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Nutrients and biomass dynamics in photo-sequencing batch reactors treating wastewater with high nutrients loadings

Dulce María Arias*, Enrica Uggetti, María Jesús García-Galán, Joan García

GEMMA – Group of Environmental Engineering and Microbiology, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya•BarcelonaTech, c/ Jordi Girona 1-3, Building D1, E-08034 Barcelona, Spain

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ABSTRACT

The present study investigates different strategies for the treatment of a mixture of digestate from an anaerobic digester diluted and secondary effluent from a high rate algal pond. To this aim, the performance of two photo-sequencing batch reactors (PSBRs) operated at high nutrients loading rates and different solids retention times (SRTs) were compared with a semi-continuous photobioreactor (SC). Performances were evaluated in terms of wastewater treatment, biomass composition and biopolymers accumulation during 30 days of operation. PSBRs were operated at a hydraulic retention time (HRT) of 2 days and SRTs of 10 and 5 days ($PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$, respectively), whereas the semi-continuous reactor was operated at a coupled HRT/SRT of 10 days ($SC_{10\text{-}10}$). Results showed that $PSBR_{2\text{-}5}$ achieved the highest removal rates in terms of TN (6.7 mg L^{-1} d^{-1}), TP (0.31 mg L^{-1} d^{-1}), TOC (29.32 mg L^{-1} d^{-1}) and TIC (3.91 mg L^{-1} d^{-1}). These results were in general 3–6 times higher than the removal rates obtained in the $SC_{10\text{-}10}$ (TN 29.74 mg L^{-1} d^{-1}, TP 0.96 mg L^{-1} d^{-1}, TOC 29.32 mg L^{-1} d^{-1} and TIC 3.91 mg L^{-1} d^{-1}). Furthermore, both PSBRs were able to produce biomass up to 0.09 g L^{-1} d^{-1}, more than twofold the biomass produced by the semi-continuous reactor (0.04 g L^{-1} d^{-1}), and achieved a biomass settleability of 86–92%. This study also demonstrated that the microbial composition could be controlled by the nutrients loads, since the three reactors were dominated by different species depending on the nutritional conditions. Concerning biopolymers accumulation, carbohydrates concentration achieved similar values in the three reactors (11%), whereas < 0.5% of polyhydrohybutyrates (PHB) was produced. These low values in biopolymers production could be related to the lack of microorganisms as cyanobacteria that are able to accumulate carbohydrates/PHB.

1. Introduction

Wastewater treatment with microalgae is regarded as an economical and environmentally friendly process with the additional advantage that the biomass produced can be reused, allowing an efficient nutrient recycling (Rawat et al., 2011; Honda et al., 2012). In this process, microalgae work in association with aerobic heterotrophic bacteria so that photosynthetic microorganisms produce molecular oxygen that is used as electron acceptor by bacteria to degrade organic matter (Abed et al., 2009; Borde et al., 2003). In return, bacteria release carbon dioxide during the mineralization process and complete the photosynthetic cycle (Muñoz and Guieysse, 2006). This wastewater treatment process has been successfully used for a range of purposes such as removal of nutrients and other compounds (i.e. heavy metals) and also to reduce the load of organic matter (Abdel-Raouf et al., 2012; de Godos et al., 2009; Honda et al., 2012; Wang et al., 2010). Furthermore, wastewater is nowadays considered the only economically viable source of water and nutrients for the production of microalgae biomass that can then be used for valuable by-products generation (Pittman et al., 2011; Uggetti et al., 2014).

In spite of the benefits, microalgae-based wastewater treatment technologies face operational limitations and challenges, such as the high costs derived from biomass separation from the treated wastewater (Renuka et al., 2013; Trivedi et al., 2015; Udom et al., 2013). Indeed, an efficient separation requires the use of biomass harvesting processes which can increase the production cost by 20–30% (Molina-Grima et al., 2003; Renuka et al., 2013; Yaakob et al., 2014). Recently, several studies have proposed to include a sedimentation period in the operational mode in order to increase spontaneous flocculation and the subsequent formation of large flocs (Valigore et al., 2012; Van Den Hende et al., 2016, 2014). This process can be carried out in a photo-sequencing batch reactor (PSBR), where hydraulic retention time (HRT) and solids retention time (SRT) are uncoupled, similarly to activated sludge systems (Wang et al., 2015). This way, the cells are forced to

* Corresponding author.
E-mail address: dulce.maria.arias@upc.edu (D.M. Arias).

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form flocs that settle faster, whereas unsettled cells are removed from the supernatant (Valigore et al., 2012). Contrary to conventional operations, which do not promote extensive spontaneous flocculation (i.e. continuous, semi-continuous and batch), this approach can avoid additional intensive harvesting process. In addition, uncoupled HRT/SRT could influence nutritional dynamics and biomass composition. This can cause biochemical changes in microalgal biomass, affecting the accumulation of valuable biopolymers such as carbohydrates, lipids and, in the case of cyanobacteria, polyhydroxybutyrates (PHBs) (Arcila and Buitrón, 2016; Arias et al., 2018a). All these compounds have obtained an increasing attention due to their potential use as biodiesel substrate and as bioplastics in the case of PHBs. The information of such promising alternative is still insufficient and all the aspects concerning nutrients dynamics in this kind of systems need to be addressed.

In a previous work by Arias et al., (2017), it was demonstrated that nutrients dynamics in a semi-continuous reactor used for a wastewater tertiary treatment played an important role in the biomass composition during a long term study. In that study, the use of digestate from an anaerobic digester diluted with secondary wastewater from a high rate algal pond proved to be suitable for the growth of a selective culture of cyanobacteria. All in all, the present study aims to evaluate the performance of different photo-sequencing batch reactors during tertiary treatment of digestate diluted with secondary wastewater, comparing the dynamics with a conventional semi-continuous reactor (SC) in terms of wastewater treatment, biomass composition and biopolymers accumulation.

2. Material and methods

2.1. Inoculum

A mixed culture composed by green algae, cyanobacteria, bacteria, protozoa and small metazoa was used as inoculum. It was collected as thickened biomass (100 mL) from a harvesting tank connected to a pilot closed-photobioreactor (30 L) already used as tertiary wastewater treatment (Arias et al., 2017).

2.2. Experimental set-up

Experiments were performed at lab-scale in three photobioreactors consisting of a closed polymethacrylate cylinder with an inner diameter of 11 cm, a total volume of 3 L and a working volume of 2.5 L each. Experiments were carried out during 30 days, and all of them were submitted to light/dark cycles of 12 h each. Illumination during the light phase was supplied by two external halogen lamp (60 W) placed at opposite sides of each reactor and providing 220 μmol m^{-2} s^{-1} of light. Reactors were continuously agitated (with the exception of settling periods) with a magnetic stirrer (Selecta, Spain) set at 250 rpm. Temperature was continuously measured by a probe inserted in the PBR (ABRA, Canada) and kept constant at 27 (± 2) °C by means of a water jacket around the reactor. pH was continuously monitoring with a pH sensor (HI1001, HANNA, USA) and kept at 8.5 with a pH controller (HI 8711, HANNA, USA) by the automated addition of HCl 0.1 N or NaOH 0.1 N. A diagram of the process of each reactor is presented in Fig. 1.

Two of the reactors were operated in a sequencing batch operation mode at a HRT of 2 days. One of these photo-sequencing batch reactors (PSBR), named $PSBR_{2\text{-}10}$, was operated at a SRT of 10 days. This means that 0.25 L of mixed liquor were discharged at the end of the dark phase, then the agitation was stopped and biomass was allowed to settle during 30 min. After this period, 1 L of the supernatant was withdrawn and then the total volume discharged (1.25 L) was replaced with the same volume of wastewater influent (Fig. 1a). The other sequencing batch reactor (named $PSBR_{2\text{-}5}$) was operated with a SRT of 5 days. Thus, 0.5 L of the mixed liquor were withdrawn at the end of the dark phase before the subsequent settling time of 30 min. After the settling period, 0.75 L of the supernatant was withdrawn and then the total volume retired (1.25 L) was replaced with the same volume of wastewater influent (Fig. 1b). The operation of these PSBRs was compared with that of a semi-continuous reactor named $SC_{10\text{-}10}$ (control reactor). This reactor was fed once a day and operated at a HRT and SRT of 10 days. This means that each day at the end of the dark phase, 0.2 L of the mixed liquor were withdrawn and subsequently this volume was replaced by 0.2 L of wastewater influent (Fig. 1c).

The influent treated in the reactors consisted on uncentrifuged digestate diluted in secondary effluent in a ratio of 1:50 (characteristics are shown in Table 1). The secondary effluent was obtained from a pilot system treating municipal wastewater which comprised a primary settler, a high rate algal pond (HRAP) and a secondary settler (Gutiérrez et al., 2016). The digestate was obtained from lab-scale anaerobic digesters (1.5 L) that produced biogas from microalgae biomass harvested from the HRAP. A detailed description of the system may be found in (Arias et al., 2018b). Mixed liquor and supernatant withdrawal, and feeding were performed by the automatic peristaltic pumps.

2.3. Analytical methods

2.3.1. Nutrients concentrations

Nutrients monitoring was carried out by analyzing samples taken from the reactors at the end of the dark phase, after settling. All parameters were determined in triplicate and analyzed from the influent (mixed digestate and secondary effluent) and the supernatant of each reactor. Note that in the case of the reactor $SC_{10\text{-}10}$, the supernatant sample was taken from the mixed liquor withdrawn and submitted to a separation process. Samples from the influent were measured once per week, and samples of supernatant were analyzed three days per week.

Nitrogen was measured as total ammoniacal nitrogen (TAN), nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$), total nitrogen (TN) and total phosphorus (TP). TAN (sum of $N\text{-}NH_3$ and $N\text{-}NH_4^+$) was determined using the colorimetric method indicated in Solorzano (1969). $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ concentrations were analyzed using an ion chromatograph DIONEX ICS1000 (Thermo-scientific, USA), while TN was analyzed by using a C/N analyzer (21005, Analytikjena, Germany). Total inorganic nitrogen (TIN) was calculated as the sum of $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and TAN. Total organic nitrogen (TON) (in dissolved and particulate form) was calculated as the difference between TN and TIN.

Phosphorus compounds analyzed were inorganic phosphorus (IP) measured as orthophosphate (dissolved reactive phosphorus) ($P\text{-}PO_4^{3-}$) and total phosphorus (TP). IP concentrations were analyzed using an ion chromatograph DIONEX ICS1000 (Thermo-scientific, USA) and total phosphorus (TP) was analyzed following the methodology described in Standard Methods (APHA-AWWA-WPCF, 2001). Total organic phosphorus (TOP) forms (dissolved and particulate) were calculated as the difference between TP and IP.

Total organic carbon (TOC), Total inorganic carbon (TIC), soluble organic carbon (OC) and soluble inorganic carbon (IC) were measured from raw and filtered samples using a C/N analyzer (21005, Analytikjena, Germany).

The volumetric load (Lv-X) of each nutrient (TOC, TIC, TAN, NO_2^-, $N\text{-}NO_3^-$, TIN, TON, TN, IP, TOP and TP) was calculated in [mg × L^{-1} d^{-1}] as shown in Eq. (1):

$$Lv\text{–}X = \frac{Q * X}{V} \quad (1)$$

where Q is the flow [L^{-1} d^{-1}], X is the nutrient influent concentration [mg × L^{-1}] and V [L^{-1}] is the volume of the reactor.

2.3.2. Biomass concentration

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured in the mixed liquor at the end of the dark phase three days per week. In $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$, two samples were taken; one from the mixed liquor right before stopping the agitation in order to evaluate the biomass production, and one from the supernatant after