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Microalgal Biomass: A Biorefinery Approach

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Abstract

The biorefinery concept has been identified as the most promising way to create a biomass-based industry, which can be defined as the sustainable biomass processing to obtain energy biofuels and high-value products through processes and equipment for biomass. Microalgae can be used as an efficient and economically viable biorefinery feedstock; microalgae could be used in different areas such as human and animal nutrition, nutraceutical and therapeutic products, fertilizers, plastics, isoprenes and biofuels and also in the treatment of wastewaters and CO_2 capture. Microalgae biomass can be used for biofuel production, such as bioelectricity, methane, biohydrogen, bioethanol and biodiesel. In this chapter, an overview of the factors that affect the production of the microalgal biomass yield and value-added by-products production is presented. Likewise, we present the results of the microalgal culture of *Scenedesmus* sp. SCX2 performed in semicontinuous culture (SCC), in 2000 raceway ponds employing Bold's Basal Medium (BBM), under greenhouse conditions. Over the SCC, we monitored biomass concentration, lipid, protein, pigments and sugar production, light irradiance, culture and greenhouse temperature and nitrate concentration in the medium.

Keywords: biomass, microalgae, *Scenedesmus* sp. SCX2, biorefinery, biofuels

1. Introduction

Microalgae have the ability to convert solar energy into chemical forms though photosynthesis, in which CO_2 is captured from the atmosphere and oxygen is produced during the process, furthermore, microalgae offers the potential to produce high-value compounds such as nutraceuticals and compounds for human consumption, and also low-value products as feed, biofertilizers and bioenergy, as well as wastewater treatment, nitrogen fixing and CO_2

mitigation [1, 2]. Microalgae have been taken into consideration as a feedstock for renewable biofuels production, such as bioelectricity, methane produced by anaerobic digestion of the algal biomass, biohydrogen produced under anaerobic conditions, bioethanol (sugar fermentation) and biodiesel derived from microalgal oil [1, 3–5]. Microalgae offer, compared to other renewable sources, higher growth rates and require less water than terrestrial crops [6]; microalgae have a very short harvesting cycle (1–10 days) compared to other land-based feedstock (which are harvested once or twice a year) [7]; algae cultures do not compete with food or arable lands [8]; their phototrophic growth has a favorable environmental impact, since the CO_2 that is released to the atmosphere during hydrocarbons combustion is recycled by microalgae in photosynthetic processes (100 biomass tons require about 183 CO_2 tons) [9]. Microalgae have higher photosynthetic efficiency than crops. Therefore, microalgae biomass cultivation will help in reducing the CO_2 in the atmosphere at a faster rate than land-based crops. Phototrophic metabolism of microalgae is mainly influenced by light irradiance, culture temperature and nutrients supply. However, in large-scale open systems as raceways, it is practically impossible to control diurnal fluctuations of irradiance and temperature [10, 11]. However, raceways are the most commonly employed technology for microalgae biomass production due to their low cost of construction and operation. Microalgae biomass production for obtaining just biofuels is not economically feasible, for which it is necessary to use high-productivity and economic reactors, as well as the use of economic and available nutrients; likewise, the maximum valorization of biomass, including the carbon fixation and wastewater treatment by integrating the concept of industrial ecology. All previous allows the conceptualization of microalgal biorefinery.

This chapter addresses the microalgal biomass production from a biorefinery point of view and describes the improvements of the microalgal biomass production in open systems raceways (RW) of 2000 L capacity operated in semicontinuous culture under greenhouse conditions.

2. Biorefineries

In a conventional refinery, the raw oil undergoes several processes after which the products are used for transportation, electricity and the further production of more complex and valuable chemicals. On the other hand, the biorefineries allow the utilization of raw materials that may be widely available at low cost and sustainable for the production of high-value products that may be considered in terms of their energetic potential. Within a biorefinery there is a wide variety of configurations and generation of several end products. An appropriate definition of biorefinery is as follows: the sustainable processing of the biomass in a wide commercial spectrum of products and energy [12]. Other definition is sustainable biomass processing to obtain energy biofuels and high-value products through processes and equipment for biomass transformation [13]. The biorefinery concept has been identified as the most promising way to create a biomass-based industry.

A biorefinery can utilize all kinds of biomass such as wood (straw and bark), agricultural crops, organic waste (derived from vegetables and animals), industrial and domestic wastes and

aquatic biomass (algae and microalgae from sea and freshwater) [14]. The microalgae present a high potential for the biodiesel production compared to the terrestrial biomass [15]. The impact on the utilization of the soil for the microalgal production is significantly lower than the generated by the agricultural biomass based on corn, canola and switchgrass [16]. The versatility of the microalgal metabolism allows the application in different areas such as human and animal nutrition, nutraceutical and therapeutic products, fertilizers, plastics, isoprenes, biofuels and also in the treatment of wastewaters and CO_2 capture [17, 18]. Algae can be used as an efficient and economically viable biorefinery feedstock. An efficient biorefinery using algae can only be constructed through its integration with other industries [19].

2.1. Microalgae as raw material for a biorefinery implementation

Algae are a polyphyletic group of organisms (including organisms that do not have the same origin, but are multiple, independent evolutionary lines) that comprise both, unicellular and multicellular forms and both, prokaryotes and eukaryotes, which are able to capture light energy through pigments (such as chlorophylls, carotenoids, anthocyanins and phycoery-thrinsphycobilins) that are necessary to carry out photosynthesis [20, 21]. The classification of algae has five main branches: chromista (brown algae, golden brown algae and diatoms), red algae, dinoflagellates, euglenids and green algae [21]. Microalgae can be eukaryotic (Chloro-phyta, Rhodophyta and Bacillariophyta) or prokaryotic (Cyanophyta) [3]. Microalgae are a large group of microscopic algae that are considered primary producers on a global scale, and are involved in all marine and freshwater ecosystems [15, 22]. Microalgae are the largest autotrophic microorganisms of plant life taxa in the world. The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 20,000–800,000 species exist of which about 40–50,000 species are described [23, 24]. The biomass produces three major biochemical components by *de novo* synthesis consisting of lipids, proteins, carbohydrates, pigments and others.

The proposal to use microalgae for biofuels production is not new and was first suggested in the 1940s–1950s. In the 1970s, the large-scale cultivation of microalgae for producing sustain-able liquid fuels was previously investigated and in the 1990s extensive research was carried out, especially at the Solar Energy Research Institute in Golden, Colorado, USA [3, 4, 9, 25].

Specifically, microalgae production offers the potential for the production of high-value compounds, products for human and animal nutrition, bioactive compounds, fluorescent pigments, stable-isotope biochemicals, drug screening, microalgal recombinant proteins, cosmetics, biofertilizers and wastewater treatment, nitrogen fixing, CO_2 mitigation and bio-energy production [2, 9, 21, 25–28]. In this manner, these microscopic organisms have been taken into consideration as a feedstock for the production of renewable biofuels such as bioelectricity, methane produced by anaerobic digestion of the algal biomass, biohydrogen produced under anaerobic conditions, bioethanol (sugar fermentation) and biodiesel de-rived from microalgal oil [3, 4, 25, 27, 29, 30]. The environmental factors such as illumina-tion, temperature, the amount of nutrients and the salinity affect the photosynthesis and the productivity of the cellular biomass by influencing the pathway and the activity of the cellular metabolism [31, 32].

2.2. Open pond culture systems

Microalgae can be grown in open ponds or in closed systems named photobioreactors. The use of open systems to produce biofuel feedstock is 2.5-times less expensive than using photobioreactors [33]. For that reason, open systems are currently the preferred option for large-scale bioprocessing. There are many types of open ponds used for microalgae cultivation such as raceway, shallow ponds or circular ponds. Raceway pond is the most common open system due to its potential to produce large quantity of microalgae. In a raceway pond system, the cultures are circulated around a race track by using paddle wheels. This will keep the microalgae suspended in the medium and allow the utilization of CO_2 from the atmosphere; CO_2 can also be injected into the medium. The pond is shallow to allow the light penetration into the pond, which in turn maximizes the light exposure of the microalgae for photosynthesis. Open systems make it possible to reduce production costs, this is mainly due to the greater investment required for closed photobioreactors, which increases the depreciation costs (making up as much as 78% of the total microalgae production cost) and also due to the greater power consumption of closed photobioreactors, which likewise increases the operational costs [34]. By using open reactors both the depreciation and the operating costs are reduced. At the same scale and under the same conditions as those considered for tubular photobioreactors, the microalgae production and the reactor costs are reduced almost 4 and 20 fold, respectively, for raceway reactors [35].

The temperature and light irradiance variations are practically impossible to control in outdoor cultures [10]. Such fluctuations can affect microalgal growth at the point to collapse the whole culture [36]. Furthermore, contamination by predators, protozoa, ciliates and other fast growing heterotrophic bacteria have restricted the commercial production of algae in open systems to only those organisms that can grow under specific conditions such as high pH [37], hyper-saline cultures [26] or where suitable irradiance is available. For all these reasons, a crucial factor for the success in culturing open systems is to choose a microalgal strain with the ability to grow in a selective medium, in outdoor environmental conditions with relatively constant productivities [38, 39].

The operational mode of the open systems is important to be considered. The main cultivation modes are in batch, fed-batch, continuous and semicontinuous culture (SCC). The SCC consists of periodic withdrawals of volume from the culture and the removed volume is reinstated with fresh medium. This culture mode is propitious for long-term outdoor cultures, because the growth is maintained in log phase, it avoids the shading effect caused by high cell density [40] and allows the inoculum and biomass availability during each harvesting cycle [41].

SCC cultures have been studied for wastewater removal [42], CO_2 fixation [43, 44] and to enhance biomass productivity in several microalgae species [40, 45, 46], but little work has been published on long-term pilot-scale open systems concerning its effect on the fatty acids methyl esters (FAMES) content, which are the feedstock for microbial biodiesel production.

2.3. Environmental factors and their effect on the microalgal growth

The microalgal growth depends on the availability and efficient utilization of light. A lack of an adequate illumination exerts a negative effect on the productivity, growth and other parameters such as lipids content. The microalgal may be in the respiration phase in the absence of light, in the limiting phase when the illumination is not enough, in a saturation phase if there is a maximum photosynthetic yield or they may be in an inhibition phase due to the suppression of the photosynthetic activity caused by an excess of light (photoinhibition) [47]. High temperatures and concentrations of dissolved O_2, low CO_2 concentrations, as well as a high intensity in illumination and a high pH in the medium, all promote an increase in the photorespiration [48].

The illumination can be supplied to the microalgal culture either by the sun light or by using artificial light such as fluorescent lamps, LEDs, optic fibers, etc. The sources of artificial light may be modified for a specific wavelength emission that leads to metabolic and physiological changes in the microalgae. For a large scale microalgal culture, the utilization of the sun light is recommended in order to operate at a low cost that allows a positive energetic balance. The utilization of artificial sources of light may be necessary for the production of high-value products, but in general, artificial light at large scale should be avoided [49].

2.3.1. Temperature

An adequate temperature is necessary for a microalgal photosynthetic activity in which there are no modifications either in the biochemistry or the physiology of the microalgae. The optimum temperature range at which the microalgal growth is between 20 and 25°C for mesophilic microalgal. The thermophilic strains can be cultured at 40°C and the psychrophilic strains can grow at 17°C. The computational modeling designed to estimate the effect of the temperature on the microalgal growth demonstrates that the temperature control at large scale is even more expensive due to the energetic requirement to control the temperature than the energy that eventually could be produced from the microalgal production (e.g. this is the case in the biodiesel production) [38]. The temperature is one of the most difficult factors to be optimized in the open culture systems due to climatic variations and the seasonal changes. This fact may cause a possible decrease in productivity [50].

2.3.2. Nutrients

A culture medium for microalgal culturing must contain all of the following: a carbon source, which makes up to 50% of the microalgal biomass. Nitrogen (N), which corresponds to approximately 5–10% dry weight basis of the biomass. Phosphorous (P), which is a component of the DNA, RNA, ATP and the cell membrane. Sulfur (S), which is a component in certain amino acids, vitamins and sulfolipids and is involved in the synthesis of proteins. Potassium (K), which is a cofactor for several enzymes and plays an important role in the osmotic regulation and the synthesis of proteins. Magnesium (Mg), which is found in the core of the chlorophyll molecule andiron (Fe), which is important during the assimilation of nitrogen and is part of the cytochromes. The trace metals, salts and organic

components in addition to the other elements mentioned above can be manipulated in order to modify the production of certain desired metabolites [51]. The utilization of wastewater that is rich in N and P as culture medium for microalgae is an economical alternative. Wastewaters can come of sectors such as domestic, commercial-service, industrial and agricultural [52]. One of the advantages that wastewater offers as a culture medium is the possibility of recovery and utilization of inorganic compounds simultaneous to the production of recycling or groundwater recharge water streams [53].

2.3.3. Salinity

An excess of sodium ions (Na^+) leads to a saline stress, whereas an osmotic stress may imply the presence or absence of sodium ions. An accumulation of Na^+ and chloride (Cl^-) ions causes an ionic imbalance in the cell. This decreases the capability to absorb minerals such as K, calcium (Ca^{2+}) and manganese (Mn^{2+}) [54]. Under a saline stress, the microalgae present change their metabolism in order to protect themselves from the osmotic damage. The salt concentration that can be tolerated by microalgae, bacteria and cyanobacteria is up to 1.7 M in marine medium [55].

2.4. Selection of the microalgal strain

The selection of the microalgal strain is of upmost importance because each strain has different and specific features. Microalgal strains may be acquired through a specialized collection or they can be isolated from extreme environments. The species isolated from local places present a better adaptation capability and often times can be adapted and grown under adverse and changing conditions and therefore, they tend to survive and grow under stressful conditions even better than the microalgae coming from specialized collections [56]. The biomass of the microalgae can be used in several forms. Biogas can be obtained from the anaerobic digestion of the biomass and the lipids are treated for a transesterification in order to produce biodiesel. The carbohydrates can be used to produce ethanol or butanol, whereas the biomass can be treated thermochemically or by gasification to obtain bio-oil [57]. The decision on the type of microalgae to use depends on its composition and the type of biofuel and other desired valuable products that need to be produced or that are of interest (**Table 1**).

Microalgal species	Mode of cultivation	BR	Biomass productivity (mg L^{-1} d^{-1})	Carbohydrates (%)	Proteins (%)	Lipids (%)	Ref.
Stichococcus sp.	Batch	Erlenmeyer flasks	167.00	40.63	26.25	11.56	[58]
Scenedesmus obliquus	Batch	1-L glass vessel	840.57	46.65	–	22.4	[59]
Scenedesmus obliquus	Semibatch	1-L glass vessel	883.80	52.90	–	–	[60]
Scenedesmus bijugatus	SCC	Vertical tubular	260.00	26.00	–	24	[61]
Chlorella vulgaris	Semibatch	Vertical tubular	268.10	–	52.2	–	[62]

Microalgal species	Mode of cultivation	BR	Biomass productivity (mg L^{-1} d^{-1})	Carbohydrates (%)	Proteins (%)	Lipids (%)	Ref.
Chlorella sp.	Batch	Vertical tubular	154.48	–	–	21.27	[63]
Chlorella ellipsoidea	Batch	Bubble column	31.55	–	41.75	–	[64]
Chlorella variabilis	Batch	Raceway	113.33	–	–	10	[65]
Chaetoceros muelleri	–	Fiberglass cylinders	–	12.7–19.3[*]	46.9–64.4[†]	22.1–33.2[‡]	[66]
Graesiella sp.	SCC	Raceway	36.00	–	–	33.4	[39]

[*]Winter, 18–19.3; spring, 15.2–15; summer, 12.7–13.6%.
[†]Winter, 49.3–46.9; spring, 54.2–56.3; summer, 59.2–64.4%.
[‡]Winter, 30.3–33.2; spring, 29.5–28.2; summer, 24, 22.1%.

Table 1. Microalgal strains for the production of biofuels and high-value products.

In this work, the marine microalgae *Scenedesmus* sp. SCX2 was grown in 2000 L raceway ponds under greenhouse conditions and was used to study the effect of semicontinuous culture mode during summer and autumn seasons on biomass, protein, pigments, sugar and lipid production.

3. Semicontinuous culture of *Scenedesmus* sp. SCX2 in RW under greenhouse conditions

3.1. Materials and methods

The cultivation system of the microalgae *Scenedesmus* sp. SCX2 was performed in semicontinuous culture in 2000 L raceway ponds employing Bold's basal medium (BBM), for 123 days (eight harvesting cycles) during summer and autumn seasons both under greenhouse conditions. Over the SCC, we monitored biomass concentration, lipid, protein, pigments and sugar production, light irradiance, culture temperature, greenhouse temperature and nitrate and ammonium concentrations in the medium as described in the following sections.

3.1.1. Microalgal strain and growth conditions

Scenedesmus sp. SCX2 strain was acquired from the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). Prior to use it, the strain was grown autotrophically and axenically in BBM medium in a 1 L photobioreactor (PBR) with a working volume of 0.9 L, and bubbled with air at a constant flow rate of 0.5 vvm and then in 20 L PBR. The PBR was illuminated with a light intensity of 180 μmol photons m^{-2} s^{-1} on the surface of the reactor by using cool white fluorescent tubes, with a photoperiod of 12 h of light:12 h of darkness and the temperature was maintained at 24 ± 1°C.

3.1.2. Raceway pond cultures

The open channel raceway reactors dimensions are: two 3.1 m channels, each of 0.72 m wide and connected by 180° bends at both ends to give a total surface area of 6.1 m². The raceways were constructed with fiberglass and the culture was circulated and mixed by a two paddle wheel system at a superficial flow velocity of 0.3 ms⁻¹. The operation volume was 1400 L, with a culture medium depth of 0.24 m. The cultures were conducted under greenhouse conditions. The light irradiance and greenhouse temperature were recorded with a data logger T&D RTR-500; the culture temperature was recorded with a data logger T&D RT-200. Additionally, temperature, pH, turbidity, NO_3^-, NH_4^+, conductivity and dissolved oxygen were continuously determined with HYDROLAB DS 5 (Hach) probe. The harvesting cycles were carried out on day 14 or 16, and to do so, half of the culture volume was removed and replaced with the same volume of fresh medium. Water losses due to evaporation were quantified and replaced periodically. The experiments were conducted under greenhouse conditions in Mexico City, Mexico (19.513°N, 99.126°W).

3.2. Analytical methods

3.2.1. Biomass determination

Biomass was determined by absorbance at 600 nm and dry biomass gravimetrically with a thermobalance. The sample was filtered through a glass microfiber membrane (Ahlstrom, 4.7 cm diameter, 0.7 μm pore size) to constant weight.

3.2.2. Determination of nitrate concentration

The nitrate concentration in the culture medium was determined as in Ref. [67] by using 0.5 mL of culture supernatant obtained after centrifugation of a microalgal culture sample at 3823×g for 10 min, then this supernatant was placed in a test tube and dried at 100°C for 12 h in an oven Riossa H62. A 0.5 mL portion of diphenyl sulfonic acid was added to the dry sample followed by the addition in four pulses of 2.2 mL of 12N KOH during constant mixing. A mix of 100 μL of supernatant with 900 μL of distilled water was read at 410 nm in a spectrophotometer GENESYS 10S UV-vis (Thermo Scientific, USA). A standard curve with the culture medium was prepared within a range of 0–500 mg L⁻¹ of NO_3^- as $NaNO_3$, to obtain the nitrate concentration.

3.2.3. Quantitative lipid determination and FAMEs determination

Cell disruption, lipid extraction, quantitative lipid and lipid composition, determined as fatty acid methyl esters (FAMEs) by gas chromatography after direct lipid transesterification, were realized as in Ref. [68].

3.2.4. Pigment content

Carotenoids and chlorophylls were measured according to the protocols and the equations obtained by Ref. [69]. Culture samples of 1.5 mL were centrifuged for 5 min at 17,000×g, after

which the supernatant was removed. The cells were suspended in 1.5 mL of methanol and incubated at 45°C in the dark during 30 min. The tubes were centrifuged (17,000×g/5 min) and the methanol extract was transferred into plastic cuvettes for measurement at 470, 653, 666 and 750 nm. Carotenoids and chlorophyll A (Chl$a$) and B (Chl$b$) concentrations were estimated according to the following equations (Eqs. 1–4):

$$\text{chl}a\left[\mu g\ mL^{-1}\right] = 15.65\left(ABS_{666}\right) - 7.34\left(ABS_{653}\right), \tag{1}$$

$$\text{chl}b\left[\mu g\ mL^{-1}\right] = 27.05\left(ABS_{653}\right) - 11.21\left(ABS_{666}\right), \tag{2}$$

$$\text{Total carotenoids}\left[\mu g\ mL^{-1}\right] = \frac{1000\left(ABS_{470}\right) - 2.86\left(\text{chl}a\right) - 129.2\left(\text{chl}b\right)}{221}, \tag{3}$$

$$\text{Total pigments}\left[\mu g\ mL^{-1}\right] = \text{chl}a + \text{chl}b + \text{total carotenoids}. \tag{4}$$

3.2.5. Total sugars

A sample of 5 mg of dried algae was added to 1 mL of 2 N HCl. Hydrolysis was achieved by heating this mixture at 100°C for 120 min. Then total sugars were quantified by the Dubois method [70].

3.2.6. Proteins

A sample of 5 mg of dried algae was added to 1 mL of 1 N NaOH. Hydrolysis was achieved by heating this mixture at 100°C for 120 min. Then proteins were quantified by the Bradford method [71].

All the samples were analyzed in triplicate. The average productivity referenced in [72] was calculated for biomass, lipid, total pigment and sugar and protein content, at the end of every cycle of culture. Productivity is defined as the milligram of product per liter of media per day.

3.3. Results and discussion

The aim of implementing an autotrophic microalgal culture in outdoor conditions was to take advantage of solar energy to convert CO_2 into organic compounds through photosynthesis and then to produce biomass, lipids, proteins, pigments and sugars. The *Scenedesmus* sp. SCX2 culture was performed using BBM medium, under semicontinuous mode, into a raceway reactor of 2000 L. The first culture cycle was considered as an adaptation period. The major growth was observed in the second and third cycles; then the growth was decreasing gradually in subsequent cycles (**Figure 1**). This was certainly due to the weather conditions; in our research group we have observed an increase in biomass with the sequenced cycles [73]. In the second and third cycles, the volumetric biomass productivities of 53 and 5 mg L^{-1} d^{-1} were

obtained, respectively, without significant differences between them. These were 15 and 11% higher than volumetric biomass productivity achieved in cycle 1 (**Figure 2**) and up to 50% higher than volumetric biomass productivity obtained in the last two cycles. Ref. [36] reported a volumetric biomass productivities of 30–260 mg L^{-1} d^{-1} with *Scenedesmus* sp. and all productivities obtained in this work are within this range. Areal biomass productivities obtained at the end of the second and third cycles were 12 and 11.5 g m^{-2} d^{-1}, respectively, which were 40% higher than that reported for *Chlorophyta* sp. and similar to the areal biomass productivity obtained with *Chlorella* sp. [74], both microalgae grown in semicontinuous mode in raceways (0.986 m^2), under greenhouse conditions. In addition, higher productivities of this work were half of that obtained by Ref. [35] with *Scenedesmus* sp. cultivated in semicontinuous mode in a raceway of 32 m^2 and CO_2-enriched aeration.
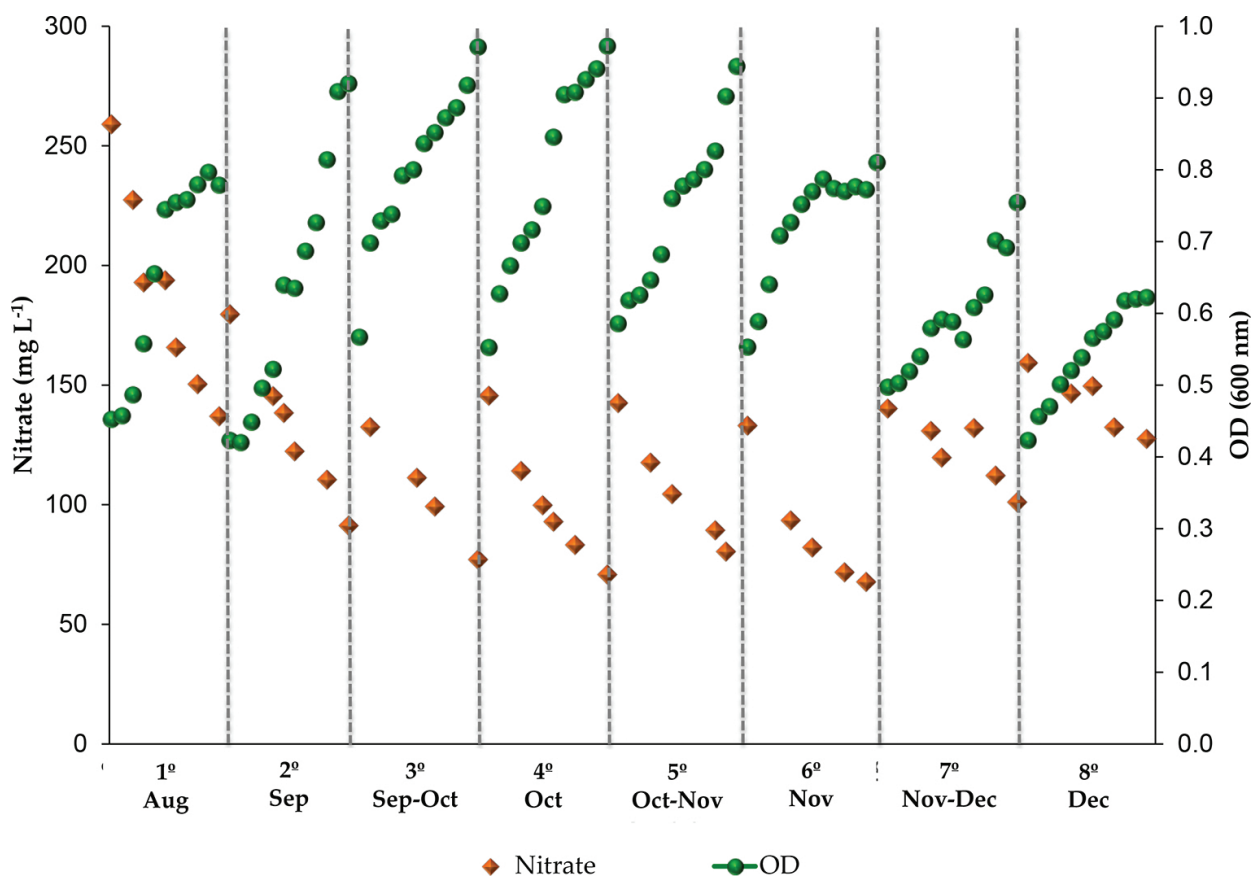

Figure 1. *Scenedesmus* sp. SCX2 growth and nitrate consumption during the eight cycles of culturing under semicontinuous mode in a raceway of 2 m^3 with 1.4 m^3 of medium, under greenhouse conditions.

The first two cycles were developed in the late summer while the other cycles were developed in the autumn, which explains the gradual decrease in both irradiance culture temperatures (**Table 2**); which decreased the biomass productivities (**Figure 2**). Temperatures of the culture of this work were within the range reported by Ref. [75] for the culturing of *Scenedesmus* sp. between 10 and 30°C.
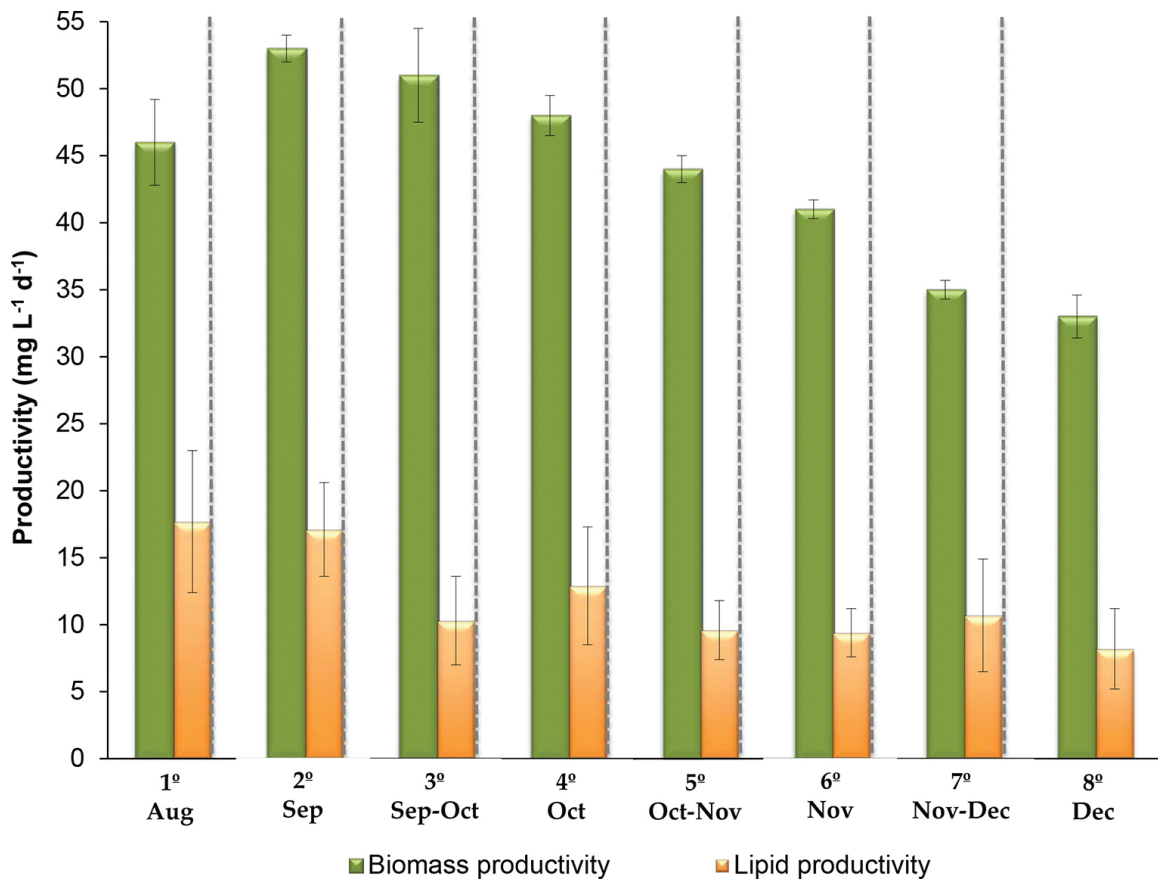
Figure 2. Biomass and lipid productivities from *Scenedesmus* sp. SCX2 cultivated during the eight cycles of culturing under semicontinuous mode in a raceway of 2 m³ with 1.4 m³ of medium, under greenhouse conditions.

Cycle	Month (2015)	Maximal irradiance ($\mu E\ m^{-2}\ s^{-1}$)	Average temperature (°C)	Protein productivity ($mg\ L^{-1}\ d^{-1}$)	Sugar productivity ($mg\ L^{-1}\ d^{-1}$)	Pigment productivity ($mg\ L^{-1}\ d^{-1}$)
1	Aug	1251.1	22.0	2.5 ± 0.02	19.2 ± 2.3	1.1 ± 0.07
2	Sep	1279.3	24.7	3.0 ± 0.01	14.8 ± 2.5	1.4 ± 0.11
3	Sep–Oct	1076.3	18.9	2.9 ± 0.01	11.9 ± 1.0	1.3 ± 0.17
4	Oct	1053.5	18.9	2.4 ± 0.02	10.8 ± 0.7	0.8 ± 0.00
5	Oct–Nov	1090.4	18.5	1.1 ± 0.02	12.2 ± 3.5	0.8 ± 0.03
6	Nov	1066.8	18.5	0.8 ± 0.02	9.0 ± 0.9	0.5 ± 0.07
7	Nov–Dec	894.3	17.0	0.8 ± 0.01	11.3 ± 2.0	0.5 ± 0.03
8	Dec	893.2	15.6	0.8 ± 0.01	5.6 ± 0.3	0.4 ± 0.01

Table 2. Irradiances, temperatures and productivities corresponding to the eight cycles of *Scenedesmus* sp. SCX2 culture in the semicontinuous mode.

Lipid production is very important, because lipids are the raw material for biodiesel synthesis by the transesterification reaction. Higher lipid productivities were obtained in the first and second cycles with 17.7 and 17.1 mg L^{-1} d^{-1} (**Figure 2**). The maximum lipid content in the biomass was obtained in the first cycle with 38.4%, which was threefold higher than lipid content obtained by *Chlorella variabilis* grown in a raceway with working volume 450 L [65] and 30% higher than that reported by [76], who cultivated *Scenedesmus* sp. in Erlenmeyer flasks with an operation volume of 800 mL and irradiance of 400 μE m^{-2} s^{-1} at 23°C, using culture media supplemented with nitrogen and phosphate sources, and even vitamins, in different concentrations. In the other culture cycles, the lipid productivities were lower without significant differences among them, with an average lipid productivity of 10.9 mg L^{-1} d^{-1}.

After carbon, nitrogen is the most important nutrient for the microalgae, which is assimilated in the form of NO_3^- or NH_4^+ [77–79]. During the semicontinuous mode, the NO_3^- was always present in the culture medium, and it was not fully assimilated in any cycle (**Figure 1**). Approximately 50% of the initially added NO_3^- for each cycle was consumed in the first six cycles; therefore, none of the cycles reached nitrogen limitation. Nitrate concentration never was less than 68 mg L^{-1} (**Figure 1**). In general, no effect between NO_3^- uptake and lipid productivity was observed, insomuch as the NO_3^- uptake was practically the same in each cycle and the lipid productivity decreased as the semicontinuous culture was progressing. The most significant change was in the last two cycles, when the NO_3^- uptake by *Scenedesmus* sp. was only 44% (**Figure 1**) and the lower productivities of biomass, lipids (**Figure 2**), proteins, total pigments and sugars were obtained (**Table 2**).

The pH of the culture is influenced by several factors such as algal productivity, photosynthesis and ionic composition of the culture medium [79, 80]. The pH is one of the most important factors in the microalgal culture because it determines the solubility and availability of CO_2 and essential nutrients, which has a significant impact on the microalgal metabolism [81]. Generally, a proportional relationship between biomass and pH was observed. In the first three cycles, a pH range of 9.1–11.3 was obtained, which is an advantage insomuch as with high pH values. Only few microorganisms can grow in these pH ranges and a non-contaminated microalgal culture could be guaranteed; however, with pH values above 8.5 the availability of inorganic carbon (CO_2 and HCO_3^-) is limited and the growth of microalgae is affected [82]. In this work, higher pH values (around 11) were achieved in the first two cycles; nevertheless, the pH had no negative effect on microalgal growth. From the third cycle forward, the pH oscillated in a range of 8.7–10.5, which allowed a greater availability of CO_2; however, it was not reflected in the biomass increase.

In regard to the protein content, it was proportional to the biomass content. The higher protein productivities were obtained in the first four cycles; the maximum productivity was achieved in the second cycle with 3.0 mg L^{-1} d^{-1}. From the fifth cycle forward, the protein productivity decreased 61% and in the last two cycles it decreased until 75% from the productivity reached in the second cycle (**Table 2**). It was report that *Scenedesmus dimorphus* has a protein content from 8 to 18% (dry weight basis) [83], and in this work the highest protein content was 5.6%, this was achieved at the end of the second cycle. Ref. [84] obtained six and four times more protein with *Scenedesmus* sp., respectively, that protein content achieved in this work, at the

end of the second cycle. *Scenedesmus* sp. was cultured in asbestos tanks, with an operation volume of 150 L and natural lighting, using fertilizer (Nitrofoska Foliar) and residual water fish.

The maximum total sugar productivity (19.2 mg L^{-1} d^{-1}) was obtained in the first cycle (**Table 2**). Total sugar productivities of the other cycles were declining gradually over time. It has been reported that *Scenedesmus* sp. can present a total sugar content of 50% (dry weight basis) [85], similar to what it has been achieved in this work (41.7%). Total pigments presented a similar behavior to the microalgal biomass, insomuch as the first three cycles presented higher productivities. The highest total pigments productivity was achieved in the second cycle (1.4 mg L^{-1} d^{-1}). For the fourth cycle, the total pigment content decreased by 40% and continued to decrease during the subsequent cycles to reach a productivity of 0.4 mg L^{-1} d^{-1} in the eighth cycle (**Table 2**).

The most abundant FAMES were palmitic acid (C16:0), stearic acid (C18:0) and palmitoleic acid (C16:1 Δ9). In several studies C18:0 is usually found in low concentration in microalgae and C16:0, C18:1 and C18:3 are the major fatty acids [86–88].

3.4. Conclusions

Higher productivities of biomass, lipids, proteins, total sugars and pigments were obtained in the first two and three cycles of cultivation, which covered a period of culturing between late summer and early autumn, when the irradiation and temperatures stimulated the *Scenedesmus* sp. SCX2 growth. A *Scenedesmus* sp. SCX2 culture was maintained under semicontinuous mode during 123 days (eight cycles) without NO_3^- limitation, with an average volumetric biomass productivity of 44 mg L^{-1} d^{-1}. The maximum content of protein, sugars, lipids and pigments was 5.6, 41.7, 38.4 and 2.4%, respectively. The lipid productivity decreased after the third cycle by almost 42%. The lowest lipid productivity was obtained in the eighth cycle and decreased by 53% compared with the highest lipid productivity. The total sugar productivity varied throughout of the experiment and decreased by 70% in the eighth cycle, compared with the highest productivity obtained in the first cycle. The maximum productivities of lipids and total sugars were obtained in the first cycle at 22°C and an irradiance of 1251.1 μE m^{-2} s^{-1}. The frequency of renewal of the semicontinuous culture can be reduced to increase the productivities and diminishing the contamination of the culture due to other microorganisms.

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