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ORIGINAL ARTICLE

Lipid production by the filamentous cyanobacterium *Limnothrix* sp. growing in synthetic wastewater in suspended- and attached-growth photobioreactor systems

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Abstract The main objective of this study was the production of biotechnological oil/biodiesel from a filamentous cyanobacterium *Limnothrix* sp. with simultaneous treatment of a model wastewater. A novel attached-growth photobioreactor was designed to facilitate the harvesting of cyanobacterial biomass and to maximize biomass and lipid production compared to suspended-growth cultivation systems. Kinetic experiments with different initial nitrate and phosphate concentrations were performed in both suspended- and attached-growth cultivation modes to define the biomass and lipid concentration as well as the capability of *Limnothrix* sp. to remove nutrients from the artificial wastewater. The removal of nitrate and phosphate was high in both suspended- and attached-growth systems. The results of this study also demonstrated that the proposed attached-growth photobioreactor system ensured higher biomass productivity compared to the suspended-growth cultivation system. The absence of long aliphatic chain fatty acids as well as the high amount of saturated and monounsaturated fatty acids (almost 80 %) in cyanobacterial lipid make the oil produced a promising feedstock for biodiesel production.

Keywords Cyanobacteria · Lipids · Wastewater · *Limnothrix* sp. · Attached-growth system · Novel photobioreactor

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Introduction

Oleaginous microorganisms (microalgae, fungi, yeast, and bacteria), with their ability to produce significant amounts of lipids, are an attractive source of oil suitable for biodiesel production (Ratledge 2004; Papanikolaou and Aggelis 2009; Economou et al. 2010, 2011; Bellou et al. 2014), especially since their fatty acid composition is often similar to that of common plants currently used as feedstock in biodiesel manufacturing (Meng et al. 2008; Vicente et al. 2009). Microalgae, which are unicellular photosynthetic organisms, are most commonly studied for this purpose, since they can grow easily under phototrophic, heterotrophic, or mixotrophic conditions in a wide variety of waters unsuitable for human consumption. Additionally, these microorganisms can grow faster and have more efficient light energy conversion than energy crops, and do not compete with food production (Mata et al. 2010; Amaro et al. 2011). It has been demonstrated that biodiesel production yield from microalgae can be 10 to 20 times higher than that obtained from oleaginous seeds (Chisti 2007).

Cyanobacteria (formerly blue-green algae or Cyanophyceae) form a group of photosynthetic organisms that includes single-celled, colonial, and filamentous-forming

organisms (Castenholz 2001). They are a ubiquitous and abundant component of phytoplankton and key biomass producers, and grow extensively in nutrient-rich water bodies (Moustaka-Gouni et al. 2006). Moreover, cyanobacteria, like microalgae, have the ability to grow under heterotrophic and mixotrophic conditions. For example, *Arthrospira* (*Spirulina*) *platensis* and *Anabaena variabilis* can grow in the dark in heterotrophic environments by using organic compounds such as glucose and fructose as a carbon source instead of CO_2. Additionally, these strains can grow under phototrophic and heterotrophic conditions (mixotrophy cultivation), depending on light availability and organic carbon concentration (Wolk and Shaffer 1976; Haury and Spiller 1981; Marquez et al. 1993).

The primary components of cyanobacterial biomass are proteins, carbohydrates, and lipids (Sialve et al. 2009). Some species, such as *Spirulina* sp. and *Planktothrix* (*Oscillatoria*) *rubescens*, are suitable for protein-rich biomass production, since their protein content is very high (Piorreck et al. 1984; Demirbas and Demirbas 2011). Furthermore, some cyanobacteria—for example, *Nostoc muscorum*—are able to accumulate starch in high concentrations, and thus can be used as feedstock for hydrogen production (Rodjaroen et al. 2007). While the accumulation of lipids in cyanobacterial biomass is fairly low, their fatty acid composition is suitable for biodiesel production (Sialve et al. 2009; Demirbas and Demirbas 2011).

Filamentous cyanobacteria have been investigated for the removal of nutrients (mainly nitrogen and phosphorus) from diluted agro-industrial waste and wastewater effluents (e.g., cattle, dairy, poultry, swine), as they can produce high amounts of biomass and can be harvested more easily than other microalgae species (Markou and Georgakakis 2011). In natural lakes, dominance of the filamentous *Limnothrix* species has been associated with nutrient enrichment due to the inflow of sewage effluents (Moustaka-Gouni et al. 2007).

In recent years, there has been a growing interest in the use of attached-growth algae systems for the biological treatment of wastewater, given their high biomass production and easy harvest of algal biomass at low cost. Attached-growth systems have been used for both the removal of nitrogen and phosphorus from wastewater (Pizarro et al. 2002; Kebede-Westhead et al. 2003, 2006; Mulbry et al. 2008) as well as the production of lipids (Johnson and Wen 2010). Additionally, attached-growth systems have been scaled up to highly productive large-scale units (Adey et al. 2011).

In this study, a novel attached-growth photobioreactor system was designed to facilitate the harvesting of cyanobacterial biomass and to maximize biomass and lipid production compared to suspended-growth cultivation systems. The biomass and lipid productivity of the filamentous cyanobacterium *Limnothrix* (*Oscillatoria*) sp. and its ability to remove nutrients from synthetic wastewater was evaluated in suspended- and attached-growth systems. Finally, the fatty acid composition of the produced cyanobacterial lipid was determined.

Materials and methods

Biological material and culture conditions

Cyanobacteria batch cultures were established using surface water samples obtained from Lake Ozeros (38° 39 N, 21° 13 E), which is a meso-eutrophic ecosystem in Western Greece (Chalkia and Kehayias 2013). The water samples were filtered through a 50 μm mesh net to remove all zooplankton. Inorganic nutrients were then added to the water to boost the growth of the remaining phytoplankton. Cyanobacteria cultivation was performed in 250 mL Erlenmeyer flasks with 100 mL working volume of artificial wastewater in which a mineral medium/artificial wastewater [containing (in mg/L) KNO_3, 200; $MgSO_4{\cdot}7H_2O$, 100; $CaCl_2{\cdot}2H_2O$, 50; K_2HPO_4, 108; KH_2PO_4, 56] was added as substrate for algae/cyanobacteria growth (Wang et al. 2010). The cultures were incubated under continuous artificial illumination at 12,000 lux with atmospheric CO_2 as carbon source. The temperature and pH ranged from 23 °C to 27 °C and 7 to 9, respectively, during incubation. After one month, the photosynthetic microorganisms became visible in the flasks.

Phytoplankton in lake water samples was dominated by the chlorophyte *Planktonema lauterbornii* and the cyanobacterium *Anabaena affinis*, whereas several species of chlorophytes, cyanobacteria, and diatoms contributed less to the total biomass. The initial mixture of phytoplankton microorganisms in culture shifted to a single species dominance (>99 % of the total phytoplankton biomass) of an oscillatorealean cyanobacterium, while heterotrophic bacteria were also conspicuous in the culture. The oscillatorealean cyanobacterium was identified as *Limnothrix* sp. using a Nikon ECLIPSE TE2000-S fluorescence microscope. Trichome, vegetative cell, and apical cell morphology were used as criteria to identify the organism to the level of genus. The *Limnothrix* culture was used as inoculum for the kinetic experiments performed in aquariums in suspended- and attached-growth modes, working under conditions similar to those described above.

Experimental design

Two types of photobioreactors (PBRs) (both with working volume of 3.5 L) were used in this study (Fig. 1). Both were glass aquariums (length, 29.5 cm; width, 11 cm; height, 15 cm). The first was used for the suspended-growth culture. The second was a novel photobioreactor equipped with 67 cylindrical glass rods. Each rod had a diameter of 0.5 cm and a surface area of 19.04 cm^2 (total rod surface in aquarium, 1276 cm^2), thus providing a significant surface area for cyanobacteria attachment and growth. At the same time, the transparent glass rods allowed light penetration across the whole PBR. The glass rods were kept in vertical position by

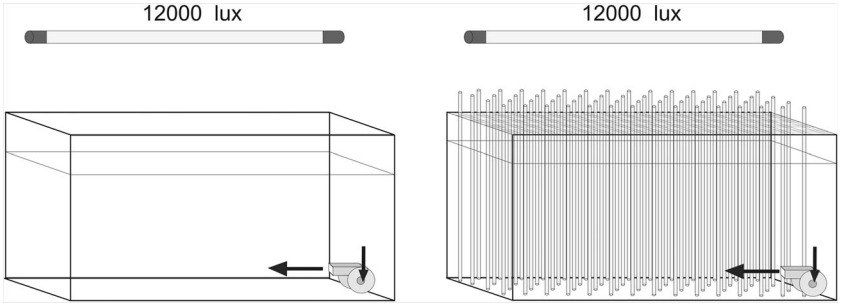

Fig. 1 Scheme of suspended (*left*) and attached (*right*) cyanobacteria growth systems

a supporting metallic grid placed on the surface of the aquarium (Fig. 1). This setup allowed the easy removal of each single rod from the photobioreactor and subsequent biomass harvesting. The growth medium used was the same as described above for strain isolation and had a composition similar to that of wastewater produced after secondary treatment (Wang et al. 2010).

For all kinetic experiments, freshwater taken from Lake Ozeros was filtered through grade GF/A Whatman filters and used to provide micronutrients to the culture. All experiments were conducted under non-aseptic conditions and constant illumination (12,000 lux), with atmospheric CO_2 as carbon source. For all kinetic experiments, two lots of independent cultures were conducted. The temperature and pH ranged from 23 °C to 27 °C and 7 to 9, respectively, and mixing was ensured by means of a continuous centrifugal pump placed into each PBR (see Fig. 1).

Analytical methods

Cyanobacterial biomass was harvested from the suspended-growth cultures by centrifuging 100 mL of culture volume for each sampling. The cyanobacterial biomass attached to the supporting rods was harvested by scraping two randomly selected glass rods for each sampling. After harvesting, the cyanobacterial biomass of both growth systems was dried at 105 °C until constant weight and then gravimetrically determined.

Oil extraction from dry cyanobacteria cells was performed according to the method described by Folch et al. (1957), in a 2:1 (v/v) mixture of chloroform and methanol, then washed with an 0.88 % (w/v) KCl solution to remove non-lipid components (lipoproteins, pigments), and dried over anhydrous Na_2SO_4. Lastly, the solvent was removed by evaporation and the produced oil weighed (Bellou and Aggelis 2012).

Fatty acid composition of the produced oil was determined by gas chromatography using an Agilent 7890A device (Agilent Technologies, Santa Clara, CA, USA) following conversion of the fatty acids to methyl esters according to the AFNOR method (AFNOR 1984). Methyl esters were separated in an HP-88 capillary column (model 112-8867: Agilent Technologies) with a length of 60 m and internal diameter of 0.32 mm, while helium (at a flow rate of 1 mL/min) was used as the carrier gas. The analysis was performed at 200 °C. Peaks were detected in a flame ionization detector (FID) working at 280 °C and identified by reference to authentic standards.

Nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{-3}) concentrations were measured according to Standard Methods for the Examination of Water and Wastewater (APHA 1998). Total sugars (expressed as starch equivalents) were determined in the cyanobacteria culture according to Dubois et al. (1956).

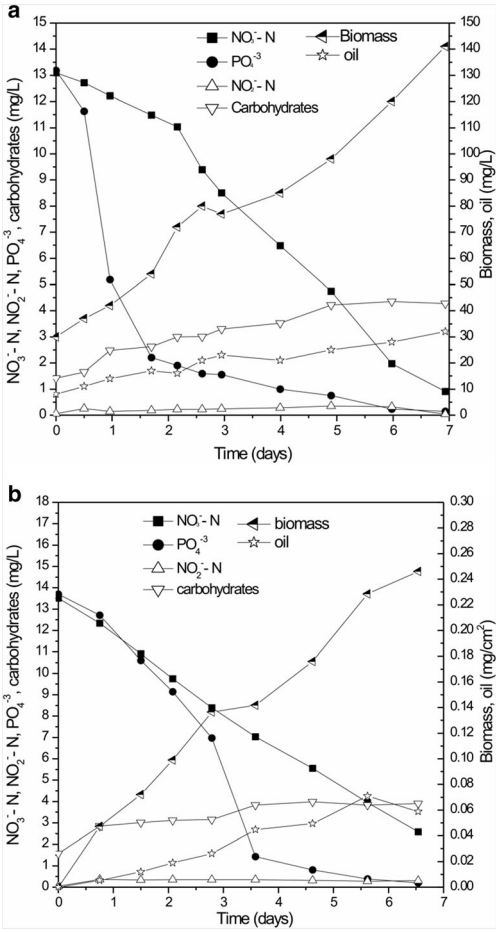

Fig. 2 Kinetic experiments of *Limnothrix* sp. **a**) in the suspended-growth system (initial nitrate and phosphate concentrations of 13.1 mg/L and 13.18 mg/L, respectively) and **b**) in the attached-growth system (initial nitrate and phosphate concentrations of 13.51 mg/L and 13.7 mg/L, respectively). Two lots of independent cultures were conducted

Results

Kinetic experiments in suspended- and attached-growth systems

A series of batch kinetic experiments were performed in suspended- and attached-growth cultivation modes in order to define the biomass and lipid concentration as well as the capability of *Limnothrix* sp. to remove nutrients from artificial wastewater.

Initially, kinetic experiments with low initial nitrate and phosphate concentrations (13.1 mg/L and 13.18 mg/L, respectively) were conducted in batch operation. The initial biomass concentration was 30 mg dry weight/L. Figure 2a shows the consumption of nitrate and phosphate and the biomass and lipid production in the suspended-growth cultivation mode over time. After 7 days of *Limnothrix* sp. cultivation, the consumption of nitrate and phosphate reached 93.05 % and 98.86 % of their initial values, respectively. Also, on day 7, the maximum concentration of biomass was 141 mg dry weight/L, while maximum lipid production was 32 mg/L. An average lipid content in the cyanobacterial biomass of 26.89 % was observed during this experiment.

Figure 2b represents the behavior of *Limnothrix* sp. in the attached-growth system under conditions similar to those the above. The initial concentrations of nitrate and phosphate were 13.51 mg/L and 13.7 mg/L, respectively, while the initial biomass concentration was 30 mg dry weight/L. In this kinetic experiment, the biomass was attached to the cylindrical glass rods, walls, and base of the PBR. In Fig. 2b, the biomass and lipid production was expressed as mg per cm^2 of glass rod surface only. However, at the end of the experiment, the total PBR attached biomass was evaluated (Table 1). In this experiment, the maximum nitrate and phosphate removal percentages were 80.9 % and 98.54 %, respectively, at day 6.5. The maximum biomass concentration on the glass rods reached 2.5 g dry weight/m^2, while the average lipid content in the attached biomass was 24.14 %.

Subsequently, kinetic experiments with higher initial nitrate and phosphate concentrations (27.8 mg/L and 29.16 mg/L, respectively) were conducted in order to ascertain the efficiency of biomass and oil productivity in the present attached-growth system at high pollutant concentrations. The maximum biomass concentration recorded in the suspended-growth cultivation was 291 mg dry weight/L, while lipid concentration reached at 43 mg/L after 13 days of cultivation (Fig. 3a). The average lipid percentage in the biomass was 16.8 %, while the total removal of nitrate and phosphate was 96.69 % and 93.14 %, respectively.

Table 1 Comparison of performance of suspended- and attached-growth systems

	Suspended system[a]	Attached system[a]	Suspended system[b]	Attached system[b]
Maximum biomass[*]	141 mg/L	2.5 g/m^2 (89.68 mg/L)	291 mg/L	5.2 g/m^2 (190.5 mg/L)
Total biomass in PBR	141 mg/L	7.24 g/m^{2**} (252 mg/L)[**]	291 mg/L	22.12 g/m^{2**} (770 mg/L)[**]
Maximum oil[*]	32 mg/L	0.6 g/m^2 (21.44 mg/L)	43 mg/L	0.5 g/m^2 (17.47 mg/L)
Total oil in PBR	32 mg/L	1.52 g/m^{2***} (52.94 g/L)[***]	43 mg/L	1.58 g/m^{2***} 54.46 mg/L[***]
Oil in biomass on glass rods (%)	–	24.14	–	14.12
Oil in total biomass of PBR (%)	26.89	21.01	16.8	7.07
Total biomass productivity (mg/L day)	20.14	38.77	22.38	35.00
Total biomass productivity (g/m^2·day)	–	1.11	–	1.01
Total oil productivity (mg/L day)	4.57	8.14	3.31	2.48
Total oil productivity (g/m^2 day)	–	0.23	–	0.07
NO_3^- removal (%)	93.05	80.90	96.69	96.92
PO_4^{-2} removal (%)	98.86	98.54	93.14	98.81
Total surface of glass rods (m^2)	–	0.1276	–	0.1276
Total surface of PBR walls (m^2)	–	0.1199	–	0.1199
Total surface of PBR (rods and walls) (m^2)	–	0.2475	–	0.2475
Volume of PBR (mL)	3500	3500	3500	3500
Cultivation time (days)	7	6.5	13	22

[a] Kinetic experiment under low nutrient concentrations (Fig. 2)

[b] Kinetic experiment under high nutrient concentrations (Fig. 3)

[*] Concentration at the end of the experiment. In the case of suspended cultivation, maximum biomass is equal to total biomass and maximum oil is equal to total oil.

[**] Sum of attached biomass on glass rods and PBR walls

[***] Sum of produced oil on glass rods and PBR walls

In attached-growth cultivation mode, the initial concentrations of nitrate and phosphate were 27.9 mg/L and 28.53 mg/L, respectively, and these values decreased to less than 1 mg/L after 22 days, corresponding to a maximum removal of 96.92 % and 98.81 % for nitrate and phosphate, respectively (Fig. 3b). In this kinetic experiment, the initial biomass concentration was 60 mg dry weight/L and the average lipid percentage of the biomass attached to the glass rods was 14.12 %.

In all of the above-referenced kinetic experiments, the nitrite concentration was very low (<0.5 mg/L) during *Limnothrix* sp. cultivation. Additionally, the carbohydrate concentration in the liquid medium of all PBRs was lower than 4.5 mg/L.

Lipid analysis

Lipid analysis of *Limnothrix* sp. biomass was performed at the end of the kinetic experiments in both suspended- and attached-growth systems with higher nitrate and phosphate concentrations (Fig. 3a and b). It was observed that the fatty acid composition of the total lipids was similar in both growth systems (data not presented). Figure 4 presents the fatty acid composition from the kinetic experiment in suspended-growth cultivation (Fig. 3a). The identified fatty acids contained 10 to 18 atoms of carbon (Fig. 4). The predominant fatty acids were palmitic (C16:0) and oleic (C18:1) acids, accounting for 26 % and 17 %, respectively. Lauric (C12:0), stearic (C18:0), linoleic (C18:2), and alpha linolenic (C18:3) acids were also present in significant percentages (12.7 %, 11.5 %, 11 %, and 10.7 %, respectively). In addition, capric (C10:0), myristic (C14:0), myristoleic (C14:1), and palmitoleic (16:1) acids were detected in small quantities in the total lipid (1.4 %, 1.4 %, 4.6 %, and 3.3 %, respectively). The absence of long aliphatic chain fatty acids and the high amount of saturated and monounsaturated fatty acids (almost 80%) in cyanobacterial lipid indicate that the oil produced is suitable for biodiesel production.

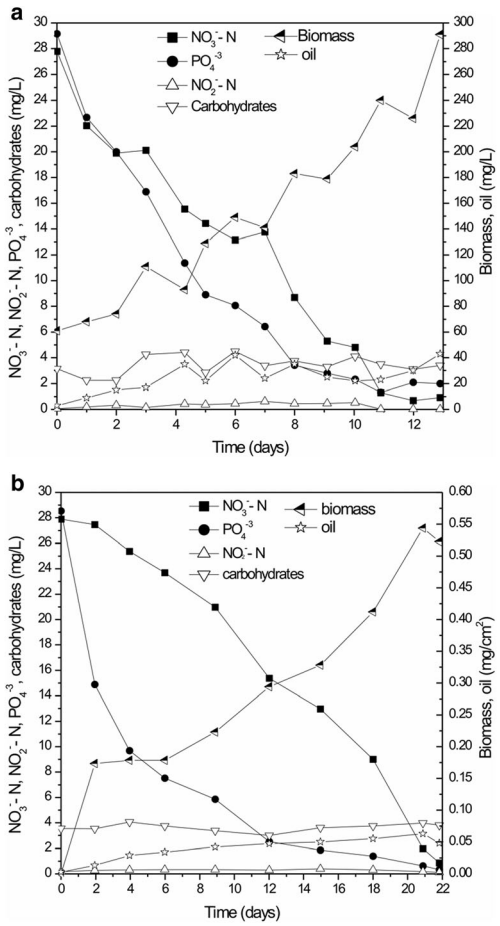

Fig. 3 Kinetic experiments of *Limnothrix* sp. **a**) in the suspended-growth system (initial nitrate and phosphate concentrations of 27.8 mg/L and 29.16 mg/L, respectively) and **b**) in the attached-growth system (initial nitrate and phosphate concentrations of 27.9 mg/L and 28.53 mg/L, respectively). Two lots of independent cultures were conducted

Discussion

Photosynthetic microorganisms can produce higher amounts of biodiesel per year than energy crops, and therefore constitute the most promising source for biodiesel production that

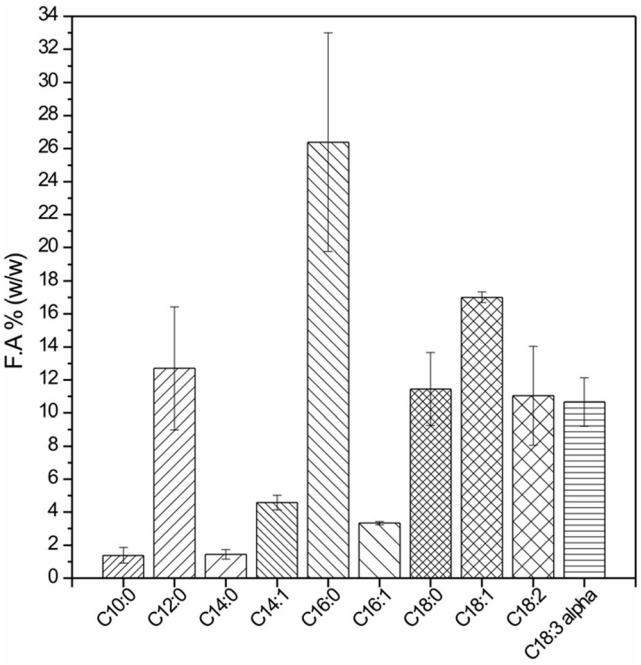

Fig. 4 Fatty acid composition of *Limnothrix* sp. lipid produced in the suspended-growth cultivation system with higher nitrate and phosphate concentrations (for details, see text). Data are presented as mean values from duplicate experiments; *error bars* show standard deviation

can significantly displace conventional diesel. Furthermore, as these organisms use energy from the sun and fix CO_2 from the atmosphere, they can contribute to the reduction of atmospheric pollution and improvement of human health (Chisti 2007). One of the major drawbacks of photosynthetic microorganism cultivation on a commercial scale is the high cost of biomass harvesting. The primary advantage of filamentous cyanobacteria compared to microalgae, however, is that they can be harvested easily from the liquid medium due to their large-sized filaments. Thus, expensive methods for biomass harvesting such as flocculation, centrifugation, or filtration are avoided (Hashimoto and Furukawa 1989; de la Noue et al. 1992; Markou and Georgakakis 2011).

The main objective of this work was to produce biomass and lipid from cyanobacteria in suspended- and attached-growth PBRs. Of note, the design of the proposed attached-growth system is a novel approach for efficient cyanobacterial biomass harvest from the perspective of minimizing the cost of oil production coupled with the ability of *Limnothrix* sp. to remove nutrients/pollutants from growth media. The key advantages of the novel photobioreactor over the suspended-growth system are its significantly larger surface area available for biomass growth, simultaneously ensuring adequate light penetration and easy biomass harvest.

Kinetic experiments with different initial nitrate and phosphate concentrations were conducted in batch operation using synthetic wastewater as the nutrient source for *Limnothrix* growth. During these experiments, the trichomes of *Limnothrix* sp. attached to the PBR walls. In the suspended-growth cultivation system, the adherent trichomes of *Limnothrix* sp. were removed mechanically from the PBR walls during cultivation. This was not possible in the attached-growth system, however, given the design of the PBR, which caused low velocity of the medium, resulting in the attachment of the filamentous cyanobacterium onto the glass rods, PBR walls, and particularly on the PBR base. This phenomenon is likely attributable to the lower mixing speed in the attached-growth system, causing the long, entangled filaments of *Limnothrix* sp. to gather at the bottom of the bioreactor. The total biomass concentration, which corresponds to the sum of the attached biomass on both glass rods and PBR walls, was estimated at the end of each kinetic experiment (Table 1). It is worth mentioning that the concentration of dissolved carbohydrates exuded by *Limnothrix* sp. did not decrease during the kinetic experiments (Figs. 2 and 3), suggesting that there was no significant bacterial contamination in the PBRs (Hulatt and Thomas 2010).

In the kinetic experiment with the lower nitrate and phosphate concentrations (Fig. 2), both biomass and oil concentrations in the suspended-growth system were almost equal to those recorded from just the walls of the PBR in the attached-growth system (Table 1). Moreover, maximum nitrate and phosphate removal were achieved in the same length of time (i.e., within 7 days). In the kinetic experiment with the highest nitrate and phosphate concentrations (Fig. 3), the oil concentration in the suspended-growth cultivation was almost equal to that obtained from the PBR walls of the attached-growth system. In contrast, the biomass concentration on the PBR walls in the attached-growth system was double that of the suspended-growth cultivation. It was observed that *Limnothrix* sp. needed 9 more days to remove nutrients/pollutants from the medium in the attached-growth system compared to the suspended-growth culture (Fig. 3b). This may have occurred because suspended cells are more efficiently "exposed" to nutrients than attached cells. The total biomass concentration in the attached-growth system was 770 mg/L, and was achieved after 22 days of cultivation (Table 1).

In previous studies, suspended cultures of *Oscillatoria* sp. (which is closely related to *Limnothrix* sp.) produced 260 mg/L biomass after 20 days of cultivation (Rodjaroen et al. 2007); when *Oscillatoria* sp. was cultivated in 13 mg/L initial nitrate concentration in suspended cultures, the biomass reached 166 mg/L within 99 days (Piorreck et al. 1984). *Limnothrix* sp., while similar to *Limnothrix redekei* (formerly *Oscillatoria redekei*), differs in its longer entangled trichomes lacking gas vacuoles. These morphological differences reflect the different conditions prevailing in culture systems—and particularly in the attached-growth system—compared to the natural environment and planktic life cycle in suspension (Gkelis et al. 2005).

The results of this study demonstrated that the proposed attached-growth system ensured a high surface area for the attachment and growth of *Limnothrix* sp., leading to high biomass productivity. Johnson and Wen (2010) also recorded higher biomass concentrations for *Chlorella* sp. cultivated in the attached-growth mode compared to the suspended-growth cultivation system. In their study, the rates of biomass and oil productivity ranged from 0.58 g/m to 2.57 g/m^2 day and from 0.06 g/m to 0.23 g/m^2 day, respectively. As such, the rates of oil productivity were similar to those obtained in the attached-growth system proposed in the present study (0.07 and 0.23 g/m^2 day).

With regard to wastewater treatment effluents, attached-growth systems with algal turf scrubbers have demonstrated much higher biomass productivity (Kebede-Westhead et al. 2006; Mulbry et al. 2008). For example, when a laboratory algal turf scrubber was used for treatment of swine manure effluent using filamentous green algae, biomass productivity ranged from 7.1 g/m to 9.4 g/m^2 day (Kebede-Westhead et al. 2006). Mulbry et al. (2008) demonstrated that algal productivity in pilot-scale algal turf scrubbers increased from 2.5 g/m to 25 g/m^2 day with an increasing loading rate of dairy manure effluent. In addition, Kebede-Westhead et al. (2003) showed that biomass productivity in algal turf scrubbers increased

with increasing loading rate and irradiance, from 7.6 g/m to 19.1 g/m^2 day. In the above studies, algal turf scrubbers were operated in semi-continuous modes. Although *Limnothrix* sp. did not achieve high biomass production, it can be used for advanced wastewater treatment, as filamentous cyanobacteria are able to efficiently remove inorganic pollutants found in high concentrations in the growth environment (Hashimoto and Furukawa 1989).

In the present study, the removal of nitrate and phosphate was high in both suspended and attached-growth systems. However, the kinetic experiments with the lower initial nutrient concentrations (Fig. 2) showed higher oil productivity. This phenomenon occurred as a result of the increased protein content in cyanobacterial cells grown at higher nitrogen concentrations. Similar phenomena have been observed in previous studies, in which it was demonstrated that an increased concentration of nitrogen in the medium leads to reduced lipid yields (Piorreck et al. 1984; Sassano et al. 2010; Bellou et al. 2014).

The produced oil contained fatty acids with 10–18 carbon atoms, while the predominant fatty acids were palmitic and oleic. Similar fatty acid profiles have been observed in other studies (Piorreck et al. 1984; Pel et al. 2004; Akoto et al. 2005). Likewise, high amounts of saturated and monounsaturated fatty acids were detected in cyanobacterial oil, indicating its suitability for the production of high-quality biodiesel (Demirbas and Demirbas 2011).

The remaining cyanobacterial biomass of the above process is rich in nitrogen and phosphorous and could be utilized in various ways, such as a low-cost bio-fertilizer (Thajuddin and Subramanian 2005) if produced by non-toxic cyanobacteria, or as feedstock for biogas production through anaerobic digestion (Sialve et al. 2009).

The aim of this study was cyanobacterial oil/biodiesel production in suspended- and attached-growth cultivation systems, with simultaneous nitrate and phosphate removal from synthetic wastewater, using the filamentous cyanobacterium *Limnothrix* sp. Biomass productivity, lipid content, and lipid productivity were evaluated in each growth system, and the proposed novel attached-growth system was demonstrated to achieve greater biomass and oil productivity than the suspended-growth cultivation system. The proposed system could be a cost-effective method for biofuel production and wastewater treatment. Further research is necessary, however, to increase the attachment surface area of PBRs using various materials.

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