Evaluation of Different Routes for Obtaining Biofuels and High Value Products from Microalgae under Biorefinery Concept

Angel González[1], Alexander Guzman[2], Viatcheslav Kafarov[1]*

[1]Industrial University of Santander, Chemical Engineering Department, Carrera 27 Calle 9, AA678 Bucaramanga, Colombia
[2]Colombian Petroleum Institute, Piedecuesta, Colombia
cisyc@uis.edu.co

A biorefinery uses all biomass components for obtaining high value usable products. In the present study, the authors defines routes for obtaining monosaccharides and lipids from microalgae biomass of the *Amphiprora* sp. and *Navicula* sp., The routes were evaluated and compared based on the extraction efficiency and sugars concentration obtained. For acid hydrolysis -Soxhlet extraction, several times of acid hydrolysis and extraction were evaluated. For Organosolv-Soxhlet extraction route, lipid efficiency obtained was 48% and for Polyfunctional Process the highest percentage of reducing sugars was 1.67 mg/mL. FTIR was used for compare oil extracted and biodiesel. Under biorefinery concept, Polyfunctional process route shows the best performance for obtaining several products of microalgae.

1. Introduction

As in an oil refinery, a biorefinery uses all biomass components for obtaining several biofuels and high value products. In this study, three routes for obtaining microalgal lipids and monosaccharides, are defined and evaluated, the routes are chemical cell disruption followed by soxhlet extraction, organosolv pretreatment followed by soxhlet extraction, and simultaneous cell disruption, extraction and in situ transesterification.

2. Materials and Methods

Microalgae biomass of *Amphiprora* sp. and *Navicula* sp. were provided by Morrosquillo Corporation (Punta Bolívar, Colombia), total reducing sugars obtained from microalgae were quantified using the (DNS) method.

2.1 Hydrolysis – Soxhlet Extraction

In each experiment, biomass was hydrolyzed according with the methodology developed by Meza and Sepulveda. (2010). The mixture was stirred during 30, 60 and 120 minutes. Lipids in hydrolyzed biomass were extracted using soxhlet equipment with hexane as solvent, oil yield was calculated with Equation 1. With the total lipid percentage of these genres was calculated the extraction efficiency using Equation 2.

$$Oil\ yield(\%) = \frac{Lipid\ extract\ weight}{Biomass\ weight} X\ 100 \quad (1)$$

$$Efficiency(\%) = \frac{Oil\ yield}{Total\ oil\ amount} X\ 100 \quad (2)$$

2.2 Organosolv - Soxhlet Extraction

Organosolv pretreatment was carried out according with the method developed by Meza and Sepúlveda (2010), after that, total reducing sugars concentration of liquid phase was measured, the hydrolyzed biomass was dried and its lipids were extracted as is explained above.

2.3 Polyfunctional Process

Based on total lipid amount of *Navicula* sp. a biomass/ethanol ratio of 1:6 was defined, sulfuric acid was used as catalyst in a oil/acid mass ratio of 1:1, these conditions were defined based on previous works of authors (Gonzalez and Kafarov. 2010, Plata et al. 2010) and similar reaction systems proposed (Ehimen *et al.* 2010, Johnson and Wen. 2009). The system was stirred at 60 ^{o}C, hexane was added for separation and analyzed with infrared spectroscopy using a Shimadzu FTIR-8400S.

3. Results

3.1 Effect of hydrolysis time on oil yield

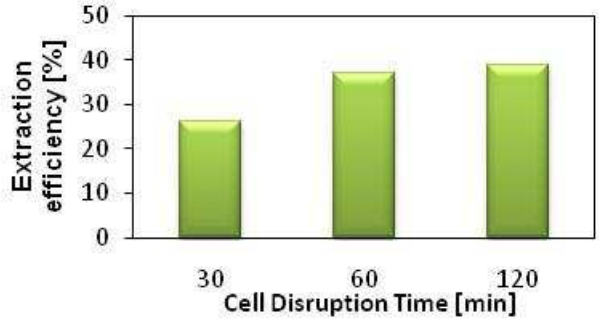

Figure 1. Effect of acid hydrolysis time on extraction efficiency for the microalgae Amphiprora sp.

Figure 1 shows differences in the efficiencies, this represents the level of cell disruption which allows that intracellular lipids of the microalgae are exposed in higher or lower intensity to the extraction solvent, taking into account these results, the acid hydrolysis time of 120 minutes was chosen for upcoming experiments.

3.2 Effect of solvent extraction time on oil yield

Using an extraction time of 16 h the process had an efficiency of 39 %, higher in comparison with times of 8 and 12 h, however, Figure 2 shows that there is no a linear relationship between extraction time and lipid efficiency, this behavior is related with the purity of the solvent after long time of contact with biomass.

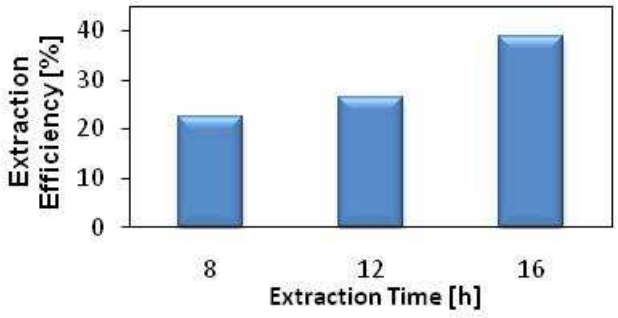

Figure 2. Effect of extraction time on the extraction efficiency for the microalgae Amphiprora sp.

Taking into account the results above, the operating conditions for hydrolysis/solvent extraction were defined with reaction times of 2 h for cell disruption and 16 h for solvent extraction, this route was also used for lipid extraction of *Navicula* sp. obtaining an extraction efficiency of 40%.

3.3 Lipid release and transesterification

Figure 3 shows an increase in the area of the peak corresponding to the (C=O) bond on 1750 cm^{-1} and the band corresponding to aliphatic chains between 2800 and 3000 cm^{-1} from 2 h of reaction for the Polyfunctional Process route. These are bands linked to esters, showing the growing presence of esters with time for the system evaluated, Figure 3 shows the changes of the spectra for the reaction system at several times.

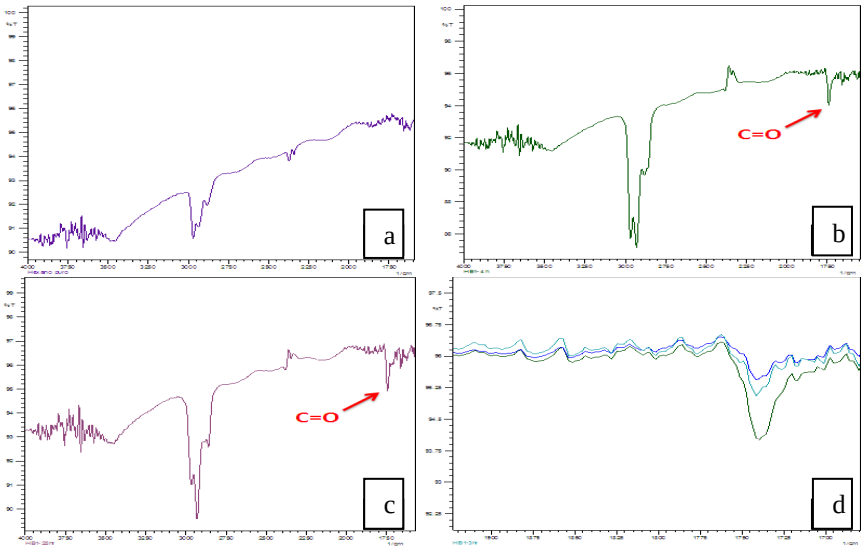

Figure 3. FTIR spectra of hexanic phase for Polyfunctional Process of Navicula sp. a) Hexane at the beginning. b) Hexane after 4 h. c) Hexane after 28 h d) comparison of spectra after 2, 3 and 4 h.

FTIR was also applied to samples obtained by hydrolysis/Soxhlet extraction in order to compare the products obtained (Figure 4). (C=O) bond appears as in biodiesel as lipid spectra, this bond for our lipid extract was found at 1704 cm^{-1} and for the hexane phase after *Polyfunctional Process* at 1750 cm^{-1}. In the *Polyfunctional Process* spectrum (Figure 4b) appears a peak at 3400 cm^{-1}, corresponding to the OH bond that is common in glycerol spectra (Ooi et al., 2001). The shape of this spectra is owing to the origin of the sample which is product of a multifunctional process when there was no a rigorous purification of the biodiesel obtained.

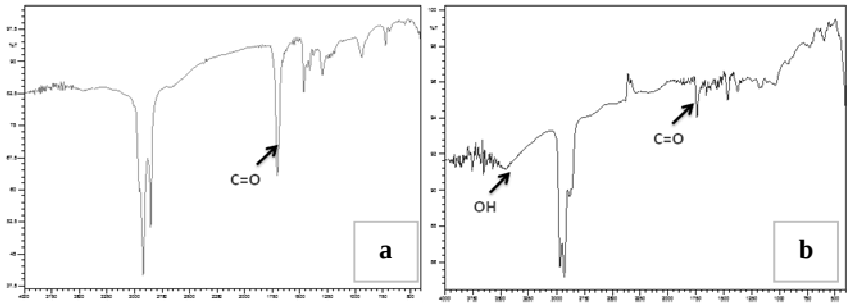

Figure 4. Comparison of infrared spectra for products obtained from Navicula sp. a) lipidic extract obtained by Acid hydrolysis/Soxhlet extraction route. b) hexanic phase obtained by Polyfunctional Process route.

Table 1. Comparison of IR spectra of lipids and biodiesel of different sources.

	Region [cm^{-1}]	*Vibration*	*Lipids*	*Ethyl Esters*	*Source*
Microalgae	3025-2954	CH3 and CH2	Present	Absent	Laurens and Wolfrum, 2010
	2918	Aliphatic chains	Present	Present	This work
	1746-1654	(C=O) bond	Present	Absent	Laurens and Wolfrum, 2010
	1704	*(C=O) bond	Present	Absent	This work
	1750	**(C=O) bond	Absent	Present	This work
	3400	(OH) bond, glycerol	Absent	Present	This work
Palm	1750	(C=O) bond	Present	Present	Martinez et al., 2007
	2800, 3000	Aliphatic chains	Present	Present	Martinez et al., 2007

* (C=O) bond for lipids, ** (C=O) bond for biodiesel

Table 1 compares peaks of biodiesel and oil from different sources. The (C=O) bond for microalgae lipids is located between 1746 and 1654 $cm^{-1,}$ in the IR spectra for ethyl esters from palm oil and *Navicula* sp., the Carbonyl bond was identified at 1750 cm^{-1}.

3.4 Comparison of routes by product

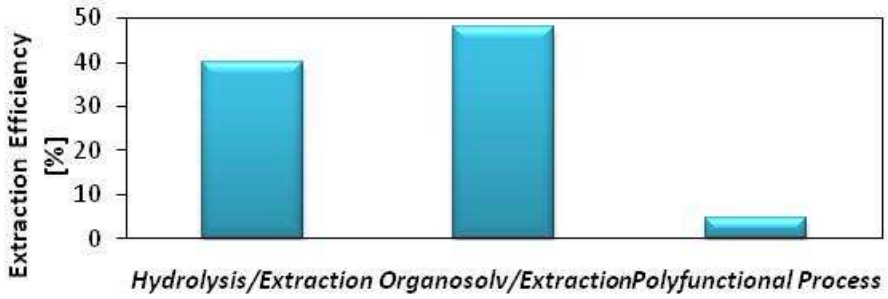

Figure 5. Comparison of lipid extraction efficiencies for three routes evaluated after solvent extraction for Navicula sp.

Extraction efficiencies for microalgae lipids using the three routes were compared (Figure 5). Organosolv/Soxhlet extraction route shows the highest oil yield with an efficiency of 48 %, although the energy requirements for the Organosolv/Soxhlet route are many times bigger than the acid hydrolysis/Soxhlet route, there is no a big difference between the extraction efficiencies. On the other hand, the residual biomass exposed to a solvent extraction after multifunctional process shown the presence of residues of algae oil, obtaining an extraction efficiency of 5 %, this means that most of oil contained into the microalgae biomass was released with the multifunctional process evaluated.

Table 2. Comparison of reducing sugars production for three routes evaluated on the microalgae Navicula sp.

Reducing sugars concentration (mg/ml)			
	Hydrolysis Extraction	*Organosolv Extraction*	*Polyfunctional Process*
Navicula sp.	0.29	0.98	1.67

Table 2. Shows the comparison of the maximum reducing sugars concentration obtained with the three routes evaluated. Using the *Polyfunctional Process* route, the highest reducing sugars concentration was obtained in comparison with Organosolv/Soxhlet extraction and acid hydrolysis/Soxhlet extraction, in this case there are significant differences in the reducing sugars yield depending of the method used, is shown that the methods with the best performance for reducing sugars yield are those in which the time of contact with the disruptor reagent is longer.

4. Conclusions

2 h of cell wall hydrolysis and 16 h of solvent extraction were chosen for the microalgae genres *Amphiprora* sp. and *Navicula* sp. FTIR spectra shows the direct biodiesel production using the Polyfunctional Process route.

The comparison of three routes as a function of the products obtained shows that organosolv/Soxhlet process gives the highest oil yield followed by acid hydrolysis/Soxhlet extraction which operation temperature and time are lower, Polyfunctional Process gives the highest reducing sugars yield and produces biodiesel, taking into account the biorefinery concept, Polyfunctional Process route proposed gives the best approach in comparison with the other routes obtaining biodiesel, algae oil, and sugars that can be used for bioethanol production.

Acknowledgements

The authors thanks to the Colombian Agriculture and Rural Development Ministry, the Colombian Department of Science, Technology and Innovation COLCIENCIAS, the Biomass lab extraction groups, the Iberoamerican Science and Technology Development Programme CYTED, the Morrosquillo Corporation and the Colombian Petroleum Institute ICP-ECOPETROL.

References

Ehimen, E. A., Sun, Z. F., and Carrington, C. G. 2010. Variables affecting the in situ transesterification of microalgae lipids. Fuel 89, 677-684.

González, A. D., and Kafarov, V. 2010. Design of a multifunctional reactor for third generation biofuels production. Chemical Engineering Transactions, Vol. 21, 1297-1302.

Johnson, M. B., and Wen, Z. 2009. Production of Biodiesel Fuel from the Microalga Schizochytrium limacinum by Direct Transesterification of Algal Biomass. Energy Fuels 23, 5179-5183.

Laurens, L. M. and Wolfrum, E. J., 2010. Feasibility of Spectroscopic Characterization of Algal Lipids: Chemometric Correlation of NIR and FTIR Spectra with Exogenous Lipids in Algal Biomass. Bioenergy Research DOI 10.1007/s12155-010-9098-y

Martínez, O. M., Sánchez, F. J., and Suárez, O. Y. 2007. Production of ethylic esters from RBD palm oil. Ingeniería e investigación Vol. 27 (2). 34-43. (in Spanish)

Meza, P., and Sepúlveda, A. 2010 Study of methanol-acid pretreatment of the microalgae *Chaetoceros gracilis* for total reducing sugars production, B. Sc. dissertation, UIS, Bucaramanga, Colombia (in Spanish).

Ooi, T. L., Yong, K. C., Dzulkefly, K, Wan Yunus, W. M. Z., and Hazimah, A. H. 2001. Crude glycerine recovery from glycerol residue waste from a palm kernel oil methyl ester plant. Journal of Oil Palm Research Vol. 13 (12). 16-22.

Plata, V., Kafarov, V., and Moreno, N. 2010. Optimization of third Generation biofuels production: Biodiesel from microalgae oil by homogeneous transesterification. Chemical Engineering Transactions, Vol. 21, 1201- 1206.