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Optimization of pilot high rate algal ponds for simultaneous nutrient removal and lipids production

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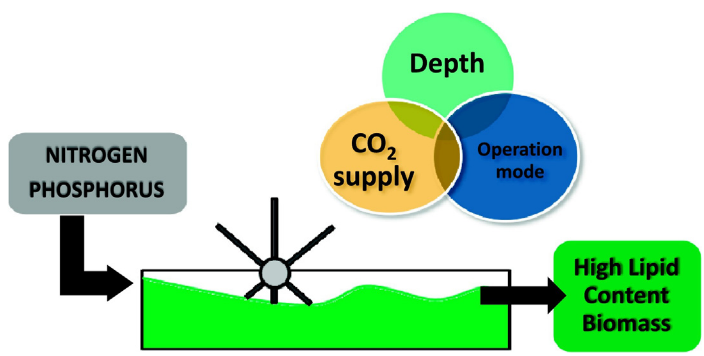
HIGHLIGHTS

- Simultaneous nutrient removal and lipid accumulation by microalgae
- Higher depth of culture affected positively biomass growth and lipid accumulation.
- Discharge limits for nitrogen and phosphorus were achieved under every condition.
- Continuous mode presented the best conditions for nutrient uptake and lipid production.

GRAPHICAL ABSTRACT



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ABSTRACT

Special attention is required to the removal of nitrogen and phosphorous in treated wastewaters. Although, there are a wide range of techniques commercially available for nutrient up-take, these processes entail high investment and operational costs. In the other hand, microalgae growth can simultaneously remove inorganic constituents of wastewater and produce energy rich biomass. Among all the cultivation technologies, High Rate Algae Ponds (HRAPs), are accepted as the most appropriate system. However, the optimization of the operation that maximizes the productivity, nutrient removal and lipid content in the biomass generated has not been established. In this study, the effect of two levels of depth and the addition of CO_2 were evaluated. Batch essays were used for the calculation of the kinetic parameters of microbial growth that determine the optimum conditions for continuous operation. Nutrient removal and lipid content of the biomass generated were analyzed. The best conditions were found at depth of 0.3 m with CO_2 addition (biomass productivity of 26.2 g TSS m^{-2} d^{-1} and a lipid productivity of 6.0 g lipids m^{-2} d^{-1}) in continuous mode. The concentration of nutrients was in all cases below discharge limits established by the most restrictive regulation for wastewater discharge.

1. Introduction

Microalgae cultivation for simultaneous wastewater treatment (mainly nitrogen and phosphorus removal) and biomass production for biofuel consists in a sequential process composed of cultivation,

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harvest, drying, oil extraction, and conversion of algal lipids into advanced biofuels. Microalgae have been identified as a possible source of new generation bio-fuels since they do not compete with food and feed crops, and can be cultivated in seawater, brackish water and in wastewater (Arbib et al., 2012; Arbib et al., 2013a, 2013b, 2013c; Ruiz et al., 2012; Tredici, 2010). Algae grown under controlled environmental conditions have shown the capability of producing 40–50 times more oil per unit land area than terrestrial oilseed crops (Sheehan et al., 1998). Even though the production of biodiesel for microalgae is technically feasible, algal biomass is at present largely too expensive to compete with petro-diesel (Tredici, 2010) and production costs must be reduced at least one order of magnitude.

The alternatives to reduce costs in production are limited, being the correct selection of cultivation technology the most important: open vs. closed systems. According to Tredici (2010), culture in closed photobioreactors may require more energy than they can provide, resulting in a negative net energy balance; therefore closed systems would be economically and technically feasible when the product of interest is highly valuable. For these reasons, the conventional high rate algal pond (HRAP), also called raceway pond, mixed with low energy input system of paddle wheels, is the main culture system used to commercially produce microalgae. HRAPs provide a cost-effective and efficient wastewater treatment and biomass production with minimal energy consumption (García et al., 2006; Heubeck et al., 2007; Arbib et al., 2013b, 2013c).

One of the major limitations of the HRAPs is the low light utilization by the cells due to the poor mixing (dark zones) and to the large light pathway (between 0.15 and 0.45 m depending on the operational depth), which results in lower volumetric productivities compared to closed systems. Since operational depth determines the light availability, this is the most relevant variable affecting the performance in terms of biomass production and lipid composition. Although this fact has been previously studied in experimental bioreactors, there is no agreement on the optimal conditions that ensure simultaneous lipid production and nutrient removal. On the one hand, it is believed that at lower depths, growth rate is promoted due to the higher light availability (Fernández et al., 2016). However, depth reduction also affects other variables that in turn affect microalgae growth, such temperature buffer capacity. Since most of the experiences reported with HRAP are addressed to specific objectives, such as evaluation of biomass productivity or wastewater treatment, the operational depth is normally fixed at 0.30–0.35 m (Park et al., 2011; Craggs et al., 2012). These figures have been reported in the very early studies dealing with the engineering aspects of microalgae cultivation (Oswald, 1988). However, this choice is made based on the efficiency of the mixing systems, rather than optimization of conditions for microalgae growth and lipids production. Therefore, comparative studies should be carried out considering the impact of depth, not only on biomass production, but also on the specific growth rate and the lipid content.

One serious drawback of microalgae cultivation in urban wastewater is the low carbon to nitrogen ratio of the wastewater. This carbon dearth involves firstly a slow rate of growth and, consequently, a deficient nutrient removal (Arbib et al., 2013b, 2013c; Park et al., 2011; Woertz et al., 2009). Therefore, the supplementation of an external carbon source (e.g. CO_2) to algal cultures enhances both the productivity and nutrient removal while avoiding pH inhibition (Arbib et al., 2013c; Heubeck et al., 2007; Kong et al., 2010; Lundquist et al., 2009; Park et al., 2011; Woertz et al., 2009). In a scenario of bioenergy production, the potential benefits of raceway carbonation should be evaluated considering the increases in biomass formation and lipid accumulation.

The effect of the operation at different depths and the addition of CO_2 in the culture of *Scenedesmus obliquus* in wastewater have been studied in experimental HRAPs. The study has been conducted using two modes of microalgae culture: batch culture and continuous operation. Biomass evolution during batch cultures was used to calculate the specific growth rate using a logistic model. The different conditions tested were evaluated in terms of biomass productivity, specific growth rate, lipid production and wastewater treatment.

2. Materials and methods

2.1. Microorganism

Freshwater *Scenedemus obliquus* (SAG 276–10) was obtained from Sammlung von Algenkulturen, Pflanzenphysiologisches Institut (Universität Göttingen, Germany). Inoculum was incubated firstly in controlled conditions in a climatic chamber in 2000 mL pyrex flasks at 20 ± 1 °C and 250 μmol m^{-2} s^{-1} of light intensity under a 14:10 h light:dark cycle in non-sterilized treated urban wastewater. After that, the inoculum was transferred to outdoor tubular photobioreactor (described in Arbib et al., 2013b, 2013c) with the main objective of obtaining enough volume of inocula for the two HRAP. Microscopic monitoring was carried out periodically and the dominant species was *S. obliquus* during the entire experimentation period.

2.2. Culture media

The effluent used for microalgae growth was the final effluent of the wastewater treatment plant (WWTP) located in Arcos de la Frontera (36°44′56.56″N, 5°47′37.12″W, Southern Spain). The wastewater passed through a preliminary screening, primary sedimentation, activated sludge and secondary sedimentation processes. Table 1 shows the average nutrient composition of the influent wastewater during the different batch and continuous periods of operation.

2.3. High rate algal ponds

Experiments were conducted using two identical high rate algal ponds (HRAPs) (referred to henceforth as $HRAP_1$ and $HRAP_2$). Both HRAPs were constructed in fiberglass, with a total working volume of 533 l when operating at 0.3 m depth and 266 l when operating at 0.15 m depth, with a surface of 1.93 m^2 (2.525 m length, 0.750 m width) (Fig. 1). The surface to volume ratio (S/V) was equal to 3.6 and 10.8 when the system was operated at 0.3 m and 0.15 m of depth, respectively. The culture was mixed mechanically with a paddle wheel with 4 blades connected to a motor working at 5 rpm, reaching a flow velocity between 0.2 and 0.3 m s^{-1}. The paddle wheel was placed over a depression (sump) on the pond bottom, this sump serves to reduce the back flow (Arbib et al., 2013b, 2013c; Dodd, 1986). Also, eccentrically placed curved walls were assembled at the end of the furthest away from the paddle wheel. This created curve zones of accelerating flow, followed by a flow expansion zone after the directional changes (Arbib et al., 2013b, 2013c; Dodd, 1986). The sole difference between both HRAPs was the depth, $HRAP_1$ was operated at 0.3 m and $HRAP_2$ at 0.15 m. In the experiments conducted with carbon dioxide supplementation this gas was injected at the bottom of the pond through a tube diffuser. The injection was controlled to maintain the pH levels during the day below a set-point of 8.

Table 1
Average total nitrogen (TN, mg L^{-1}) and total phosphorus (TP, mg L^{-1}) concentration in the influent wastewater during the different batch periods (with CO_2 and without CO_2 addition) and in the continuous mode operation.

	Batch		Continuous
	No CO_2	CO_2	CO_2
TN (mg L^{-1})	20.46 ± 0.83	20.93 ± 0.54	17.77 ± 1.00
TP (mg L^{-1})	2.14 ± 0.13	2.35 ± 0.14	1.58 ± 0.16