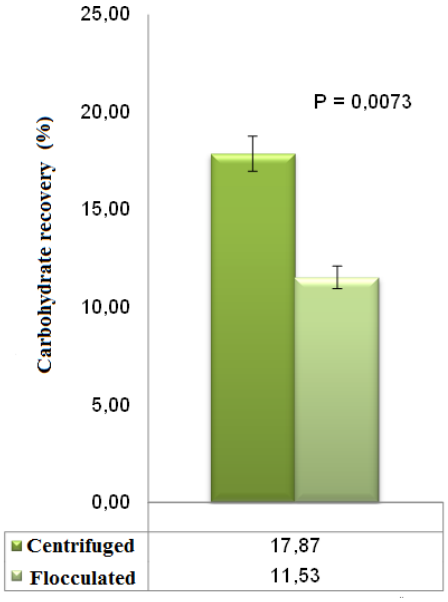

Figure 2. Carbohydrates recovery by alkaline hydrolysis using centrifuged and flocculated biomass

The carbohydrate recovery from *Nannochloropsis sp*. using alkaline hydrolysis is shown in Figure 2. The centrifuged biomass exhibited higher carbohydrate recovery (17.87 %) than flocculated biomass (11.53 %). In addition, The T test reported a value for P less than 0.05 indicating that flocculation affects this metabolite extraction with alkaline pretreatment using NaOH. It was observed that alkaline hydrolysis reduces the performance of carbohydrate extraction, hence, recovery yield also decreases. Robert et al. [17] reported that carbohydrate recovery can decrease up to 23.17% for flocculated *T. pseudonana* at specific conditions in alkaline treatment.

3.3. Protein extraction

Figure 3 shows protein recovery from both flocculated and centrifuged biomasses. It was found that 55.48% and 38.40% of protein was extracted from centrifuged and flocculated biomass, respectively. The P value was 0.004, less than 0.05, which suggested that flocculant used in culture medium reduce the efficiency of extraction. It has been reported similar results regarding the inhibitory effect of flocculants in protein extraction. Robert et al. [17] claimed that process efficiency can decrease by almost 30% when ferric chloride is used as flocculant to concentrate *T. pseudonana* biomass. The acid hydrolysis route was not considered in this section because of the HCl acid solution can degrade amino acids that make up this biomass.

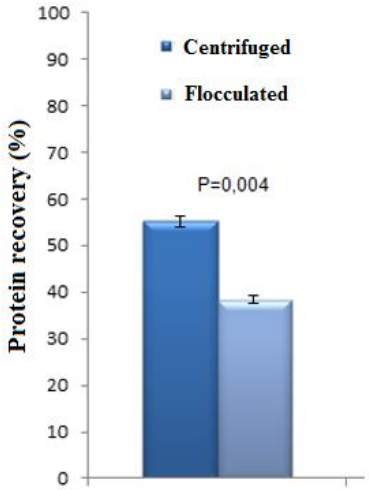

Figure 3. Protein recovery from centrifuged and flocculated biomass

3.4. Lipid extraction after acid treatment (HCl)

Figure 4 shows lipid extraction from *Nannochloropsis sp*. using acid hydrolysis pretreatment. The recovery yield corresponds to 43.45% for centrifuged biomass and 39.85 % for flocculated biomass. Talukder et al. [18] reported that 60% of lipid can be extracted from microalgae *Nannochloropsis salina* when acid hydrolysis with 5% of H_2SO_4 and Soxhlet method with hexane are used. The lower value for

lipid recovery obtained in this study in comparison to other researches might be attributed to the acid selected in acid hydrolysis, hence, it is recommended to evaluate other reagents and routes for lipid extraction.

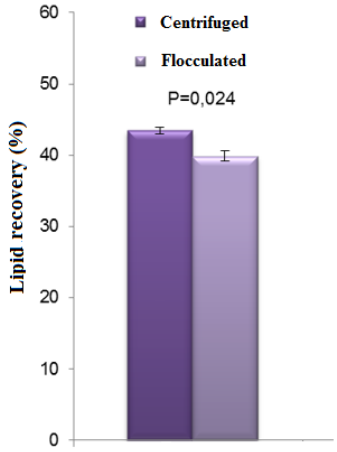

Figure 4. Lipid recovery from harvested biomass (acid treatment)

3.5. Lipid extraction after alkaline treatment (NaOH)

Figure 5 shows lipid extraction from both biomasses subjected to alkaline treatment. Centrifuged biomass reported lipid recovery higher than flocculated biomass (36.81 % and 33.03 %, respectively). Regarding statistical analysis, $P<0.05$ indicating that flocculant in culture medium affects lipid extraction procedure. Bellou & Aggelis [19] reported that *Nannochloropsis* can accumulate up to 70% of non-polar lipids, however, it depends on solvent selectivity and nature, as well as cultivation conditions [20].

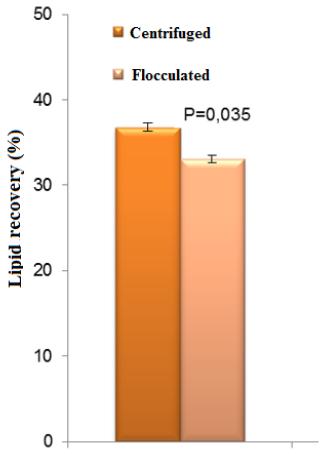

Figure 5. Lipid recovery from harvested biomass (alkaline treatment)

4 Conclusions

This study was focused on evaluate the effect of flocculant agent on metabolites extraction from *Nannochloropsis sp*. biomass. The extraction of carbohydrate, lipid and protein were higher for centrifuged biomass than for flocculated biomass, which suggested that flocculants affect negatively the performance of metabolites extraction. It was found that acid hydrolysis reported better results than alkaline hydrolysis with carbohydrate recovery of 41% and 35.39% using centrifuged and flocculated biomass, respectively. For protein extraction, it was obtained a protein recovery of 55.48% for centrifuged biomass and 38.40% for flocculated biomass. The route acid hydrolysis-solvent extraction exhibited the highest lipid recovery (43.45%) in comparison to alkaline hydrolysis-solvent extraction route (36.81%).

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