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Cell Disruption and Lipid Extraction from Microalgae *Amphiprora* sp. Using Acid Hydrolysis-Solvent Extraction Route

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Abstract

Growing global energy demand requires the development of novel alternatives for sustainable energy production and microalgae are being widely studied as an attractive source of biofuels and bioproducts. To obtain oil from microalgae biomass, pre-treatment methods are important for the product quality and production cost. In this work, lipids from microalgae *Amphiprora* sp. were obtained

using acid hydrolysis-solvent extraction route. The effect of hydrolysis and solvent extraction times on lipid extraction efficiency were evaluated in order to determine the most suitable conditions for performing both stages. In addition, morphological response of biomass after acid hydrolysis and liquid extraction was studied, as well as lipid and metabolites characterizations. The highest lipid efficiency (38.74%) was reached at 120 minutes of hydrolysis time and 16 hours of lipid extraction. The cell disruption was confirmed through the presence of remnants of cellular wall and cytoplasmic constituents and changes in colour of microalgae corresponded to alterations in cellular membrane caused by solvent extraction. The low percentage of saturated fatty acids (20.9 %) indicated that *Amphiprora* sp. is less suitable for biodiesel production in comparison with other genus widely studied for lipid extraction.

Keywords: Microalgae, biofuels, lipids, hydrolysis, extraction

1. Introduction

In recent years, microalgae has gained attention as the most promising alternative source for energy production [1, 2]. This is due to its higher growing rates than other crops (e.g. plants or trees), some of these even can double their biomass within 24 h, short life cycle and diversified cultivation conditions including marginal lands [3–5]. They just need liquid medium, some nutrients and sunlight to stimulate the growth of biomass [6] which has a higher photosynthetic efficiency compared to other biomass such as land plants [7]. Microalgae synthesize lipids, carbohydrate and proteins. Lipids have a variety of applications such as in the field of food and bioenergy [8]. In terms of biofuels, microalgae offer promising prospects as feedstock [9]. To extract lipid from this biomass, cell-wall disruption and subsequent lipid collection by solvent are performed [10]. Several methods aiming at the break of the cell walls of whole microalgae have been classified in mechanical and non-mechanical process such as high-pressure homogenization, ultrasonic treatment, hydrodynamic cavitation, acid hydrolysis, osmotic shock, among others [11]. This paper is focused on the production of lipids from microalgae *Amphiprora* sp. using acid hydrolysis technique and Soxhlet extraction, whose processing steps are similar to the ones raised by Halim et al. [12]. In addition, this work evaluates the effect of the hydrolysis and extraction times, as operating parameter, on the efficiency of liquid extraction and characterizes the cell morphology and main components of the oil extracted.

2. Materials and Methods

Microalgae biomass of *Amphiprora* sp. was provided by the Morrosquillo Corporation (Punta Bolivar, Colombia), which was harvested by flocculation and dried in an oven at 105°C for 8 hours.

Production of monosaccharides

Acid hydrolysis pretreatment was used for cellular wall disruption. According to Halim, Danquah & Webley [13], this pretreatment forces the release of intracellular lipids to the surrounding medium, thus assisting the lipid extraction process. Solutions with 10 g of dry biomass of the microalgae *Amphiprora* sp. and 150 mL of 0.5 mol L^{-1} hydrochloric acid were prepared and independently subjected to a stirring speed of 500 rpm for 30, 60 and 120 minutes at room temperature. Subsequently, vacuum filtration was performed and pH was adjusted about 6 or 7 by adding distilled water, thereby obtaining two products, hydrolyzed biomass which is used for lipid extraction and water-soluble bioproducts in acid solution that contains desired monosaccharides. Hydrolyzed biomass was dried at 102 ºC for 4 hours, by this step remaining water was removed in the biomass and after that, hydrolyzed biomass was used for lipid extraction by Soxhlet method. On the other hand, water-soluble bioproducts in acid solution were neutralized with sodium hydroxide (NaOH).

Lipid extraction

Biomass of microalgae *Amphiprora* sp. after monosaccharides production was subjected to lipid extraction by Soxhlet method according to González & Kafarov [14], using hexane as solvent and taking repeated wash times of 8, 12 and 16 hours. Subsequently, the extract was filtrated for removing biomass residues or impurities, obtaining the solvent that contains lipids from the microalgae. A fraction of the solvent was removed by simple distillation and the other was allowed to volatilize until lipids concentration, tests were not greater than 120 minutes and 16 hours due to the high energy requirements and reagent consumption.

To determine the lipid extraction efficiency, the maximum amount of removable biomass components by the combination of a nonpolar solvent (methanol) and a polar solvent (chloroform) was taken as a basis. For this extraction, the method proposed by Bastianoni was followed [15], maximum obtainable lipid extract were determined in 12% for *Amphiprora* sp. and following equations are used to calculate the percentage of lipid extraction and efficiency:

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

$$\text{Efficiency} = \frac{\%\ \text{extraction}}{\%\ \text{maximum lipid yield}} \times 100 \quad (2)$$

3. Results and Discussion

Table 1 shows the composition of *Amphiprora* sp., this microalgae synthesizes its biomass in form of lipids, carbohydrates and proteins [8]. Nevertheless, for bioenergy applications, lipids as triacylglyceride (TAG) take particular attention.

For *Amphiprora* sp. the percentage of lipids is 12%, which is within the range for lipids content of 1-85 % by dry weight [16].

Table 1. Metabolites characterization of *Amphiprora* sp.

Microalgae	Nitrogen (%)	Proteins (%)	Carbohydrates (%)	Lipids (%)	Ash[a] (%)
Amphiprora sp.	2.1	10	6.2	12	52.8

[a]. Ash percentage includes flocculant amount (150 ppm $FeCl_3$).

Acid hydrolysis-solvent extraction

The increase in contact time between hydrochloric acid and microalga biomass of *Amphiprora* sp. influenced the release of lipids after solvent extraction for 16 hours. When the cellular lysis time was 120 minutes, a lipid efficiency of 38.74% was reached, followed by 32.87 and 26.06 % when the pretreatment time was 60 and 30 minutes, respectively (Figure 1).

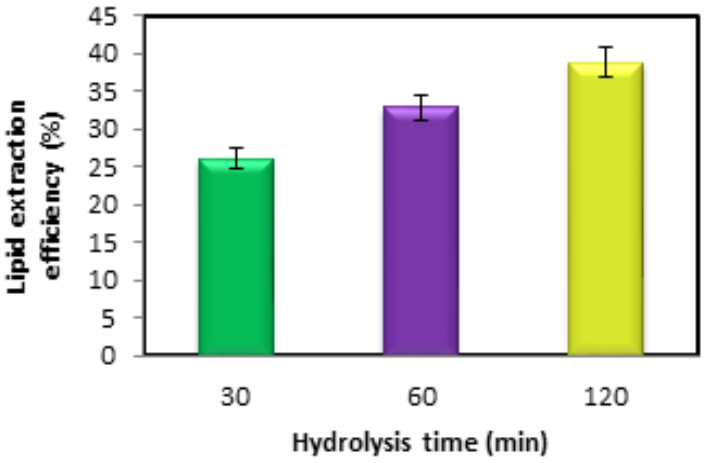

Figure 1. Effect of acid hydrolysis time on the increase of lipid efficiency for *Amphiprora* sp.

Acid hydrolysis method facilitated lipid extraction by breaking the cellular walls and allowing to the solvent to have greater access to the components of lipid constitution, which was reflected in an increase of lipid efficiency over time of contact. According to lipid extraction efficiency obtained, 120 minutes of reactions were used in subsequent treatments. Acid hydrolysis has an effect on morphology of microalgae *Amphiprora* sp.; cellular wall of this microalga is rigid and is composed by hydrated silica and proteins (Figure 2A). The rigidity of the frustules was interrupted by the action of acid pretreatment, so that, it can be seen remnants of cellular wall and cytoplasmic constituents (Figure 2B). *Amphiprora* sp. showed an increase in the volume of some cells, accompanied by the subsequent

fragmentation of the microalgae and therefore the release of intracellular content. Cellular morphology after solvent extraction also changed in comparison with the observations of the biomass after acid pretreatment (Figure 2C). Diatoms, in some degree, preserved cellular structure or conformation, turned out from a shield to an irregular appearing in *Amphiprora* sp., because of the temperature used in the procedure. The olive-gold color characteristic of this microalgae genus became golden-brown. The permeability of cellular walls and membranes allowed the lipids evacuation causing the disorder in the structure and coloration of the diatoms. In addition, there were fragments of cellular structures, which although attributed to a previous step of cellular disruption, but could also be caused by the continuous flow of solvent into the interior and exterior of each cell.

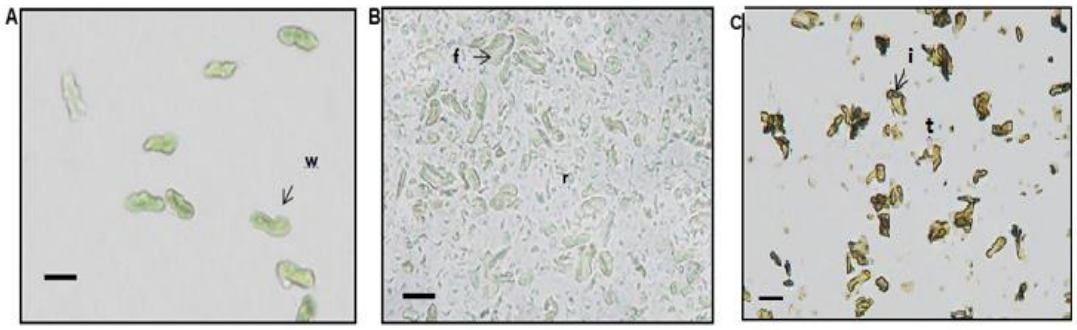

Figure 2. Morphology of: A. Dry biomass, before acid pre-treatment. B. Biomass after 120 minutes of acid hydrolysis. C. Biomass after lipid extraction. i: irregular shape; t: cellular remaining; w: Cellular wall; r: Remnants of frustules and organelles; f: Cellular fragmentation; c: Chromatophores. Scale equals to 20 μm.

Solvent extraction times of 8, 12 and 16 hours were compared using a cellular lysis time of 120 minutes. When the procedure was extended to 16 hours, lipid extraction efficiency was increased to 38.74 %, result that differs markedly for repetitive washing times of 12 and 8 hours, where the oil extraction efficiency were 26.33 and 22.5%, respectively (Figure 3). Solvent extraction causes alterations in the cellular membrane that improves the circulation of lipids to outside of the cell. Taking into account the results, it is clear that properties of cellular membrane play an important role in the process of acid hydrolysis - solvent extraction route which was established as the combination of 120 minutes of acid hydrolysis and 16 hours of solvent extraction.

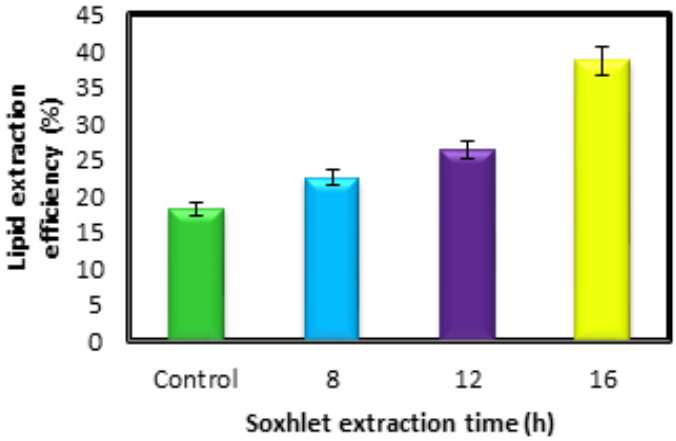

Figure 3. Comparison of lipid extraction efficiency for different times of experimentation using the microalga *Amphiprora* sp.

Table 2 shows the characterization of oil from *Amphiprora* sp. made through HPLC (High-performance liquid chromatography). A percentage of 20.9 % was obtained for saturated fatty acids. If fatty acid chain is long and highly saturated, the cetane number of the biodiesel produced will be higher [17]**.** According to Liu et al. [17], cetane number parameter is widely used as diesel fuel quality, related to the ignition delay time of fuel upon injection into the combustion chamber of a diesel engine. High cetane number indicates that the injection delay time is short. Other researches showed higher saturated fatty acids content of 53.2 and 46.35 % for *Chlorella* sp. and mixed microalgae culture (*Scenedesmus dimorphus* sp., *Chlorococcus* sp. and *Chlorella* sp.) respectively [8, 18], which indicates that *Amphiprora* sp. is less suitable for lipids production than other genus.

Table 2. Characterization oil extracted for *Amphiprora* sp. Microalgae

Fatty acid	**%**	**Fatty acid**	**%**
C14:0	9	C18:1n9t	3.3
C16:0	5.5	C18:3	4.6
C18:1	5.9	C20:2n11,14c	3.7
C18:2n9,12t	31.7	C20:4	0.8
C18:2	15.2	C22:0	0.8
C6:0	1.8	C22:2	3.9
C8:0	0.5	C23:0	0.4
C11:0	0.1	Saturated fatty acids	20.9
C12:0	0.4	Monounsaturated fatty acids	9.2
C13:0	1	Polyunsaturated fatty acids	59.9
C15:0	1.4		

4. Conclusions

Acid hydrolysis pretreatment increases the release of lipids in microalgae biomass through contact and lysis of the cellular wall with disrupting agents and catalysts of the process, such as hydrochloric. The hydrolysis and solvent extraction times affect significantly the lipid extraction efficiency due to the access of solvent to components of lipid constitution. Hence, for carrying out this route, the most suitable operating times are 120 minutes of hydrolysis and 16 hours of extraction. However, the low percentage of saturated fatty acids in *Amphiprora* sp. microalgae indicates that this genus is less convenient to produce lipids for biofuel applications in comparison with other genus as *Chlorella* sp.

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