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Using atmospheric emissions as CO_2 source in the cultivation of microalgae: Productivity and economic viability

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ABSTRACT

This study approached the use of atmospheric emissions as a source of carbon for the cultivation of microalgae in high rate ponds (HRPs), continuously fed with domestic sewage pre-treated in a septic tank. To do so, two HRPs were used: one had CO_2 at a concentration of 99.9% (HRP 1), and the other had gas from the combustion of gasoline (HRP 2). Biomass yield, sewage treatment efficiency and the economic viability of using these two sources were assessed. The results showed that the CO_2 source did not influence the domestic sewage treatment or the yield and biochemical composition of biomass, since there was no statistical difference ($p < 0.05$) between the values measured for both ponds. The mean yield values were 6.00 and 6.12 g m^{-2} day^{-1}, respectively for the HRPs 1 and 2. As for the mean concentrations of ammonia nitrogen and the percentages of removal of chemical oxygen demand (COD), they were in the average for both HRPs (26.4 mg L^{-1} and 31.2%). The negative Net Present Values (NPV) showed that in this study the investments for installing external CO_2 sources are not economically viable for any of the two studied sources, when the biomass produced in the HRPs is used for the production of biofuels and as source of protein for animal feeding. The initial investments, allied to the prices of the biomass, were the factors that mostly influenced the economic analysis, contributing to the lack of attractiveness in this scale. Despite this, the use of atmospheric emissions along with domestic sewage as a cultivation medium means the biomass produced in this study has less environmental impact when compared to similar biomasses.

1. Introduction

Carbon dioxide (CO_2) is one of the main greenhouse gases. In Brazil, the energy sector is the third largest responsible for the emission of such gas. In 2016, 18.6% of all emitted CO_2 (over 2 billion tons) in the country was generated in the energy sector, mostly by the burning of fuels (Seeg, 2016).

Microalgae stand out in this perspective due to their capacity to treat sewage as they fix CO_2 (Pires et al., 2012; Farrelly et al., 2013). With a rapid growth rate, they assimilate nutrients in their biomass, which can transform into feedstock for products such as biofuels and fertilizers (Cuellar-Bermudez et al., 2015), representing additional benefits to the process of CO_2 mitigation (Ferreira et al., 2017).

Among the factors that influence the microalgae growth in open systems using domestic sewage as a cultivation medium, the carbon limitation is one of the main disadvantages. Considering that microalgae require 1.8–2.0 kg of CO_2 to produce 1 kg of biomass (Chisti, 2007), the low C:N (carbon:nitrogen) ratio usually found in domestic sewage, allied to the low CO_2 concentration in the atmosphere and a low mass transfer coefficient between the air and the cultivation medium, can be harmful to microalgae growth and consequently contribute to an incomplete nutrient removal (Park and Craggs, 2011; Sutherland et al., 2016). To overcome this problem, CO_2 must be added to the cultivation medium. CO_2 supply not only increases the availability of carbon for the growth of microalgae, but also improves the recovery of nutrients by assimilation in their biomass, as it will prevent the increase of pH, caused by photosynthetic activity, mitigating the losses of nitrogen by the

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volatilization of ammonia, and phosphorus precipitation (Heubeck et al., 2007; Fernandez et al., 2012; Cai et al., 2013). The first studies on increase of microalgae production by the addition of CO_2 date back to the 1960s (Heubeck et al., 2007). Since then, many authors have reported a significant increase in biomass and nutrient removal when an external source of CO_2 is used in the cultivation medium (de Godos et al., 2010; Posadas et al., 2015). It can be added in high concentrations using cylinders of high purity gas (99.9%), but this is an expensive technique that can compromise the use of the system, especially on a commercial scale. In order to minimize costs, CO_2 can be added to the ponds in the form of exhaust gases. The use of atmospheric emissions, in addition to reducing costs of effluent treatment, contributes to the mitigation of greenhouse gas emissions, which are increasing every year.

In addition to CO_2, normally present between 5 and 15% (Lee et al., 2002; Li et al., 2008), the exhaust gases can contain other compounds such as NO_x, SO_x unburned hydrocarbons (C_nH_m), CO, N_2, O_2 and particulate matter (Lee et al., 2002; Van den Hende et al., 2012). Some authors have already studied the tolerance of microalgae to some of these compounds (Nagase et al., 2001; Vaz et al., 2016) and also the toxicity that can interfere in the cellular growth of some species (Lee et al., 2002). The CO_2 concentration can also interfere in the cellular growth of the microalgae, changing the competitive relationships among the species and leading to changes in relative abundance (Low-Decarie et al., 2011). These changes can directly influence the use of the final biomass, which may be destined to different purposes depending on the species present due to the different amount of protein, carbohydrates and lipids for each species (Becker, 2007).

Even with a lower price when compared to pure CO_2 (Van den Hende et al., 2012), the use of CO_2 from exhaust gases in the cultivation of microalgae has a cost. Among the several costs involved in the process, those related to the installation and maintenance of the cultivation system, as well as those of the process of capture and compression of the gas are highlighted (Thomas et al., 2016), and represent 75% of the expenses (Stewart and Hessami, 2005). Ideally, the input costs for the cultivation of microalgae are zero if exhaust gases are used together with the sewage treatment (Wang et al., 2008; Fernandez et al., 2012), since the CO_2 is the most expensive among the inputs required for cultivation (Fernandez et al., 2012). The main focus was to make explicit with numbers the difference between using each of the sources of CO_2 supplementation. Many authors state that this difference exists; that the use of CO_2 from atmospheric emissions is economically and environmentally a better option (Van den Hende et al., 2012; Judd et al., 2017), but there is a lack of literature that really demonstrate this by assessing the viability of the project as a whole, involving the initial investment, operating costs and mainly the market price of the biomass produced; and not just the costing of each of the steps involved. To exemplify this, one can cite Fernandez et al. (2012) that studied several scenarios evaluating the costs of producing algal biomass in two different culture media (water + fertilizers and wastewater) and testing two different sources of CO_2 (industrial CO_2 and atmospheric emissions) in the production of microalgae in photobioreactors. However, their study did not take into account the profit obtained with the biomass produced in each scenario and consequently if the investment was economically viable.

Thus, the objective of this study was to verify the influence of the compounds present in the exhaust gas from gasoline combustion (EGGC) in the treatment of domestic sewage and biomass productivity, and also carry out an analysis of the economic viability of adding exhaust gas as a CO_2 source in a pond system, considering the biomass produced as final income.

2. Material and methods

The experiment was carried out in the experimental area of the Sanitation and Environmental Engineering Laboratory at the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil (20°45′14″S, 42°52′54″W). The city of Viçosa is characterized by a humid climate with rainy summers and dry winters. According to the National Institute of Meteorology (INMET), considering data from 1991 to 2016, the annual mean precipitation in the city was 1272 mm and the relative air humidity was 80%. Also, for the same period, the mean temperature in the dry season (between April and September) was 19.5 °C, whereas in the rainy season (between October and March) it was 23.3 °C. It is important to highlight that, independently of the month, the climate of Viçosa is favorable for the cultivation of microalgae with mean temperatures between dry and rainy seasons within the optimal range for microalgae growth, between 15 and 30 °C (Ras et al., 2013).

2.1. Experimental unit

The experiment consisted of two high rate ponds (HRPs) continuously fed with domestic sewage that was previously treated in a septic tank. The septic tank is part of the Sewage Treatment Plant located in the neighborhood of Romão dos Reis, Viçosa – Minas Gerais, operated by the Autonomous Water and Sewage Service (SAAE – Viçosa).

The pilot-scale HRPs used in the study had the following characteristics: width of 1.28 m, length of 2.86 m, total depth of 0.5 m, useful depth of 0.3 m, surface area of 3.3 m^2 and useful volume of 1 m^3. These ponds were made of fiberglass with six-blade steel paddles, moved by a 0.5 hp electric motor. The rotation was reduced by a speed reducer coupled to the motor and controlled by a frequency inverter (WEG series CFW-10).

For the CO_2 supply, two sources were used: CO_2 in high concentration (99.9%), commercialized in high pressure cylinders (WHITE MARTINS PRAXAIR INC) (HRP 1); and CO_2 from the exhaust gas of gasoline combustion (EGGC) in an electric energy generator (Schulz S5500MG), stormed and pressurized in a compressor (3 Phase Schulz BRAVO CSL BR/100 L) for further addition (HRP 2).

The flow used in both cases was 1 L min^{-1}, controlled by flowmeters. The CO_2 addition was controlled by the pH, using a Hach controller (model SC200), with a system of electric signal compatible with a solenoid valve to maintain the pH of the effluent in the HRPs between 7.0 and 7.5. The pH, temperature, and time were registered by the controller every 30 s, which allowed the monitoring of the duration of the addition of gas during the day.

The CO_2 addition was carried out in the lower part of the HRPs, through the carbonation column, in order to allow a greater contact time of the gaseous CO_2 with the effluent. The carbonation column used in the study was designed according to Putt et al. (2011) and is

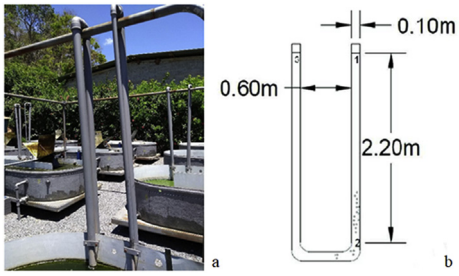

Fig. 1. Scheme of the carbonation column: (a) Experiment area (b) Column dimensions (1 – Effluent input; 2 - CO_2 input; 3 – Effluent output) (Source: Adapted from Couto et al. (2017)).

shown in Fig. 1.

Fig. 2 presents the scheme of the experiment with the two described ponds.

2.2. Operation and monitoring

The HRPs were operated from July to December 2017, in two periods: Period I (hydraulic retention time – HRT = 5 days), from July to August; and Period II (HRT = 8 days), from September to December. Period I was used for the production and adaptation of the microalgae and the stabilization of the system; therefore, all the reported results are related to Period II. The samples were collected once a week in each of the two ponds. The variables total kjeldahl nitrogen (TKN), ammonia nitrogen ($N-NH_4^+$), nitrate ($N-NO_3^-$), soluble phosphorus (P_s), oxygen chemical demand (COD – soluble and total), total soluble organic carbon (TOC_s), and volatile suspended solids (VSS) were measured using the composite samples collected every 2 h throughout the day (8 a.m.–6 p.m.). The analysis of such variables was carried out according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The TOC_s was determined using the Shimadzu TOC 5000. Temperature, pH, and dissolved oxygen (DO), in addition to the photosynthetically active radiation (PAR) were measured in loco every 2 h, using a Hach meter (model HQ40d; Luminescent Dissolved Oxygen for dissolved oxygen). The PAR was determined using the LI-COR - LI-193 Underwater Spherical Quantum Sensor. For determination of chlorophyll-*a* (chl *a*) and *Escherichia coli* (*E. coli*), the samples were collected once a day, at 12 p.m. The chlorophyll-*a* was measured by hot extraction with 80% ethanol as described in the Netherlands Norms (Nederlands Norm, 1981), based on Nush (1980). The chromogenic/fluorogenic method (Colilert®) was used to analyze *E. coli.*

2.3. EGGC analysis

The EGGC analysis was carried out in a gas analyzer - *Wuhan Cubic Optoeletronics/Gasboard 3100* for the determination of the concentration percent in terms of volume of CO_2, CO, CH_4, C_nH_m, H_2 and O_2. For the collection of EGGC, a Tedlar SKC 5-L sample bag with a single polypropylene valve was used. The sampling was carried out in a meter located before the flowmeter for three consecutive days, and three samples were collected each day: at 9 a.m., 1 p.m. and 5 p.m. The samples were collected and analyzed in sequence.

During the analysis, the flow was maintained at 0.7 L min^{-1}, and each analysis lasted around 600 s. The equipment consisted of a chiller (for cooling the gas to 4 °C) and a hydrophilic membrane (0.22 μm pore size, 25 mm diameter). The final result of the gas characterization was obtained by the mean of all values during stable reading.

2.4. Phytoplankton community characterization

At the beginning of Period I, beginning of Period II (after two additions of gas) and the end of Period II, samples were collected for the characterization of phytoplankton community in each of the HRPs.

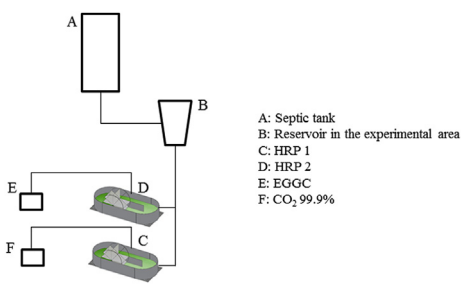

Fig. 2. Scheme of the experiment.

The phytoplankton community was characterized in qualitative and quantitative terms. In qualitative analysis, the identification was carried out using an inverted optic microscope (Olympus CK2), according to the Utermöl (1958) method and identification keys. The density of organisms per sample was determined using the criteria described in APHA (2005). For the more abundant organisms, the calculus of the biovolume was carried out according to the equation proposed by Wetzel and Likens (1991).

2.5. Biomass characterization

The biomass was collected every week throughout the last month of operation. This biomass was concentrated using a high rotation refrigerated centrifuge (HITACHI CR21GIII) at 10,000 rpm for 10 min and then frozen. The frozen biomass was then lyophilized and characterized in terms of carbohydrates, neutral lipids, proteins, moisture and ash contents.

The determination of moisture was carried out by weighing the sample, after drying for 1 h in a drying chamber at 105 °C, until constant mass (EN 14771–1:2009). The moisture content was calculated by the difference between the masses before and after drying. The ash content was measured after combustion of the sample in a muffle at 550 °C for 1 h (EN 14775:2009), and was determined by the difference of masses before and after combustion.

The carbohydrates present in the biomass were determined from the quantitative acid hydrolysis and measured by the phenol-sulfuric acid method. The analysis of the neutral lipid content consisted of breaking the cellular wall with 3M hydrochloric acid and subsequent petroleum ether and methanol extraction. After the extraction, the extracted oil was washed with 4% lead acetate to remove impurities and pigments. The lipid content was determined gravimetrically. The determination of the protein content was carried out indirectly by the total kjeldahl nitrogen (TKN) method (APHA, 2012) using the conversion factor 6.25 g g^{-1} (Brasil, 2001).

2.6. Investments analysis

The economic viability of the process, represented by the Net Present Value (NPV), was calculated by electronic spreadsheets using Microsoft® Excel.

The Minimum Attractiveness Rate of Return (MARR) used was 12%, which is the rate adopted in the Program for the Modernization of the Sanitation Sector of the Brazilian Federal Government (Wagner and Belloto, 2008).

The period considered for the investment analysis was one year. Thus, the data for the operation period were extended for the entire year and we considered that the operation, environmental and productivity conditions were the same. Even knowing the variability of algal biomass production during the year due to the seasons and their climate patterns, this was necessary since the calculations for carrying the investment analysis involve annual data.

The investments analysis started with the hypothesis that the entire treatment infrastructure already exists, taking into account only the investments with the addition of gas. Three quotes were requested in order to obtain the mean acquisition value. For industrial CO_2, we considered the purchase of the cylinder plus the regulators and manometer required for their operation, and for the EGGC, only the purchase of the compressor was taken into account.

The operation costs involved for each source were: costs with the recharge of the CO_2 cylinder (based on the mass of CO_2 added to

the culture during the operation) and the cost with electric energy for the functioning of the compressor.

The CO_2 flow (1 L min^{-1}) and the records in the pH controller indirectly gave the mass of CO_2 provided to the algal culture, which allowed us to discover how long the gas addition lasted. With the time of addition and the flow, we obtained the added volume. From the CO_2 density (1.977 kg m^{-3}) the mass was obtained and from that, the expenses for the recharge of the cylinder. This value was also obtained through a price survey, and was US$ 67.38, corresponding to the recharge of a 25 kg cylinder.

For calculating the annual profit in a 20-year horizon, the market prices of algal biomass in applications that enable the use of domestic sewage as input, as a protein source for animal feeding and biofuel production (Wijffels et al., 2010; Fernandez et al., 2012; Zhu, 2015; Ruiz et al., 2016) and the cost of the kilogram of the produced biomass were used.

The cost of the biomass produced in this research in each of the HRPs was calculated by dividing the total costs of the investment by the total annual biomass production (kg/year) (Zardo, 2011), calculated based on the VSS production for each of the sources. The equation used to calculate the annual cost per kg of biomass produced in each of the HRPs is presented in Equation (1).

$$\frac{\textit{Total costs of the investment (US\$)}}{\textit{total annual biomass production (kg/year)}} = \textit{Annual cost per kg of biomass (US\$. year/kg)} \quad (1)$$

2.7. Statistical analysis

The R© software was used to assess the differences between the mean values of the variables measured in the HRPs. The statistical differences among the experimental groups were assessed through variance analysis (Tukey test), at a 95% significance level. For the graphics, Microsoft® Excel and R© version 64 3.3.3 were used.

3. Results and discussion

3.1. Influence of the compounds present in the EGGC

Table 1 presents the results of the EGGC analysis used as an external source of carbon in HRP 2. The rest of the gas composition (80.24%) is considered mostly N_2 and nitrogen oxide in small amounts (Motor Vehicle Exhaust Emissions, 2017).

If the gasoline combustion had been complete, the exhaust gas would consist of: 15.33% of CO_2, 1–2% of CO, unburned hydrocarbonates and NO_x, approximately 13% would be water and the rest would be N_2 (Motor Vehicle Exhaust Emissions, 2017). The gasoline burning conditions in the study did not allow the complete combustion, thus the reference values aforementioned were not observed. The motor used in the study had to be with the throttle pulled in order to start, and this condition provided a rich air/fuel ($\lambda < 1$) mixture, i.e., a larger amount of fuel than oxidizer (O_2). This non-stoichiometric ratio led to the incomplete combustion.

The CO_2 is the main component of interest of the EGGC. Compared to the other components in the gas, CO_2 is the most soluble with 1.7 g CO_2 L^{-1} of water at 20 °C and 1 atm.

Despite being present in amounts almost equivalent to CO_2, carbon monoxide (CO) is not a factor of concern and it does not interfere with microalgae growth due to the low solubility of this gas (0.028 g CO L^{-1} of water, 60 times lower than the solubility of the CO_2). Thus, it is expected that the presence of CO in the EGGC will not interfere in microalgae growth. The same situation is expected for the presence (in small amounts) and low solubility of the compounds CH_4 (0.024 g CH_4 L^{-1} of water at 20 °C and 1 atm), H_2 (0.0016 g H_2 L^{-1} of water at 20 °C and 1 atm), and O_2. With respect to nitrogen oxides, despite being present in small amounts, some of these compounds are highly soluble, and in general, the NO_x species consist of 95% of NO (0.032 g NO L^{-1} of water at 20 °C and 1 atm) and 5% of NO_2 (213.0 g NO_2 L^{-1} of water at 20 °C and 1 atm) (Wang et al., 2008). When any of these compounds dissolve in water, nitric or nitrous acid is formed, which can contribute to a decrease in the pH of the medium. On the other hand, the dissolved NO will be available as a nitrogen source for the microalgae (Nagase et al., 2001).

Some authors have already reported that the cultivation of microalgae is not influenced by the composition of the exhaust gases used as an external carbon source. Talec et al. (2013) evaluated the tolerance of four species of microalgae to exhaust gases from cement industry and concluded that the growth for the four species is not affected by the cement flue gases. Doucha et al. (2005) used the emission of the combustion of natural gas in the *Chlorella sp.* cultivation and concluded that the presence of nitrogen oxides (up to 45 mg m^{-3}) and carbon monoxide (3 mg m^{-3}) in the emission gas did not negatively influence microalgae growth. Tastan et al. (2013) studied the effects of addition of emissions from the combustion of liquefied petroleum gas on growth of microalgae *Phormidium sp.* and *Chlorella sp.* The results showed that both cultures were able to tolerate the conditions imposed by the emissions used in the study, reaching productivity of 1.331 g L^{-1} for *Phormidium sp.* and 1.636 g L^{-1} for *Chlorella sp.*

Table 1
EGGC characterization.

Compound	Concentration (%)
CO	5.92 (0.15)
CO_2	5.87 (0.17)
CH_4	0.15 (0.02)
C_nH_m	0.02 (0.01)
H_2	3.71 (0.13)
O_2	4.09 (0.32)

3.2. Environmental conditions

Fig. 3 presents the diurnal behavior of the parameters (mean of every sampling day) in Period II: PAR (μmol m^{-2} s^{-1}), temperature of the effluent in the HRPs (°C), % of saturation of dissolved oxygen, % of DO in the HRPs, and pH.

Fig. 3(a) shows the variation of the PAR during the day, which, on average reached its peak (1661.96 μmol m^{-2} s^{-1}) at 12 p.m. During the entire operation, the PAR was kept between 259.24 and 1661.96 μmol m^{-2} s^{-1} on average, and the lower value was measured at 6 p.m. The temperature of the effluent in the ponds ranged between 19.68 and 26.68 °C (Fig. 3(b)), and was within the optimal range pointed out in the literature for most microalgae species (Ras et al., 2013).

The maximum values for the mean DO saturation percent for HRPs 1 and 2 were 110.16% and 114.33%, respectively, both measured at 2 p.m. During the operation, HRP 1 reached a maximum of 146.62% of saturation and HRP 2 reached 184.25%, indicating more photosynthetic activity in HRP 2.

As observed in Fig. 3(d), the decrease in pH in HRPs 1 and 2, followed by pH increase, is related to the moments of gas addition (pH starts to lower) and when addition is over (pH goes up again). The pH decrease lasts longer in HRP 2 due to the low CO_2 concentration in the exhaust gas, which increases the time for reestablishing the chemical balance of the system.

Fig. 4(a) and (b) show the behavior of the pH values in HRPs 1 and 2 during all sampling days for period II. In November, the pH was lower due to the rainy conditions and low incidence of solar radiation.

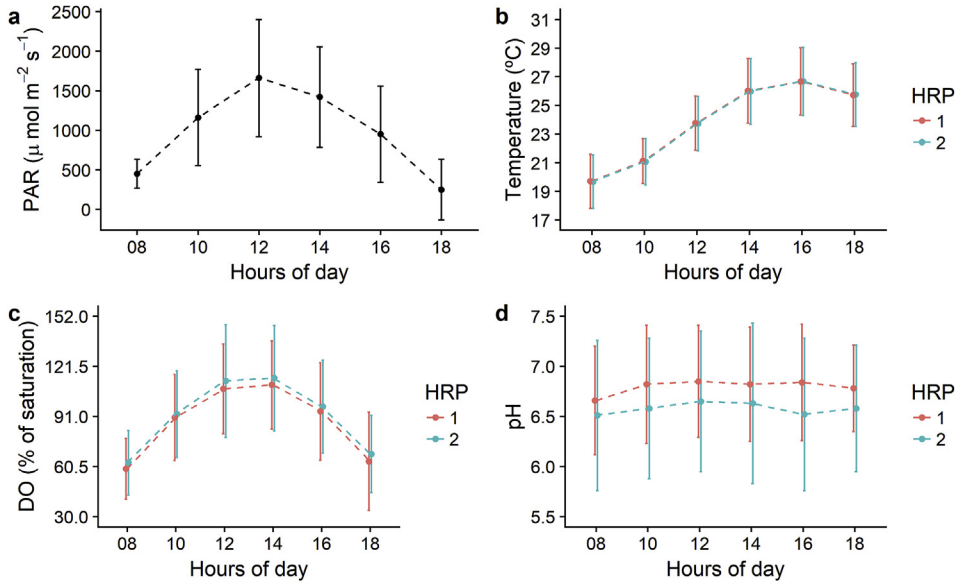

Fig. 3. Diurnal behavior of the parameters (mean of each sampling day): (a) PAR; (b) temperature; (c) % of DO; and (d) pH. n (number of samples) = 12. Vertical bars represent the standard deviation.

Fig. 4(a) and (b) show the moments of pH decrease at 7.5, with the activation of the solenoid valve and gas addition. When pH 7.0 is reached and the gas supply stops, the CO_2 consumption due to photosynthetic activity causes pH to increase again. Even if kept in the same control range of pH, it is usually lower in HRP 2 than HRP 1 (Fig. 3 (d)), which can be explained due to the presence of nitrogen oxides in the EGGC. The NO_x, despite being present in a small percentage, can interfere in the pH of the effluent. When dissolved in water, these oxides form the nitric acid (HNO_3) or nitrous acid (HNO_2) that can contribute to the pH decrease in the medium.

3.3. Removal of organic matter and nutrients

Table 2 presents the characterization of the domestic sewage affluent to the HRPs, the mean results (mg L^{-1}), the variation

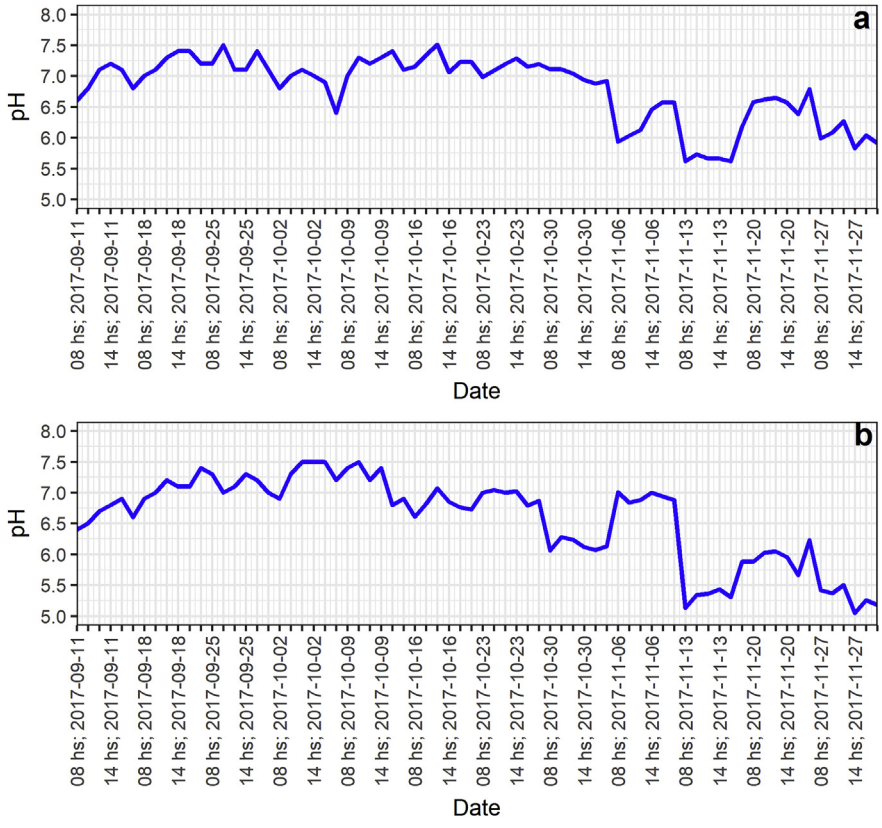

Fig. 4. (a) pH behavior in HRP 1 during the sampling days of Period II and (b) pH behavior in HRP 2 during the sampling days of Period II.

coefficients (%), and the percentages of removal for the variables CODs, TOCs, $N-NH_4^+$, $N-NO_3^-$, TKN, P_s and *E. coli* in each of the HRPs.

The statistical analysis showed that there was no difference between the treatments ($p < 0.05$) for all measured variables. Thus, the CO_2 source in high concentrations or in the form of exhaust gas, did not influence nutrient removal, organic matter and indicators of fecal contamination. The percentages of COD removal were 31.7 and 30.8%, and the percentages of TOC_s removal were 23.5 and 24.6%, respectively for HRPs 1 and 2.

The increase in nitrate concentration and the high removals of ammonia nitrogen, indicate the occurrence of nitrification. The values presented in Table 2 show that in any of the ponds, the final concentration of ammonia nitrogen satisfied the maximum value established in the Brazilian Resolution CONAMA nº 430 (20 mg L^{-1}). It is important to highlight the fact that the anaerobically treated sewage has high concentrations of $N-NH_4^+$, which makes the observed removal considerably satisfactory. With respect to the phosphorus removal, the results presented in Table 2 show that the treatment was not able to remove the dissolved phosphorus, which actually increased in both ponds. As high pH values were not reached in this study, phosphorus removals via chemical precipitation were not expected. The increase can be attributed to phosphorus, both particulate and dissolved, present in the excretion and mortality of aquatic organisms (Jr et al., 2009; Tundisi and Tundisi, 2016).

With respect to pathogens removal, both HRPs presented a removal of 2 log units of *E. coli*, which is coherent with other studies that found similar removals (Santiago et al., 2013).

The use of domestic sewage previously treated in a septic tank was favorable since the high concentration of organic matter (influent CODt) required the addition of CO_2 to the cultivation medium in low frequencies (see Fig. 4a and b), resulting in the economy of the system and indicating that the breathing of the heterotrophic bacteria was enough to supply the demand for inorganic carbon in the microalgae for a significant part of the operation period. Therefore, the characteristic of the wastewater influent to the HRPs, more specifically, the concentration of the carbonaceous organic matter, is essential for the performance of this system with respect to the need for carbon supply.

3.4. Characterization of the phytoplankton community

The relative abundance (RA %) of the phytoplankton community in each of the HRPs in (a) the beginning of Period I, (b) beginning of Period II, and (c) end of Period II, is presented in Fig. 5.

During the entire operation, the predominant gender in both HRPs was *Chlorella* sp., and the most abundant species was *Chlorella vulgaris*. The presence of the gender *Chlorella* sp. was already expected and the abundance is justified by the preference of this gender for nutrient-rich environments with high luminous intensity, in addition to being able to support high organic loads (Sutherland et al., 2014; Assemany et al., 2015).

At the beginning of the operation, the density of microorganisms in HRPs 1 and 2 were 1.96×10^6 and 1.26×10^6 respectively. In HRP2, 16.6% of abundance was related to the gender *Navicula* sp. that belongs to the clado *Bacillariophyta* (diatoms). The addition of CO_2, regardless of the source, enabled the maintenance of the initial density in both HRPs (2.24×10^6 and 1.98×10^6), which decreased only at the end of Period II, when environmental conditions such as rainfall, temperature and incident radiation did not allow the CO_2 addition. For this reason, the density of microorganisms per mL of the sample decreased, compared to the two samples collected at the beginning of Period I and the beginning of Period II: 2.45×10^5 and 8.89×10^4.

The results of the algal biovolume for the samples collected in the beginning of Period I, beginning of Period II and end of Period II are presented in Fig. 6.

The predominant genera used for the calculation of the biovolume were *Chlorella*, *Acutodesmus*, *Navicula* and *Fragilaria.* The analysis of Fig. 6 shows that the CO_2 source did not influence the biovolume in HRPs 1 and 2.

Some authors state that the relative abundance of phytoplankton organisms significantly differ when the medium is submitted to different CO_2 concentrations (Tortell et al., 2002; Low-Decarie et al., 2011; Low-Decarie et al., 2015; Sutherland et al., 2016). The physiological mechanisms of each clado can influence the capacity of the organisms to absorb and use CO_2 (Raven, 1991; Tortell, 2000; Kardol et al., 2010), which alters the competitive capacity of each species, further leading to taxonomic changes in the community.

3.5. Biomass characterization

Table 3 presents the composition of the biomass produced in both HRPs.

None of the measured variables statistically differ ($p < 0.05$) when both HRPs were compared, i.e., the CO_2 source did not influence the biomass biochemical composition. Depending on the biochemical composition, the microalgae biomass can be used for several purposes: as food supplement in animal and human food, production of drugs and cosmetics, fertilizers and raw material for biofuel production. The biochemical characterization and the fact that domestic sewage has been used as culture medium limit the use of the biomass produced in this study for animal feed and as raw material for the production of biofuels. As supplementation in animal feed, mainly in aquaculture, the microalgae biomass can be

Table 2
Final concentrations and percentages of removal of organic matter and nutrients in each of the HRPs.

	Domestic sewage	HRP 1	% Removal	HRP 2	% Removal
COD_T (mg L^{-1})	233.9 (109.8)				
CODs (mg L^{-1})	174.5 (63.8)	110.2 (15.1)	31.7	110.8 (34.2)	30.8
TOCs (mg L^{-1})	46.1 (23.1)	31.2 (2.9)	23.5	30.7 (1.9)	24.6
$N-NH_4^+$ (mg L^{-1})	77.4 (8.9)	26.7 (3.9)	65.1	26.1 (3.3)	65.7
$N-NO_3^-$ (mg L^{-1})	1.2 (0.9)	93.6 (84.1)	–	80.2 (77.5)	–
TKN (mg L^{-1})	87.8 (9.9)	32.3 (10.8)	40.9	36.2 (6.2)	37.9
Ps (mg L^{-1})	12.3 (1.9)	13.2 (2.0)	−8.4	13.5 (1.6)	−11.3
E. coli (MNP 100 mL^{-1})	7.4×10^4 (3.1×10^5)	5.8×10^2 (2.1×10^4)	2 log unit [a]	8.9×10^2 (6.2×10^3)	2 log unit [a]

Standard deviation in parenthesis.
Negative removal results indicate an increase of the variable.
[a] Removal in unit logs.
n (number of samples) = 12.

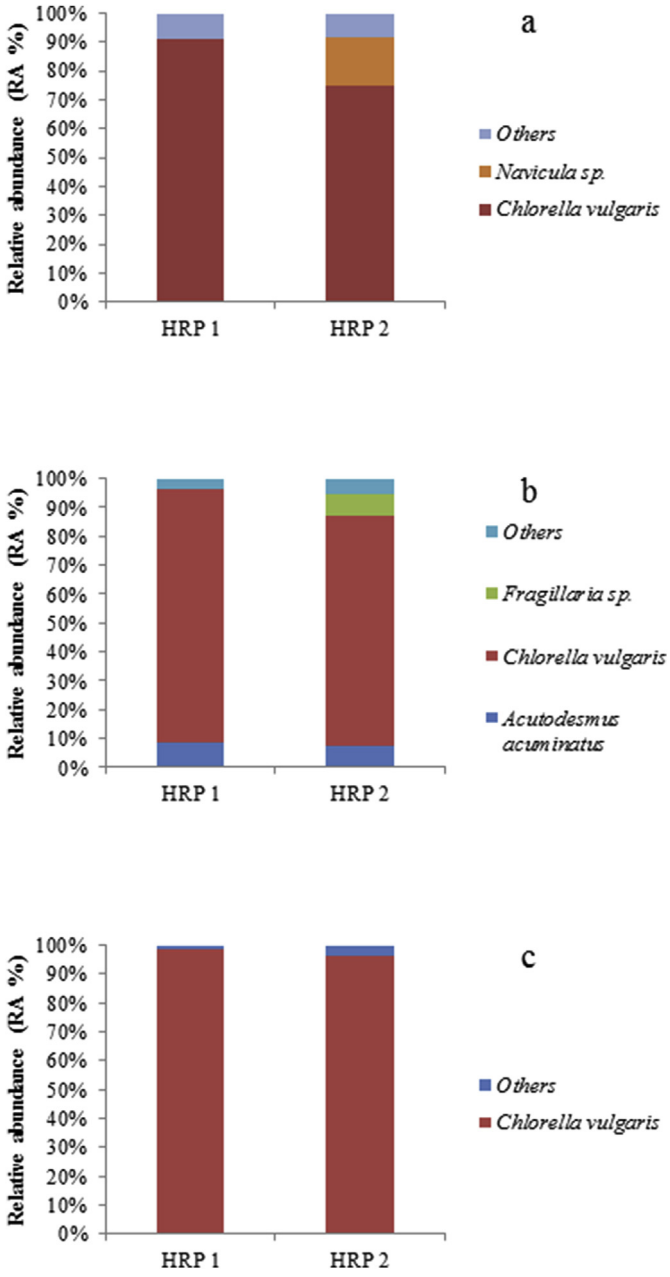

Fig. 5. Relative abundance (RA %) of the phytoplankton community in (a) the beginning of Period I, (b) beginning of Period II, and (c) end of Period II. Species that presented RA lower than 5% were accounted as "Others".

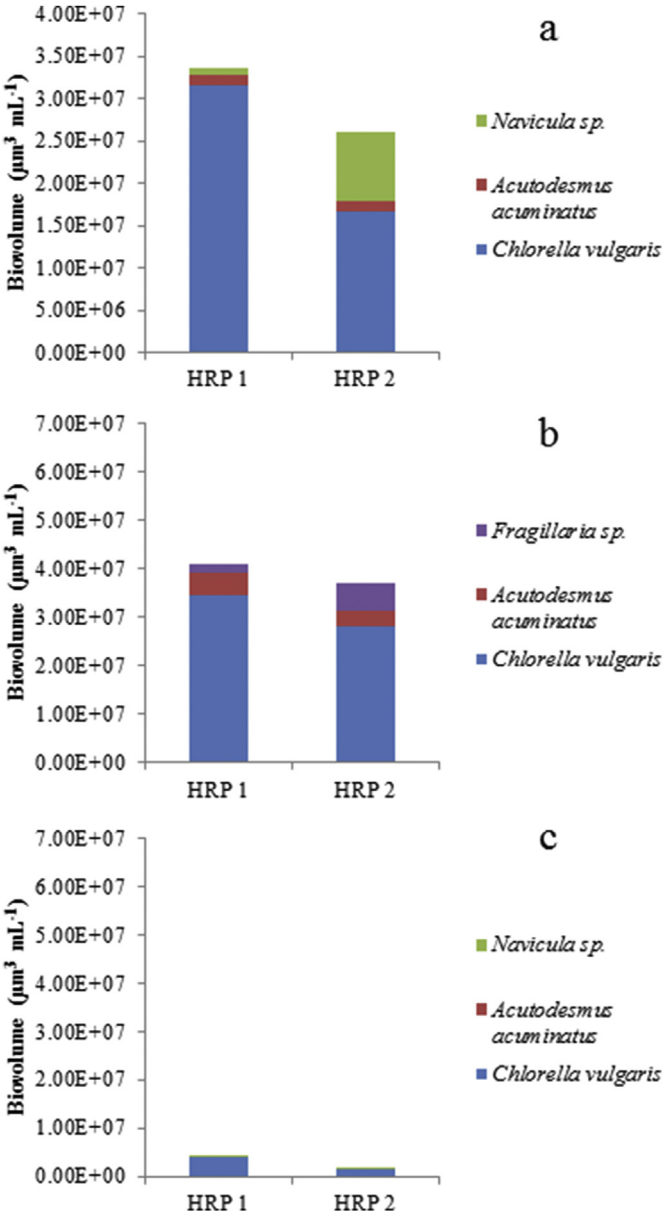

Fig. 6. (a) Biovolume of the phytoplankton organisms present at the beginning of Period I, (b) beginning of Period II, and (c) end of Period II.

used to grow zooplankton, which serves as food for crustaceans and fish (Pires et al., 2012). The ash content is below the one normally found in biomass produced in effluents at 30–50% (Yu, 2012), as well as the carbohydrate content. Posadas et al. (2015) investigated the influence of pH (7, 8 and 9) and CO_2 source (pure or exhaust gas) in the treatment of domestic sewage, production and composition of algal biomass in pilot-scale HRPs. The results showed that the pH and the CO_2 source did not influence the protein content of the biomass, which remained constant in all experiments (38.2 ± 3.3%). The lipid and carbohydrate contents ranged between 5.8 and 23% and 38 and 61.2%, respectively. The highest lipid content was found for pH 9, using exhaust gas as a carbon source, and the highest carbohydrate content was found in pH 7, using pure CO_2 as a carbon source.

Table 3
Composition of the biomass produced in both HRPs.

	HRP 1	HRP 2
Immediate composition (%)		
Moisture	5.8 (0.7)	5.5 (0.0)
Ash	23.7 (0.1)	18.8 (0.1)
Biochemical composition (% dry basis)		
Neutral lipids	5.5 (1.3)	6.0 (2.1)
Carbohydrates	15.8 (6.4)	14.6 (0.7)
Proteins	37.2 (0.1)	35.3 (0.8)

3.6. Investments analysis

Table 4 presents the mean values of chlorophyll-*a* concentrations (mg L^{-1}), VSS (g m^{-3}), total daily yield (g m^{-2} day^{-1}), total annual yield (kg m^{-2} $year^{-1}$) and annual biomass yield (kg $year^{-1}$)

for each of the HRPs. The annual yield was used in the calculations of the cost of a kilogram of biomass.

The statistical analysis showed that the productivity of variables chlorophyll-*a* and VSS did not differ ($p < 0.05$) between the two treatments (HRPs 1 and 2). Posadas et al. (2015) studied the influence of pH and CO_2 source (pure or exhaust gas) in the treatment of secondary domestic sewage and in the yield of biomass in pilot-scale HRPs for six months, in the same manner as the present study. The results found by those authors showed that the CO_2 source did not influence the biomass yield.

The annual biomass production (kg year^{-1}) was used in Equations (2) and (3) to determine the annual cost of 1 kg of biomass produced in HRPs 1 and 2, respectively.

For industrial CO_2:

$$Cost\ per\ kg\ of\ biomass = \frac{US\$317.61}{7.23\ kg/year} = US\$\ 43.93 \quad (2)$$

For the EGGC:

$$Cost\ per\ kg\ of\ biomass = \frac{US\$\ 575.76}{7.36\ kg/year} = US\$\ 78.23 \quad (3)$$

Tables 5 and 6 contain the investments and annual operation costs for when each source is used.

When using EGGC as a CO_2 source, the investment that corresponds to the purchase of the compressor is US$ 575.76. When the source is CO_2 99.9%, the investment with the purchase of the cylinder plus the regulators and pressure gauges is US$ 317.61. With respect to operation costs, only the electric energy was accounted for as an operation cost for EGGC. This calculation was carried out based on consumption (kwh), hours of functioning of the compressor, and price of the kwh, resulting in an annual expense of US$ 0.031 for the production of 1 kg of biomass. For CO_2 99.9%, the operation cost is US$ 4.58 per year, due to the recharge of the cylinder.

Table 7 presents the results of the investment analysis for both HRPs with external CO_2 supply, taking into account the use of biomass for energy purposes and as a protein source for animal feeding. The spreadsheets used in the calculations are presented as Supplementary Material.

The negative NPV indicates that, in the context for this experiment, the investment will not be viable for any of the CO_2 sources in a 20-year horizon. For application in the energy sector, the NPV is higher when the EGGC is used; when the purpose is to use the biomass as a source for animal feeding, the NPV is higher when industrial CO_2 is used. These results indicate that for a biomass with lower market value (energy), it is more viable to use a source of gas with lower operation costs. The opposite is also true, biomass with higher market prices enable the use of gas sources that have higher operation costs.

The viability of the project can be reached with the increase of scale, which would provide greater biomass productivity. The higher productivity, considering the same investment, would result in a lower annual cost of 1 kg of biomass. Fig. 7(a) and (b) present the behavior of the NPV with respect to the scale of the experimental unit, for both sources of gas addition. We assessed the scale used in this study, and 10, 20, 30, 40 and 50 times that scale.

The analysis of Fig. 7(a) and (b) show that there is a more significant increase in NPV when the scale is increased 10 times, after that the NPV remains practically constant. The increase is more pronounced when industrial CO_2 is used for the production of biomass for energy purposes: 44% increase. In the other cases, the increase does not reach 22%. This difference in the increase of the NPV between the two sources is related to the cost of the biomass, which is higher when the EGGC is used. The factors that influenced the NPV to remain negative, even with increasing scale and consequently productivity were the initial investment and market price of biomass.

Due to several rainy days, there was lower solar radiation incidence, lower temperature, and lower photosynthetic activity, and the high concentration of organic matter in the sewage from the

Table 7
Investment analysis for HRPs 1 and 2.

Source	Biomass application	NPV (US$)
CO_2 99.9%	Energy	−658.34
	Protein/animal feeding	−199.51
EGGC	Energy	−638.67
	Protein/animal feeding	−452.24

Table 4
Mean concentration values for chlorophyll-*a* (mg L^{-1}), VSS (g m^{-3}), total daily yield (g m^{-2} day^{-1}), total annual yield (kg m^{-2} year^{-1}) and annual biomass yield (kg year^{-1}) for each of the HRPs.

HRP	Chlorophyll-*a* (mg L^{-1})	VSS (g m^{-3})	Total daily yield (g m^{-2} day^{-1})	Total annual yield (kg m^{-2} year^{-1})	Annual biomass yield (kg year^{-1})
1	2.37 (0.99)	160.06 (76.83)	6.00 (2.88)	2.19 (1.05)	7.23 (1.05)
2	2.42 (0.84)	163.11 (85.28)	6.12 (3.20)	2.23 (1.17)	7.36 (1.17)

Standard deviation in parenthesis.
n (number of samples) = 12.

Table 5
Investment and annual operation costs when EGGC was used for the production of 1 kg of biomass.

Source: Exhaust gas from gasoline combustion						
Equipment	Dimension (m^3 h^{-1})	Investment (US$)	Hours per day (h)	Total hours in a year (h)	Total annual consumption (kwh)	Price of the kwh (US$)
Compressor	16.99	575.76	0.03036	11.08	16.62	0.031

Table 6
Investment and annual operation costs when CO_2 99.9% was used for the production of 1 kg of biomass.

Source: CO_2 99.9%					
Equipment	Dimension (kg)	Investment (US$)	Mass added per day (kg)	Mass added per year (kg)	Price of the recharge/year (US$)
Cylinder and regulators	25	317.61	0.0354	12.92	4.58

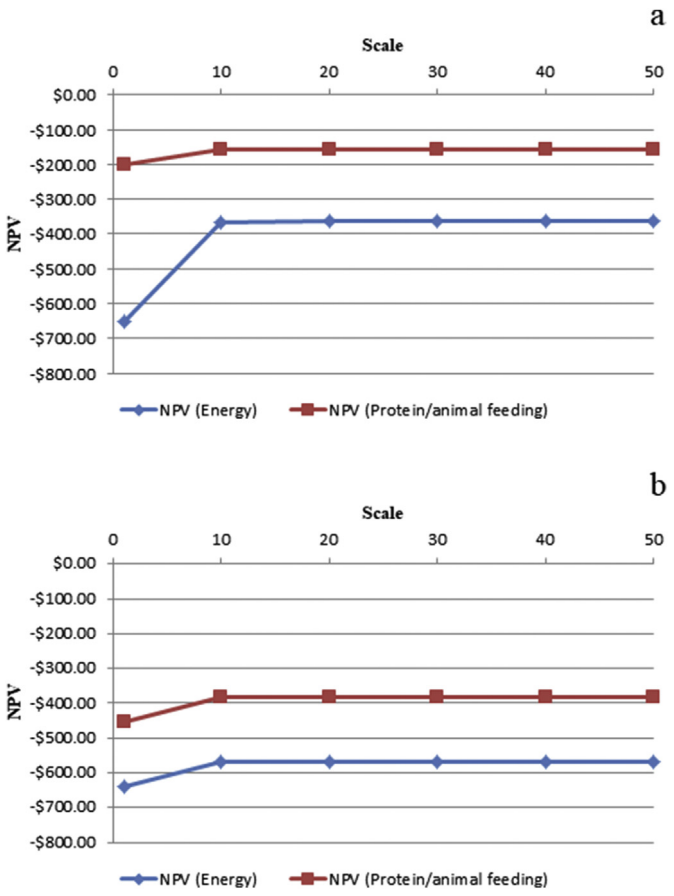

Fig. 7. (a) NPV behavior with respect to the scale of the experimental unit, when industrial CO_2 is used as a CO_2 source, (b) NPV behavior with respect to the scale of the experimental unit, when EGGC is used as a CO_2 source.

septic tank contributed to the low frequency of CO_2 addition. When comparing the data of the characterization of the domestic sewage used in this study with data from domestic sewage pre-treated by a UASB (Upflow Anaerobic Sludge Blanket) reactor, a difference mostly in terms of concentration of organic matter can be observed. When compared to the sewage pre-treated by the UASB reactor, the sewage previously treated by a septic tank has a higher organic load. Some studies carried out using domestic sewage from a UASB reactor as a cultivation medium for the production of microalgae in HRPs enable us to suggest this. Couto et al. (2017) used sewage with total COD concentration of 183.1 mg L^{-1}, whereas Assis et al. (2017) worked with a total concentration of 116 mg L^{-1}. When comparing this data to that obtained in this study, the ones found here were higher. These authors reported that pH increase was related to the consumption and consequently, the demand for CO_2 due to the photosynthetic activity of the microalgae. In the HRPs without CO_2 addition, Couto (2016) measured a maximum pH of 11.3. Santiago et al. (2013), who also worked with domestic sewage pre-treated by the UASB reactor, found pH values above 8.0. We highlight that the studies were carried out in the same location and with the same HRPs used in the present study, but in different seasons of the year.

Despite the fact that the economic analysis showed that in this study it was not economically viable to use atmospheric emissions in the cultivation of microalgae, it is important to highlight that the system used in this study, in addition to treating an environmental liability (sewage), also avoids the emission of greenhouse gases to the atmosphere. This makes algal biomass production of less importance when compared to similar biomasses produced using nitrogen and phosphorus and industrial CO_2. Thus, the final product can be commercialized with a green seal, which in addition to reflecting the sustainable practices used in the production of biomass, can be used as a marketing tool, adding more commercial value to the product. It is also important to highlight that depending on the exhaust gas used, the other gases present can impose extreme conditions to the microalgae, and a treatment of the gas may be necessary, which would make the process more expensive.

4. Conclusions

The results showed that it is possible to use exhaust gases from the combustion of gasoline as an external source of CO_2, since the compounds in the gas were not able to influence the microalgae growth due to the low solubilities. This was except for the nitrogen oxides, which contributed to the decrease of the pH of the medium, but that still did not influence the growth of these microorganisms. The results also showed that the CO_2 source did not influence the relative abundance, the algal biovolume, the treatment efficiency and the biomass yield, or the composition of such biomass.

The cultivation of microalgae in domestic sewage with exhaust gases is valid from an environmental perspective, since it avoids releasing these gases to the atmosphere. On the other hand, for the conditions of this study, it was not viable from an economic perspective because the high investment was not compensated when the produced biomass was used for the production of biofuels and as a protein source in animal feeding. The results also showed that depending on the purpose of the biomass, CO_2 sources with higher or lower operational costs can be used. That is, when the biomass produced is used for a purpose where the market price is smaller, it is more viable to use EGGC, since the operation costs of this source are smaller. When the biomass produced is used for a purpose where the market price is higher, it is more viable to use industrial CO_2.

The main limitation of the experiment was the low carbon demand due to the high concentration of organic matter in the domestic sewage used, which caused few additions of gas in the medium. To circumvent this, it is suggested that rather than added CO_2 on demand, be added throughout the day or at short intervals. Continuous addition throughout the day can be carried out without concern for the drop in pH of the medium once the pH reaches constant chemical equilibrium (Cheng et al., 2015). In addition, environmental benefits can be included in investment analysis. In this way, the biomass could be marketed with a green seal, which would valorize the product, raising its commercial price and consequently increasing the profit, which could make the process economically more viable. On the other hand, another future possibility is the charge for the sale of CO_2. This is the case of industrial CO_2-trading, that according to Rockström et al. (2017) starting prices are about of 50US$/ton CO_2.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2019.01.093.

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