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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)):

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

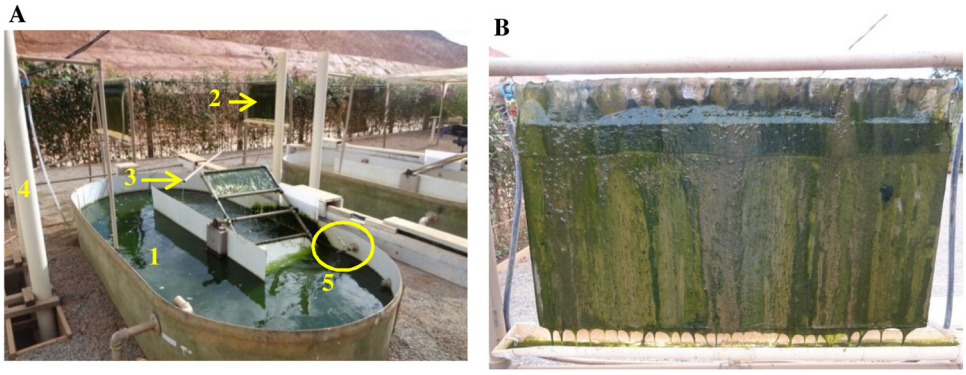

Fig. 1. (A) Overview of the hybrid system: high-rate pond (1), biofilm reactor (2), effluent recirculation (3), carbonation column (4), continuous feed of domestic sewage (5); (B) biofilm reactor.

where Pr is the weighed production/daily productivity rate of the hybrid system ($g\,m^{-2}/g\,m^{-2}\,day^{-1}$), Pr_{HRP} is the production/daily productivity rate of the HRP ($g\,m^{-2}/g\,m^{-2}\,day^{-1}$), Pr_{BR} is the maximum production/daily productivity rate of the BR ($g\,m^{-2}/g\,m^{-2}\,day^{-1}$), 3.3 is the HRP surface area (m^2), 1.0 is the BR total area (m^2), and 4.3 is the total growth area of biomass in the hybrid system (m^2).

Attached biomass growth occurred cumulatively in the BRs. In the HRPs, biomass grew in suspension and was continuously renewed due to the inherent flow through of the culture medium. Therefore, for the BRs and the HRPs, Eq. (1) was evaluated using the production and production rates at the ends of growth cycles and the mean production and production rates, respectively.

2.3. Chemical composition of the attached biomass

The humidity, carbon (Walkley and Black, 1934), nitrogen (APHA, 2012) and phosphorus (Valderrama, 1981) contents of the biomass attached to the panels were determined at the end of the experiment. The protein content was calculated using a nitrogen conversion factor of 5.95 (González López et al., 2010). The ash content was quantified based on a ratio between the total fixed solids (TFS) (APHA, 2012) and the total biomass collected.

To determine the neutral lipid content, the collected attached biomass was dried in an oven at 50 °C for 24 h and macerated. The lipid content analysis consisted of disrupting the cell wall using 3 M hydrochloric acid and further solvent extraction with petroleum ether and methanol. After the extraction, the extracted oil was washed with 4% plumbic acetate to remove the impurities and pigments. The lipid content was determined gravimetrically. The lipid production rate was obtained by multiplying the total biomass production rate ($g\,TSV\,m^{-2}\,day^{-1}$) by the lipid content (%).

2.4. Phytoplankton community

A total of 200 mL of samples from the HRP surfaces and 25 cm^3 from the six positions of each panel was collected at the end of the experiment. These samples were diluted in 100 mL of distilled water. The samples were kept in 4% formaldehyde solution for further phytoplankton characterisation. The characterisation was performed at the genus level, and for the dominant genus, the species present in the samples were identified.

In quantitative analyses, individual counts were performed in a sedimentation chamber using an inverted microscope according to the method described by Uthermöl (1958). Organism density was determined based on APHA (2012) criteria. Biovolume calculation was performed for the most abundant organisms as described by Wetzel and Likens (1991). In qualitative analyses, identification was performed using an inverted microscope as described by Parra et al. (1982) and Komárek and Foot (1983).

2.5. Statistical analysis

The *Assistat* program, version 7.7 beta (2016), developed by Silva and Azevedo (2016), was utilised to assess the differences among the mean values of the variables measured in the HRPs. A variance analysis (ANOVA) was also performed. The experiment used an entirely randomised design for *t*-tests with 5% probability.

3. Results and discussion

3.1. Biomass growth

Productivity rates of the three production systems are shown in Table 1. The concentration of the biomass attached to the BRs was higher than that of the suspended biomass produced in the HRPs. This difference occurred because the growth of the attached biomass was cumulative in the BRs, as opposed to the growth of the biomass suspended in the HRPs, which was kept under a continuous flow of culture medium. Additionally, the direct contact with solar radiation, atmospheric air and the adhering support medium favoured more biomass growth in the BRs. Thus, the production of volatile solids in HS1 and in HS2 was approximately three and two times larger than in the conventional HRP, respectively, and the total productivity rates of both hybrid systems were close to that of the conventional HRP. The total productivity rate of hybrid system HS1 ($6.79\,g\,m^{-2}\,day^{-1}$) was lower than its BR ($9.99\,g\,m^{-2}\,day^{-1}$), whereas for HS2 ($6.10\,g\,m^{-2}\,day^{-1}$) was lower than that of the HRP with suspended biomass ($6.17\,g\,m^{-2}\,day^{-1}$).

The production of chlorophyll-*a* in the three culture systems was similar. To better assess the indication of microalgae production, the ratios between chlorophyll-*a* and volatile solids were considered in each culture system. According to Veloso et al. (1991), ratios between chlorophyll-*a* and volatile solids that are in the 1–1.5% range indicate a good proportion of algal and total biomass culture. Lower ratios indicate an algal biomass under stress, with competition for nutrients and predation. The highest proportions of microalgae in biomass occurred in the HRPs of HS1 (2.2%) and HS2 (2.06%) and in the conventional HRP (2.04%). The BRs of HS1 and HS2 had ratios of 0.27% and 0.35%, respectively, which indicated a higher contribution of heterotrophic organisms, such as bacteria and protozoa. Therefore, the conventional HRP had the highest microalgal concentration when compared with HS1 (0.67%) and HS2 (0.96%).

The advantages of the hybrid systems, such as higher biomass concentrations, simple biomass physical separation and easy har-

Table 1
Chlorophyll-*a*, volatile solids and total productivity of each production system.

Culture System		Chlorophyll-*a* ($g\,m^{-2}$)	Volatile Solids ($g\,m^{-2}$)	Total Productivity ($g\,m^{-2}\,day^{-1}$)
HS1	Suspended	0.60 (0.35)	29.11 (10.65)	5.82 (2.13)
	Attached	0.94	339.55	9.99
	Total	0.68	101.31	6.79
HS2	Suspended	0.68 (0.32)	30.87 (12.25)	6.17 (2.45)
	Attached	0.75	211.41	5.87
	Total	0.70	72.86	6.10
HRP	Suspended	0.64 (0.32)	31.37 (11.76)	6.27 (2.35)
	Attached	–	–	–
	Total	0.64	31.37	6.27

Note: Maximum values of chlorophyll-*a* and volatile solids at the end of the growth cycle were considered for the attached systems (BRs) and mean values were considered for the suspended systems (HRPs). For the hybrid systems, the total values were determined from weighted calculations based on the surface areas of the HRPs and the biofilm reactors, as described in Eq. (1). The standard deviations are shown in parentheses.

vesting, make them promising culture systems. Additionally, the absence of CO_2 supplementation in HS1 did not compromise biomass production and therefore, gas addition was not required.

Fig. 2 compares the biomass growth curves of the hybrid systems and the conventional HRP. The HS1 had the highest production of attached biomass in its BR, i.e., 339.55 $g\,m^{-2}$ in 34 days. The HRP of the HS1 had a production of 29.11 $g\,m^{-2}$, corresponding to a daily production rate of 6.79 $g\,m^{-2}\,day^{-1}$. In the HS2, the BR reached a maximum production of attached biomass of 211.41 $g\,m^{-2}$ in 36 days of culture, and the HRP reached an average production of suspended biomass of 30.87 $g\,m^{-2}$, which corresponded to a daily production rate of 6.10 $g\,m^{-2}\,day^{-1}$ for the hybrid system with CO_2 addition.

In general, biomass productivities obtained in this study (Table 1) were close to those reported in other works that assessed attached biomass cultures in HRPs. Gross et al. (2013) studying the attached growth of microalgae on a cotton fabric surface (3.5 m^2) with synthetic growth in a HRP (24 m^2) reached a productivity of the hybrid system that varied between 8.1 and 14.1 $g\,m^{-2}\,day^{-1}$. Regarding the production rate of the total attached material, the average results were between 1.9 and 4.3 $g\,m^{-2}\,day^{-1}$. Christenson and Sims (2012) achieved after 20 days, a total productivity of 20 $g\,m^{-2}\,day^{-1}$, using cotton braids as support media for microalgal adhesion in a rotating algal biofilm reactor adapted to a HRP (2.72 m^2 surface area) with domestic sewage as culture media. Lee at al. (2014) assessing an algal system attached to different nylon meshes (33.1 m^2 total area) inserted inside an HRP (20.6 m^2) during a batch treatment of domestic sewage for 18 days, reported a maximum productivity of the attached system of 13.5 $g\,m^{-2}\,day^{-1}$, 4.2 times higher than the average production rate of a conventional HRP.

3.2. Composition of biomass grown in biofilms

Table 2 shows the chemical composition of the attached biomass grown in biofilms. A comparison of both biofilm compositions showed that the BR of HS1 had a higher neutral lipid content. However, the neutral lipid content was low in both systems, indicating that the biofilm production in hybrid systems using domestic sewage as culture media was not favourable having the production of neutral lipid-based biofuels, such as biodiesel, as the final destination. The diversity of microorganisms with low lipid content, such as bacteria, results in a reduced lipid content of biomass grown in wastewater (Shen et al., 2015).

The biomass concentration and the humidity content are related and are important in determining a method to harvest and separate the biomass. The humidity content of both biofilms were similar and close to a humidity content of 93.75% reported by Johnson and Wen (2010) in attached biomass growth systems. The authors compared their results to those of a suspended biomass system after centrifugation that had a 1.5% lower humidity content than that of the attached culture system.

The solid content of the BRs of HS1 and HS2 found in this study, 8.4% and 11.7%, respectively, met the required humidity specifications for energy use via anaerobic digestion (McKendry, 2002); therefore, a biomass concentration stage was not necessary. Additionally, those contents were within the ranges of results of studies that used different algal biomass dehydration technologies, such as flotation by dissolved air, 3–10% (Wiley et al., 2011); centrifugation, 1–22% (Dassey and Theegala, 2013; Grima et al., 2003); and filtration membranes, 5–27% (Grima et al., 2003; Cooney et al., 2011; Ríos et al., 2012).

As was performed by Johnson and Wen (2010), in this study, the attached biomass was collected by scraping and did not require chemicals or energy. Moreover, it presented a dewatering efficiency similar to a suspension culture system, after a centrifugation stage. Therefore, the attached growth system could also be considered a unit of biomass physical separation.

Carbon (C) and nitrogen (N) contents of the BR of HS2 were higher than those of the HS1, whereas the P contents were the same in both systems. Boelee et al. (2011) studying nitrogen and phosphorus removal from municipal wastewater through algal biofilms with pH control by CO_2 addition, reported a biomass with 5% of nitrogen and 1% phosphorus contents. In the study by Zamalloa et al. (2013), the biofilm composition was grown in domestic sewage, but without CO_2 supplementation, and had 3.8% nitrogen and 0.83% phosphorus contents on the 130th day of growth.

Woertz et al. (2009) assessed the use of effluents with different N/P ratios and obtained high removal efficiencies in all cases. According to these authors, algae have stoichiometric flexibility, which allows the nitrogen and phosphorus concentrations in their cells to be proportional to the N/P ratio of the culture medium. Therefore, the different nutrient contents found in studies of algal biofilm production are associated with the culture media and are indications of the quantity of N and P present in algal cells. The ash content was also associated with the culture media, and its amount can be related with the fixed solids presented in the domestic sewage.

The protein contents were kept between 20 and 30%. These results were close to the range reported by Gross et al. (2013) of 32–38% protein content in attached algal biomass. The authors considered this ratio as promising to produce animal feed and for fish farming.

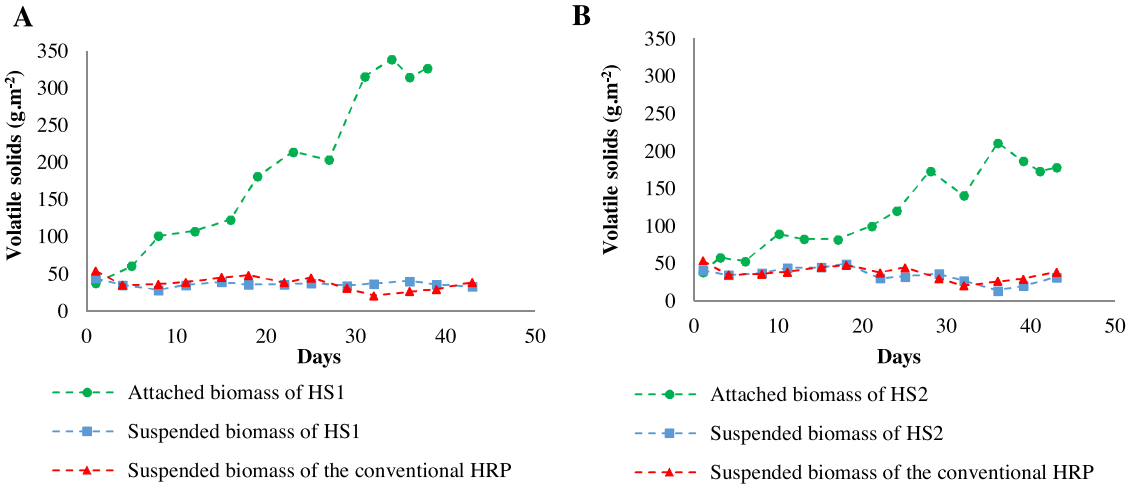

Fig. 2. Biomass production: (A) hybrid system without CO_2 (HS1) and conventional HRP with CO_2; (B) hybrid system with CO_2 (HS2) and conventional HRP with CO_2.

Table 2
Composition of the biofilms.

Biofilm reactor	C (%)	N (%)	P (%)	Humidity (%)[a]	Neutral lipid (%)	Protein (%)	Ash (%)
HS1	25.6	4.0	1.1	91.6	5.6	23.6	21.0
HS2	31.7	4.9	1.1	88.3	2.9	29.0	29.1

[a] Humidity relative to the harvested biomass.

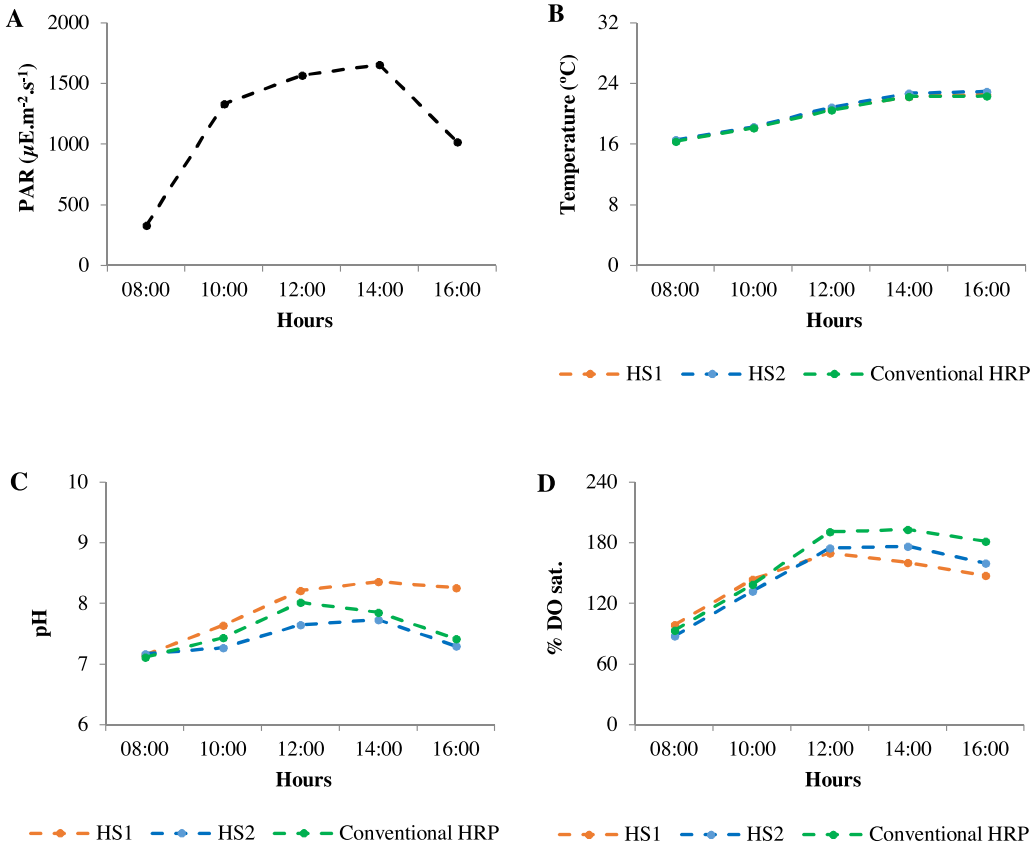

Fig. 3. Daytime behaviour (average of all collection days): (A) photosynthetically active radiation (PAR); (B) temperature; (C) pH; (D) dissolved oxygen (DO) saturation.

3.3. Characterisation of environmental and sewage treatment conditions

Fig. 3 shows the PAR, temperature, DO and pH behaviours of the HRP culture media of the three assessed systems. PAR was highest between 12:00 P.M. and 2:00 P.M., with maximum values of 1571 and 1655 $\mu E\, m^{-2}\, s^{-1}$, respectively. The temperature, OD and pH curves had similar behaviours in all systems. Most of those variables were influenced by the PAR and had the highest values during periods of intense solar radiation and lower values at the start and end of the day, except for the pH of the hybrid system without CO_2.

Temperatures of the culture media of the HRPs of the three systems remained between 15 and 25 °C, which, according to Ras et al. (2013), were within ranges considered adequate for the development of many species able to perform photosynthesis and cell division.

The limited availability of carbon in the culture medium is shown by increased pH. This occurs due to the intense photosynthetic activity of algae that consume inorganic carbon in wastewater. The HS1 kept its pH higher than 8, especially in the late afternoon. The systems with CO_2 supplementation showed different pH variation. The conventional HRP reached maximum values close to 8 at 12:00 P.M., whereas the HS2 had less pH variation, with a 7.7 maximum at 2:00 P.M. The presence of the BR may have had an influence. The exposure of biomass to atmospheric CO_2 resulted in a lower consumption of inorganic carbon in the HRP culture medium. Additionally, as previously discussed, the conventional HRP had a richer algal biomass than the other systems. Thus, the CO_2 consumption of this system was higher due to photosynthetic activity.

The conventional HRP had a DO maximum concentration of 204% of saturation, whereas that of HS2 was 195%. The highest CO_2 consumption by autotrophic microorganisms in the systems with CO_2 supplementation resulted in more O_2 generation as a photosynthesis product. HS1 had a maximum concentration of 155% of saturation.

Table 3 shows the characteristics of the domestic sewage before and after treatment and removal values in each of the systems. The organic matter removal rates of the systems were assessed based on COD_s and TOC_s. The COD_s removal rates were 33%, 27% and 46%, and the TOC_s removal rates were 58%, 45% and 53%, for HS1, HS2 and the conventional HRP, respectively. No significant differences were observed ($p > 0.05$). These results indicated a direct relationship of bacterial-microalgae consortia in the improvement of the biodegradation activity. The DO content confirmed this conclusion, since all systems showed DO saturation (Maza-Marquez et al., 2017).

Regarding nutrients, the P_s removal rates ranged between 20 and 30% in all systems, with no significant differences among them ($p > 0.05$). The N-NH_3 removal rates were not significantly different ($p > 0.05$), presenting values of 84%, 79% and 69% for HS1, HS2 and the conventional HRP, respectively. These data indicated that synergistic microalgae-bacteria consortium was successfully applied to the treatment of domestic sewage, as could be seen by nutrients removal rates.

Mean N-NO_3^- values statistically differ among the three systems ($0.01 < p < 0.05$), with final concentration of N-NO_3^- in the HS2 statistically different from the final value in the conventional HRP and statistically equal to the HS1. The value of the conventional HRP was also equal to that of the HS1. These results indicated that the BR interfered in the development of nitrifying bacteria. Similarly, Babu et al. (2007) and Mulbry and Wilkie (2001) also associated the presence of algal microfilms with increased nitrifying rates observed in stabilisation ponds treating synthetic wastewater and dairy effluents, respectively. Nitrifying bacteria prefer static environments and rarely live as suspended and free bacteria (Hammer and Knight, 1994).

Table 3
Domestic sewage treatment and removal values in each of the systems.

	Domestic sewage	HS1	Removal of HS1	HS2	Removal of HS2	Conventional HRP	Removal of HRP
COD_s (mg L^{-1})	116.0 (111.5)	78.0 (74.9)	33%	85.0 (65.4)	27%	62.2 (26.5)	46%
TOC_s (mg L^{-1})	43.1 (17.0)	18.3 (4.3)	58%	23.8 (20.7)	45%	20.2 (6.0)	53%
N-NH_3 (mg L^{-1})	37.3 (13.9)	6.1 (6.1)	84%	7.9 (10.4)	79%	11.5 (11.0)	69%
N-NO_3^- (mg L^{-1})	1.6 (1.2)	29.9 (24.1)	−1769%	42.0 (41.5)	−2525%	17.8 (21.8)	−1013%
P_s (mg L^{-1})	5.2 (0.9)	4.1 (1.0)	21%	3.9 (1.0)	25%	3.8 (1.0)	27%
E. coli (MPN.100 mL^{-1})	$2.6 \times 10^5 (6.5 \times 10^5)$[a]	$7.0 \times 10^3 (9.9 \times 10^5)$[a]	2 log unit[b]	$5.2 \times 10^3 (2.5 \times 10^6)$[a]	2 log unit[b]	$1.2 \times 10^4 (2.5 \times 10^6)$[a]	1 log unit[b]

Number of samples: n = 24.
[a] Geometric mean. Standard deviation values between parentheses.
[b] Removal in logarithmic units.
Negative % removal values indicate an increase in the variable.

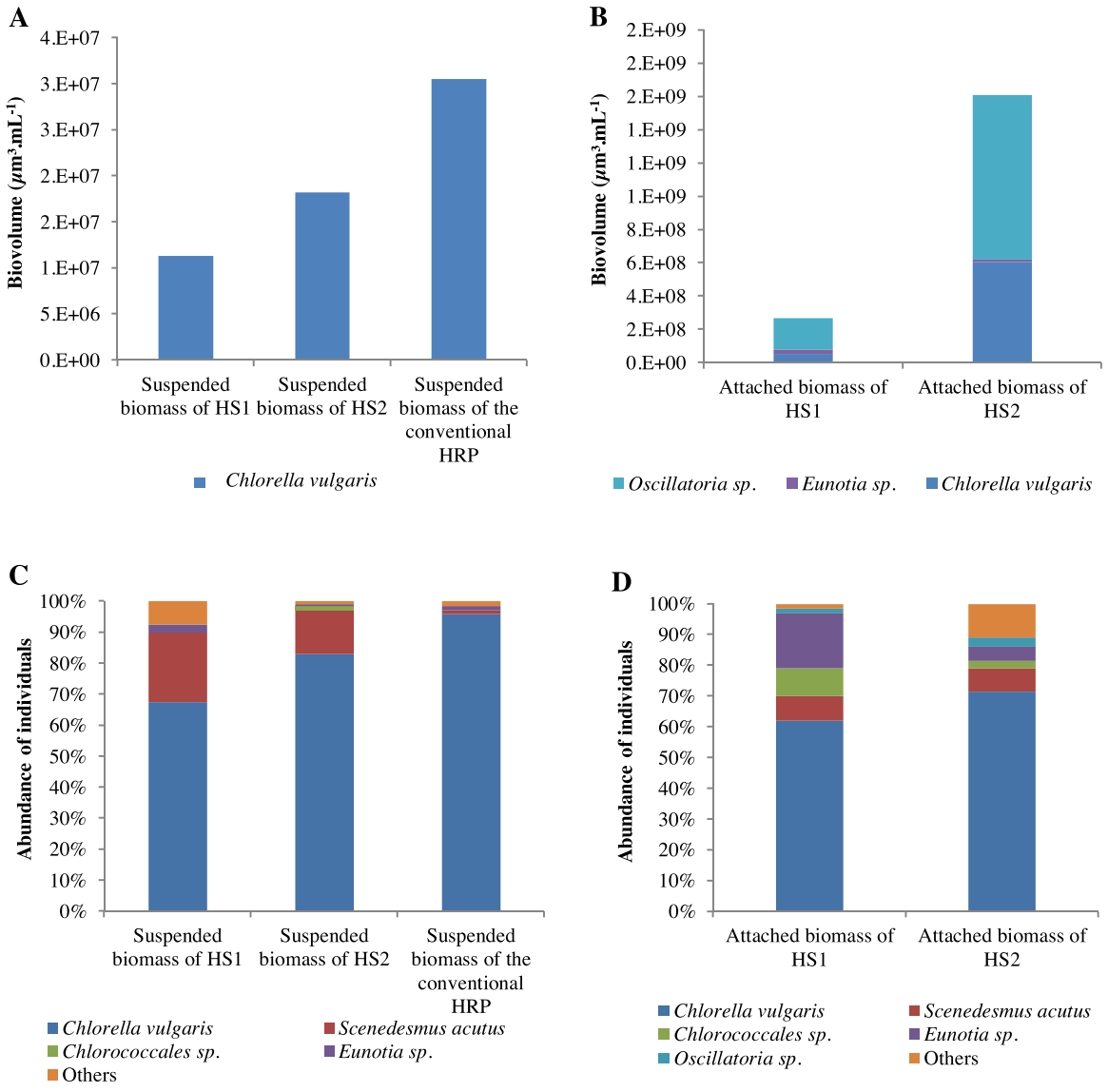

Fig. 4. Total biovolumes in the HRPs (A) and in the BRs (B). Phytoplankton communities in the HRPs (C) and in the BRs (D) (abundance of individuals, %).

E. coli concentrations did not show significant differences among the systems. The removal rates in the HS1 and HS2 systems were two logarithmic units. The removal rate in the conventional HRP was one logarithmic unit.

3.4. Phytoplankton community

The total biovolume and the relative abundance of the main phytoplankton species are shown in Fig. 4. Algal size varies for different species, within the same species and during different growth phases. The algal biovolume, i.e., the algal size relative to its biomass, is correlated with species of different maximum linear dimensions in the structure of a community (Bellinger and Sigee, 2010). A single individual of one planktonic specie may contribute to the equivalent of several individuals of other specie (Figueredo and Giani, 2001; Fonseca et al., 2014).

Chlorella vulgaris was dominant in the HRP of HS2 (83%) and in the conventional HRP (96%) and predominant in the HRP of HS1 (67%). The biovolume of all HRPs was associated with this microalgal specie only. *Chlorella vulgaris* was also the predominant microalgal specie in the BRs, with relative abundance of 71% and 62% for HS1 and HS2, respectively. The dominance and predominance of this specie in all the suspended production systems can be related to the use of domestic sewage as culture medium. According to Kim et al. (2010), of all green microalgae, *Chlorella vulgaris* is characterised by its high growth potential and ability to easily absorb nitrogen and phosphorus present in wastewater.

Unlike HRP biovolumes, the BR of HS2 had higher biovolumes of *Chlorella vulgaris* and cyanobacteria *Oscillatoria* sp. due to CO_2 supplementation. According to Sutherland et al. (2015), biomass concentration and biovolume increase with CO_2 addition to culture media. The authors investigated the effect of CO_2 supplementation on the performance of microalgae grown in wastewater compared with a culture medium without CO_2 addition. Increases in chlorophyll-*a* biomass concentration (pH = 6.5) of up to 96% and 18% and an increase in microalgal biovolume (pH = 6.5) of 560% and 104% in New Zealand in summer and winter, respectively, were reported. Likewise, in this study, the total biovolume of HS2 was 1171% and 1038% higher than those of HS1 and the conventional HRP, respectively.

Biomass cultivated in the BRs was directly in contact with solar radiation and possibly high temperatures were experienced. On the other hand, the suspended biomass from HRPs was alternately exposed to solar radiation, due to paddlewheels rotation. Despite

these differences between the reactors, it was observed that direct contact with solar radiation did not influence the populations of microalgae that developed in BR, since the only genus other than LAT that developed in BR was *Oscillatoria* sp. The cyanobacteria *Oscillatoria* sp. prefers static environments since it has non-ramified simple filaments (Azeredo, 2012). Despite its lower abundance in the BRs, its size relative to biofilm biomass was larger than the size of *Chlorella vulgaris*, which was more abundant in the biofilms.

4. Conclusions

The use of hybrid systems for biomass production and domestic sewage treatment under bacterial-microalgae consortia provided an efficient nutrient recovery. These systems also demonstrated the ability to grow attached biomass that was easily collected and thereby minimised separation and collection costs. Carbon supplementation did not increase production rates or the treatment efficiency of the hybrid systems. As the biofilm reactors were in direct contact with atmospheric air and solar radiation, the carbon requirements of the algal biomass were met, therefore, additional carbon supplementation was not necessary.

Future works should focus on optimising hybrid reactors and consider other biofilm growth reactor configurations, other types of support materials, recirculation flowrates and panel inclination and positioning relative to HRPs.

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