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Selenastrum Capricornutum: Harvesting and Oil Extraction, for Biodiesel Production

Leticia Pérez[1], Ángeles Cancela[1], Rocío Maceiras[2], J.L. Salgueiro[1], Ángel Sánchez[1]

Abstract
An alternative for biodiesel production is the use of lipids from microalgae. Although all steps to obtain this biofuel are important, harvesting and extraction are the most important. Advances in these areas are necessary in order to obtain third-generation fuels. The purpose of the present study is to compare different methods of lipids extraction and harvesting for freshwater Selenastrum Capricornutum microalgae. The method used for harvesting was flocculation with inorganic agent. Copper (II) Sulphate (CuSO4) was used as flocculant, resulting in the best percentage of recovery (76%) for a dose of 2 g/L. Previously oil extraction, the collected samples were homogenized and their moisture content was analysed obtaining values of 55-96%. Two extraction methods were used: Soxhlet and ultrasound. The use of ultrasound favours cell disruption and increases the extraction yield. In extraction methods, polar, non-polar solvents, and mixes of solvents were used. N-hexane and acetone were used as solvents for Soxhlet extraction, and ethanol, acetone, methanol, chloroform, and a mixture of chloroform and methanol in 1:2 and 2:1 ratios, for extraction ultrasound assisted method. The use of the methanol-assisted ultrasound is the most efficient method for lipids extraction for Selenastrum Capricornutum biomass.

Keywords: microalgae, extraction, ultrasound, Soxhlet, solvent, harvesting

1. Introduction

The need of energy is increasing continuously, because of rising in industrialization and population. The main sources of this energy are petroleum, natural gas, coal, hydro and nuclear. The major disadvantage of using petroleum based fuels is atmospheric pollution created by the use of petroleum diesel. Petroleum diesel combustion is a major source of greenhouse gas (Yin, Yaakob, Ali, Min, & Wa, 2011). Accordingly, nowadays, the replacement of fossil fuels with renewable alternatives is of great importance because of decreasing oil dependency, being an instrument in the fight against environmental degradation. Biodiesel is designated as renewable, biodegradable, less CO_2 and NOx emission fuel as compared to petroleum sourced fuel. Biodiesel from microalgae has emerged as one of the promising sources to displace the petrodiesel (Francisco, Neves, Lopes, & Franco, 2009). The lipid content of microalgae cells can vary from 2 to 77% depending on the species and environmental conditions / growth (Souza, et al., 2014).
In the production of biodiesel from microalgae the next steps are necessary: culture, harvesting, extraction of lipids and transesterification reaction. This paper will focus on harvesting and lipid extraction. There are various methods to extract lipids from microalgae, such as mechanical methods, microwave assisted extraction, solvent extraction and ultrasonic extraction. Some methods are usually used in combination with an organic solvent. In this paper, two different methods for lipids extraction from *Selenastrum Capricornutum* microalgae were studied (Soxhlet and ultrasound). In harvesting method, an inorganic coagulant was used.

[1]Chemical Engineering Department, EEI, University of Vigo, 36310 Vigo, Spain
[2] Defense University Center, Escuela Naval Militar, Plaza de España 2, 36920 Marín, Spain

2. Materials and Methods

2.1 Microalgae

The microalgae *Selenastrum Capricornutum* was cultivated in fresh water at a temperature of 19.9 ºC for 10 days in a photobioreactor of 30 L made of polyethylene. 14/10 light/dark cycles were used and the approximate pH of the microalgae cultures was 8.63. The measured concentration from the culture was 9000 cells/microliter. Growth characteristics are very important, these properties can affect the microalgae dewatering and the algae lipid content. Algal biomass was stored at room temperature after harvesting. All experiments were performed with cells from a single harvest.

The culture medium consists on the mixture of two different macronutrients solutions. One containing $NaNO_3$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and Na_2CO_3, and the other $MgCl_2$ $6H_2O$, $CaCl_2$ $2H_2O$, H_3BO_3, $MnCl_2$ $4H_2O$, $ZnCl_2$, $FeCl_3$ $6H_2O$, $CoSO_4$ $7H_2O$, Na_2MoO_4 $2H_2O$, $CuSO_4$ $5\ H_2O$ and Na_2EDTA $2H_2O$.

2.2 Coagulation Harvesting

Coagulation is a process in which flocs are formed, which are aggregates of particles of solute. The flocculation is produced by addition of a chemical substance, which helps the solute particles adhered together. These substances are called flocculants. This process has lower energy requirements than centrifugation and it is able to increase the biomass concentration. Coagulation may be induced by application of various forms of flocculants such as inorganics compounds. Chemical flocculation experiments were carried out in 1L batch glass cylinder using copper (II) sulphate ($CuSO_4.5H_2O$) solution. Three different doses (0,5, 1 and 2 g/L) were added to the microalgae to analyse the influence of coagulant.

After the addition of coagulant, the mixture was stirred in two steps. In the first one, the mixture was mixed quickly at 200 rpm during 1 min, with the aim of distributing the coagulant through the water. In the second step, flocculation occurred, so it was necessary a low mix at 50 rpm during 3 min. After a while, the microalgae settled down and the absorbance was measured at 550 nm with a digital spectrophotometer Spectro 22 (Labomed, USA).

2.3 Humidity

Drying the biomass after harvesting is a vital step because improves the lipid extraction efficiency and other value-added compounds extraction (Viswanathan, Mani, Das, S.Chinnasamy, & Bhatnagar, 2011). So the influence of moisture content in the extraction has been analysed. The humidity content was measured according to UNE-EN 14774-1:2010.

To determine moisture content, 1 g of microalgae was introduced into an oven at 105 ºC and the sample was weighed at different time intervals until constant weight. When the sample was dried, part of it was used to perform some of the experiences and the remainder was preserved in the refrigerator. Days later, the moisture content of the sample was in the refrigerator was analysed again.

2.4 Ultrasound Lipid Extraction

According to its frequency, ultrasound is classified into high-frequency (2–10 MHz) and low-frequency (20–100 kHz) ultrasound (TJ & JP, 2002). Nowadays, low-frequency ultrasound is more and more present in all areas of chemistry and chemical technologies. The energy of ultrasonic irradiation can activate various mechanisms to affect positively chemical reactions and processes. These positive effects of ultrasound are generally attributed to so-called ultrasonic cavitation, which means the formation, growth and implosive collapse of bubbles (cavities) in a liquid irradiated with ultrasound. When ultrasound passes through the liquid, it consists of both

expansion (negative pressure) and compression (positive pressure) waves (Wei, et al., 2008). These form bubbles, filled with solvent and solute vapour and dissolved gases, which grow and recompress. Under proper conditions, ultrasonic cavitation leads to implosive cavitation bubble collapse, producing intense local heating, high pressures and very short lifetimes (Veljkovic, M.Avramovic, & Stamenkovic, 2012).

In this paper, all experiences were made twice and carried out in an Ultrasound bath (Model S 300H from Elmasonic). Power rating was 300W and irradiation frequency of 37 kHz. Acetone, ethanol, methanol, chloroform and a mixture of chloroform-methanol in ratios 1:2 and 2:1 were used as solvents for the ultrasound extraction method. In all experiments, crude dried *Selenastrum Capricornutum* (1 g) was mixed with solvent (50 mL in the case of ethanol and 15 mL for the remaining solvent) in flasks at room temperature (25 ºC) for lipid extraction for a period of 30 minutes. These flasks were placed on a metal support, preventing it coming into direct contact with the bottom of the ultrasound bath. Throughout the process, the temperature was measured by a thermometer inserted in the flask. After reaction, the samples were vacuum filtered and the resultant liquid fraction was dried to constant weight in an oven at a temperature of 65 ºC (G.S.Araujo, et al., 2013).

2.5 Soxhlet Lipid Extraction

The extraction microalgae oil was performed in a round-bottomed flask using Soxhlet equipment. For this, a sample of 3 g of *Selenastrum Capricornutum* microalgae and 150 mL solvent were used. Two solvents were used: acetone and n-hexane. The reflux period was 4.5 h. After the extraction, a mixture of oil and solvent was obtained. In order to remove the solvent, distillation at boiling the solvent temperature was carried out. The resultant lipid samples were dried to constant weight in an oven.

3. Results and Discussion

3.1 Harvesting

The technique employed was coagulation. Copper (II) sulphate was used as flocculant and three different doses were used (0.5 g/L, 1g/ L and 2 g/L). Figure 1 shows the obtained results. The efficiency of the process was analysed in basis to the microalgae concentration with the time. Data show that higher doses of flocculant give higher conversions due to the higher availability of flocculant molecules to attach to microalgae cells. It can be observed in Figure 2.

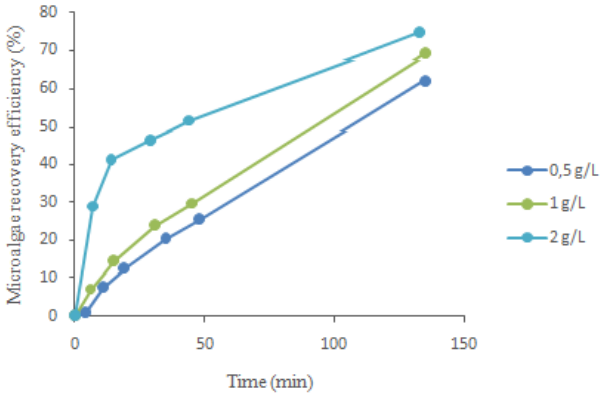

Figure 1. Absorbance of solution with different doses of flocculant.

Finally, the efficiency of the flocculation process was determined by the presence of microalgae at the bottom of the jar tests in terms of absorbance. It was observed after 24 hours of sedimentation that the microalgae recovery was successful with values among 63 and 76% (Figure 2). Definitely, it can be said that the copper (II) sulphate in a doses of 2 g/L is suitable for recovering *Selenastrum Tricornutum* microalgae.

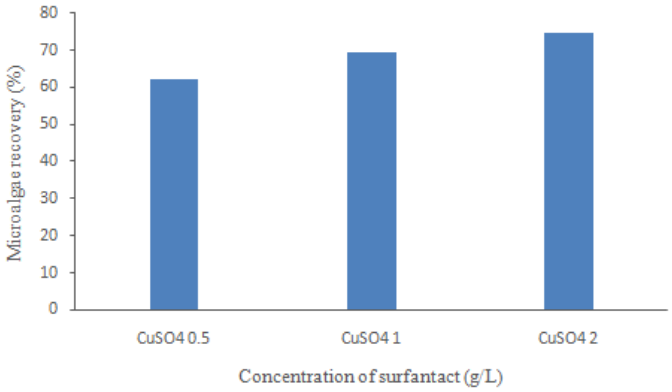

Figure 2. Microalgae recovery (%) for different doses of copper (II) sulphate.

3.2 Humidity

The moisture content is an important parameter since a greater presence of water in the sample lead to a worse extraction, obtaining fewer lipids. Therefore, the moisture content was analysed and the humidity curves for *Selenastrum Capricornutum* microalgae are shown in Figure 3 and 4.

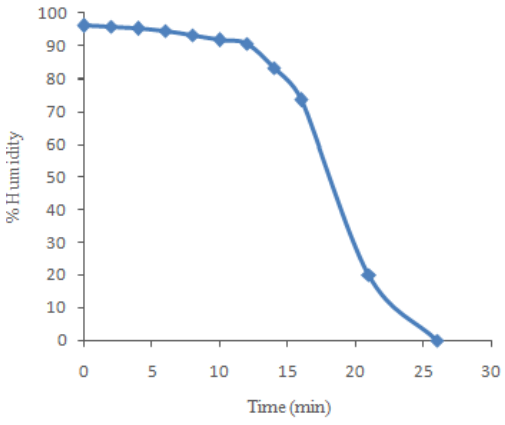

Figure 3. Humidity curve of Selenastrum *Capricornutum sample.*

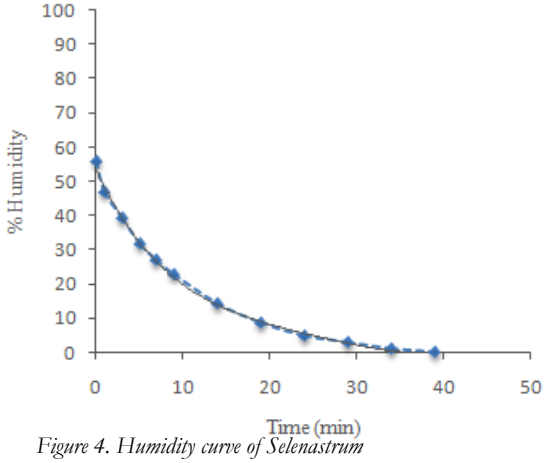

Figure 4. Humidity curve of Selenastrum Capricornutum sample kept in the refrigerator.

Figure 3 shows the humidity curve for a drying the biomass sample after it has been harvested. This sample presents a moisture content quite high, around 96%. However if the same sample is preserved in refrigerator its moisture content decreases. This is because the sample dries inside the refrigerator. This effect is depicted in Figure 4 where humidity percentage has decreased going from 96% to 55%.

3.3 Lipid extraction

The lipid content extracted by two different methods: Ultrasound and Soxhlet, expressed as % for *Selenastrum Capricornutum* is presented in Table 1. Based on these experiences, the best technique for extracting lipids was ultrasound-assisted extraction with methanol, achieving 95% of extracted lipids per dried microalgae. On the other hand, ultrasound-assisted extraction with acetone recovered the fewest amount of lipids, around 10%. Chloroform-methanol combination showed worse lipid recoveries than using only methanol as solvent. While ultrasonic assisted extraction causes the disruption of cells, Soxhlet extraction process is based on mass transfer. When acetone was used as extracting agent, the results were better in Soxhlet extraction (11.30%) than in ultrasound (9.92%).

Table 1. Percentage extracted lipids per Soxhlet and Ultrasound.

Method	**Amount of extracted lipids**		
	Solvent	***% Humidity***	***% w/w***
Soxhlet	N-hexane	96	25.84
	Acetone	55	11.30
Ultrasound	Chloroform-methanol 1:2	96	50.49
	Chloroform-methanol 2:1	96	78.03
	Methanol	96	95.00
	Chloroform	96	45.58
	Ethanol	55	31.03
	Acetone	55	9.92

Throughout ultrasound-assisted extraction, the temperature was measured by a thermometer inserted in the flask. It can be seen that temperature increased in all experiments (Figure 5). All used solvents presented similar temperature variations of the order of 4.5 to 5 ºC. However, ethanol presented a slightly greater variations (6.5 ºC) as well as acetone (6 ºC). As was remarked above, smaller temperature variations are produced because ultrasonic cavitation leads that implosive cavitation bubble collapse, producing intense local heating.

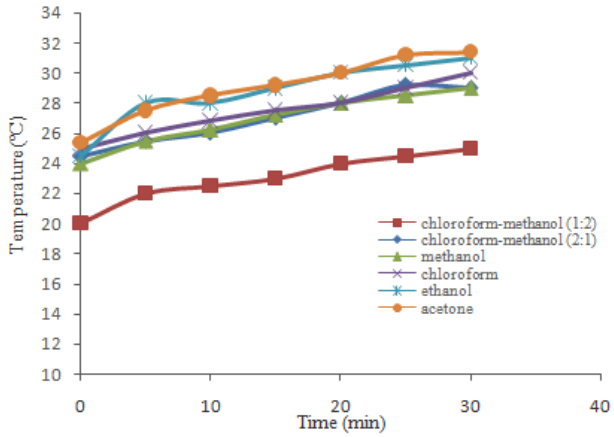

Figure 5. Temperature curve of ultrasound-assisted extraction process.

4. Conclusions

The present study investigated the effects of different doses of coagulants and the best results were obtained with Copper (II) sulphate as coagulant in doses of 2 g/L achieving a recovering *Selenastrum Capricornutum* microalgae of 76%. Fewer doses imply lower recoveries.
On the other hand, results revealed that the use of the methanol-assisted ultrasound is the most efficient method for lipids extraction for *Selenastrum Capricornutum* biomass. And finally, it can be said that the use of ultrasound favours cell disruption and increases the extraction yield.

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