Research Paper

Microalgae population dynamics in photobioreactors with secondary sewage effluent as culture medium

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Abstract

Nitrogen and phosphorus present in sewage can be used for microalgae growth, possibiliting cost reduction in the production of microalgae at the same time that it decreases the eutrophication potential of the effluent. This research aimed at monitoring the native community of microalgae and coliform bacteria in a secondary effluent from anaerobic municipal sewage treatment. Two treatments (aerated and non-aerated) were performed to grow microalgae under semi-controlled conditions in semi-closed photobioreactors in a greenhouse. The results showed no significant pH and coliforms (total and *Escherichia coli*) variation between treatments. Nutrient concentrations were reduced supporting microalgae growth up to 10^7 cells.mL^{-1} independent of aeration. Exponential growth was obtained from the first day for the non-aerated, but a 5 day lag phase of growth was obtained for the aerated. *Chlorella vulgaris* was the dominant microalgae (99.9%) in both treatments. In the aerated, 5 algae classes were detected (Chlorophyceae, Cyanophyceae, Chrysophyceae, Bacillariophyceae and Euglenophyceae), with 12 taxa, whereas in the non-aerated, 2 classes were identified (Chlorophyceae and Cyanophyceae), with 5 taxa. We concluded that effluent is viable for microalgae growth, especially *Chlorella vulgaris,* at the same time that the eutrophication potential and coliforms are decreased, contributing for better quality of the final effluent.

Key words: microalgae, effluent treatment, coliforms, *Escherichia coli*, *Chlorella vulgaris*.

Introduction

Secondary effluents generated from anaerobic treatment of domestic sewage are commonly disposed of in water bodies although they may not have ecologically acceptable physical, chemical and/or biological composition. Most often they contain organic matter, nutrients, metals and pathogens, leading to the pollution and contamination of aquatic environments (Zanetti *et al.*, 2006). Usually, the continuous discharge of such effluents is a cause of accelerated eutrophication. Thus, there is a need for tertiary treatments to further reduce nutrients concentration and pollution potential of secondary sewage effluents.

According to Hammouda *et al.* (1995), Villaverde (2004) and Weismann *et al.* (2007), anaerobic sewage treatment does not remove efficiently nitrogen (N) and phosphorus (P), remaining available for the phytoplankton community. Released into the environment, this can support microalgae growth, leading to excessive biomass increase that will end up, decomposed by heterotrophic microorganisms, causing oxygen deficit, and all the other negative effects of eutrophication such as the death of aquatic animals (Oswald, 1988; Olguín, 2003). The mean concentration of N (40 mg.L^{-1}) and P (8 mg.L^{-1}) per liter of effluent is sufficient to produce 0.6 g of microalgae with a productivity of 77,600,000 kg.day^{-1} (Klausmeier *et al.*, 2004). The N:P ratio ideal for microalgae growth according to the Redfield ratio is around 16:1, representing an average ratio, that can vary among species, from 8:1 to 45:1 (Klaus-

meier *et al.*, 2004; Christenson and Sims, 2011; Perez-Garcia *et al.*, 2011).

Growing microalgae in such effluents has several advantages, besides reducing the eutrophication potential and the number of bacteria in the effluent (de La Noüe *et al.*, 1992; Hammouda *et al.*, 1995; Hoffmann, 1998). According to Oswald (1998), de Bashan *et al.* (2004) and Shantala *et al.* (2009), heterotrophic bacteria in the effluent will decompose biodegradable organic matter, produce carbon dioxide, ammonium, nitrates and phosphates for microalgae use (Olguín, 2003). Through photosynthesis, microalgae produce oxygen, supporting the degradation of organic matter and reduction of the biochemical oxygen demand in the effluent by aerobic bacteria. Also, the oxygen and pH variation induced by microalgae photosynthesis help reduce coliform and other pathogenic bacteria in the effluent (Pearson, 1986; Oswald, 1988; Mayo and Noike, 1994; Meiring *et al.*, 1994; Davies-Colley *et al.*, 1997; Aksu 1998; Metcalf and Eddy, 2003; Kiso *et al.*, 2005).

According von Sperling (1996), some algal groups dominate over others depending on the physical (solids, turbidity, absorbance, temperature, electrical conductivity), chemical (pH, chloride, alkalinity, nitrogen, phosphorus, gases, metals, organic compounds, among others) and biological (bacteria, funghi, virus, protozoans, larvae) features of the effluent (von Sperling, 1996; Metcalf and Eddy, 2003). The genera most commonly found in effluents from wastewater treatment plants (WWTPs) are: *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Micractinium*, *Euglena*, *Ankistrodesmus*, *Oscillatoria*, *Microcystis*, *Nitzchia*, *Navicula* and *Stigeoclonium* (Palmer, 1969; Mara and Pearson, 1998; Amengual-Morro *et al.*, 2012).

Commercial production of microalgae is expensive, and the addition of nutrients in culture media contributes to its high cost. Therefore, the growth of microalgae in wastewater effluents can be a way to reduce algal cultures costs. Biofertilizers and biofuel do not require high purity cultures as required for applications of microalgae in pharmaceutical or food industry (Mayo and Noike, 1994; Cho *et al.*, 2011), so wastewater effluents may suffice for such algae production. Understanding the behavior and growth of indigenous microalgae in the effluent over time is important to define the suitability of the effluent and algae growth conditions for biotechnological interest focusing on a large scale algal production system (Oswald, 1988).

This study investigated the composition of indigenous phytoplankton present in a secondary effluent from anaerobic sewage treatment that collects and treats altogether the sewage from an aircroft maintanance stablishmens and domestic sewage. Microalgae growth reduction of nutrients (N and P), as well as the reduction of coliform bacteria (total and *Escherichia coli*). The potential of such secondary effluent from anaerobic sewage treatment for the production of algal biomass while reducing nutrients and coliform bacteria concentrations are discussed.

Materials and Methods

Samples and experimental conditions

Secondary effluent (50 L) from anaerobic treatment whose sewage is originated from domestic and aircraft maintenance stablisiment was obtained before its discharge into the receiving water body at the wastewater treatment plant of Água Vermelha district (São Carlos, SP, Brazil). The wastewater treatment plant treat approximately 8 L of sewage per second from a population of approximately 3,500 people plus the residues from the aircraft maintenance stablishment.

The experiments consisted of eight bioreactors used in partially sealed system with 8 L capacity containing 5 L of the secondary sewage effluent each. The bioreactors consisted of transparent plastic cylindrical reservoirs, internally coated with transparent low density polyethylene bags that were covered with PVC film, measuring 26 cm high by 27 cm diameter. Of these, 4 bioreactors received artificial aeration through air pump (Regent Air Pump, Model 8500), refereed as *aerated treatment* and 4 were left without aeration (*non-aerated treatment*). Thus, treatments were performed with 4 experimental replicates. Samples for the determination of the initial conditions of the effluent were obtained immediately after arrival of the effluent in the laboratory and before incubation. The photobioreactors were incubated during 30 days in a greenhouse at the Botany Department, Federal University of São Carlos (Brazil). Temperature in the greenhouse varied from 23 °C at night up to 30 °C at midday. Light intensity varied from 4.6 $\mu mol.m^{-2}.s^{-1}$ at 8:00 h to a maximum of 37 $\mu mol.m^{-2}.s^{-1}$ at noon (12:00) with natural sunlight photoperiod during 30 days between autumn 2012. In this time of year, the climate in sub-tropical weather has the greater thermic oscilation of the year.

pH and chlorophyll *a*

Bioreactors were monitored for pH (pHmeter - pHep®, Brazil) and chlorophyll *a* concentration on alternative days. Chlorophyll *a* was determined by *in vivo* fluorescence using a fluorimeter (Turner Designs, Model Trilogy - U.S.A.) and its concentrations were obtained from a calibration curve performed through fluorescence intensity vs concentration of chlorophyll *a* extracted from exponentially growing cultures of *Chlorella sorokiniana*.

N and P concentrations

Total nitrogen (N) and phosphorus (P), and dissolved nutrients (nitrate, nitrite, ammonium and phosphate) were determined according to APHA (2005) at the beginning and end of the experiment. For the determination of dissolved nutrients samples were first filtered through glass fiber filter (3.0 μm pore diameter) and then through 0.45 μm pore diameter cellulose acetate membrane (Sartorius).

Phytoplanktonic community

Qualitative and quantitative analysis of the phytoplankton community were performed on samples collected every 5 days. All bioreactors, aerated and non-aerated, were homogenized manually before sampling. For the qualitative analysis samples were preserved in 4% formaldehyde solution and analyzed using a Zeiss light microscope with maximum magnification of 2560 times. Algae identification was made to the taxonomic level as detailed as possible by consulting specialized literature (Komárek and Fott, 1983; Anagnostidis and Komárek, 1989; Komárek and Anagnostidis, 1999; Bicudo and Menezes, 2005; Komárek and Anagnostidis, 2005). For the quantitative analysis, 90 mL sample was collected and preserved with Lugol's acid solution. Microalgae populations were counted under an inverted microscope (Zeiss, Axiovert 200), with 400x magnification using the method described in Utermöhl (1958). Depending on the concentration of organisms, samples ranging within 10 and 50 mL were allowed to settle for at least 3 h (Wetzel and Likens, 1991). Individuals (cells, colonies, filaments and/or coenobia) were counted in randomized fields and densities calculated according to APHA (2005), and expressed as cells.mL^{-1}.

Total microalgae productivity (P) in the bioreactors is reported as cell.mL^{-1}.day^{-1} was obtained according to Eq. (1) as described below:

P = (final cell density - inicial cell density) / incubation time (days)

Coliforms analyses

Total coliforms and *Escherichia coli* samples were obtained from each biorreactor and quantified on alternate days until colonies were no longer detected. Samples (1 mL) were diluted in phosphate buffered saline solution (PBS) to the decimal scale 10^{-3}. Each dilution was inoculated in duplicates into sterile and disposable Petri dishes by the Pour Plate Method according to APHA (2005). For this, the culture medium Cromocult® Coliform Agar (Merck KGaA, Germany) was used. The Petri dishes were then incubated under controlled conditions at 36 °C for 24 h in the dark. The colonies were counted and the results expressed as colony forming units per volume (CFU.mL^{-1}).

Data analyses

The results were analyzed using *t*-test to compare mean values obtained from the aerated and non-aerated treatments. For analysis of the results within each treatment ANOVA (Hammer *et al.*, 2001) was used.

Results

pH and chlorophyll *a*

Figure 1 reports pH values as function of time. It shows that pH was maintained within 8.5 and 9.5 up to the 18th incubation day and while algae were growing, after which microalgae growth decreased and so did the pH, to near 6.0 at the end of the experiment (30th day). No significant pH variations were observed between the aerated and non-aerated treatments (*t*-test, p > 0.05).

Microalgae growth, reported as chlorophyll a concentrations and cell.mL^{-1} as function of time are shown in Figure 2. It is observed that the aeration process resulted in an extended adaptation or lag growth phase in the first 5 days, possibly due to the modification of the conditions imposed by the air bubbling. In the non-aerated treatment, cells grew exponentially since the beginning of the experiment. According to equation 1, the productivity (cells.mL^{-1}day^{-1}) was 3.4 times higher in the aerated (2.4×10^5 cells.mL^{-1}.day^{-1}) than in the non-aerated treatment (7.1×10^4 cells.mL^{-1}.day^{-1}) treatment. Although higher productivity was obtained for the aerated treatment, significant differences (p < 0.05) for chlorophyll *a* concentrations between the two treatments were obtained for the 5, 25 and 30th experimental days only.
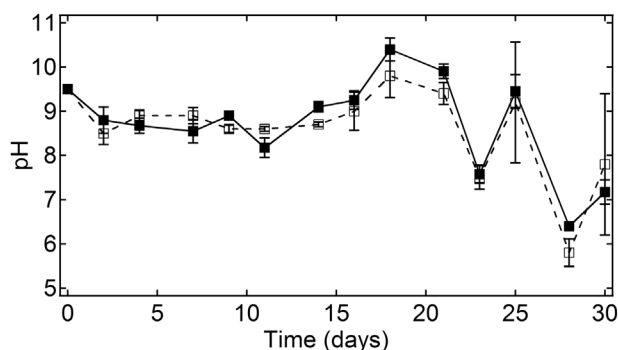
N and P concentrations

Table 1 shows the concentration of nutrients in absolute and relative values for both aerated and non-aerated treatments. Higher nutrient reduction was obtained for the aerated in comparison with the non-aerated treatment.

Phytoplanktonic community

Taxonomic composition and relative abundance of each taxon detected in the experiments are presented in Tables 2 and 3 for the aerated and non-aerated treatment, respectively. Dominance was observed for the Chlorophyceae *Chlorella vulgaris* in both treatments. This species amounted more than 99% of the total algae biomass.

Other taxa belonging to the Chlorophyceae were also observed, but at lower densities. *Chlamydomonas* sp (1.8%), *Scenedesmus acuminatus* (0.04%), *Oocystis* sp (0.1%), *Desmodesmus quadricauda* (0.33%) and *Desmodesmus* sp (0.01%). Cyanophyceae was present in approximately 36.6% of the total algae biomass, compris-

Figure 1 - Variation of pH in photobioreactors as a function of time in aerated (filled squares) and non-aerated (open squares) treatments.
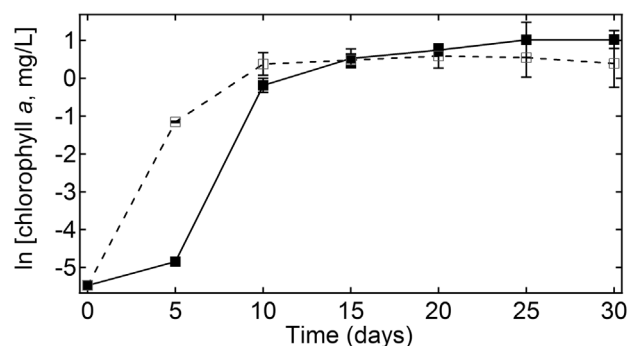
Figure 2 - Variations in the concentration of chlorophyll (mg/L) presented in natural log (ln) in photobioreactors as a function of time in aerated (filled squares) and non-aerated (open squares) treatments.

ing two genera, *Pseudanabaena* sp1 (35.6%) in both treatments, and *Pseudanabaena* sp2 (0.28%) and *Spirulina* sp (0.71%) in the aerated treatment only. The class Euglenophyceae (*Euglena* sp) was detected on the 1st experimental day in both treatments, while the classes Chrysophyceae and Bacillariophyceae (*Navicula* sp) occurred in the aerated treatment only and in reduced percentiles.

Figure 3 synthesizes the distribution and succession of the microalgae groups throughout the experiment, in aerated (Fig 3a) and non-aerated (Fig 3b) treatments. They show that the highest density were for the class Chlorophyceae, which was present throughout the experimental period, in both bioassays. The class Cyanophyceae was detected from the 5th to the 15th day in the non-aerated

Table 1 - Mean values (± standard deviation) of the nutrient concentration ($\mu g.L^{-1}$) in the initial and end samples of the experiments (aerated and non-aerated treatments). TKN = total Kjeldahl nitrogen.

Nutrient (µg/L)	Initial	Aerated		Non-Aerated	
		Total value	Reduction	Total value	Reduction
Nitrite	23.69	7.53 (± 2.23)	68%	764.03 (± 124.5)	N.R.*
Nitrate	79.64	36.73 (± 0.29)	54%	8950.67 (± 2203.94)	N.R.*
Ammonium	13.05	5.86 (± 2.36)	45%	144.98 (± 32.45)	N.R.*
TKN	30330.00	2570.00 (± 0.00)	92%	18790.00 (± 6550.0)	38%
Total Phosphorus	2387.10	1699.58 (± 276.18)	29%	2250.58 (± 201.17)	21%
Phosphate	1342.90	78.19 (± 17.63)	94%	1062.00 (± 154.11)	18%
Total Dissolved Phosphorus	1464.80	530.74 (± 52.75)	64%	1196.48 (± 137.67)	6%

*N.R. = no reduction.

Table 2 - Taxonomic composition and relative abundance (%) of algal taxa obtained in the aerated treatment during the experimental period. Samples obtained on experimental days 1, 5, 10, 15, 20, 25 and 30.

Aerated treatment	1	5	10	15	20	25	30
Chlorophyceae							
Chlorella sp	97.90	62.50	99.70	99.60	99.60	99.60	99.30
Chlamydomonas sp	-	1.83	0.18	0.33	0.23	0.23	0.47
Desmodesmus quadricauda	-	-	-	0.33	0.01	0.05	0.04
Desmodesmus sp	-	-	-	0.01	-	0.01	-
Oocystis sp	-	-	-	-	0.04	0.03	0.10
Scenedesmus acuminatus	-	-	-	0.02	0.02	-	0.04
Cyanophyceae							
Pseudanabaena sp1	-	35.80	0.09	-	-	-	-
Pseudanabaena sp2	-	0.28	-	-	-	-	-
Spirulina sp	-	0.71	-	-	-	-	-
Chrysophyceae							
Alga unidentified	-	-	-	0.01	0.03	0.06	0.01
Euglenophyceae							
Euglena sp	2.10	-	-	-	-	-	-
Bacillariophyceae							
Navicula sp	-	0.01	0.04	-	-	-	-

Table 3 - Taxonomic composition and relative abundance (%) of algal taxa obtained in the non-aerated treatment during the experimental period. Samples obtained on days 1, 5, 10, 15, 20, 25 and 30.

Non-aerated treatment	1	5	10	15	20	15	30
Chlorophyceae							
Chlorella sp	100.00	87.30	99.40	99.30	99.80	99.90	99.90
Chlamydomonas sp	-	-	0.29	0.32	0.20	0.10	0.07
Desmodesmus quadricauda	-	-	-	-	-	-	0.01
Oocystis sp	-	-	-	-	-	-	0.01
Cyanophyceae							
Pseudanabaena sp1	-	12.70	0.30	0.39	-	-	-

bioassays. The classes Chrysophyceae, Bacillariophyceae and Euglenophyceae were present in trace amounts and therefore were grouped altogether within *other groups*. The class Euglenophyceae was observed in both treatments, while the classes Bacillariophyceae and Chrysophyceae in the aerated treatment only.

Coliforms analyses

Results obtained for the coliform group of bacteria are reported in Figure 4a (total coliforms) and 4b (*E. coli*). They show that the reduction of colony forming units for both total coliforms and for *E. coli* occurred abruptly in the

first 2 days, no longer detected after the 18th day and the 11th day for total coliforms and after the 11th day for *E. coli*, regardless of the treatment.

Discussion

pH and chlorophyll *a*

The pH ~ 8.0 in the beginning of the experiments can be attributed to the methanogenesis occurring during the anaerobic treatment. According Jeris and McCarty (1965) and Weimer and Zeikus (1978), methanogenic archaea convert H^+ ions and organic acids in CH_4, H_2O and HCO_3^-,
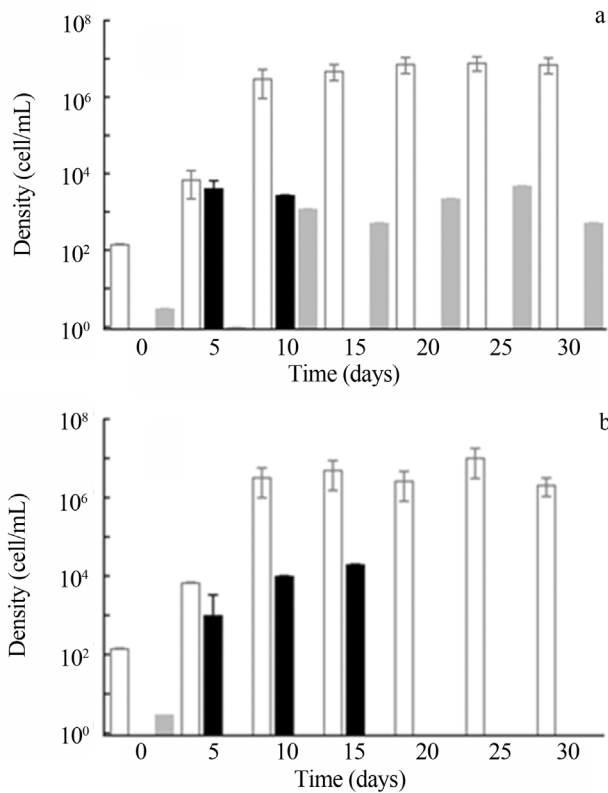
Figure 3 - Phytoplanktonic groups density (cells/mL) in the aerated (a) and non-aerated (b) treatments throughout the experimental period. White columns represent the class Chlorophyceae, the class Cyanophyceae black, and gray Other Groups (classes Chrysophyceae).
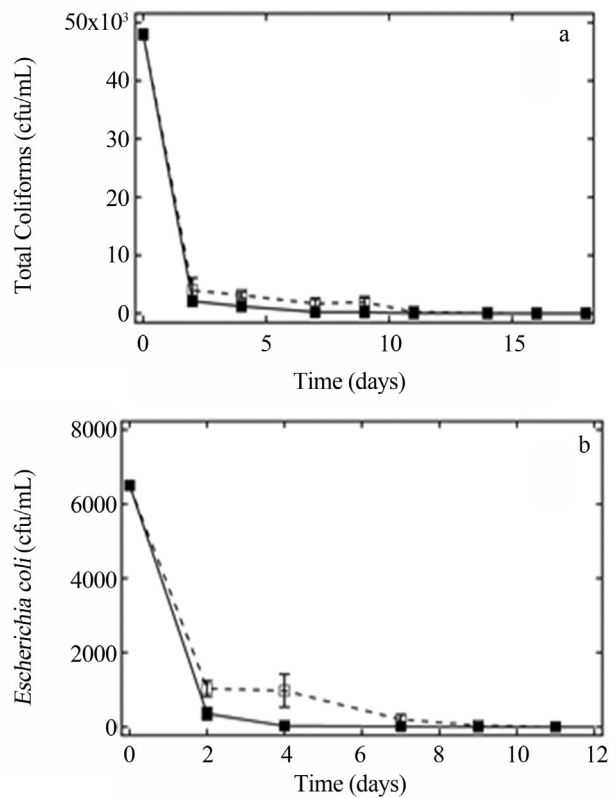
Figure 4 - Density of total coliforms (a) and Escherichia coli in aerated (filled squares) and non-aerated (open squares) treatments over the experimental period reported in colony forming units per mL (CFU/mL).

what helps maintain basic pHs. This fact, alied to algae growth that consumes nitrate and inorganic carbon, can be responsible for the pH maintenance (Reynolds, 2006). The reduction of pH at the end of the experiment for both treatments can be a result of the decrease of microalgae growth, as detected after the 10th experimental day and shown as the stationary growth phase in Figure 2. Besides this, bacteria degradation in the effluent, as discussed in von Sperling (1996), Arauzo *et al.* (2000), Bitton (2005), Amengual-Morro *et al.* (2012) may have contributed to pH decrease in the treatment.

Phytoplankton growth in both aerated and non-aerated treatments, resulted in increased chlorophyll *a* and cell density. The initial lag phase observed just for the aerated treatment also was found by Bernal *et al.* (2008) and Zhang *et al.* (2011) that lasted at least 8 days when investigating changes in the structure and dynamics of the phytoplankton community in domestic effluents from primary treatment. It is known that aeration in microalgae cultures helps create turbulence, so decreasing microalgae self-shading (Larsdotter, 2006) and furnishing the cultures with CO_2, an essential nutrient for photosynthetic microalgae (Fontes *et al.*, 1987; Becker, 1994). In the present research, we showed that although aeration was an important factor for the development of algae and the combination of algae growth, nutrient decrease and bacteria reduction, no significant difference was observed for the maximum cell density between the aerated and non-aerated treatments within 10 to 15 days of incubation.

N and P concentrations

Phosphate reduction, more intense in the aerated than in non-aerated treatment, can be associated with consumption by microalgae (Larsdotter, 2006; Boelee *et al.*, 2011; Zhang *et al.*, 2011). Boelee *et al.* (2011) showed that microalgae are effective at removing phosphate when used as tertiary biological treatment of wastewater. Several microalgae can assimilate and store P as polyphosphate granules inside the cells through the P luxurious consumption (Larsdotter, 2006), what can help in the reduction of P from the effluent.

The significant N reduction observed in the aerated treatment in the present research has also been obtained in other investigations and can be related to the sum of processes occurring simultaneously while bubbling, *e.g.*, nitrification, consumption of NH_4^+ by microalgae and elimination of NH_3-N to the atmosphere (Mayo and Noike, 1994; Zhang *et al.*, 2011; Ray *et al.*, 2012). Although the non-aerated treatment had their N concentration decreased, the percent reduction (~38%) was much lower than the aerated (~92%). This difference can be due to different transformations of N in the two treatments, such as the oxidation of NH_4^+ to N_2 under the aerated conditions. In addition, the lower cell density detected at the end of the non-aerated treatment as compared with the aerated may have lead to N

accumulation, which was then quantified at the end of the treatment as NO_2^-, NO_3^- and NH_4^+. According to Raven (1988), Borowitzka *et al.* (1998) and Wood *et al.* (1999) aeration has the important function of system homogenization, exposure of microalgae to light and nutrients, thus enabling photosynthesis, population growth and nutrient consumption. The reduction in the concentration of nutrients (N and P) during the experiment shows the effectiveness and importance of tertiary treatment with microalgae to the improve the quality of the final effluent, reducing the environmental impact after its discharge into receiving water bodies.

Phytoplanktonic community

In the present study, qualitative analysis of the phytoplankton community in both treatments confirmed the presence of microalgae typical of environments with high content of organic materials and a greater diversity for the aerated in comparisom with the non-aerated treatment. This agree with other studies (Palmer, 1980; König, 1984; König *et al.*, 2005), that showed that phytoplankton diversity in sewage effluent is influenced by factors such as organic loading, hydraulic retention time, temperature, pH, and nutrient concentration (Hosetti and Frost, 1998; Kayombo *et al.*, 2002; Zanotelli *et al.*, 2002; Ahmad *et al.*, 2005). According to Palmer (1969; 1980) and König (1984), the composition of phytoplankton community is strongly related with the concentration of nutrients and organic materials.

The Chlorophyceae dominance in comparison with other classes obtained in the present study has been observed by Palmer (1969) and Bernal *et al.* (2008). Such dominance can be due to the resistance and adaptation of the Chlorophyceae for hughly eutrophic environments. In our study, the species *Chlorella vulgaris* dominated in all treatments, with approximately 99% over the other microalgae. Chu *et al.* (2009) and Bhatnagar *et al.* (2010), also found dominance of the genus *Chlorella* in sewage treatment effluents. Shanthala *et al.* (2009) evaluated phytoplankton diversity in stabilization ponds and obtained dominance of *Chlorella* sp and *Scenedesmus* sp; König *et al.* (2002) studied stabilization ponds in the state of Paraíba (Brazil) and also had low contribution of taxa other than *Chlorella* sp. According Salomoni (1997), organisms such as the species *Chlorella vulgaris*, which survive in environments rich in nutrients and organic materials exhibit adaptive features such as small size and high growth rate that enable them to dominate in such environments. It is known that the smaller the size, greater the efficiency in the absorption and assimilation of nutrients (r-strategists organisms) due to the increased surface/volume ratio. In addition, there is evidence that sodium triphosphate, a common constituent of synthetic detergent present in domestic sewage, can stimulate the growth of *Chlorella vulgaris* (Palmer, 1980; Granado, 2004). Another aspect that may have favored *C. vulgaris* in relation to other species is the

production of chloreline, a substance that has bactericidal properties and is capable of inhibiting growth, respiration and photosynthesis of other algae, besides affecting the metabolism of other organisms (Pratt, 1944; Ryther, 1954).

The species *C. vulgaris* has great potential for the production of lipids, which can be converted into biodiesel, a substitute for fossil fuels (Christenson and Sims, 2011; Perez-Garcia *et al.*, 2011). In relation to this biotechnological potential, the present study demonstrated that effluent from anaerobic sewage treatment can be used as culture media for the production of *C. vulgaris*, which has come to represent over 99% of all microalgae in the experiment.

The class Cyanophyceae is, in general, resistant to organic and inorganic pollution, sewage effluents and anaerobic environments with high organic loads (Ahmand *et al.*, 2005; Komárek and Anagnostidis, 2005; Tucci *et al.*, 2006; Escorihuela *et al.*, 2007). However, they can be sensitive to turbidity (Havens *et al.*, 2004), what may have accounted for the low percent contribution of the Cyanophyceae (36%) in the present investigation. The N:P ratio can also influence the presence/absence of specific groups of phytoplankton. According Pearsall (1930) and Arauzo *et al.* (2000) the N:P ratio in anaerobic effluent is not favorable to Cyanophyceae. Havens *et al.* (2004) claim that N:P ratios < 29 favor Cyanophyceae in detriment to other classes, which may be favored in N:P ratios > 29 such as the Chlorophyceae. Considering the values of total N (30.4 mg.L^{-1}) and P (2.4 mg.L^{-1}) quantified in the beginning of the experiments, the N:P ratio in the anaerobic effluent of this study was 12.7. Although lower than 29, it seemed not favorable to the Cyanophyceae. Farina (2011), studying the population dynamics of microalgae in secondary effluent from domestic aerobic treatment, found dominance of Cyanophyceae and greater variety of phytoplankton groups than observed in the present research. According to the author, this was a consequence of low N:P ratios found in effluents of aerobic wastewater treatments, a fact also observed by Ahmadi *et al.* (2005) and Bernal *et al.* (2008). The most abundant genus of Cyanobacteria in this work, *Pseudanabaena* sp, was observed in both aerated and non-aerated treatments. This genus is common in eutrophic environments, and dominant in effluent from pulp and paper industry (Kirkwood *et al.*, 2003; Wehr and Sheath, 2003; Komárek and Anagnostidis, 2005).

In the aerated treatment, the classes Bacillariophyceae and Chrysophyceae were present in low proportion compared with the Chlorophyceae, what is in agreement with other works about biodiversity in effluents (Mendes *et al.*, 1995; Sukias *et al.*, 2001; Tharavathi and Hosetti, 2003; Bernal *et al.*, 2008).

As reported by Roche (1995), individuals of the class Euglenophyceae are scarce in effluents and the mixotrophic genus *Euglena* sp (Pearson, 1986) is sensitive to variation in environmental conditions, what may have accounted for its presence just on the 1st day of sampling, both in the aerated and non-aerated treatments.

Coliforms analyses

The reduction of total coliforms and *E. coli,* and their absence after ~10 days of incubation confirms other literature studies (Pearson *et al.*, 1987; Dixo *et al.*, 1995; Hammouda *et al.*, 1995; Tangeby *et al.*, 1996; Davies-Colley *et al.*, 1997; Kiso *et al.*, 2005; Bernal *et al.*, 2008) that have shown reduction of these organisms in effluent treatments employing microalgae. Hanajima *et al.* (2011) reported that air bubbling can reduce populations of fecal coliforms. The initial presence and reduction of colony forming units of coliform and *E. coli* in both aerated and non-aerated treatments confirm that using microalgar as tertiary treatment improves the quality of the sewage effluent and can be adopted as routine procedure in sewage treatment plants before discarding the effluent, making the microalgal treatment sustainable and efficient in the reduction of coliforms.

However, most literature that report on *E.* coli reduction, shows it is less intense than what we obtained in the present research, a reduction of 4 logs times (99.9% of the bacteria were reduced) for *E. coli* was detected, whereas according Kassab *et al.* (2010) anaerobic treatment can be responsible for a 1 log reduction of *E. coli* only. According to literature, the significant reduction of coliform bacteria and *E. coli* we obtained can be attributed to the pH variation in the effluent as result of algae photosynthesis in the light and respiration in the dark (natural light cycle). In fact, our results showed significant variation of pH when comparing pHs for each experimental day. This is confirmed by the experiments of König *et al.* (1999) and Amengual-Morro *et al.* (2012), who showed that the microlagae photosynthetic activity in sewage treatment effluents overcomes bacteria respiration during daytime, leading to an imbalance in pH between day and night. This leads to the inactivation of coliform and other bacteria forms. According Metcalf and Eddy (2003), there are bacteria that do not support pH above 9.5 or below 4.0, with the optimal range of pH between 6.5 and 7.5. In addition, the low depth of the bioreactors favored light penetration, which may have contributed to bacteria reduction (Pearson *et al.*, 1987; von Sperling, 1996; Cavalcanti *et al.*, 2001; Cordero *et al.*, 2010).

We showed that the effluent from an anaerobic sewage treatment (constituted by domestic and aircraft maintenance operation) supported the growth of native *Chlorella vulgaris* with a productivity 2.4x10^5 cels.mL^{-1}.day^{-1} better than did the same effluent, but in non-aerated conditions. The growth of *C. vulgaris* in such aerated effluent improved its quality, reducing in 99% the colony forming units of total coliform bacteria and *Escherichia coli*, at the same time that the concentration of nitrogen and phosphorus were reduced. This study confirmed the sustainable character of using microalgae for tertiary treatments of wastewater: it reduced N and P and pathogenic bacteria, so

decreasing the potential impact the discharge of secondary effluent can cause the receiving water body, at the same time that serves for the production of microalgal biomass.

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