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Evaluation of Two Pre-Treatments for Improving Lipid Extraction from Microalgae *Navicula* sp.

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Abstract

The rapid growth of human population and technological advances have led to an urgent need for alternative sources of renewable energy and microalgae have attracted much global attention in recent years. In this work, production of oil and reducing sugars from microalgae *Navicula* sp. were studied through the design and experimental evaluation of two sequential routes based on pre-treatments (acid hydrolysis and organosolv) and lipid extraction. The effect of these procedures on microalgae cell structure was evaluated through changes in morphology of this biomass. For Organosolv-Soxhlet extraction route, lipid efficiency was 46.96%, higher than for acid hydrolysis-Soxhlet extraction route (39.98%) that is attributed to a greater degree of cell lysis due to the operating conditions of Organosolv pre-treatment. In addition, total reducing sugar (RS) measurement corresponded to 0.45 and 1.47 mg/mL for acid hydrolylisis and organosolv, respectively, which result significatly affected by contact time of biomass with disruptor agents. From characterization of oil extracted, content of saturated fatty acids was 45.1%, this value indicates that *Navicula* sp. is suitable as feedstock for biofuel production and Organosolv-Soxhlet extraction could be applied as an efficient route for this purpose.

Keywords: Microalgae, extraction, hydrolysis, organosolv

1. Introduction

Nowadays, microalgae have emerged as a promising eco-friendly alternative source of renewable energy due to the advantages of photosynthetic efficiency, biomass productivity and oil content [1]. In order to face the growing of global demand for energy, depletion of resources and environmental pollution problems, they have been identified as suitable feedstocks for biofuels production [2, 3]. Photosynthesis drives the first stage of all processes of biofuels production, solar energy captured and stored as chemical energy such as oil and starch. The increase in light collection efficiency is a significant innovation in the development of the entire production of biofuels [4]. To obtain competitive biofuels from microalgae, technological challenges have been assumed including strains development, cultivation methods, and improvement of lipid extraction methods and optimization of conversion processes [5]. Currently, production of biofuels from biomass-derived feedstock has been studied via different routes that comprise pretreatment and extraction methods [6]. Several pretreatment methods are widely used on microalgae such as: physical, chemical and enzymatic hydrolysis [7]. This paper is intended to evaluate two sequential routes for obtaining valuable metabolites of microalgae *Navicula* sp., which consist on pretreatments and lipid extraction. In addition, the effect of both routes on lipid extraction efficiency, cell morphology and total reducing sugar concentration is studied for determining the most suitable pretreatment (acid hydrolysis or organosolv) for biofuel production.

2. Materials and Methods

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

Production of monosaccharides

Acid hydrolysis was carried out based on the procedure described by Halim, Danquah & Webley [8] for 120 minutes at room temperature, which assists lipid extraction process through the release of intracellular lipids and it is shown in Figure 1.

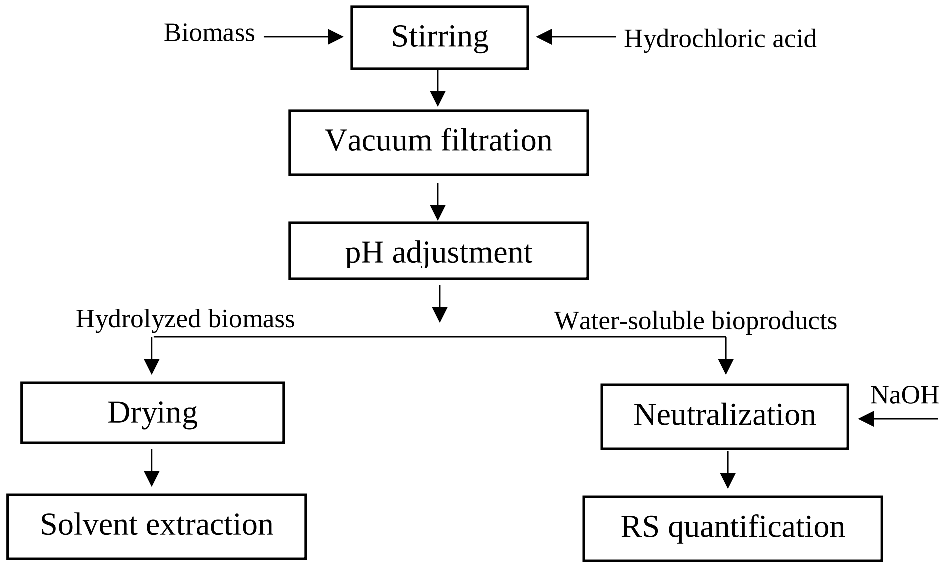

Figure 1. Experimental procedure for acid hydrolysis pretreatment.

Organosolv pretreatment experiments were performed following a methodology previously developed by authors which includes the use of sulfuric acid and organic solvents [9]. After pretreatment, two phases were obtained, pretreated biomass and pretreatment liquor, which contains the desired monosaccharides, pretreatment liquor was separated of pretreated biomass by vacuum filtration. Then, pretreated biomass was used in the same way as is described in section of lipid extraction. Neutralization of pretreatment liquor was made by adding calcium carbonate ($CaCO_3$) at pH of 5 or 6, after that total reducing sugars were quantified.

Quantification of total reducing sugars

This step for evaluating monosaccharide production alternative of both routes was performed using the method of dinitrosalicylic acid (DNS) proposed by Miller [10]**.** For its quantification, base 10 dilutions were used for each of the samples taken by water-soluble bioproducts in acid solution and pretreatment liquor. Absorbance measurements were performed at 540 nm on a spectrophotometer MERCK Spectroquant®Pharo 300.

Lipid extraction

The lipid extraction by HBE method was carried out base on the methodology described by González & Kafarov [11]. The hexane was used as solvent and 16 hours was selected as extraction time. In order to eliminate biomass residues or impurities, extract was filtrated and a portion of solvent was removed using simple distillation and the rest of this was sent to volatilize and obtain concentrated lipid extract. The procedure proposed by Bastianoni was followed to calculate lipid extraction efficiency using Equations 1 and 2 [12], therefore, maximum obtainable lipid extract were determined in 13.2%:

$$\%\ \text{extraction} = \frac{\text{oil weight}}{\text{biomass weight}} \times 100 \tag{1}$$

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

3. Results and Discussion

The metabolites characterization of microalgae *Navicula* sp. is shown in Table *1*. One of the main criteria for applying microalgae species as suitable feedstocks for biofuel production is the lipid contents [2]. Most microalgae could accumulate more than 20% total lipid of their dry biomass and it depends on species and environmental/growth conditions [1]. In this case, microalgae *Navicula* sp. presents 13.2 % of lipids that are mostly triacylglycerides.

Table 1. Metabolites characterization of Navicula sp.

Microalgae	**Nitrogen (%)**	**Proteins (%)**	**Carbohydrates (%)**	**Lipids (%)**	**Ash[a] (%)**
Navicula sp.	3.2	15.1	5.2	13.2	51.6

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

Figure *2* shows the effect of acid hydrolysis and lipid extraction by HBE method on morphology of microalgae *Navicula* sp.; biomass before pretreatment presented a rigid carbohydrate-rich cell-wall that preserves its biomechanical stabilities and protects it against invaders and harsh environment [13] (Figure 2A). However, when it gets in touch with acid, many small sized cell fragments were formed and less distinct cell walls were observed [14] (Figure 2B). After carrying out lipid extraction, it is observed a change in cellular morphology compared with hydrolyzed biomass, moreover the color characteristic of *Navicula* sp. became from olive gold to pale olive (Figure 2C). These alterations in structure and coloration of the diatoms are attributed to the liquids evacuations caused by degradation of pigments through acids and permeability of cell walls and membranes [14].

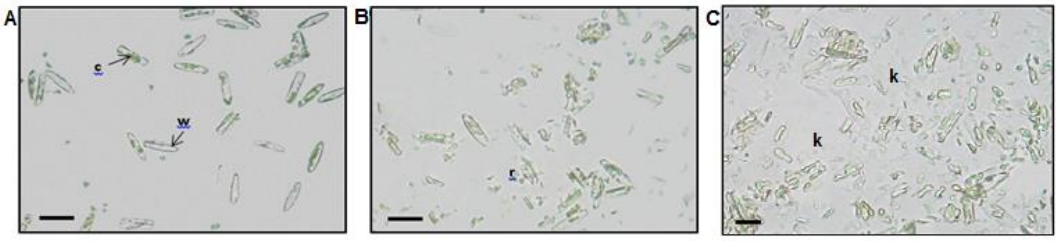

Figure 2. Morphology of: A. Microalgae biomass before cell lysis. B. Hydrolyzed biomass, after 120 minutes of reaction. C. Biomass after lipid extraction; w: Cell wall; c: chromatophores; r: Remnants of frustules and organelles; k: Cell remaining. Scale of A and B equal to 50 μm. For C, scale of 20 μm.

The oil extracted from *Navicula* sp. was characterized by HPLC (High-performance liquid chromatography) technique and the results are shown in Table 2. A criteria to identify biofuels quality is the amount of saturated fatty acids (%) that includes palmitic acid (C16:0) and stearic (C18:0), because of long and highly saturated fatty acid chain present higher cetane number of biodiesel [15]. Cetane number is based on ignition delay from starting of injection and includes both physical (vaporization) and chemical components [16]. T. According to other researches, The high percentage of saturated fatty acids of this oil (45.1%) suggested that microalgae *Navicula* sp. can be widely used as a suitable feedstock for biofuel production.

Table 2. Characterization oil extracted from microalgae *Navicula* sp.

Fatty acid	**%**	**Fatty acid**	**%**
C14:0	18.1	C18:1n9t	1
C16:0	14.3	C18:3	2.4
C18:1	4.3	C20:2n11,14c	3.8
C18:2n9,12t	11.9	C20:4	0.4
C18:2	17.5	C22:0	1.8
C6:0	0	C22:2	1.7
C8:0	0	C23:0	1
C11:0	1.3	Saturated fatty acids (%)	45.1
C12:0	1.1	Monounsaturated fatty acids (%)	5.3
C13:0	4.3	Polyunsaturated fatty acids (%)	37.7
C15:0	3.2		

Evaluation of Organosolv pretreatment - solvent extraction route

Organosolv pretreatment applied to the microalga Navicula sp. increased the lipid extraction efficiency compared with acid hydrolysis. Through this procedure, an efficiency of 47.68 % was reached as it is shown in Figure 3. The increase in the production of oils was given to the operating conditions of the method, as the increase in pressure, temperature and contact time of biomass with disrupting agents such as methanol and sulfuric acid, caused a greater degree of cell lysis. Richmond [17] states that the results obtained by autoclave and mechanical disruption biomass tripled the performance of it compared to other methods. One advantage of mechanical pretreatment is the non-production of inhibitors that disrupt the fermentation process. About vapor explosion, this process has lower power requirements than mechanical process, less corrosion and limited use of chemicals, only water is used [18]. This pretreatment caused an aggressive effect on the cell wall of Navicula sp. (Figure 4). The remains of microalgal organelles after solvent extraction confirms the effectiveness of this route.

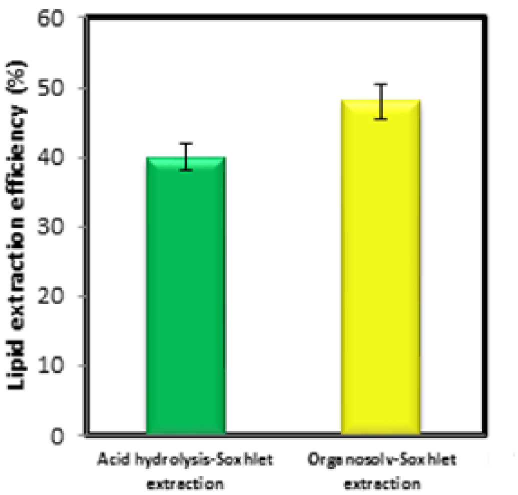

Figure 3. Comparison of routes evaluated in terms of lipid extraction efficiency

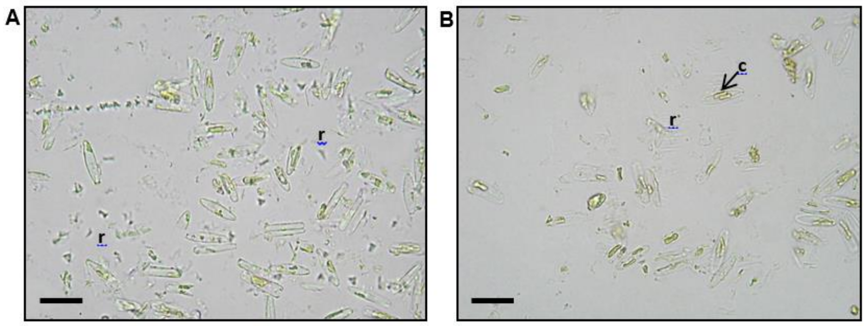

Figure 4. Cell structure of Navicula sp. A. After pretreatment Organosolv. B. After lipid extraction. r: Remnants of cell structures; c: Chromatophores. Scale represents 50 µm

Quantification of total reducing sugar

Concentration of total reducing sugars is shown in Table 3 for both routes. In the acid hydrolysis and Organosolv pretreatments, the contact time of biomass with disruptor agent affected the production of reducing sugars. According to Ferrer et al. [19], for sugar cane bagasse, the concentration of RS ranged from 2.58 to 20.45 mg/mL, with an overall average of 10.53 mg/mL. Although the production of RS is greater through the use of this raw material, the application of microalgae has the advantage of avoiding large tracts of land for cultivation, besides being in direct contact with water, getting a wide range of products interest, among others.

Table 3. Spectrum characteristic regions of lipids, biodiesel and petrodiesel.

	Concentration of total reducing sugars (mg/mL)	
	Acid hydrolysis	**Organosolv**
Navicula sp.	0.45	1.47

4. Conclusions

Pretreatments Organosolv and acid hydrolysis affect the morphology of biomass cells due to the disruption of cell wall that facilities the solvent-extraction of lipids, thereby increases lipid extraction efficiency. The high value of saturated fatty acids in the oil extracted (45.1 %) indicates that *Navicula* sp. could be widely applied as feedstock for biofuel production. The lipid extraction efficiency for both routes suggests that Organosolv is more suitable for this purpose than acid hydrolysis because of its operating conditions for cell lysis such as temperature, pressure, time of operation, among others, which allow releasing higher amounts of lipids in microalgae biomass.

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