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Evaluation of In-Situ Transient Simultaneous Cell Disruption and Transesterification of Microalgae

Ángel Darío González-Delgado

University of Cartagena
Chemical Engineering Department
Avenida del Consulado Calle 30 No. 48-152
Cartagena, Colombia

Andrés Fernando Barajas-Solano

Universidad Francisco de Paula Santander UFPS
Facultad de Ciencias del Medio Ambiente y Vida
Av. Gran Colombia No. 12E-96
Cúcuta, Colombia

Yeimmy Yolima Peralta-Ruíz

Universidad del Atlántico
Agroindustrial Engineering Department
Km. 7 Vía a Puerto Colombia
Barranquilla, Colombia

Abstract

Microalgae has recently been highlighted as source of valuable products including biofuel. The production process of biofuels from microalgae involves mass cultivation, harvesting, deep dewatering, lipid extraction and biofuel conversion. In this work, lipids from microalgae *Navicula* sp. were obtained using multifunctional process that consists of acid hydrolysis or cellular disruption, oil extraction and in situ transesterification. The effect of alcohol added to produce ethyl and methyl esters on lipid extraction efficiency was evaluated using methanol and ethanol in order to determine the most suitable route for obtaining the high values of lipids

and total reducing sugar. The highest lipid extraction efficiency and total reducing sugar (7.72 % and 2.63 mg/ml, respectively) was obtained for methanol. The low lipid extraction efficiency of multifuctional process is due to transesterification of lipids that gradually released into the system. The formation of alkyl esters was confirmed by FTIR with an increase in carbonyl peak as the reaction progressed, thus muntifuctional process reduce cost of alkyl esters production by eliminating the step of lipid extraction by solvent.

Keywords: Microalgae, biofuels, lipids, cell disruption, transesterification

1. Introduction

Biofuels have gained great attention as the most sustainable and environmental friendly energy source given their advantages of reproducibility, resource abundance, and lower environmental impacts [1–3]. Microalgae is an attractive feedstock for biofuel production due to its high photosynthetic efficiency to convert solar energy and carbon dioxide into oxygen and biomass and its growth on a non-arable land [4]. These unicellular organisms synthesize lipids, carbohydrate and proteins [5]. Lipid content in microalgae can vary widely according to culture conditions and organism's particular metabolic mechanism [6]. *Navicula* sp. is characterized by producing a mucilage with a high content of extracellular polymeric substances included lipids and polysaccharides [7]. Biofuel production from microalgae has been studied through different pretreatment and extraction methods [8]. Biofuels obtained from microalgae usually requires the following steps: cultivation, harvesting, lipid extraction, and conversion [9]. Lipid extraction is a critical step; hence, it is necessary to develop efficient and cost effective approaches to implement a process that will support the separation of algae from its components [10, 11]. The extraction of lipid content of biomass involves the use of different solvents such as ethanol and methanol [12]. This work focus on implementing a multifunctional process with hydrolysis, extraction and transesterification using methanol and ethanol in order to evaluate the efficiency of lipid extraction of both routes.

2. Materials and Methods

Microalgae biomass of *Navicula* sp. was obtained from Morrosquillo Corporation (Punta Bolivar, Colombia) followed by harvesting and drying at 105°C for 8 hours.

Multifunctional process

This process is based on procedure described by González & Kafarov [13] that involves acid hydrolysis or cellular disruption, oil extraction and in situ transesterification. Multifunctional process led two simultaneous systems due to the use of two different alcohol: ethanol and methanol. According to the maximum lipid

yield of microalga *Navicula* sp. and in order to promote the reaction towards ethyl and methyl esters, the ratio biomass-alcohol was estimated of 1:6, by weight (Modified from Ehimen et al. [14] and Johnson & Wen [15])**.** Sulfuric acid was used as catalyst for transesterification with oil-acid mass ratio of 1:1. These reactions were subjected to a stirring speed of 500 rpm for 10 hours at 60 ºC and 1 mL of samples were taken at different time intervals. Samples were centrifuged for 10 min to separate hydrolyzed and water-soluble biomass. In this step, 0.5 mL of liquor per sample were obtained, each of them was neutralized by adding 50 mL of sodium hydroxide (NaOH) 1 N to adjust pH near to 7. Subsequently, 1.5 mL of hexane and 0.5 mL of distilled water were added to obtain a three-phase system consisting of hexane phase, residual biomass and hydro-alcoholic (Modified from Johnson & Wen [15] and Plata, Kafarov & Moreno [16]).

The addition of hexane and water to each sample allowed separating hydrophobic components as lipids and alkyl esters of residual biomass and hydro-alcoholic phase that contents reducing sugar (RS) and other polar components. Those elements that migrate to the hexane phase were tested using Infrared Spectroscopy. The spectra were performed on a Shimadzu FTIR-8400S (Fourier Transform Infrared Spectrophotometer) in the wavelength range of 400-4000 cm^{-1}, using a cell of 2.5 cm of diameter. On the other hand, the hydro-alcoholic phase was treated according to the DNS method. For remaining biomass in each system, lipid extraction was carried out in order to quantify non-extracted and/or trans esterified lipids.

3. Results and Discussion

Evaluation of multifunctional-process route

Multifunctional process involved treatment of acid hydrolysis or cellular disruption, lipid extraction and in situ transesterification. Cell wall breaking of microalga *Navicula* sp. released polysaccharides and lipids from walls and cytoplasm and operating conditions allows carrying out the hydrolysis and transesterification reaction reactions. Triglyceride molecules released from the cellular disruption step reacted with ethanol or methanol, under the catalytic action of sulfuric acid, yielding reducing sugars, fatty acids esters and glycerin. In this multifunctional process, sulfuric acid served the important function of acting as a catalyst for hydrolysis and in situ transesterification reactions in the system.

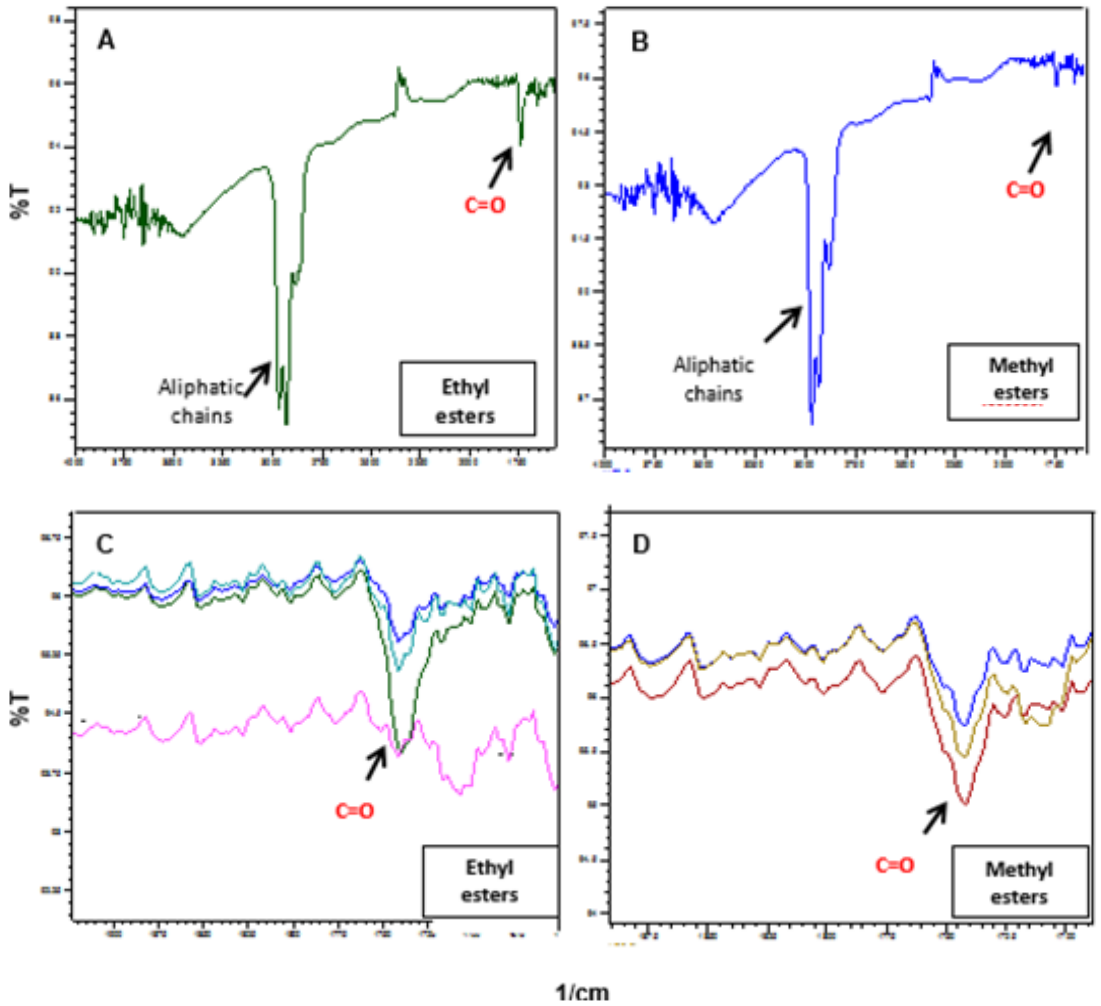

Figure 1. FTIR spectra of the hexane phase of the samples taken from the multifunctional process. A. sample of 4 hours for the system with ethanol and B. methanol.C. Expansion of C=O bands for samples 2, 3 and 4 hours in the process with ethanol and D. 1, 3 and 5 hours with methanol.

By measurements of mid-infrared transmittance, it indicated the presence of alkyl esters or biodiesel in the hexane phase of the samples taken during the development of multifunctional process. The spectra obtained by FTIR showed an increase in the band area corresponding to the carbonyl bond (C=O) about 1750 cm^{-1} and the strip forming the aliphatic chains between 2800 and 3000 cm^{-1}, for 2 hours of reaction in the system with ethanol and 1 hour with methanol (Figure 1 A and B). Carbonyl peak, characteristic of esters increased as the reaction progressed; hence, it was attributed to the formation of alkyl esters or biodiesel (Figure 1 C and D). The biofuel production was possible in this system through cell disruption holding sulfuric acid, consequently lipids inside the microalga *Navicula* sp. were released and led to the alkyl esters formation by transesterification with ethanol or methanol.

This process eased the direct conversion of microalgae biomass to alkyl esters, eliminating the step of lipid extraction by solvent, which is necessary to obtain oil by conventional method. Therefore, the multifunction system dramatically reduced the cost of alkyl esters production. Figure 2 shows the hexane phase spectrum of sample for 10 hours of reaction time. The ester bond has been reported in both lipids and biodiesel [17, 18], specifically carbonyl bond (C=O) was found at 1750 cm^{-1}. The band of aliphatic chains was identified at 2970 cm^{-1} for ethyl esters. Ethyl and methyl esters exhibited a peak at 3400 cm^{-1}, which corresponds to the OH bond, characteristic spectra of glycerin, according to Ooi et al. [19]**.** This was attributed to the source of biodiesel, which comes from a multifunctional system where there was not a process of purification of products.

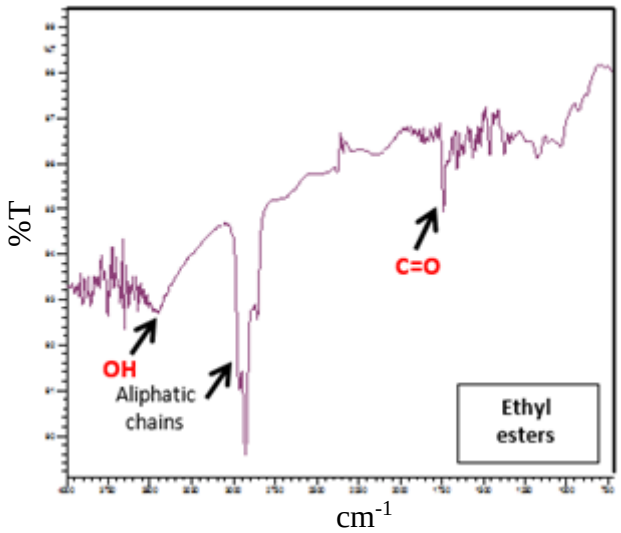

Figure 2. FTIR spectra of hexane phase of sample for 10 hours of reaction time in multifunctional process.

The effect of this route over the cellular structure of the microalga *Navicula* sp. allowed obtaining monosaccharides and biodiesel through extraction and in situ transesterification of lipids. Monosaccharides are abundant constituents of polysaccharides in the cellular wall and cytoplasm of *Navicula* sp., main sugars are glucose, galactose, mannose, ribose and others in different amounts [20]. These sugars related to the structural polysaccharides of diatoms were released by contacting with acid catalyst through hydrolysis of the glycosidic bond that allows the progressive formation of monomers.

Figure 3 shows lipid extraction efficiency for both methanol and ethanol multifunctional routes. The extraction efficiency for this process was lower than other lipid extraction routes reported by González-Delgado et a. [8], which is due to transesterification of lipids gradually released into the system. Biomass used for Soxhlet extraction step was obtained after centrifugation of samples taken at different intervals of time and biomass remaining after completion of the process.

This biomass is separated in order to quantify the amount of lipids were not extracted and trans esterified during the reaction time of the multifunctional system. When ethanol was used in the scheme of procedure, the recovered biomass reached a lipid extraction efficiency of 4.8 % and when methanol was used in the process, lipid efficiency was 7.72%.

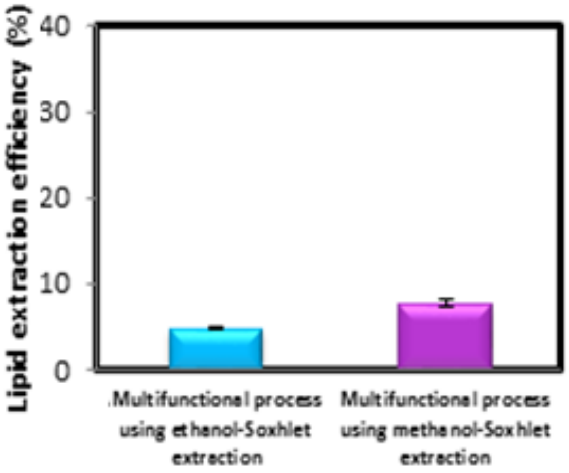

Figure 3. Comparison of multifunctional process routes evaluated in terms of lipid extraction efficiency.

Concentration of total reducing sugar was determined according to the procedure previously described and the results are shown in Table 1. Multifunctional system with methanol exhibits higher RS (2.63 mg/ml) than other routes as acid hydrolysis and organosolv (0.45 and 1.47 mg/ml, respectively) obtained in previous research [8]. In the acid hydrolysis and Organosolv pretreatments, the contact time of biomass with disruptor agent affected the production of reducing sugars. However, in the multifunctional process, biomass is further exposed to the action of acid, which is evidenced by the increased concentration of monosaccharides.

Table 1. Reducing sugar concentration for ethanol and methanol multifunctional process route

	Multifunctional process using ethanol	**Multifunctional process using methanol**
Concentration of RS (mg/ml)	2.5	2.63

4. Conclusions

Multifunctional process allows the production of monosaccharides and fatty acid esters by dissociation of cellular structure, extraction and transesterification of

lipids released in the process. The change of alcohol in the system affects the production of total reducing sugars, since the use of methanol in the process increases their concentration. On the other hand, highest lipid extraction efficiency was obtained using methanol (7.72%). The versatility of bioproducts and operating conditions through this route represent important aspects for the scaling of biofuels production from *Navicula* sp. microalgae.

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