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Microalgae biomass and lipid production using primary treated wastewater

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ABSTRACT

The outdoor algal cultures are common in wastewater treatment, but the selection and investigation of microalgae species for efficient nutrient removal are more demanding, nowadays. The autochthonous algae are well adapted to the local environmental conditions, which may result in more efficient nutrient removal. This paper evaluates the potential of autochthonous microalgae for the removal of organics and nutrients from primary effluent and biomass production. The process parameters were investigated under batch, fill and draw, and continuous operation mode, at two different radiation intensities (100 and 200 μmol/m^{2}s). The maximum biomass concentration (450 mg/L) was observed in the continuous operation mode. Phosphate concentration in the influent ranged from 0.60 to 1.57 mg P/L, while in the effluent was, in most cases, almost zero and was the limiting factor for algal growth. The growth rate of microalgae and their lipid content were depended on the concentration of nutrients in the influent. Specifically, the nitrates in the influent ranged from 0.47 to 20.87 mg NO_3^-/L and were the main factor for the algal lipid content. The highest lipid content was observed when the system was operated in continuous mode with low nutrient content of wastewater and was up to 15% of the dry weight.

Keywords: Microalgae; Primary treated wastewater; Lipid production; Nutrient removal

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1. Introduction

Biological wastewater treatment through cultivation of microalgae is particularly attractive due to the photosynthetic ability of algae to capture and store solar energy into useful biomass and to remove nutrients such as nitrogen and phosphorus, which can cause eutrophication [1]. The most common use of microalgae in wastewater treatment is the stabilization ponds [2]. Stabilization ponds are low-cost natural systems for the treatment of municipal and industrial wastewater and are classified into facultative, maturation and high-rate algal ponds [3–5]. Facultative ponds, with typical depths 1.2–2.5 m and hydraulic retention time (HRT) from 5 to 20 d, are most frequently applied for organic matter and solid removals and pathogen control [6,7]. Increased levels of nutrient removal can be obtained at higher ambient temperatures in well-designed stabilization ponds, which include shallow maturation ponds [8]. Recently, various attempts have been directed to the potential of algal–bacterial symbiotic process for the treatment of primary effluent [9,10], secondary effluent [10,11], or other type of wastewater [10,12,13].

Microalgae is a broad category of photosynthetic microorganisms consisting of eukaryotic algae and prokaryotic cyanobacteria. The concentration of nitrogen and phosphorus in wastewater is an essential factor, which has a direct effect on algal growth rate, and thus on nutrient removal and lipids accumulation [14]. Many factors affect the performance of algal systems such as nutrient concentration, CO_2, pH, aeration rate, light conditions, and temperature [10,12,15,16]. Nutrients assimilated by algal biomass can be recycled through the production of fertilizers, while algal biomass can be also used for the production of bioenergy or production of pharmaceutical substances or food [17,18]. Moreover, their capacity to remove heavy metals, as well as some toxic organic compounds avoiding secondary pollution makes microalgae a sustainable alternative for wastewater treatment [2,4].

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The selection of algal strain is one of the most important factors in wastewater treatment. Many studies have reported that using autochthonous algal strains was the best way to get more efficient results [19–21]. Since the outdoor culture is a prerequisite method in wastewater treatment with microalgae, the quest of local algal strains is more demanding. The autochthonous algae are well adapted to the local environmental conditions, which may result to more efficient nutrient removal [20]. Finally, local mix-microalgae cultures have been reported to yield higher lipid content than single algal cultures [10].

This paper presents the findings of an experimental study, which investigated the treatment of primary effluent with a laboratory-scale algal pond, using autochthonous algal strains. The treatment was conducted with mix-microalgae cultures, which were isolated in the local wastewater treatment plant. The performance of the reactor was evaluated under batch and continuous feeding mode for biomass production, nutrients and organic matter removal, and lipid production.

2. Materials and methods

2.1. Experimental system

Raw wastewater was collected from the wastewater treatment plant located at the University of Patras. The wastewater was settled for 1 h and the supernatant was used to feed the pond. Algal precultures were prepared by mixing 5 L of tap water, 0.5 L of primary treated wastewater and 10 L of BG-11 medium in a glass bottle. The bottle was placed in the laboratory close to the window with constant aeration.

One pilot oxidation pond was used in this study and was placed in a walk-in incubator room under controlled environmental conditions at 20°C, in order to investigate the microalgae biomass production, the efficiency of the pond to remove nutrients, and the lipid content of algal cells. The dimensions of pond were 50 × 50 × 25 cm (L × W × H) and the working volume was 30 L. The experiments were carried out in six phases (Table 1). In order to evaluate the algal growth, different operating conditions were examined such as batch, fill and draw, and continuous operation mode, at two different radiation intensities 100 and 200 μmol/m^{2}s at a 12:12 h light:dark photoperiod.

The first set was carried out in batch conditions and the second one under fill and draw conditions using 1 L/d primary treated sewage. In the fill and draw mode, the liquid content of the pond was mixed and 1 L of the mixed liquor was removed and replaced with 1 L of primary treated wastewater. In the third experiment, the pond was continuously fed with primary effluent using a peristaltic pump. The fourth and fifth experiments were conducted under batch conditions. At the beginning of the fifth set 0.55 g of K_2HPO_4 (1.5 mg P/L of pond) was added in the pond. The final experimental set was conducted under continuous operation mode and the radiation intensity was increased to 200 μmol/m^{2}s.

Table 1
Cultivation of microalgae in primary effluent – experimental conditions

Phase	Operation mode	HRT (d)	Radiation intensity (μmol/m^{2}s)	Organic loading (kg COD/m^{3}d)
1	Batch	–	100	–
2	Fill and draw	30	100	0.396
3	Continuous	30	100	0.231
4	Batch	–	100	–
5	Batch[a]	–	100	–
6	Continuous	30	200	0.217

[a]Phosphates were added in the pond.

2.2. Analytical methods

Microalgal biomass was determined by the measurement of total suspended solids (TSSs) according to standard methods [22]. Total phosphorus (Total-P) and soluble Total-P (STotal-P) were determined by the ascorbic acid method after digestion of the sample with ammonium persulfate [22]. Total nitrogen was determined spectrophotometrically by the method of 2,6-dimethylphenol [23]. Nitrates and phosphates were determined by using ion chromatography (Dionex DX500, Dionex Corporation, Sunnyvale, CA). Chemical oxygen demand (COD) was determined with the closed reflux colorimetric method using COD digester according to standard methods [22]. Soluble non-purgeable organic carbon (SNPOC) was measured by the combustion–infrared method using a TOC analyzer (TOC-5000, Shimadzu Corporation, Japan), and the pH was measured electrometrically. All the measurements were performed in duplicate except for the algal lipid content, due to the high algal biomass demand. The algal lipid content of microalgae was measured by the modified method of Folch et al. [24]. A measured quantity of dry algal biomass (approximately 100 mg) was homogenized and extracted three times with a chloroform:methanol (2:1) mixture. The biomass was removed by filtration through a filter paper and the extracted lipids transferred quantitatively to a tared Erlenmeyer flask. The procedure was repeated three times in order to extract all the lipids. Weight measurements were made on a precision analytical balance (AE200, Mettler Instrumente AG, Zurich, Switzerland). The flask was placed in an oven at 90°C until all reagents were removed. The flask was allowed to cool to ambient temperature in a desiccator and then was weighed. The weight difference corresponded to intracellular lipids.

3. Results and discussion

3.1. Biomass production

Biomass production was determined by the dry weight (Fig. 1(A)) and chlorophyll a (chl-a; Fig. 1(B)). The results of microalgae biomass production showed that biomass growth was affected by light intensity. Specifically, biomass concentration increased with the increase of the radiation intensity from 100 to 200 μmol/m^{2}s, in phase 3 and 6, respectively (Fig. 1(A)). Similar results were observed for chl-a concentration (Fig. 1(B)), and the highest concentration was observed at the 200 μmol/m^{2}s radiation intensity period. The maximum biomass concentration of 450 mg/L was observed under continuous mode and high radiation intensity in phase 6.

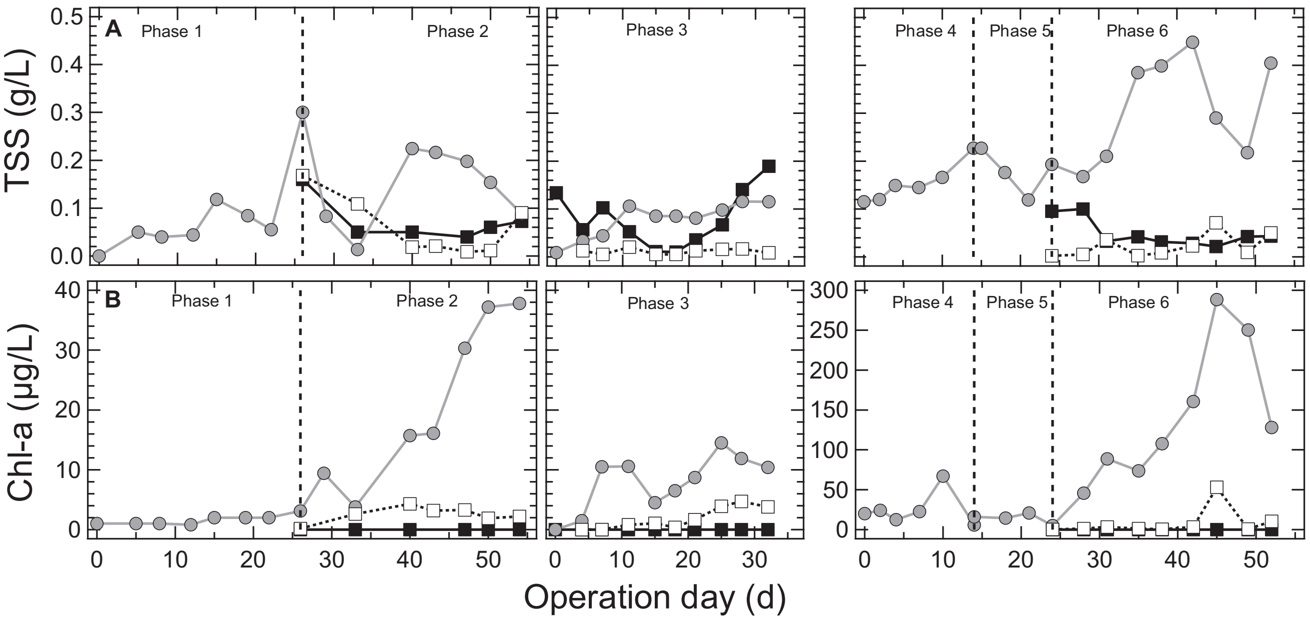

Fig. 1. Solids (A) and Chl-a (B) variation in the influent (■), mixed liquor (●), and effluent (□) of the pond.

Although the light intensity is an important factor for algal growth, the nutrient concentration, which was fed in the pond, is more important. In phases 1, 4, and 5 under batch mode of operation, the algal growth was relatively stable, especially in phases 4 and 5. The continuous supply of substrate into the pond with primary effluent (phase 1 to phase 2) resulted initially to the decrease of algae biomass in the pond. Similar results were reported in other studies [9,25], which mentioned that after the transition of their system operation from batch to continuous mode, the algal concentration was reduced.

During the fill and draw mode of operation (phase 2), the TSSs concentration varied significantly compared with the continuous feeding period (phase 3). The higher biomass concentration was observed during the fill and draw mode, even though that the average biomass concentration in each operation mode (phases 2 and 3) was actually the same, 106 mg/L. Similar observations have also been reported by other investigators who employed a 0.25-m^2 open pond for the cultivation of blue-green algae *Anabaena variabilis* at a dry weight concentration of 0.2–0.3 and 0.1–0.2 g/L in summer and winter conditions, respectively [26]. As it was mentioned earlier, the supply of nutrients in the pond is a major factor for algal growth, so this high biomass concentration (450 mg/L) during the phase 2 should be due to the high phosphate content in the influent wastewater during that period.

The results of chl-a measurements are presented in Fig. 1(B). Chl-a concentration in phases 1, 4, and 5 during the batch mode conditions presented lower concentrations than in fill and draw, and continuous feeding mode (phases 2, 3, and 6). Moreover, the increase of radiation intensity resulted in high chlorophyll concentrations during that period. Specifically, in phase 2 with radiation of 100 μmol/m^{2}s, the higher chl-a concentration was 38 μg/L, whereas in phase 6 with the increased radiation (200 μmol/m^{2}s), the highest chl-a concentration was up to 288 μg/L. The chl-a concentration, in the effluent of the pond, was low and in most cases almost zero. In the cases where the turbidity in the pond was high, chl-a in the effluent was not zero due to escape of algal cells in the effluent. Chl-a is an important measurement in these systems, since representing the photosynthetic activity of algae, which depends on nutrient removal. On the other hand, chl-a is reported as an unreliable indicator of algae concentration, since the photosynthetic activity of the algae strongly depends on the environmental conditions and time of sampling [27]. Chl-a values were not analogous to TSSs, since the latter represent the TSSs in the pond including the debris and dead algal cells, organic and inert material.

3.2. Nutrient removal

Microalgae can assimilate a significant amount of nutrients in excess of the immediate metabolic needs [28]. The nitrogen and phosphorus removal efficiency of natural systems depends on the selection of appropriate microalgae species since the algae growth controls directly and indirectly the nutrients removal [29]. In this study, autochthonous microalgae were cultivated in primary wastewater treatment, in order to investigate their ability for nutrient removal, biomass and lipid production. The nitrate concentration in influent, effluent, and inside the pond are shown in Fig. 2.

In phase 1 (batch conditions), there was not observed any significant decrease in the concentration of nitrates in the pond, due to the low concentration of phosphates in the influent wastewater. The higher concentration of phosphates in the influent, in phase 2, resulted in a prompt decrease of nitrates, while in phase 3, nitrate concentration was similar in the pond and the effluent. The nitrate removal was satisfactory, and the maximum decrease of nitrates (52%) was observed the same day with the external addition of phosphorus on day 14 (phase 5). The uptake of ammonium and nitrate by microalgae is important in nitrogen removal because nitrogen often exists as ammonium in wastewater especially in primary treated wastewater, which was used in this study. It should be noted that even the nitrate concentration in the influent was almost zero, high concentration of nitrates was observed in the pond. This is attributed to the conversion of ammonium to nitrates in the pond [30,31], which results in the increased concentration of nitrates in the pond. In order to explain the nitrogen conversion in the pond, ammonia nitrogen concentration was measured in phase 6. Ammonia nitrogen was completely removed from the pond even though ammonia concentration in the influent ranged from 23 to 29 mg N/L.

Phosphorus and nitrogen are essential nutrients for biomass growth since they are used by algae cells for the synthesis of proteins, nucleic acids, and phospholipids [28]. Phosphate concentration in the influent ranged from 0.60 to 1.57 mg P/L and in the effluent was almost zero, implying the complete removal of phosphates. On the other hand, the STotal-P in the effluent ranged from 0.0 to 0.9 mg/L. The determination of STotal-P referred to organic phosphates and orthophosphates. Organic phosphates can be converted to orthophosphates by phosphatases at the algal cell surface, and this occurs especially when inorganic phosphate is in short supply [28]. Phosphate consumption is performed in synergy with nitrates. The experimental results (Fig. 3) revealed that phosphates were the limiting factor for biomass growth in continuous mode conditions, since the phosphate removal approached 100% in all operation modes. Because of the microalgal photosynthetic ability and the simultaneous oxygen production, pH reached high values up to 8.75, which did not affect phosphorus removal. Other studies [9,17] have also reported that microalgae can grow and efficiently remove nutrients from primary settled sewage.

3.3. Organic matter removal

The experimental results of SNPOC are shown in Fig. 4(A). The system worked satisfactorily to remove SNPOC, especially during the phase 2, in which the primary treated wastewater had the highest SNPOC concentration and the highest organic loading (0.396 COD kg/m^{3}d). Specifically, when the system was operated in fill and draw mode, the SNPOC removal was up to 90%. The decrease of SNPOC in the effluent of the

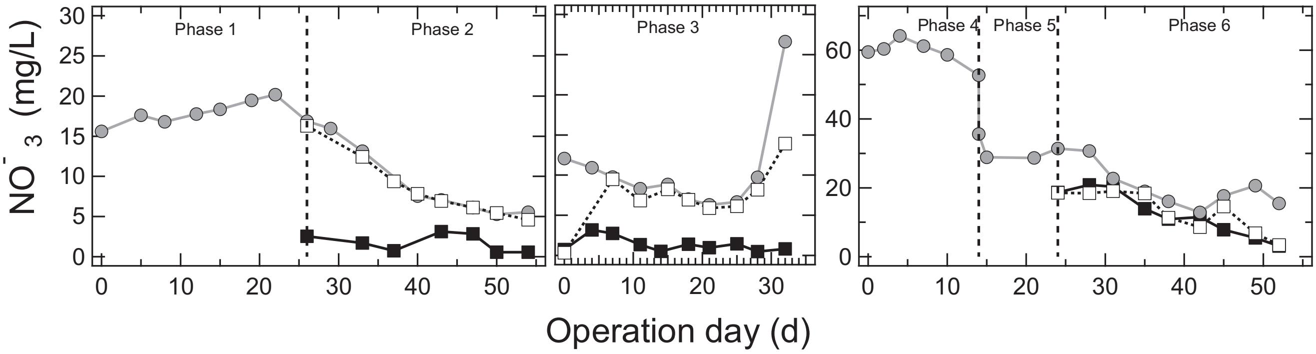

Fig. 2. Nitrate variation in the influent (■), mixed liquor (●), and effluent (□) of the pond.

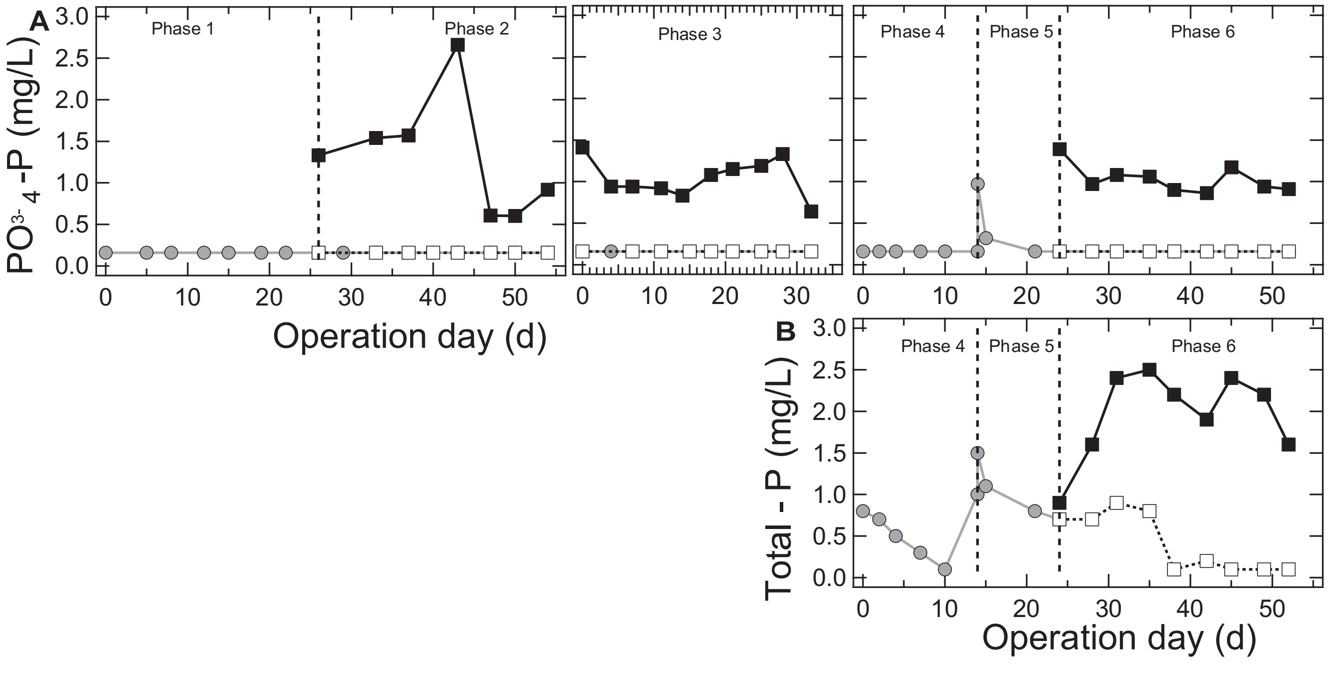

Fig. 3. Phosphorus variation in the influent (■), mixed liquor (●), and effluent (□) of the pond: (A) phosphates and (B) soluble total-P.

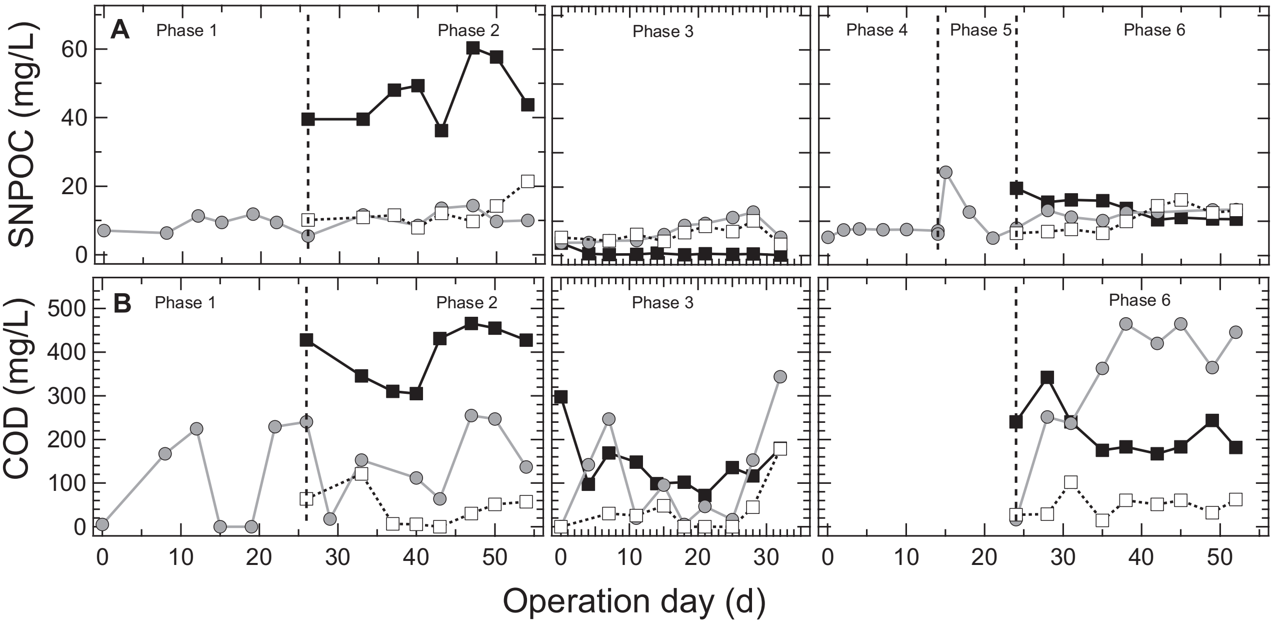

Fig. 4. SNPOC (A) and COD (B) variation in the influent (■), mixed liquor (●), and effluent (□) of the pond.

pond implies the presence bacteria and other heterotrophic microorganisms, which attributed to the removal of organic matter. Similar results were reported by Manariotis and Grigoropoulos [32], who used anaerobically pretreated wastewater with similar organic loading. In phases 3 and 6, the SNPOC in the effluent was higher than in the influent, despite that the SNPOC of the influent was very lower than in phase 2. The increase of SNPOC in the effluent may be caused by algal biomass, and especially by the algal debris, which is a source of organic carbon. In batch mode experiments, the SNPOC concentration was mostly stable, even though at the beginning of sixth phase was increased probably due to the increase of biomass in the pond and algal cell lysis.

The COD was efficiently removed as it is seen in Fig. 4(B). In phase 2, the removal of COD ranged from 65% to 100%, despite of the high initial concentration in the influent (305 to 466 mg/L), while in phases 3 and 6, COD removal was up to 95% and 91%, respectively. The decrease of COD implies the presence of bacteria and heterotrophic microalgae. Recent studies have reported that easily biodegradable organic matter may be used either by aerobic bacteria or by heterotrophic microalgae [13]. The biodegradation of the organic matter by aerobic bacteria would produce CO_2, or smaller organic molecules ready to be taken up for autotrophic and heterotrophic microalgae growth, respectively [13].

3.4. pH monitoring

The pH was monitored during the entire operation period (data not shown). In phase 1, pH was increased rapidly from 7.1 to 8.7 from the 5th day of operation. This increase of pH is due to the photosynthetic activity of microalgae [33], and their metabolism actions, which change the composition of the environment in the pond. After that period, the pH values were stable, and ranged from 8.2 to 8.9. Similar pH values were observed by Gonzalez-Fernadez et al. [13], who studied the treatment of pig slurry in an open stabilization pond.

3.5. Microalgae lipid content

It is important to highlight the reverse relationship between the nitrate concentration and the lipid content. The lipid content was affected by the nutrient concentration in the influent, and higher values were observed with low nitrate concentration in the pond. The results of this study showed that the nutrient removal and the impact of nutrient concentration on the lipid content of algal cells are essential steps before the scale-up of biomass and lipid production by microalgae. The experimental data of lipid content and nitrate concentration are presented in Fig. 5.

The highest lipid content of the dry algal biomass was 15% and was occurred at the end of phase 5 when the microalgae were exposed to nutrient starvation. The lowest lipid content was observed at the end of third phase when the nitrate concentration increased rapidly. It seemed that lipid content was not depended on the operation mode but on nutrient concentration in the influent. Similar observations that the lipid accumulation was affected by the initial nutrient concentration have also been reported in the literature [9,10,34]. Other factors that affect the lipid content of the algae include the temperature [20], the substrate type [20], the initial algal concentration [10,16], and the algal species [14]. Nutrient starvation or extreme environmental conditions before biomass harvesting have been proposed to enhance the lipid accumulation in algal cells [35,36].

The low lipid content of the autochthonous algal strains may make economically unfeasible the production of biofuel. For this reason, more studies should be focused on these strains and their use in alternative products such as fertilizers, pigments, proteins, biomass pellets, biomethane

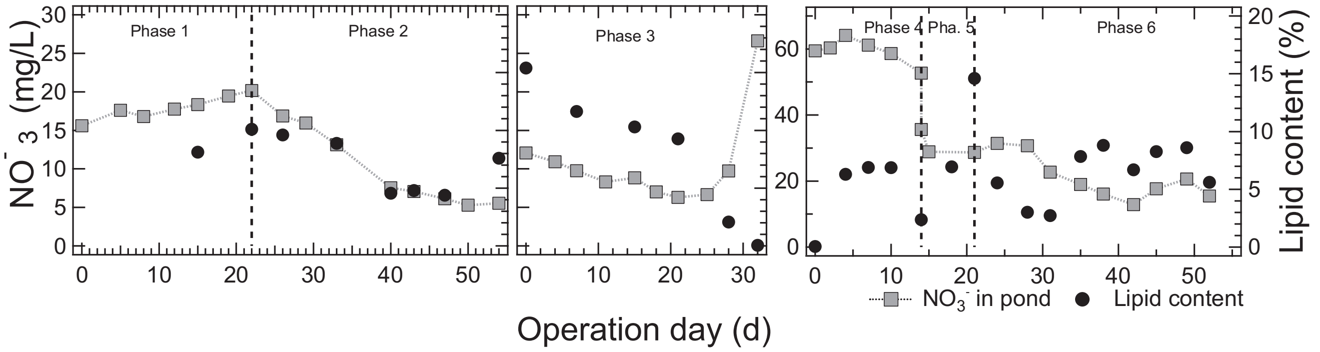

Fig. 5. Variation of nitrate concentration and algal lipid content in the pond.

production, and biocrude oil via hydrothermal liquefaction [17,18,36–39]. It should be noted that the incorporation of anaerobic digestion to algal biomass handling is expected to improve the viability of biodiesel production [37].

4. Conclusions

This research work combines wastewater treatment with cultivation of mixed microalgae culture for potential lipid production. The results of this study revealed that autochthonous microalgae, treating primary wastewater treatment, were able to reach a lipid content at 15%, similar to a single-microalgae culture. Furthermore, microalgal growth was affected by phosphate concentration and irradiation intensity. The nutrient removal in the pond reached values up to 52% and 100%, for nitrates and phosphates, respectively. The results of this work could be useful for the study of autochthonous microalgae in large-scale wastewater treatment plants.

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