Microalgae biomass growth and lipid production using primary treated wastewater

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Abstract
Microalgae are considered to be the most promising new source of biomass and biofuels, since their lipid content in some cases would be up to 70%. The algal growth and its metabolism processes are essential in wastewater treatment with many economical prospects. The aim of this work was to evaluate the algal production in a laboratory scale open pond. The pond had a working volume of 30 L and was fed with primary treated wastewater. The behavior of the pond was 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 was 449 mg/L, and was observed in the continuous operation mode. Phosphates concentration in the influent ranged from 0.60 to 1.57 mg P/L, while in the effluent was in most cases almost zero. The growth rate of microalgae and the algal lipid content were depended on the concentration of nutrients in the influent. Specifically, the nitrates were the limiting factor for the algal lipid content in each operation mode. The highest lipid content was observed when the system was operated in continuous mode with low nutrient supply of wastewater and was up to 15% of the dry weight.

Keywords
Microalgae; wastewater treatment; primary effluent; nutrient removal; lipid content

INTRODUCTION

Biological wastewater treatment with microalgae is particularly attractive due to the photosynthetic ability of algae to convert solar energy into useful biomass, and to incorporate nutrients such as nitrogen and phosphorus, which cause eutrophication (De la Noüe and De Pauw, 1988). The most common use of microalgae in wastewater treatment is the stabilization ponds (Aravantinou and Manariotis, 2014). Stabilization ponds are low-cost natural systems for the treatment of municipal and industrial wastewater. The stabilization ponds are classified to facultative, maturation and high-rate algal ponds. Facultative ponds, with typical depths 1.2 to 2.5 m and hydraulic retention HRT 5 to 20 d, are most frequently applied for organic matter and solids removal and pathogen control (WEF, 1990; Manariotis and Grigoropoulos, 2002). Increased levels of nutrient removal can be obtained at higher ambient temperatures in well-designed stabilization ponds, which include shallow maturation ponds (Silva et al., 1995; Soares et al., 1996). Recently, various attempts have been directed to the potential of algal-bacterial symbiotic process for the treatment of primary effluent (Aravantinou et al., 2016), secondary effluent (Conclaves et al., 2016) or other type of wastewater (Gonzalez-Fernandez et al., 2011).

Microalgae is a broad category of photosynthetic microorganisms consisting of eucaryotic microalgae and procaryotic 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 (Aravantinou et al., 2013). Many factors affect, the performance of algal systems such as nutrient concentration, CO_2, pH, aeration rate, light conditions, and temperature (Aslan and Kapdan, 2006; Aravantinou and Manariotis, 2016). 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 (Rawat et al., 2011; Grobbelaar, 1982).

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 (Aravantinou et al., 2013). This paper presents the findings of a study that investigated the treatment of primary effluent with an algal pond. The reactor performance was evaluated under batch and continuous feeding mode for biomass production, nutrients and organic matter removal, and lipid production.

MATERIAL AND METHODS

Experimental system

Raw wastewater was collected from the University of Patras campus wastewater treatment plant. The wastewater was settled for 1 h and the supernatant was used as the influent of the pond. Algal precultures were prepared by mixing 5 L of tap water, 0.5 L 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^{o}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 pond's dimensions were 50x50x25 cm (LxWxH) and the working volume was 30 L. The pond was fed with primary treated wastewater. 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. The third experiment was under continuous operating mode, and the pond was fed with 1 L/d by a peristaltic pump. The forth and fifth experiments were conducted under batch conditions; at the beginning of the fifth set 0.55 g of K_2HPO_4 were 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	Flow rate (L/d)	Radiation intensity (μmol/m^{2}s)
1	Batch	-	100
2	Fill and draw	1	100
3	Continuous	1	100
4	Batch	-	100
5	Batch*	-	100
6	Continuous	1	200

* Phosphates were added in the pond

Analytical methods
Microalgal biomass was determined by the measurement of total suspended solids (TSS) according to standard methods (APHA et al., 1998). 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 (APHA et al., 1998). Total nitrogen (TN) was determined spectrophotometrically by the method of 2.6 – dimethylofenolis (ISO, 1986). Nitrates and phosphates were determined by using ion chromatography (Dionex DX500, Dionex Corporation, Sunnyvale, CA). Soluble nonpurgeable 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. The algal lipid content of microalgae was measured by the modified method of Folch et al. (1957). 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^{o}C until all reagents was removed. The flask was allowed to cool to ambient temperature in a desiccator and then was weighed. The weight difference corresponded to intracellular lipids.

RESULTS AND DISCUSSION

Biomass production
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 (Figure 1). Similar results were observed for chlorophyll-a concentration (data not shown), and the highest concentration was observed at the 200 μmol/m^{2}s radiation intensity period. The maximum biomass concentration of 449 mg/L was observed under continuous mode and high radiation intensity in phase 6.

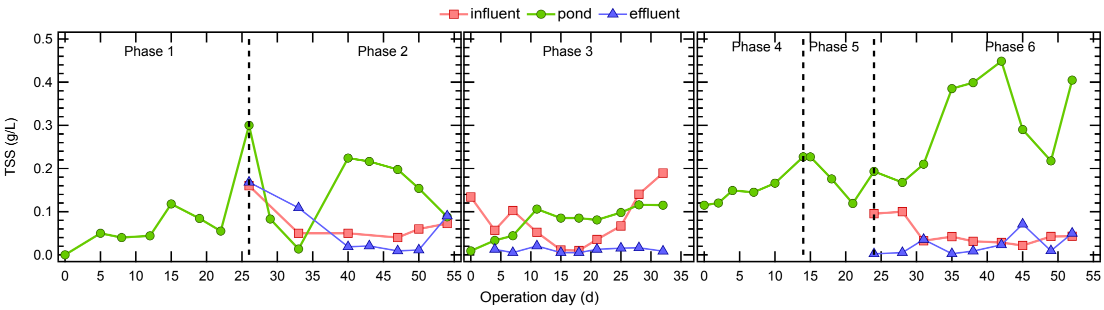

Figure 1. Solids variation in the influent, mixed liquor, and effluent of the pond.

Although the light intensity is an important factor in algae growth, the nutrient concentration, which was fed in the pond, is more important for algae growth. In phases 1, 4 and 5 the batch mode of operation directed the algae growth to be stable especially in phase 4 and 5. The continuous supply of substrate into the pond with primary treated wastewater resulted in the reduction of microalgae biomass in the pond. Similar results were reported in other studies (Aravantinou et al. 2016; Bellou and Aggelis, 2013), which mentioned that after the transition of their system operation from batch mode to continuous mode the biomass concentration of microalgae was reduced.

Between phase 2 and 3, the biomass concentration demonstrated similar growth despite the fact that there was difference in the operation mode. The higher biomass concentration was observed during the fill and draw mode even though that the average biomass concentration during the operation of each mode was actually the same 106 mg/L. As it was mentioned before, the supply of nutrients in the pond is a major factor for algal growth, so this high biomass concentration during the phase 2 should be due to the high phosphates concentration in the influent wastewater during that period. This leads to the conclusion that the microalgae which were cultivated were affected mostly by phosphates concentration in the culture.

Nutrient removal

The selection of appropriate microalgae species is critical since the algae growth controls directly and indirectly the nitrogen and phosphorus removal efficiency (Olguin 2003). In this study autochthonous microalgae from primary wastewater treatment were cultivated, in order to investigate their ability for nutrient removal, biomass and lipid production. Microalgae can assimilate a significant amount of nutrients in excess of the immediate metabolic needs (Larsdotter 2006), because they require high amounts of nitrogen and phosphorus for proteins (45-60 % of algal dry weight), nucleic acids and phospholipids synthesis. The nitrates concentration in influent, effluent, and inside the pond are shown in Figure 2.

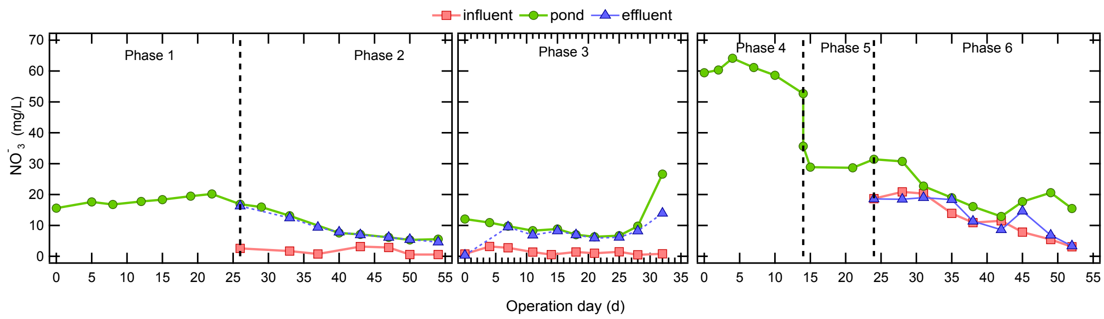

Figure 2. Nitrates variation in the influent, mixed liquor, and effluent of the pond.

In phase 1 (batch conditions), there was not observed any decrease in the concentration of nitrates in the pond, due to the low concentration of phosphates in the influent wastewater. The increased concentration of phosphates in the influent, in phase 2, resulted in a prompt decrease of nitrates, while in phase 3 nitrates concentration was similar in the pond and the effluent. The nitrate removal was satisfactory, and the maximum decrease of nitrates concentration (76%) was observed the same day with the external addition of phosphorus on day 14 (Phase 5). The uptake of ammonium and nitrate is important in microalgal nitrogen removal because nitrogen often exists as ammonium in wastewater especially in primary treated wastewater which were used in this study. It should be noted that even that the nitrates concentration in the influent was almost zero a high concentration of nitrates was observed in the pond. This phenomenon is because during the life cycles of algal cells in the pond the ammonium converted to nitrates (de-Bashan et al., 2004), thus increasing the nitrates concentration in the effluent.

Phosphates as well as nitrates are essential nutrients for biomass growth. Phosphates concentration in the influent ranged from 0.60 to 1.57 mg P/L and in the effluent their concentration was almost zero, implying the complete removal of phosphates. On the other hand the STotal-P in the effluent was ranged from 0.0 to 0.9 mg/L; this meant that STotal-P was not completely removed. Phosphates consumption is performed in synergy with nitrates. The experimental results (Figure 3) revealed that phosphates were the limiting factor for biomass growth in continuous mode conditions; phosphates 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 affected phosphorus removal. Other studies (Aravantinou et al., 2016) have also shown that microalgae can grow and efficiently remove nutrients from primary settled sewage.

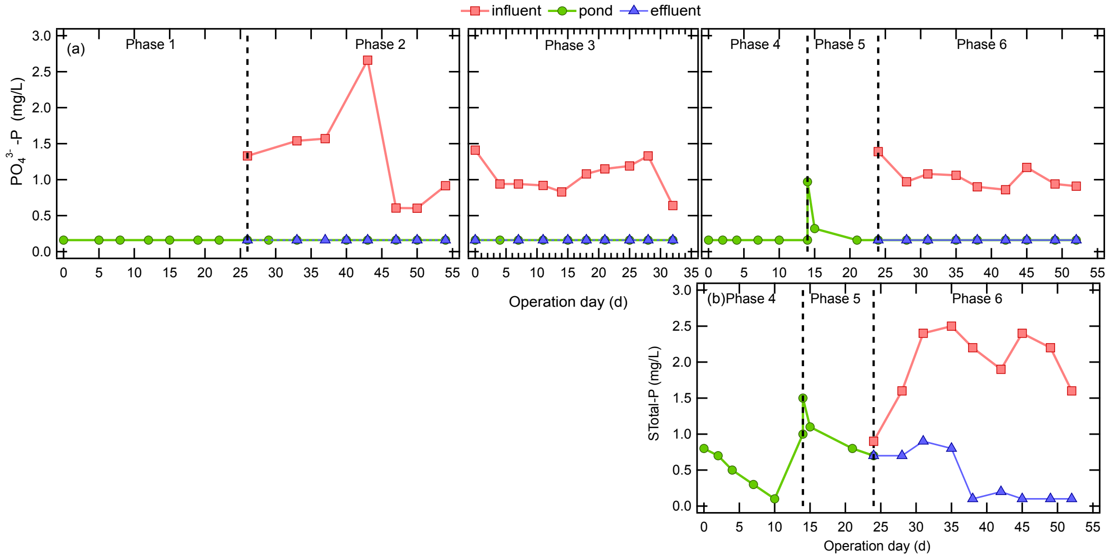

Figure 3. Phosphorus variation in the influent, mixed liquor, and effluent of the pond: (a) Phosphates and (b) Soluble Total-P.

The experimental data with the primary treated wastewater showed that in batch experiments, the organic matter concentration in the pond was increased probably due to the increase of biomass in the pond, and the mortality of microalgae. The latter resulted to cells lysis, and the release of nutrients in the liquid. In the experiments with continuous feeding with primary treated wastewater, the SNPOC in the effluent was higher than in the influent in some case, despite that the SNPOC of the effluent was very low. 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. On the other hand, when the system was operated in fill and draw mode, the removal of SNPOC was up to 90%. The decrease of SNPOC in the effluent of the pond might be attributed to the symbiotic action of bacteria and other heterotrophic microorganisms.

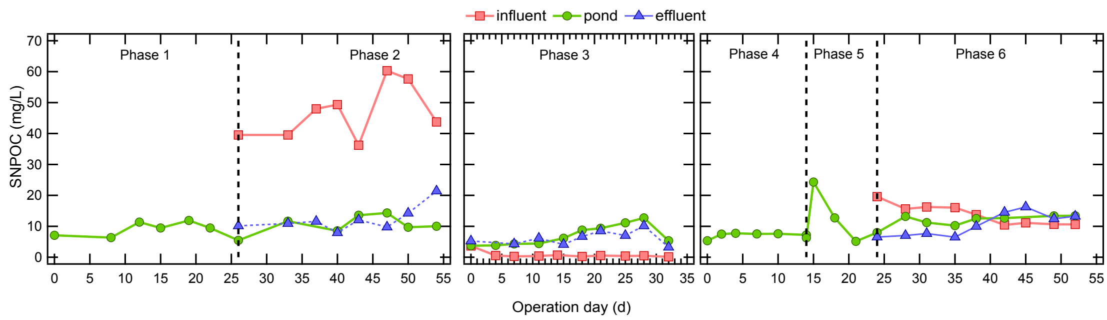

Figure 4. SNPOC variation in the influent, mixed liquor, and effluent of the pond.

Lipid content

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

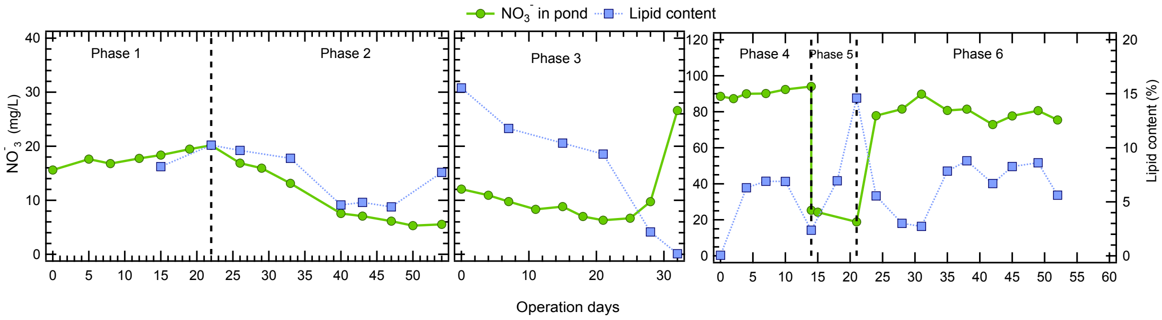

Figure 5. Variation of nitrates concentration and algal lipid content in the pond.

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 3rd phase when the nitrates concentration increased abruptly. It seemed that the lipid content was not depended on the operation mode but was affected by the nutrient concentration in the influent.

CONCLUSIONS

This research work combines wastewater treatment with microalgae for potential lipid production. The algal production was studied in a laboratory open pond, which was fed with primary treated wastewater. Microalgal growth was affected by phosphates concentration and irradiation intensity. The efficiency of microalgae to remove nitrates and phosphates was satisfactory, and reached removals of 76% and 100%, respectively. Finally, the highest lipid content was 15% when the microalgae faced starvation conditions.

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