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Microalgal biomass production and nutrients removal from domestic sewage in a hybrid high-rate pond with biofilm reactor

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ABSTRACT

In this study, biomass production and domestic sewage treatment in hybrid systems under bacterial-microalga consortia were assessed. Biomass was grown suspended in the growth media of high-rate ponds (HRPs) and attached in biofilm reactors (BRs). These hybrid systems were operated with and without the addition of CO_2 (HS2 and HS1, respectively) in the HRP growth media. The performances of these systems were compared with that of a conventional HRP with CO_2 supplementation. Regarding sewage treatment with microalgae and bacteria consortia, the three systems showed no significant differences in the removal of organisms associated with faecal contamination, organic matter and most nutrients. However, nitrate levels were increased in the hybrid systems due to the presence of BRs. There were no differences in algal biomass production among the three systems, which remained in the 0.6–0.7 g m^{-2} range. HS1 showed the highest total biomass production of 101.31 g m^{-2} at a production rate of 6.79 g m^{-2} day^{-1}. The BR of HS1 was able to supply the necessary CO_2 and therefore no additional gas supplementation was required. This result indicates that a conventional HRP with CO_2 supplementation can be replaced by a hybrid system with biofilm reactor, with additional advantages of resources saving, operational simplicity and easier harvesting.

1. Introduction

Microalgae and bacteria consortia have important remediation role in wastewater treatment. Through photosynthesis, microalgae release oxygen improve the aerobic degradation of the organic matter, while heterotrophic bacteria release CO_2 that contributes to the growth of microalgae (Prajapati et al., 2013; Subashchandrabose et al., 2011). Treated effluent discharged in water bodies typically has higher oxygen concentrations (Arbib et al., 2013) and nutrients recovered as biomass during treatment can be turned into different bio-products, such as biofertilisers, animal feed supplements and third-generation biofuels (Brennan and Owende, 2010; Chisti, 2007).

High-rate ponds (HRPs) are an efficient and inexpensive technology used to treat wastewater and produce microalgae. However, they require large land areas to collect sufficient amounts of algal biomass, since concentrations of algal biomass are typically lower than 10 g L^{-1}, more than 99% of culture volumes is water instead of algal biomass (Zhang et al., 2014). Thus, collection and separation processes are the weakest point of the entire production chain, representing close to 20–30% of the production costs of microalgal biofuels (Grimma et al., 2013).

The use of biofilms in HRPs is a potential solution to reduce the required land area (Johnson and Mara, 2005; Xia et al., 2008) and improve nutrient removal (Babu et al., 2010). Biofilms also provide other advantages, such as increased biomass concentrations, reduced sensitivity to toxicity, accelerated biomass formation and reduced biomass separation costs (Johnson and Wen, 2010; Ozkan et al., 2012; Gross et al., 2013; Zamalloa et al., 2013; Schnurr et al., 2013; Liu et al., 2013).

Laboratory-scale studies have been developed to optimise algal collection through the immobilisation of biomass on support media with wastewater as culture media (Johnson and Wen, 2010; Posadas et al., 2013; Shi et al., 2014). Studies by Christenson and Sims (2012), Gross et al. (2013) and Lee et al. (2014) have demonstrated that the integration of suspended and attached culture systems results in higher algal yields compared with conventional HRPs. Furthermore, biomass collection is simpler and cheaper in these hybrid systems.

In this study, the concept of attached biomass growth in pilot scale under continuous operation was applied during domestic

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sewage treatment by bacterial-microalgae consortia. Biofilm reactors (BRs) were integrated with the HRPs, which created a hybrid system for biomass growth (biomass was grown suspended in HRPs and attached in BRs). The biomass production and wastewater treatment results of the hybrid systems were compared with those of a conventional suspended-growth HRP with CO_2 supplementation. The main objective of this study was to demonstrate the potential use of BRs in producing and physically separating biomass and to show that these BR configurations did not require additional gas supplementation of the microalgal culture media.

2. Materials and methods

2.1. Experimental unit

This study was conducted at the experimental area of the Federal University of Viçosa (Universidade Federal de Viçosa; UFV), located in Viçosa, Minas Gerais, Brazil (20°45′14″S, 42°52′54″W), during the winter, from June to August, 2015. The average altitude of the location is 648 m. The climate is classified as highland tropical with hot and rainy summers and cool and dry winters. The mean annual precipitation is approximately 1221 mm, and the mean annual temperature is between 19 °C and 20 °C (Rocha et al., 2012).

Three biomass production systems were operated: a hybrid system without CO_2 supplementation (HS1), a hybrid system with CO_2 supplementation (HS2) and a conventional high-rate pond with CO_2 supplementation (HRP). The hybrid systems combined two different types of biomass growth, i.e., those attached to the biofilm reactor and those suspended in the HRP. HS1 consisted of a HRP adapted with a BR, whereas HS2 consisted of a HRP adapted with a BR plus CO_2 supplementation. Both hybrid systems were compared with a conventional HRP with CO_2 supplementation. The influence of BRs on biomass production and sewage treatment was assessed. Additionally, the hybrid systems were compared to evaluate the differences in the system due to CO_2 supplementation, since the direct contact with atmospheric air and solar radiation can supply the need for addition of gases in the culture medium.

The HRPs were made from fiberglass and had the following dimensions: width = 1.28 m, length = 2.86 m, total depth = 0.5 m, useful depth = 0.3 m, surface area = 3.3 m^2, useful volume = 1 m^3. The inlet flow was manually regulated to 0.2 $m^3 \cdot day^{-1}$ to maintain a hydraulic retention time (HRT) of 5 days. The paddlewheels were driven by a 1 hp electric motor. Rotation was reduced by a reduction gear coupled to the motor and controlled by a frequency inverter (WEG, series CFW-10) to provide a mean horizontal water velocity of approximately 0.10–0.15 m s^{-1}. The culture medium used in the study was domestic sewage pre-treated in a real-scale upflow anaerobic sludge blanket (UASB) reactor. The UASB reactor was operated with an average effluent flow of 115 m^3 day^{-1} and a HRT of 7 h.

The HRPs were supplemented with CO_2 using a cylinder of 99% CO_2 purity. The CO_2 supplementation was controlled based on media pH, which was kept between 7 and 8. A carbonation column was built using PVC and designed according to Putt et al. (2011). The column had a height of 2.20 m and a diameter of 0.10 m. The effluent recirculation flow rate through the carbonation column was 4 L min^{-1} and was controlled using an underwater pump (Sarlobetter SB 1000A). The gas was added at a flow of 1 L min^{-1}, controlled by flow meters with a 0–15 L min^{-1} capacity.

The BRs were made of flat acrylic panels and have the following characteristics: total surface area of 1.0 m^2, with each panel measuring 1.0 m in width and 0.5 m in length. The panels were kept in direct contact with atmospheric air and solar radiation. They were installed vertically next to the HRPs and supported on PVC pipes 0.85 m from the ground. To enable biomass attached growth, each panel was lined with an interlace (Entrevin, E460, 100% cotton), a support material normally used in clothing manufacturing. The reactor material and type were based on Vicente (2010). Fig. 1 shows the hybrid system with CO_2 addition.

In both hybrid systems, the HRP effluents were recirculated to the BRs during 10 h per day, i.e. the useful volume of the pond was recirculated 10 times in a day, with an underwater pump (Sarlobetter SB 1000A) at a 1 m^3 h^{-1} flow. After being pumped, effluents were percolated through the panel surfaces by dripping, collected in a gutter and returned to the HRPs by gravity.

2.2. Monitoring

Samples totalizing a volume of 1.250 L were collected from the HRPs every 3 days, from 8:00 A.M. to 4:00 P.M. To characterise the domestic sewage inflow to the HRPs, and to determine chlorophyll-*a* and *Escherichia coli (E. coli)* samples were collected at 4:00 P.M. only. Additionally, dissolved oxygen (DO), temperature and pH were monitored in the culture media of all HRPs using a Hach HQ40d probe (Luminescent Dissolved Oxygen–LDO for DO). Photosynthetically active radiation (PAR) was measured with a LI-COR LI-193 Underwater Spherical Quantum Sensor radiometer. These measurements followed a 2-h collection interval.

The following chemical and physical variables were determined: total suspended solids (TSS; 2540D), volatile suspended solids (VSS; 2540E), soluble chemical oxygen demand (COD_s; 5220D; the samples were filtered through a 0.45-μm filter), ammoniacal nitrogen (N-NH_3; 4500 – NH_3C), nitrate (N-NO_3^-; 4500-NO3A) and soluble phosphorus (P_s; 4500 P C; the samples were filtered through a 0.45-μm filter). The analyses were performed as recommended by the Standard Methods for the Examination of Water and Wastewater (APHA, 2012), and the methods used for analysis of each variable are between parentheses. Soluble organic carbon (TOC_s) was obtained with a Shimadzu TOC 5000 analyser (from samples filtered through a 0.45-μm filter). The chromogenic-fluorogenic method (Colilert®) was used to measure *E. coli* levels. Chlorophyll-*a* was extracted with 80% ethanol (Nush, 1980) and measured by spectrophotometry (APHA, 2012); concentrations were determined following equations provided in Dutch standard (NEN 6520, 1981).

In the hybrid systems, the BRs were operated until layers of attached biomass were formed on the panels, typically 40 days. Then, the biomass layers were scraped and collected, and the remaining cells were kept on the adherent material as inoculum for the next growth cycle. Subsequently, the panels were scraped with a spatula every 48 h to assess biomass growth. Compounded samples from six positions (right side, left side and middle of the front and back of each panel) of each panel surface were scraped.

The samples that were scraped from the panels were used to quantify the biomass in the BRs. The total biomass was determined by total volatile solids (TVS) analysis (APHA, 2012). Each scraped area on the panels was 6.25 cm^2. To quantify chlorophyll-*a*, an area of 1.0 cm^2 areas was scraped at each position and were diluted in 15 mL of distilled water. Chlorophyll-*a* of the attached biomass was extracted with 80% ethanol (Nush, 1980), and measurements were obtained by spectrophotometry (APHA, 2012). Calculations were performed using equations described by Schwarzbold et al. (2013), adapted from Marker et al. (1980) and Sartory and Grobbelaar (1984).

Weighted calculations of the productivity rates were applied for the hybrid system based on the biomass growth areas in the HRPs and the BRs (Eq. (1)):

$$Pr = \frac{(3.3 * Pr_{HRP}) + (1,0 * Pr_{BR})}{4.3} \quad (1)$$