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A CYLINDRICAL-CONICAL PHOTOBIOREACTOR AND A SLUDGE DRYING BED AS AN EFFICIENT SYSTEM FOR CULTIVATION OF THE GREEN MICROALGAE *Coelastrum* sp. AND DRY BIOMASS RECOVERY

FOTOBIORREACTOR TRONCO-CÓNICO Y ERA DE SECADO COMO SISTEMA EFICIENTE PARA EL CULTIVO Y RECUPERACIÓN DE LA BIOMASA SECA DE LA MICROALGA VERDE *Coelastrum* sp.

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Abstract

Microalgae are photosynthetic microorganisms that are studied due to the variety of metabolites they produce. However, if cultivated in photobioreactors, these devices should be easy to scale up. Also, harvesting and drying the microalgal biomass should be economically viable at industrial scale. Based on this point of view, in this work a 1.5 L cylindrical-conical photobioreactor was designed to cultivate and harvest the microalga *Coelastrum* sp. under nitrogen limitation. A cell density of 23.47 × 106 cells mL^{-1} was attained. Biomass and lipid productivities after 5 days of nitrogen limitation were 266.24 mg L^{-1} d^{-1} and 137.51 mg L^{-1} d^{-1} respectively. A lipid content of 45.6% (w/w dry biomass) was attained. A sludge drying bed was also designed to dry the microalgal biomass. The retention filter used in the sludge drying bed allowed an 86.8% of cell retention efficiency, and the final water content of the dried biomass was 7.8% ± 1.99 which allowed the lipid extraction without further drying. The lipid profile after transesterification of triacylglycerides was [C14:0 (3%), C16:0 (35.5%), C18:0 (42.5%), C18:1 (14.3%), C18:2 (4.6%)] which indicates that the lipid extract is more suitable for green diesel production than biodiesel.

Keywords: harvest, dewatering, nutrient depletion, lipid content, biofuels.

Resumen

Las microalgas son microorganismos fotosintéticos de interés biotecnológico debido a la variedad de metabolitos que producen. Cuando son cultivadas en fotobiorreactores, estos sistemas deben de ser fácilmente escalables. Por otro lado, la cosecha y el secado de la biomasa producida deben ser económicamente viables a escala comercial. Por ello, en este trabajo se diseñó un fotobiorreactor tronco-cónico de 1.5 L para cultivar y cosechar la biomasa de *Coelastrum* sp. Se alcanzó una concentración celular de 23.47 × 106 células mL^{-1}. Las productividades de biomasa y lípidos fueron de 266.24 mg L^{-1} d^{-1} y 137.51 mg L^{-1} d^{-1} respectivamente, después de 5 días de limitación de la fuente de nitrógeno. El contenido de lípidos totales fue de 45.6% en base seca. Se diseñó una era de secado que permitió una eficiencia en la retención de biomasa del 86.8%, con un contenido de humedad del 7.8% lo que permitió la extracción de los lípidos sin necesidad de una etapa de secado posterior. El perfil de lípidos después de la transesterificación fue [C14:0 (3%), C16:0 (35.5%), C18:0 (42.5%), C18:1 (14.3%), C18:2 (4.6%)] lo que indica que el extracto lipídico es más adecuado para producir diésel verde que biodiesel.

Palabras clave: cosecha, secado, limitación de nutriente, contenido lipídico, biocombustibles.

1 Introduction

Among the eligible sources for generating renewable energy, microalgae are notable for their versatility, given that they can be used as feedstock to produce a variety of biofuels: biodiesel, bioethanol, biomethane, bio-oil, biojet fuel or biohydrogen. Also notable is their capacity for wastewater bioremediation (Lam and Lee, 2012). Since the 1970s, microalgae have

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been considered as an alternative energy source due to their significant advantages compared to oil crops. Nevertheless, when large-scale biofuel production is considered, many technological barriers appear.

The cultivation systems used for their development, as well as methods for harvesting (including biomass concentration and dehydration) and oil extraction are highly energy consuming (Su *et al*., 2017) and need to be improved if commercial exploitation is intended. Microalgae are 10% more efficient at converting light (compared to higher plants) to synthesize starch, oils and sugars that can be used for biofuel production. Additionally, they present better assimilation of CO_2, water and other nutrients, meaning that they are capable of producing 30 times more oil per unit area than oil crops (Khan *et al*., 2009; Pandey *et al*., 2011). It is estimated that there are around 200,000 species of microalgae of which only 35,000 have been studied. Their great variety makes them a source of different products of economic importance, such as carotenoids, antioxidants, fatty acids, polymers, peptides, sterols, and enzymes, among other valuable compounds (Hu *et al*., 2008; Pulz, 2001; Spolaore *et al*., 2006). According to the literature, the microalga *Coelastrum* sp. has high potential for oil production (Minillo *et al*., 2013) and its high lipid productivity and adequate fatty acid profile have demonstrated its economic potential as a raw material for biofuel production (Zhiyong *et al*., 2013).

Having selected the microalgal strain, it is necessary to develop systems that facilitate its cultivation. As such, the design and optimization of bioreactors for the growth of these microorganisms is crucial (Dragone *et al*., 2010). High volumetric productivity, minimal investment and maintenance costs, simple control of the different culture parameters (agitation, temperature, pH, etc.), as well as high technological reliability are key factors that should be studied (Olaizola, 2013). There are two types of microalgae growth systems: open and closed bioreactors. In general terms, open systems present advantages such as ease of construction and operation, and they are capable of using sunlight and wastewater from treatment plants (Dragone *et al*., 2010). However, they also present a series of disadvantages, such as a limited number of cultivable microalgae species and evaporation. The latter factor complicates the preservation of water content in the bioreactor and also permits the loss of CO_2 (only 13-20% is absorbed by the cultures). Furthermore, they require large areas of land (0.2 to 0.5 ha) and productivity is lower than in closed systems (0.117 kg m^{-3} d^{-1}) (Darzins *et al*., 2010; Borowitzka, 1999; Pushparaj *et al*., 1997; Su, 2008; Chiaramonti *et al*., 2012). In light of these problems, competitive closed systems have been developed for cultivating microalgae. Photobioreactors are mainly used for unialgal cultures (Chisti, 2007). They are characterized by virtually complete regulation and control of the biotechnological parameters involved in the process (pH, illumination and agitation), maintaining low risks of contamination, they do not present CO_2 losses, and their hydrodynamics and temperature are controllable (Pulz, 2001). Likewise, they present high productivity with respect to reactor volume and they reduce media evaporation, making them appropriate systems for cultivating microalgae (Schenck *et al*., 2008). Disadvantages such as water condensation, biological contamination (Chaumont, 1993), and reduced light penetration due to the material used for their construction are some of the problems with this equipment, meaning that it is important for them to have a high volume-surface area ratio, which allows for better photosynthetic efficiency of the species, resulting in higher productivity (1.535 kg m^{-3} d^{-1}) (Carvalho *et al*., 2006; Chisti, 2007).

One of the most challenging steps in the biofuel production process is the harvesting of microalgal biomass. This is due to the microscopic size of the cells, meaning that this stage accounts for 20-30% of the total production cost. The harvesting method depends on the biomass suspension density and the characteristics of the selected strain (Grima *et al*., 2003; Greenwell *et al*., 2010; Mata *et al*., 2010; Uduman *et al*., 2010). Flocculation is a harvesting process covering a broad range of techniques: autoflocculation, which is produced by the presence of magnesium and calcium ions in the medium at a high pH; bioflocculation uses biodegradable polymers such as chitosan and sodium alginate (González and Ballesteros, 2010); electroflocculation is a complex electrical process involving chemical species such as metallic ions (Mollah *et al*., 2004); organic flocculation is the addition of a high-molecular-weight substance that leads to a greater increase in the size of the flocculate (Lertsutthiwong, 2009), and inorganic flocculation, in which compounds such as iron chloride, aluminum sulfate and ferric sulfate are used, being the most efficient. The disadvantage of these flocculants is that they contaminate the biomass (Shelef and Soeder 1980; Janssen *et al*., 2003; Cui *et al*., 2014). Flocculant efficiency is affected by the pH

of the medium, biomass concentration, the types of polymers used, the charge density of the molecules, and the initial strength and mixing degree of the fluids (Grima *et al*., 2003). Flotation is the process by which microalgal cells are captured using micro-bubble air diffusers. In contrast to flocculation, it does not require the addition of chemicals (Laamanen *et al*., 2016). This method is faster than sedimentation because it only requires a couple of minutes rather than hours for collection (Uduman *et al*., 2010; Bruton *et al*., 2009). Centrifuging is an accelerated sedimentation process that operates by rotating the walls of a container or by means of fixed-wall systems called hydrocyclones.

Biomass recovery by this method depends on the sedimentation characteristics of the cells, their residence time in the centrifuge and the sedimentation depth in the equipment (Grima *et al*., 2003; Dassey and Theegala, 2013). This is the preferred method for collecting microalgae, given that it does not require the addition of flocculants. However, it does require a greater supply of energy, making it too expensive on a large scale. Biomass collection by this method presents an efficiency of over 95% (Heasman *et al*., 2000). Filtration is ideal when dealing with relatively large microalgae ($> 70\ \mu$m). However, for species with bacterial dimensions ($< 30\ \mu$m) it is inadequate, meaning that it is recommended to use microfiltration and ultra-filtration membranes as collection devices (Hwang *et al*., 2015).

Drying is an important step, because the water content present in the algal biomass inhibits the lipid-extraction and transesterification processes (Lam and Lee, 2012). As such, drying of the raw material is advantageous for stable storage. Drying accounts for 70-75% of the total cost of the process. Although the majority of methods for drying sludge can also be used for biomass treatment, not all of them are appropriate for processing microalgal sludge (Ryan, 2009; Show *et al*., 2013). In the case of wastewater, there is a variation on this method called a drying bed, which consists of a bed of sand and gravel over which the material to be dried is dispersed. This variation can also be applied to algal biomass (Ramalho, 1996), but reports in the literature using this material are scarce. Sun-drying has also been used as a method for drying microalgal biomass in recent years (ViajayaVenkataRaman *et al*., 2012). Dehydration is complete when the material has a moisture content of 10%, and the exposure time can be just one day (Show *et al*., 2015). Sand improves the separation of biomass water content, increasing the drying area and therefore increasing drying speed, whilst solar radiation provides the energy to evaporate the moisture (Brennan and Owende, 2010; Brink and Marx, 2013).

The notable advantages of the sun-drying method include the reduced investment in equipment, the lack of dependence on fossil fuels, the use of marginal lands as drying areas, and the use of solar energy that has zero cost and is non-polluting. This drying method is highly advantageous, not just because of its economic viability, but also because it is a clean and sustainable process. The main disadvantages of this method are its dependence on regional climate and the need for large areas of land (Ryan, 2009; Brennan and Owende, 2010; Show *et al*., 2013).

The objective of this study was to determine the efficiency of a 1L cylindrical-conical photobioreactor coupled to a sludge drying bed in the harvesting and drying of *Coelastrum* sp. cell biomass.

2 Materials and methods

2.1 Strain

The strain *Coelastrum* sp. (UADY-PRIORI-014-FMVZ-05) was provided by the Facultad de Medicina Veterinaria y Zootecnia of Universidad Autónoma de Yucatán (Yucatán, Mexico).

2.2 Culture medium

Growth and lipid production cultures were carried out in complete TAP medium (Gorman and Levine, 1965) in 250 mL Celstir flasks with mechanical agitation as a reference and in 1.5 L photobioreactors with aeration. Nitrogen limitation was attained by feeding the 250 mL Celstir flask and the photobioreactor with 20 mL of concentrated TAP medium (the concentration was calculated to obtain a final concentration equal to TAP 1X when diluted in the flask and photobioreactor) without NH_4Cl, but maintaining the $Mo_7O_{24}(NH_4)_6 \bullet 4H_2O$ in the Hutner solution (0.011 mg L^{-1} nitrogen). Media were sterilized at 121 °C and 1.1 atm for 15 min (Herrera *et al*., 2011).

2.3 Photobioreactor design and culture conditions

The photobioreactor was designed following the principle of a circular bubble column bioreactor, as reported by Chisti (1989).

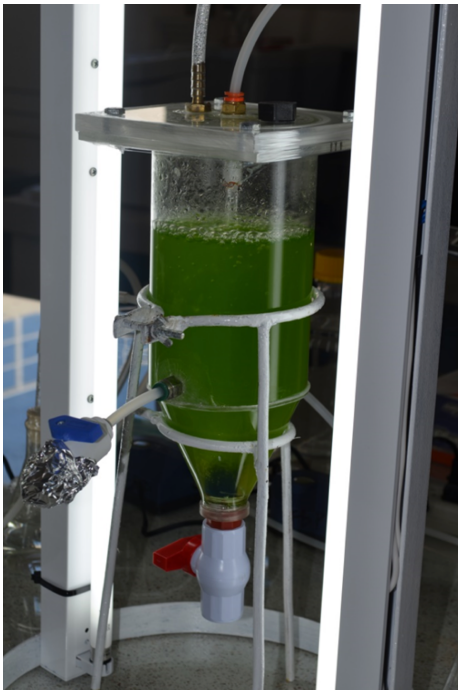

Fig. 1. Conical-cylindrical photobioreactor.

It was made of commercial acrylic plastic tube and consisted of a cylindrical section 16 cm in height (10 cm in diameter) and a conical section with a bottom diameter of 2.5 cm (Fig. 1).

Total volume inside the photobioreactor was 1.5 L and the working volume was 900 mL of culture medium. Aeration was provided by an inner tube with an air sparger at the end of it. A flat flange on the top of the photobioreactor allowed the use of a flat top lid of the same material. After biomass sedimentation, a valve on one side of the cylinder allowed the clarified medium to be discharged and another valve at the bottom of the bioreactor allowed the biomass sludge to be recovered. Ambient air (1.6 vvm) was supplied by an Elite® 800 commercial aquarium air pump. The air was humidified by bubbling it through sterile distilled water. The photobioreactor was equipped with two 24 W fluorescent lamps providing light intensities of 81 and 38 μmol m^{-2} s^{-1} outside and inside the photobioreactor, respectively. A 16:8 (light:dark) photoperiod was used. The photobioreactor was disinfected with 10% sodium hypochlorite solution for 1 hour and rinsed with sterile water. It was maintained under UV radiation for 1.5 h before culturing. Cultures were carried out at laboratory temperature: 25 ± 2 °C. A 10% (v/v) inoculum from a 4-day-old culture was used.

2.4 *Sludge drying bed design*

The sludge drying bed design was based on the method reported by Ramalho (1996). The container was made of commercial acrylic plastic with a cubic shape (7.5 × 11 × 27 cm). A 2 cm high upper layer of sand (particle diameter = 0.3-2 mm) was placed over an 18 cm layer of gravel (particle diameter = 3-25 mm). Both layers were supported by a grill to allow evacuation of the filtrate. To retain the biomass, a commercial layered fabric (blackout curtain fabric) was used as a filtration device on top of the sand layer.

2.5 *Growth evaluation*

Cell concentration was determined using a Neubauer hemocytometer and a Nikon Eclipse E200 microscope at 40X objective magnification. To determine dry biomass weight, 5 mL samples were collected from the photobioreactor every 24 h and centrifuged at 4,000 rpm for 10 min at 4 °C. Pellets were cooled to −80 °C and freeze-dried for 24 h. At the end of the experiments, the cultures were left to settle overnight. In the case of the Celstir flasks, the clarified medium was eliminated and the microalgal sludge was centrifuged at 4,000 rpm for 10 min and 4 °C. The pellet was then freeze-dried for 24 h. With the photobioreactor, the clarified medium was eliminated via the spent medium valve and the microalgal sludge was recovered via the biomass recovery valve. The sludge was poured onto the sludge drying bed and left to dry for 4 days at room temperature. No further drying was needed for lipid extraction. The duration of the exponential growth phase was 4 days.

2.6 *Lipid extraction*

Total lipids were extracted with a chloroform:methanol (2:1 v/v) mixture. 15 mL of solvent mixture was used for 38 mg of dry biomass. After extraction (5 h at 150 rpm and 38 °C, 3x), the solvent was vacuum evaporated to obtain the crude lipid extract.

2.7 *Transesterification reaction*

In accordance with Herrera *et al*. (2011), the crude lipid extract was dissolved in 2 mL of n-heptane and 300 μL of a sodium methoxide solution (0.35% w/w of crude extract) and 300 μL of ethyl acetate were added. The reaction temperature was 60 °C and it was carried out under agitation for 1.5 h. After transesterification, the reaction medium was let to settle.

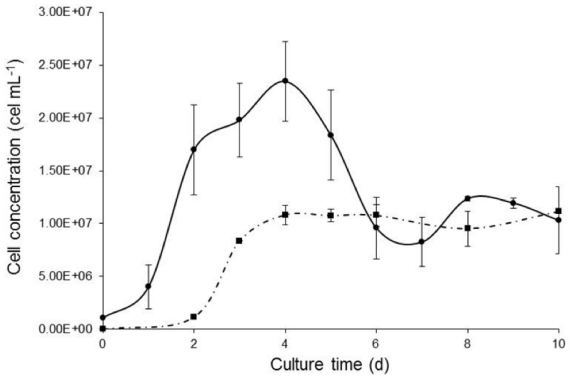

Fig. 2. Growth curves of the microalgae *Coelastrum* sp. in 1L cylindrical-conical photobioreactor (●) and 250 mL Celstir flask (■)

The upper phase (fatty acid methyl esters, FAME) was passed through an ion-exchange zeolite to retain traces of glycerol that was formed during the reaction and other impurities.

2.8 Gas chromatography analysis

GC analysis was carried out in a Perkin-Elmer Clarus gas chromatograph equipped with a FID detector. A Varian Select^{TM} Biodiesel for FAME capillary column (30 m × 0.32 mm ID × 0.25 μm film thickness) was used to quantify FAME. The carrier gas was N_2 at 7 psi and 80 mL min^{-1}. Temperature conditions were 180 °C for 2 min and a 5 °C min^{-1} ramp to 250 °C for 25 min. The injector temperature was 270 °C and the FID detector temperature was 300 °C. The sample injection volume was 1 μL. The external standard technique and the following standards were used for quantification: miristic acid methyl ester, palmitic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester and linolenic acid methyl ester (Sigma-Aldrich).

2.9 Statistical analysis

All experiments were carried out in triplicate (n = 3). For statistical analysis, a one-way ANOVA ($p \leq 0.05$) was performed with Excel 2013 (Microsoft Corporation, Redmond, WA) software.

3 Results and discussion

3.1 Growth curves

Growth curves were carried out in 250 mL Celstir flasks and the 1.5 L photobioreactor (Fig. 2). Inoculum in the flask was 10,000 cells mL^{-1}. A two-day adaptation phase was observed and then a two-day exponential growth phase occurred, followed by a stationary phase until day 10 of culture. The photobioreactor was first inoculated with 10,000 cells mL^{-1}, but important bacterial growth was detected because the photobioreactor was only disinfected (the flask was sterilized). A higher cell concentration (1.082 × 106 cells mL^{-1}) was found to be adequate to inoculate the photobioreactor in order to avoid predominance of bacterial growth. The adaptation phase in the photobioreactor was one-day long, followed by a three-day exponential growth phase; then a decline phase was observed. At day 4 of culture, cell concentration in the photobioreactor was double that of the 250 mL Celstir flask, but cell concentrations were similar in both culture systems after the decline phase in the photobioreactor. It has been reported that the microalga Scenedesmus obliquus showed an increase in cell concentrations at aeration rates of 1.34 vvm (Leupold *et al*., 2013). When *Chlorella*, *Spirulina* sp. and *Scenedesmus obliquus* were cultivated in a tubular photobioreactor with aeration and CO_2 (6%), biomass production was double that obtained in Erlenmeyer flasks (de Morais and Costa, 2007). The increase in cell concentration in the photobioreactor is due to a higher inoculum volume and enhanced mixing in the medium that promotes higher growth rates and higher photosynthetic yields (more light is available for the cells) as reported by Robles-Heredia *et al*. (2016). Higher growth rates could result in economically viable processes at industrial scale (Henrard *et al*., 2011). The presence of a decline phase in the photobioreactor can be explained by the faster growth of the biomass, which contributes to a faster decay in the nutrient concentration of the culture medium and a decrease in viable cells. The end of exponential growth for the 250 mL Celstir flask and the photobioreactor was established at day 4 of culture and this was the point when more nutrients were added (without nitrogen source) to begin the nitrogen limitation phase, which was carried out for 6 more days, giving a total culture time of 10 days.

Table 1. Dry biomass and lipid productivities of the microalga *Coelastrum* sp.

Bioreactor	Culture time (d)	Biomass productivity ($mg{\cdot}L\text{-}1{\cdot}d^{-1}$)	Lipid Productivity ($mg{\cdot}L\text{-}1{\cdot}d^{-1}$)
250 mL Flask	4[a]	103.6 ± 28.6	48.6 ± 17.2
	10[b]	229 ± 13.1	89.1 ± 3
1.5 L photobioreactor	4[a]	146.1 ± 4.7	49.4 ± 2.9
	10[b]	266.2 ± 18.3	137.5 ± 4.3

[a]End of exponential growth phase; [b]End of nitrogen limitation

3.2 Biomass and lipid productivity

Samples taken at the end of the exponential growth and nitrogen limitation phases were analyzed for biomass and lipid productivity. Dry biomass production at the end of exponential growth reached 0.52 ± 0.14 and 0.68 ± 0.02 g L^{-1} in the 250 mL Celstir flask and 1.5 L photobioreactor, respectively. After 6 days of nitrogen limitation, dry biomass increased to 1.14 ± 0.06 and 2.01 ± 0.07 g L^{-1}, respectively.

It has been reported that a 44.77% reduction in biomass production of the strain *Scenedesmus* sp. CCNM 1077 was observed when the nitrogen concentration in the culture medium was reduced from 247 to 0 mg L^{-1} (Pancha *et al*., 2014). Lipid production at the end of exponential growth was 0.24 ± 0.08 g L^{-1} in the 250 mL flask and increased to 0.44 ± 0.01 g L^{-1} after nitrogen limitation. In the photobioreactor, lipid production also increased after nitrogen limitation, showing values of 0.22 ± 0.01 g L^{-1} at the end of the exponential growth phase and 0.91 ± 0.03 g L^{-1} after nitrogen limitation. The increase in lipid production by microalgae after nutrient stress is well reported. In *Chlamydomonas reinhardtii* cultures, an increase in neutral lipids, triacylglycerides and carbohydrates after 4 days under nitrogen or sulfur limitation has been reported (Cakmak *et al*., 2012). Cultures of *Chlorella pyrenoidosa* showed a slight decrease in biomass concentration due to the absence of a nitrogen source ($NaNO_3$), but an increase in lipid content which resulted in productivities of 115 mg L^{-1} d^{-1} (Han *et al*., 2013). Dry biomass productivity was higher in the photobioreactor (Table 1) both at the end of exponential growth and the end of nitrogen limitation. In the case of lipid productivity, no statistical difference was found at the end of exponential growth between the 250 mL Celstir flask and the photobioreactor, but lipid productivity was higher after nitrogen limitation in the photobioreactor.

3.3 Sludge drying bed efficiency

The biomass recovery efficiency of the blackout curtain fabric was accomplished by determining the amount of biomass retained in the fabric and the loss was calculated using centrifuged biomass as a 100% recovery reference. After overnight sedimentation in the photobioreactor, approximately 650-700 mL of clarified culture medium and 350-700 mL of concentrated microalgal sludge were obtained (initial working culture volume was 900 mL). The biomass sludge from the photobioreactor was poured onto the fabric and left to dry (Fig. 3a). After 3 days, the dried biomass detached easily from the fabric (Fig. 3b and c). Biomass recovery efficiency using the fabric was 86.8 ± 7.4%. From the concentrated microalgal sludge, 1.25 ± 0.43 g of dry biomass with a final water content of 7.82% ± 1.99 was obtained. Drying efficiency was 92.1 ± 1.9%. This method has been used to dry *Spirulina* biomass in Sde Boker, Israel, resulting in dry biomass with 10% moisture content in 1 day (Ryan, 2009). In India, the same species was dried using this methodology with a drying time of 5-6 h, obtaining a final product with 4-8% moisture content (Show *et al*., 2013). When the biomass sludge was centrifuged, a biomass pellet of 6 ± 1.51 g with a moisture content of 79% was obtained. This water content is very high for the next lipid extraction step, making an additional drying step necessary. In this study, the biomass from the sludge drying bed was directly used for lipid extraction without further drying due to the low water content.

3.4 Fatty acid methyl esters (FAME) profile

FAME profiles at the end of the exponential growth phase and after nitrogen limitation are reported in Table 2. It has been reported that nutrient and culture conditions significantly affect fatty acid composition in microalgae (Gao *et al*., 2013; Navarro-Peraza *et al*., 2017). Fatty acid composition also depends on the microalgae strain (Wu and Miao, 2014). Ratha *et al*. (2013) reported that the microalga *Chlorella*

sp. MIC-G4, cultivated using NaNO3 as nitrogen source and then subjected to nutrient limitation, showed an improvement in lipid accumulation. The fatty acids produced by this strain were C16:0 (19.5%), C18:2 (32.1%) and C18:3 (18.0%). This distribution varied when the strain MIC-G6 of the same microalga was used, producing 27.3, 6.5 and 43.7% of the same compounds respectively. When another microalga, *Scenedesmus* sp. MIC-G8, was cultivated under the same conditions, the profile obtained was C16:0 (22.3%), C18:2 (26.7%) and C18:3 (15.5%). In another work, the microalga *Scenedesmus incrassatulus* cultivated under nutrient limitation produced a different profile: C16:0 (24.9%), C18:1 (45.4%) and C18:3 (5.9%) (Arias-Peñaranda *et al*., 2013). The distribution of FAMEs within the lipid extract of microalgae can be an indicator of the type of biofuel that can be synthesized. In the case of biodiesel, the presence of C16:0 and C18:0 chains contributes to a higher cetane number of the mixture and a higher oxidative stability, whereas C18:1 and C18:2 induce better behavior of the biofuel at low temperatures (Ganduglia *et al*., 2009). There is no quantitative profile for the perfect balance of FAMEs for biodiesel production. The best mixture of components in the lipid extract would be the one that provides a biodiesel that complies with the international standards. The microalga *Coelastrum* sp. used in this work showed a lipid profile of 78.5% and 21.5% of saturated and unsaturated FAMEs, respectively, at the end of the exponential growth phase in the photobioreactor (4 days). After a 5-day nitrogen limitation phase, the amount of saturated FAMEs increased to 81.1% and unsaturated FAMEs were 18.9%. A small amount of the di-unsaturated linoleic acid methyl ester was also observed. This increase of saturated FAME after nitrogen limitation has already been reported (Valdez *et al*., 2015). These results are consistent with those reported in the literature, showing that the use of a sludge drying bed does not affect the lipid extraction step and the lipid content.

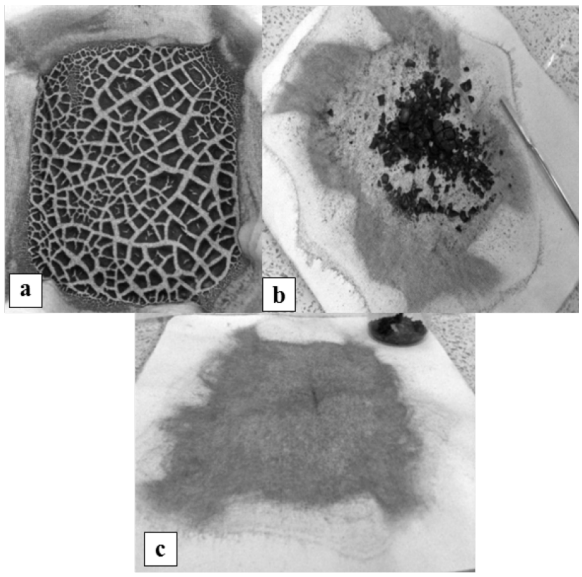

Fig. 3. Retention filter with (a): dried biomass; (b): recovered biomass; (c): used filter.

Table 2. FAME profiles of the microalga *Coelastrum* sp.

		% Total FAME	
	FAME	End of exponential growth	After nitrogen limitation
250 mL Flask	C14:0	19.6	11.7
	C16:0	9.7	38.7
	C18:0	47.8	4.5
	C18:1	0	1.7
	C18:2	22.9	43.4
1.5 L photobioreactor	C14:0	37.8	3.1
	C16:0	38.3	35.5
	C18:0	2.4	42.5
	C18:1	21.5	14.3
	C18:2	0	4.6

Conclusions

The use of an aerated photobioreactor for the cultivation of the microalga *Coelastrum* sp. resulted in an increase in biomass and lipid productivities compared to the cultures carried out in 250 mL Celstir flasks. As the photobioreactor could not be sterilized, only disinfected, a 10% (v/v) inoculum was necessary to prevent the growth of other microorganisms in the culture media. These results are interesting because the conditions in the photobioreactor are more suitable for scaling-up of the culture system. The conical bottom of the photobioreactor facilitated the settlement of the cells without the use of flocculants, which is an advantage of the process, because the extract will be free of such chemicals. The microalgal slurry was easily recovered from the photobioreactor and the use of the sludge drying bed avoided the energy consuming centrifugation step. However, it was noticed that the drying of the microalgal slurry should not take more than two days at room temperature or the biomass begin to decompose. More studies are required to shorten the microalgal biomass drying time in the drying bed so it can be scaled-up. Finally, the lipid content of the microalga *Coelastrum* sp. make it suitable for biofuels production.

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