Diseño y ajuste de métodos acoplados de extracción de aceite de microalgas para el desarrollo de una topología de biorefinería

Design and adjustment of coupled microalgae oil extraction methods for the development of a topology of biorefinery

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RESUMEN

La biomasa de microalgas tiene el potencial de producir una gran cantidad de biodiesel por unidad de área debido a su alto contenido lipídico. Para lograr una producción sostenible de biodiesel a partir de microalgas, es necesario tener en cuenta el concepto de biorefinería, este concepto puede ser aplicado a la biomasa de microalgas para la obtención de biocombustibles y otros productos de alto valor agregado. La extracción del aceite de microalgas es un paso importante para el desarrollo de una topología de biorefinería. En este trabajo, tres métodos acoplados de extracción de aceite de algas fueron diseñados y ajustados a condiciones locales. Para los experimentos de disrupción celular, el efecto del autoclave, hidrólisis y pretratamiento organosolv sobre la eficiencia de extracción fueron evaluados, así como tres metodologías de extracción basadas en solventes orgánicos fueron diseñadas y ajustadas, extracción con la mezcla etanol/hexano, extracción con solvente de reflujo continuo y extracción con solventes asistida con homogenización a alta velocidad.

Las cepas utilizadas fueron Closterium sp., Navicula sp., Amphiprora sp., Guinardia sp., Botryococcus sp., Desmodesmus sp. y Tetraselmis sp., los resultados muestran que la hidrólisis y el pretratamiento organosolv como etapas de disrupción celular son las que mas incrementan la eficiencia de la extracción, mientras que para la extracción con solvent de reflujo continuo los mejores resultados son obtenidos al utilizar hexano durante 8 horas y para la extracción con solventes asistida con homogenización a alta velocidad las mayores eficiencias se obtienen a bajos tiempos y frecuencias se 5000 rpm.

Palabras clave: Microalgas, Biocombustibles, Biorefinería, Disrupción celular, Extracción de aceite

ABSTRACT

Microalgae have the potential to produce a big amount of biodiesel per area unit owing to its high lipid content. However, for a sustainable biodiesel production from microalgae, the concept of biorefinery must be taken into account; this concept can be applied to microalgae biomass for the production of biofuels and high added value products owing to different components present in microalgae strains. Microalgae oil extraction is an important step to take into account for the development of a topology of biorefinery. The main objective of this work is to establish different coupled methods for cell disruption and oil extraction of bioprospected microalgae. Three methods were designed and adjusted to local conditions. For cell disruption experiments, effect of autoclave, hydrolysis and organosolv pretreatment on the increase of extraction efficiency was evaluated. For oil extraction, three solvent based methodologies were developed, ethanol/hexane mixture, continuous reflux solvent extraction and solvent extraction with high speed homogenization.

Microalgae used in experimental development were Closterium sp., Navicula sp., Amphiprora sp., Guinardia sp., Botryococcus sp., Desmodesmus sp. and Tetraselmis sp. microalgae strains. Cell disruption procedures that presents better results were acid hydrolysis and organosolv pretreatment, best results for continuous reflux solvent extraction were reached when extraction time was 8 hours and hexane is used as extraction solvent, while solvent extraction with high speed homogenization shows higher oil yields when are used times around 14 minutes and frequencies of 5000 rpm.

Keywords: microalgae, biofuels, biorefinery, cell disruption, oil extraction
1. INTRODUCTION

Microalgae has the potential to produce a big amount of biodiesel per area unit owing to its high lipid content which exceeds lipid content of all biodiesel sources used currently [1], in addition, microalgae are cultivated in photobioreactors and open ponds which only needs water, some nutrients and sunlight to stimulate growing, these culture conditions makes feasible the using of non-crop lands for photobioreactor or open pond assembly. The use of microalgae for biodiesel production is an advantageous alternative because of the high lipid content and fatty acid profiles that suitable offers.

The biorefinery concept has been identified as the most promising way for the creation of an industry based on biomass. This concept can be applied microalgae biomass for the production of biofuels and high added value products based on the composition of promising species, a microalgae based biorefinery must take into account several issues for its sustainability as water requirements, production costs, environmental impacts and process efficiency [2].

Studies about microalgae oil extraction for biodiesel production are taking significance because the efficiency of biodiesel production chain from microalgae depends in a great way of the oil extraction efficiency. In previous works, authors explain several physical, chemical and enzymatic microalgae oil extraction methods [3]. Solvent-based lipid extraction methods as Folch, and Bligh and Dyer’s method [4], has been used for obtaining lipids from microalgae. Using a mixture hexane-ethanol, can be extracted around of 80% of fatty acids presents into biomass [5], hexane is frequently used for soxhlet extraction using microalgae biomass as a raw material [6], hexane is cheap, easy to recovery after extraction and is selective to neutral lipids, Ethanol with acid has been used for simultaneous cell disruption and lipid extraction using microalgae strains Amphiprora sp. and Navicula sp. [7].

The main objective of this work is to establish different solvent based high detailed methodologies for the cell wall disruption and lipid extraction of microalgae for the development of a topology of biorefinery through the evaluation of operating conditions for each step in each method, after that, best operating conditions as cell disruption as solvent extraction are assembled and adjusted to a coupled extraction method for future integration in a microalgae based biorefinery concept.

2. METHODOLOGY

Microalgae biomass was provided by Corporación Instituto de Morrosquillo (Punta Bolivar, Colombia), algae was cultured in F/2 medium, grown in open ponds and harvested by flocculation (150 ppm FeCl₃), biomass was sun-dried and frozen until using.

2.1 Cell disruption experiments

General methodology for cell disruption experiments is shown in Figure 1, all raw materials supplied were dried in a convection digital oven (make WTC binder) at 105 °C for 4 hours, based on the standard NREL/TP-510-42621, and then, the sample was homogenized. A 25 L Autoclave was used for thermal cell disruption experiments, microalgae biomass was exposed at autoclaving conditions of 394.15 K and 103410 Pa. by 1 and 3 hours.

For solvent-based cell disruption, an organosolv pretreatment previously developed by authors was performed using a mixture of water, an organic solvent and an acid at high temperatures [8]. For cell disruption using acid hydrolysis, microalgal biomass were dried in an oven at 378 K for 4 hours, after that, 3g of biomass were mixed with different HCl solutions at concentrations of 0.1 mol L⁻¹, 0.5 mol L⁻¹, 1 mol L⁻¹ and 3 mol L⁻¹ with an exposure time of 0.5 hours with magnetic stirring at room temperature. Solid and liquid phases were separated by filtration and biomass was washed with distilled water, biomass was dried and submitted to lipid extraction, all cell disruption experiments were made by triplicate.

After pretreatment, biomass was separated from the liquor by vacuum filtration. Separate biomass was washed with distilled water and dried in oven at 378.15 K for 4 hours. For measurements of the effect of pretreatments on oil yield, lipid extraction using the mixture ethanol/hexane method (EHE) described in Figure 2 was used, biomass was mixed with ethanol using a ratio of 1:5, mixture was stirred by 14 hours at 500 rpm. After that, the mixture was
filtered and solid phase was stirred again with fresh ethanol, two liquid phases were combined, hexane and water were added for two liquid phases formation, the phases were separated and fresh hexane is added again to hydroalcoholic phase, this process is repeated two times, the four hexane phases were mixed and lipid extract is separates of hexane by distillation. The quantification of lipid extract was determined with the aim of evaluating the performance of the process and obtains an indirect measure of the effect caused by pretreatment of cells. Deeper explanation of development and results of this method can be seen in dissertation presented by Sarmiento y Amaya in which is based this subsection [9].

2.2 Continuous reflux solvent extraction (CRSE)

For continuous reflux solvent extraction evaluation, a typical Soxhlet extractor with 45/50 outer/upper and 24/40 lower/inner joint, for 250 mL capacity was used, each experiment was performed with 5 grams of dry treated biomass, three commonly used extraction solvents were evaluated; hexane, cyclohexane and ethanol. These solvents were chosen taking into account their low boiling point, costs, safety factors and toxicity. In the next phase, after selecting the cell disruption method and the solvent for lipid extraction, the extraction time was evaluated, using values of 4, 6 and 8 hours (based on literature review [6]).

During solvent extraction, the amount of biomass and the ratio biomass/solvent were kept constant. After extraction, extract-solvent mixture was filtered, distilled and the remnant solvent was evaporated. Total lipids were also quantified gravimetrically, in the final phase, the best experimental conditions for the oil yield were applied to the three genera studied, figure 3 shows the methodology proposed. Deeper explanation of development and results of this method can be seen in dissertation presented by Cordoba y Lopez in which is based this subsection [10].

**Figure 2.** Lipid extraction methodology for cell disruption experiments on microalgae biomass (EHE method).

**Figure 2.** Metodología para la extracción de lípidos en la evaluación de métodos de disrupción celular (método EHE).

**Figure 3.** Methodology for continuous reflux solvent extraction adjustment (CRSE).

**Figure 3.** Metodología para el ajuste del método de extracción con solventes de reflejo continuo (CRSE).
2.3 Solvent extraction with high speed homogenization (SHE)

The solvent extraction method combined with high speed homogenization is based on Folch and Bligh & Dyer's method, solvents chosen were methanol and chloroform, the methodology to adjust is shown in Figure 4, and includes the steps of stirring, centrifugation, separation and volatilization. In the stirring phase, two rates of biomass/solvent were evaluated 1:10 and 1:20 based on preliminary test results, the effect of adding water in the first part of homogenization and the effect of time and frequency of homogenization, accord to an experimental design. Centrifugation was carried out for 15 minutes and it was assessed a frequencies of 2500 and 3400 rpm. The phase separation was performed by removing the upper phase methanol/water from the centrifuge tube while lower biomass/lipids Chloroform, was filtered by gravity. Finally, the lipid extract was allowed to volatilize to constant weight for its measurement.

Figure 4. Methodology for adjustment of Solvent extraction with high speed homogenization (SHE).

For each experiment, 5 g. of disrupted biomass were mixed with methanol and chloroform in a ratio 2:1 three homogenization frequencies were evaluated (5000, 8000 and 11000 rpm.) using a Heidolph® SilentCrusher homogenizer. variables were evaluated following a 2^2 central composite experimental design, phases were separated by centrifugation and filtration and lipids were recovered from chloroform phase by evaporation, statistical analysis of main effects was made using STATISTICA 7.0 software taking as a response variable the lipid extract yield concentration. Oil yield for all experiments was measured by gravimetric method. Each experiment was performed by triplicate in order to give reproducible results. Deeper explanation of development and results of this method can be seen in dissertation presented by González y Galindo in which is based this subsection [11].

3. RESULTS AND DISCUSSION

3.1 Design and adjustment of cell disruption methods

3.1.1 Effect of moisture on cell disruption efficiency. Table 1 shows the yields obtained from the lipid extraction process using wet and dry biomass. These tests were performed with a mixture of Navicula sp. and Amphiprora sp., it is shown that the water content in the sample is not favorable for the extraction of lipids due to two reasons; Presence of water in the sample decreases the concentration of ethanol in the biomass / solvent mixture during the first stage of the process, reducing the efficiency of solvent extraction of crude oil.

Table 1. Effect of moisture on cell disruption: L_r: (%) lipid recovery in the total biomass; Y: (%) losses of biomass by handling (strains: mixture of Navicula sp. and Amphiprora sp.).

<table>
<thead>
<tr>
<th>Moisture (%)</th>
<th>Extracts Weight (g)</th>
<th>Standard deviation</th>
<th>( L_r ) (%)</th>
<th>Y (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.0337</td>
<td>0.0028</td>
<td>1.5</td>
<td>30.8</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0.0629</td>
<td>0.0033</td>
<td>2.8</td>
<td>9.4</td>
</tr>
</tbody>
</table>

3.1.2 Effect of autoclaving time. Thermal pretreatment results for microalgae strain Amphiprora sp. are shown in Figure 5. Although cell disruption process shows a significant increase in the recovery rate of lipids for an autoclaving time of 3 hours, failed to overcome any of the results obtained with the other pretreatments. The recovery percentages for autoclave times evaluated do not differs more than 1.2 % w/w despite increased exposure time to 2 hours. This allows inferring that long times represents large and unnecessary energy expenditure.

For microalgae strain Amphiprora sp.

Figure 5. Effect of autoclave time on the recovery rate of lipids for microalgae strain Amphiprora sp. Figura 5. Efecto del tiempo de autoclave sobre la separación de lipidos utilizando la cepa Amphiprora sp.
3.1.3 Effect of HCl concentration. Effects of hydrochloric acid concentration on the extraction yield were also evaluated, using concentrations of 0.1 mol L$^{-1}$, 0.5 mol L$^{-1}$, 1 mol L$^{-1}$ and 3 mol L$^{-1}$. Figure 6 shows that the extraction yield increases when acid concentration is also increased within the range set but at concentrations higher than 0.5 mol L$^{-1}$, this effect is less pronounced with a tendency to stabilize.

Figure 6. Effect of acid concentration on the recovery rate of lipids using microalgae biomass of *Amphiprora* sp.

Cell disruption with HCl 3 mol L$^{-1}$ presents the highest percentage of lipids recovery, however, this concentration involves the increase of acid amount several times for very little yield increase in comparison with the oil yield obtained with an acid concentration of 0.5 mol L$^{-1}$, corresponding to 11.78 % w/w. In addition, higher concentrations of hydrochloric acid might increase the levels of corrosion in the equipment involved throughout the process. Therefore, a solution of 0.5 mol L$^{-1}$ hydrochloric acid was the most suitable for pretreatment of biomass, 250 % decline in spending on chemical agent worked to the maximum concentration, without affecting performance deeply.

3.1.4 Organosolv Pretreatment. Results obtained of applying organosolv pretreatment to microalgae biomass of *Amphiprora* sp. are shown in Table 2, although organosolv pretreatment increased the recovery rate of lipids in more than 3 % w/w over 3 hours of autoclave treatment, did not surpass the results obtained with HCl 0.5 mol L$^{-1}$. In addition, this pretreatment involves high energy costs, a longer exposure time and use of more chemicals making it inconvenient to use as pretreatment method prior to cell disruption with ethanol-hexane if the only one product desired is microalgae crude oil.

3.2 Comparison of cell disruption methods

Values obtained in the response variable ($L_R$) shows clearly that the chemical treatment with HCl 0.5 mol L$^{-1}$ and organosolv pretreatment gives the highest oil yield when ethanol/hexane method is used for microalgae oil extraction (Figure 7).

Method selected for oil extraction in this section presents lower yields compared to traditional procedures for the recovery of lipids, but the product obtained is mainly composed of neutral lipids due to the selectivity of hexane, this being the most suitable fraction for later processes of esterification, transesterification or hydro-treatment, taking into account this results, acid hydrolysis and organosolv pretreatment were taken into account as cell disruption methods in further sections of this work.

3.3 Adjustment of continuous reflux solvent extraction coupled with cell disruption (CRSE)

Best cell disruption methods obtained in previous section were applied to microalgae biomass and a continuous reflux solvent extraction was applied for lipids recovery. Highest oil yield was obtained with organosolv pretreatment (6.8%) in comparison with HCl 0.5 mol L$^{-1}$. In addition, when organosolv pretreatment was used, the oil yield was increased three times in comparison with the control. This difference can be attributed by the degree of hydrolysis of the cellulosic cell wall components of microalgae according to each disruptor agent and operation conditions of treatment. Then, the lipids are exposed to higher or lower proportion to the solvent extraction and the oil yield is affected. Efficiencies of the extraction process using cell disruption methods are shown in Table 3, it can be seen also that all extraction efficiencies using continuous reflux solvent extraction are higher than efficiencies obtained using ethanol/hexane method.

Table 2. Organosolv pretreatment results for *Amphiprora* sp. strain: $L_R$: % w/w lipid recovery in the total biomass; $L_{Rc}$: % w/w % w/w Biomass unrecovered.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>$L_R$ (%)</th>
<th>$L_{Rc}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organosolv</td>
<td>9.55</td>
<td>71.6</td>
</tr>
</tbody>
</table>

Figure 7. Comparison of microalgae biomass cell disruption methods for *Amphiprora* sp. biomass.

Figura 7. Comparación de los métodos ajustados de disrupción celular utilizando la microalga *Amphiprora* sp.
Table 3. Comparison of best adjusted cell disruption methods using continuous reflux solvent extraction (CRSE) for microalgae biomass of *Amphiprora* sp.

<table>
<thead>
<tr>
<th>Cell disruption method</th>
<th>Extraction efficiency (%)</th>
<th>Standard deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.0</td>
<td>2.4947</td>
</tr>
<tr>
<td>Organosolv</td>
<td>56.5</td>
<td>2.5495</td>
</tr>
<tr>
<td>Hydrochloric acid 0.5 mol L⁻¹</td>
<td>37.9</td>
<td>1.7802</td>
</tr>
</tbody>
</table>

3.3.1 Solvent Selection. By using hexane, cyclohexane and ethanol as solvents in extraction process, it was shown that the hexane presents higher loss of solvent. However, as evidenced in Figure 8, this solvent produced the greatest oil yield (6.8%) relative to cyclohexane (3.2%) and ethanol (2.3%). It is also the cheapest solvent of the three tested, also is selective to neutral lipids and commonly used in solvent extraction processes chemicals. Besides, when performing the extraction with cyclohexane was obtained the second highest oil yield (3.2%), but this is the solvent most expensive of the three solvents studied.

**Figure 8.** Oil yield with different solvents for *Amphiprora* sp. microalgae biomass.

3.3.2 Effect of extraction time. Effect of extraction time is observed clearly in Figure 9, when the contact time between solvent and biomass was increased, there was a significant impact on the oil yield, because it promotes the mass transfer of lipid components into the solvent, reaching a higher oil yield (5.8%) when the sample was extracted for eight hours and with an increasing trend for higher times. In the same way when compared the results with (dark bars) and without cell disruption method (white bars), there was an increase of more than five times in the oil yield, for all operation times evaluated.

**Figure 9.** Effect of extraction time on *Amphiprora* sp. microalgae oil yield.

Furthermore, the extraction time of eight hours produced the best extraction efficiency of 53.6% as is reported in Table 5.

3.3.3 Best experimental conditions obtained. When the best experimental conditions according to the higher oil yield (organosolv pretreatment as cell disruption method, hexane as solvent and 8 hours of operation time) were applied, results obtained were the shown in Figure 10 for the three genera studied.
Table 5. Lipid extraction efficiency in relation to operation time for *Amphiprora* sp. strain.

<table>
<thead>
<tr>
<th>Cell disruption method</th>
<th>Extraction time (h)</th>
<th>Extraction efficiency (%)</th>
<th>Standard deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass without disruption (control)</td>
<td>4</td>
<td>3.7</td>
<td>0.8325</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.9</td>
<td>0.3648</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9.8</td>
<td>0.9874</td>
</tr>
<tr>
<td>Biomass with organosolv pretreatment</td>
<td>4</td>
<td>19.6</td>
<td>2.3862</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>34.3</td>
<td>2.5732</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>53.6</td>
<td>2.7439</td>
</tr>
</tbody>
</table>

Figure 10. Oil yield with the best (CRSE) conditions for the three genera of microalgae (*Amphiprora*, *Desmodesmus* and *Tetraselmis*).

These results confirm the advantage of applying a cell disruption method before extraction process, it can achieve significant increases in the oil yield for biomass without disruption, and increments of three, five and twelve times oil yield for genera *Amphiprora*, *Desmodesmus* and *Tetraselmis* respectively. Also as shown in Table 6, for all genera of microalgae was obtained a superior process efficiency to 50% using the best conditions of the variables analyzed, getting the highest value 57.6% for the genus *Tetraselmis*.

Table 6. Oil extraction efficiency with adjusted (CRSE) method for several microalgae strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Extraction efficiency (%)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphiprora</em> sp.</td>
<td>56.5</td>
<td>2.5495</td>
</tr>
<tr>
<td><em>Desmodesmus</em> sp.</td>
<td>53.6</td>
<td>2.7439</td>
</tr>
<tr>
<td><em>Tetraselmis</em> sp.</td>
<td>57.6</td>
<td>1.8656</td>
</tr>
</tbody>
</table>

3.4 Adjustment of solvent extraction with high speed homogenization (SHE)

In the stirring stage, when the biomass/solvent ratio 1:10 was initially evaluated, there was no lipid extract obtained because the rate of volatilization of chloroform was higher than the rate of filtration of the mixture, leaving all biomass retained in the filtration stage. While performing the extraction at a biomass/solvent ratio of 1:20 this problem was overcome and it was decided to maintain this ratio for further experiments. On the other hand, in the stage of centrifugation, when the frequency was adjusted according with literature in 2500 rpm, there was no a complete separation of the solvents mixture, for this reason, centrifugation frequency was increased to 3400 rpm, in this case, it was identified the biphasic system composed by a methanol and water in the upper phase and lower chloroform-lipids -biomass. Therefore, for the development of the extraction method, centrifugation stage was tuned in 3400 rpm during a time of 15 minutes.

3.4.1 Effect of cell disruption. Given that cell wall of microalgae is destroyed by the degradation of the polysaccharides present in biomass, and these and other components of the solid matrix are soluble in liquor of hydrolysis, a large percentage of the biomass subjected to the cell disruption process becomes part of the liquor, reducing the biomass used for extraction.
La hidrólisis ácida y el pretreatment orgánosolv no incrementaron el porcentaje de extracto de aceite en este método de extracción. Basado en estos resultados, el pretreatment orgánosolv fue desechado para futuros ensayos debido a bajos porcentajes de extractos lipídicos obtenidos y la dificultad en el desarrollo de extracción. Sin embargo, la disrupción celular con hidrólisis ácida, aunque reportó un 32% de disminución en el rendimiento, continuó siendo el objeto de estudio ya que facilitó los pasos de centrífugación y filtrado al extrayendo. Después de eso, nuevos ensayos se realizaron usando hidrólisis ácida en especies de *Amphiprora* (Figura 11b) para verificar que el efecto negativo de este método en otras especies todavía estaba obteniendo un 55% de reducción en el rendimiento de extracción.

El bajo rendimiento de extracción usando biomasa con disrupción celular respecto a la biomasa sin disrupción celular es debido a la extracción de componentes lipídicos con homogeneización a alta velocidad, en particular, a lipidos, también extrae cantidades significativas de componentes no lipídicos. Al aplicar previously applying the disrupción method this lipido components become parte of hydrolysis liquor thus obtaining a purer lipid extract compared with the extraction using biomass without cell disruption. That is, the application of a cell disruption method allows obtaining purer extracts after lipid extraction performed with the solvent extraction with high speed homogenization, but decreases the percentages of extraction. For third method can be concluded that use of acid hydrolysis or organosolv pretreatment is not necessary because cell disruption is performed by the high speed homogenization process.

### 3.4.2 Efecto de la adición de agua durante la primera homogenización

La cantidad de extracto de aceite obtenida para dos diferentes generes de microalgas con y sin adición de agua en la primera parte del proceso de agitación se muestra en la Tabla 7, donde se observa que la adición de agua disminuye el rendimiento de extracción para las especies *Amphiprora* sp., *Botryococcus* sp. y *Nannocloropsis* sp., por 15% y 40% respectivamente. Esto se debe a que el agua es soluble en metanol e insoluble en cloroformo y lipidos, lo que afecta la solubilidad del cloroformo - metanol y dificulta la extracción de lipidos. Basado en estos resultados se decidió que para adaptar el método de extracción con alta velocidad de homogeneización a la extracción de lipidos de las microalgas biomasa es más conveniente no agregar agua en la primera parte del agitador.

### 3.4.3 Efecto de la frecuencia de agitación

Para estudiar el efecto de la frecuencia de agitación en el rendimiento de extracción, se realizó disrupción celular mediante pretreatment orgánosolv y la extracción se realizó homogeneizando la mezcla de biomasa/solvente durante 14 minutos a frecuencias de 5000, 8000 y 11000 rpm.

La Figura 12 muestra que un aumento en la frecuencia de agitación disminuye el porcentaje de extracto de aceite, lo que coincide con lo reportado por Cravotto et al [12], quienes evaluaron la extracción asistida con ultrasónidos usando frecuencias entre 19 y 300 kHz obteniendo mayores rendimientos a menores frecuencias. Por tal razón se propuso un diseño experimental para examinar juntos las variables tiempo y frecuencia de agitación.

La tabla 7 muestra los valores de los niveles seleccionados para cada variable del diseño experimental. La matriz del diseño experimental y sus respectivas porcentajes de extracto de aceite se muestran en la Tabla 8. Los mejores resultados se obtienen en el nivel más bajo de cada variable y corresponden a los experimentos 1 y 6.
Table 8. Values and levels of the studied variables.
Tabla 8. Valores y niveles de las variables estudiadas.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time / min</td>
<td>-1</td>
</tr>
<tr>
<td>Frequency / rpm</td>
<td>5000</td>
</tr>
</tbody>
</table>

Table 9. Experimental design matrix and oil yield obtained during extraction of microalgae oil from *Amphiprora* sp.
Tabla 9. Matriz del diseño experimental y porcentaje de aceite obtenido durante la extracción de aceite de la microalga *Amphiprora* sp.

<table>
<thead>
<tr>
<th>N° Experiment</th>
<th>Frequency</th>
<th>Time</th>
<th>Oil yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>9.13</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-1</td>
<td>6.34</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>1</td>
<td>5.30</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4.46</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>6.43</td>
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<td>6</td>
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<td>0</td>
<td>5.21</td>
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</tbody>
</table>

The Pareto’s chart (Figure 13) shows that time, frequency and their interaction have significant effects on extraction, because all the blocks pass the threshold. In addition, can be inferred that the variables of time and frequency have negative effects on the performance of the extraction of lipids from microalgae, being the time the factor that mostly negatively affects the response variable. Finally, it should be noted that the combination of the independent variables has a positive effect on the response variable studied.

Figure 13. Effect of frequency and shaking time on the percentage of lipid extract from *Amphiprora* sp. strain.
Figura 13. Efecto de la frecuencia y el tiempo de agitación sobre el porcentaje de lipidos utilizando la microalga *Amphiprora* sp.

Figure 14. Effect of frequency and shaking time on the percentage of lipid extract for *Amphiprora* sp. microalgae. a) response surface plot b) contour diagram.
Figura 14. Efecto de la frecuencia y tiempo de agitación sobre el porcentaje de extracto lipídico para la microalga *Amphiprora* sp. a) diagrama de superficie de respuesta b) diagrama de contorno.
The interaction between time and frequency of homogenization can be seen in Figure 14 where the surface and the level curve show a region with the higher (bottom right side) and another with a lower percentage of lipid extract (upper left side). Finding then that the percentage of lipid extract is maximized when the variables of time and shaking are found on the lowest level within the experimented region, i.e. 14 minutes and 5000 rpm.

3.4.4 Extraction efficiency. The extraction efficiency is shown in Table 10. The Nannocloropsis genera presented the lowest yield, Closterium, and Botryococcus approached a yield of 50% and best results were obtained Amphiprora and Guinardia strains. This high performance was due to the rapid separation of the lipid extract and the solid in the filtration stage. Are also shown the differences in the efficiencies with the adjusted method and operating conditions reported in literature.

Table 10. Extraction efficiency for several species of microalgae using SHE method: a, b, c base extraction method, d method adjusted by authors.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total Lipids</th>
<th>Extracted Lipids</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nannocloropsis</td>
<td>11</td>
<td>1.50</td>
<td>13%</td>
</tr>
<tr>
<td>Botryococcus braunii</td>
<td>15</td>
<td>5.60</td>
<td>37%</td>
</tr>
<tr>
<td>Closterium</td>
<td>22</td>
<td>9.10</td>
<td>41%</td>
</tr>
<tr>
<td>Amphiprora</td>
<td>12</td>
<td>9.03</td>
<td>75%</td>
</tr>
<tr>
<td>Guinardia</td>
<td>7</td>
<td>5.80</td>
<td>87%</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

- Three ways of microalgae oil extraction by combining cell disruption and solvent based lipid removal and recuperation were designed and adjusted, towards the development of a topology of biorefinery, different alternatives for microalgal biomass rupture were evaluated, with chemical cell disruption, the recovery rate of lipids was proportional to the concentration of hydrochloric acid within the range established for the pretreatment of biomass. However, an acid concentration of 0.5 mol L⁻¹ was the most suitable for the cell disruption process, reducing by 250 % w/w reagent consumption compared to the maximum concentration worked, without significantly affecting the extraction yield, organosolv pretreatment also showed high efficiency on the increase of lipid yield for extraction methods without homogenization.

- Adjustment of continuous reflux solvent extraction also corroborate that the addition of a step of cell disruption before extraction process increases the efficiency of lipid extracted from microalgae, and the cell disruption using organosolv pretreatment (56.5%) was the most efficient. Higher oil yield was reached using hexane as solvent and an operating time of eight hours, these conditions increased significantly the efficiency of the process (56.5% and 53.6% respectively). Furthermore, using the best experimental conditions, the extraction efficiency was over 50% for the algae strains Amphiprora sp., Desmodesmus sp. and Tetraselmis sp.

- For solvent extraction with high speed homogenization best operating conditions were: Biomass/solvent ratio 1:20, homogenization frequency 5000 rpm, homogenization total time 14 minutes and centrifugation time of 3400 rpm by 15minutes. Moreover, the addition of water in the first part of stirring facilitated the filtration but decreased the percentage of oil extraction in a range between 15-40% . Use of acid hydrolysis or organosolv pretreatment is not necessary because cell disruption is performed by the high speed homogenization process.

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REFERENCES


