Microalgae cultivation in agro-industrial effluents for biodiesel application: effects of the availability of nutrients

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ABSTRACT

The present study evaluated the cultivation of microalgae in a photobioreactor using effluents from the meat-processing industry, which had been previously treated at the primary and secondary levels. *Scenedesmus* sp. was the dominant genus in the phytoplankton community in both of the evaluated effluents. The different nutritional conditions affected the production of biomass, which reached 1,160 mg/L of volatile suspended solids (VSS) and 371 mg/L of VSS with cultivation in the primary (PE) and secondary effluents (SE), respectively. In both effluents, great removal efficiencies close to quantification limits were observed for ammoniacal nitrogen and soluble phosphorus. Regarding the accumulation of lipids, there were no considerable differences between the effluents. The highest lipid productivity that was observed in the PE, which reached 3.7 g/m²·d, was attributed to its larger production of biomass as a consequence of its better nutritional condition in relation to the SE.

Key words | biomass, bubble column photobioreactor, effluent treatment, lipids, meat-processing industry

INTRODUCTION

Globally, livestock contributes 15% of total food energy and 25% of dietary protein and is one of the fastest growing parts of the agricultural economy. In order to meet rising demand, global annual meat production is expected to expand from 228 currently to 465 million tonnes by 2050 with the cattle population estimated to grow from 1.5 billion to 2.6 billion and that of goats and sheep from 1.7 billion to 2.7 billion (FAO 2009). Brazil is one of the leading countries in world meat exportation for having the largest commercial cattle herd in the world, with approximately 198 million head in 2014 (ANUALPEC 2015). Brazil is also being recognized with one of the main producers and exporters of pork and poultry. Each year, Brazilian participation in international trade is growing, particularly in the production of beef, pork, and chicken. By 2020, it is expected that the national production of meat will supply 44.5% of the world market (MAPA 2015).

Inherent to the production process and as consequence of the promising economic scenario, the meat-processing industry generates a large volume of effluents, which represent serious environmental and health problems primarily due to their biological instability and pathogen development potential (De Sena et al. 2009). Issues such as increasing demand for animal protein, environmental and health restrictions imposed by national legislation and international agreements (De Sena et al. 2009), and the search for viability and sustainability of industrial processes highlight the importance of the development of innovative effluent treatment technologies. These technologies must combine efficient and inexpensive treatments, recyclable nutrients, and the production of added-value items.

In meat production processes, during the various stages (from slaughtering to food processing and animal products), there is an immense production potential for added-value items, such as pigments and biofuels. Previous research has proven the efficiency of microalgae in the removal of nutrients and metals, in agro-industrial effluents, such as previously digested dairy effluents, slaughterhouse, piggery
(Qi et al. 2017) and tannery effluents. Despite the global importance of meat production and the great potential use of generated effluents, it was not found in the literature studies that assessed the utilization of effluents from the meat-processing stage in the cultivation of microalgae.

Microalgae can be cultivated in suspended form, in open photobioreactors (such as a high rate pond – HRP), or in enclosed photobioreactors (PBRs). Considering the use of effluents as culture media, HRPs represent a technology that is consolidated to a certain extent and has been extensively evaluated. Alternatively, PBRs have a higher productivity of biomass in comparison to HRPs due to a better control of the cultivation conditions (illumination, pH, and mixture), which reduces the area and lowers the costs associated with biomass collection.

However, this optimization also generates negative consequences, such as an increase in power consumption, which is primarily associated with the mixture and still restricts its application on a full scale. For example, a PBR, depending on its type, consumes 10–50 W/m³ (Xu et al. 2018) for its functioning versus 3.72 W/m³ for raceways ponds (Jorquera et al. 2010). However, together with airlift reactors, they are easy to scale up and have a low power consumption compared with that of other types of PBRs. These are essential characteristics when considering the treatment of large volumes of effluents and intensive production of algal biomass. However, most of the studies that have investigated the simultaneous treatment of effluents and biomass production in PBRs use production systems on a laboratory scale (Wang et al. 2010) and with synthetic or diluted culture media (Qi et al. 2017), which poorly depicts reality and makes it difficult to replicate the results on a large scale.

Considering the previous discussion, the goal of the present study is to evaluate the cultivation of microalgae in real effluents of the meat-processing industry with a focus on both the biomass production and the accumulation of lipids as well as the treatment of effluents in a pilot-scale bubble column PBR. The objective is to identify the nutritional factors that increase the production of algal biomass in primary and secondary pre-treated effluents, which would also provide support in the production of biodiesel.

METHODS

Experimental unit

The experiment was conducted in an outside area of the Laboratory of Sanitary and Environmental Engineering at the campus of the Federal University of Viçosa – UFV, Brazil (20°45′14″S, 42°52′54″W) from September to December of 2014.

As shown in Figure 1, the bubble column PBR that was used consisted of three independent acrylic tubes with diameters of 15 cm and a total volume of 16 liters each. The culture medium was mixed continuously by bubbling air (0.625 vvm, volume of injected air per minute divided by the volume of the culture medium) enriched with CO₂ (6.5%, v/v).

The air for the mixture was supplied by a diaphragm air compressor with a power of 0.25 kW and driven to each acrylic tube through a pneumatic hose followed by a PVC pipe connected to a cylindrical porous stone disperser (22 × 12 mm). To control the flows, valves and flowmeters with an accuracy of 0–15 L/min were installed.

The CO₂ supply was controlled by the pH variation in the units, which was maintained at between 6 and 8. For this automated system, a probe was used for real-time measurement of the pH and temperature (controller sc200, brand HACH and pH differential analog sensor for effluents) with an electrical signal transmission system compatible with the solenoid valve to control the addition of CO₂.

The main components of the system are presented in Figure 1(a), and the dimensions of the acrylic tubes that were used are shown in Figure 1(b).

Culture medium and inoculum

To test different nutritional conditions, primary (PE) and secondary effluents (SE) evaluated as culture media were collected from a wastewater treatment plant (WWTP) installed in a meat-processing industry after flotation treatment (PE) and after an activated sludge unit (SE). For each operation, 10% (v/v) of inoculum collected from HRP was added to the evaluated culture medium (Table 1). This HRP was preceded by an upflow anaerobic sludge blanket (UASB) reactor, which was used to treat domestic effluent, as previously studied by Santiago et al. (2013).

Monitoring of the photobioreactor

Each culture medium was evaluated during three outdoor operations of the PBR, which were performed in batches: OPR 1, OPR 2, and OPR 3 (PE) and OPR 4, OPR 5, and OPR 6 (SE). The three acrylic tubes had identical dimensions and were simultaneously operated with the same culture medium, mixture, supplement of CO₂, and percentage of inoculum to represent a single-treatment system.
The operation was interrupted when the concentration of phaeophytin exceeded the concentration of chlorophyll-\(a\) for two consecutive days. In the next operation, the culture medium was replaced, and new inoculum was added.

The incident photosynthetically active radiation (PAR, 400–700 nm) was measured at 12:00 pm by means of a radiometer (LI-COR – LI-193 Underwater Spherical Quantum Sensor). The dissolved oxygen (DO) was measured three times per day at 6:30 am, 12:00 pm, and 5:30 pm using a probe (Hach, model HQ40d, luminescent dissolved oxygen).

The culture medium was collected in composite form daily at 5:30 pm. For this purpose, simple samples of 300 mL collected from each PBR tube were mixed, forming a composite sample for analyses. The physical and chemical analyses essentially followed the provisions of Standard Methods for the Examination of Water and Wastewater (APHA 2012) and the methods used for analysis of each variable are between parentheses: total alkalinity (Alk) (2330–Alk B), chemical oxygen demand (COD) (5220–COD D) and filtered chemical oxygen demand (CODf) (5220–CODf D – sample filtered at 0.45 \(\mu\)m), total suspended solids (TSS) (2540–TSS D) and volatile suspended solids (VSS) (2540–VSS E), nitrate (N-NO\(_3\)) (4500–N-NO3 E), (N-NO\(_2\)) nitrite (4500–NO2 B), ammoniacal nitrogen (N-NH\(_4^+\)) (4500–NH3 C), total Kjeldahl nitrogen (N-TKN) (4500–Norg C), total phosphorus (Pt) (4500–FT D), and soluble phosphorus (P\(_s\)) (4500–FS D – sample filtered at 0.45 \(\mu\)m). The N-Norg was determined by the difference between the N-TKN and the N-NH\(_4^+\). The forms of total

### Table 1 | Inoculum characteristics

<table>
<thead>
<tr>
<th>Sample</th>
<th>Genus/species</th>
<th>Total density (individuals/mL)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPR 1 and 2</td>
<td><em>Scenedesmus</em> sp. (<em>Scenedesmus accuminatus</em> and <em>Scenedesmus arvensis</em>)</td>
<td>3.5 (\times) 10(^6)</td>
<td>High density of yeast and bacteria</td>
</tr>
<tr>
<td>OPR 3</td>
<td><em>Scenedesmus</em> sp.</td>
<td>1.2 (\times) 10(^6)</td>
<td>Presence of yeasts and bacteria</td>
</tr>
<tr>
<td>OPR 4</td>
<td></td>
<td>1.9 (\times) 10(^6)</td>
<td></td>
</tr>
<tr>
<td>OPR 5</td>
<td></td>
<td>6.6 (\times) 10(^5)</td>
<td></td>
</tr>
<tr>
<td>OPR 6</td>
<td><em>Scenedesmus</em> sp. (free cells)</td>
<td>1.6 (\times) 10(^6)</td>
<td>High density of bacteria</td>
</tr>
</tbody>
</table>
dissolved carbon (TDC) and dissolved organic carbon (DOC) were determined by TOC 5000 Shimadzu equipment.

The variable VSS was used as an indirect measurement of the concentration of total biomass at the end of the operations because, as it is not an axenic culture, other microorganisms develop in consortium with the microalgae. The development of microalgae was accompanied by monitoring chlorophyll-a and phaeophytin according to Nush (1980) which was modified according to NEN 6520 (1981).

**Phytoplankton community**

Samples (200 mL) of the inocula and the culture medium at the end of each operation were collected to characterize the phytoplankton community, which was performed at the genus level, and for the dominant genus, the species present were identified.

**Qualitative analysis**

The samples were preserved with a 4% formalin solution. The identification was conducted on an inverted microscope (Olympus CK2) by the method of Uthermöhl (1958). The morphological and morphometric characteristics of the vegetative and reproductive life of the taxonomic value for the species were analyzed according to the specialized literature.

**Quantitative analysis**

Due to the high density of organisms in the ponds, a 1:50 dilution of the samples was performed before quantification. The counting of the organisms was performed in sedimentation chambers with a volume of 2 mL, as described in Uthermöhl (1958), with an inverted microscope OLYMPUS CK2. The total density of the phytoplankton (individuals/mL) was calculated according to Equation (1):

$$D = \frac{C_{org} \times A_t}{A_f \times F \times V_s}$$

where: D: total density (individuals/mL); C_{org}: number of counted organisms; A_t: total area of the bottom of the sedimentation chamber (mm²); A_f: area of the counting field (mm²); F: number of counted fields; and V_s: volume of the sedimented sample (mL).

**Lipid quantification**

The lipid content was evaluated at the end of each batch by solvent extraction, as described by Assemany et al. (2014).

The lipid productivity was obtained by the product of the total biomass productivity (mg VSS/L·d and/or g VSS/m²·d) by the lipid content obtained in each operation.

**RESULTS AND DISCUSSION**

**Characterization of the culture medium**

Table 2 shows the physical and chemical characterization of the effluents used as culture media.

There was a wide variability in the physical and chemical composition registered in the operations of each effluent, which is typically observed in effluents from multiproduct factories due to the variations in the production system. Still, characteristics such as the concentrations of nitrogen and phosphorus were different in relation to the origin of the effluents and assessable for microalgal cultivation.

**Table 2 | Characterization of the effluents used as culture media**

<table>
<thead>
<tr>
<th>Characterization of the effluents used as culture media</th>
<th>PE</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.2</td>
<td>5.6</td>
</tr>
<tr>
<td>Alk (mg CaCO₃/L)</td>
<td>207.1</td>
<td>152.3</td>
</tr>
<tr>
<td>DO (mg O₂/L)</td>
<td>3.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>29.0</td>
<td>29.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>29.0</td>
<td>29.0</td>
</tr>
<tr>
<td>COD total (mg/L)</td>
<td>346.0</td>
<td>347.1</td>
</tr>
<tr>
<td>COD filtered (mg/L)</td>
<td>345.4</td>
<td>342.2</td>
</tr>
<tr>
<td>N-NH₄⁺ (mg/L)</td>
<td>22.8</td>
<td>23.1</td>
</tr>
<tr>
<td>N-Norg (mg/L)</td>
<td>83.7</td>
<td>73.5</td>
</tr>
<tr>
<td>N-NO₃⁻ (mg/L)</td>
<td>6.1</td>
<td>2.4</td>
</tr>
<tr>
<td>Pt (mg/L)</td>
<td>8.8</td>
<td>2.5</td>
</tr>
<tr>
<td>P₈ (mg/L)</td>
<td>6.0</td>
<td>1.6</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>588.3</td>
<td>381.3</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>560.3</td>
<td>333.8</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>236.6</td>
<td>125.8</td>
</tr>
<tr>
<td>TDC (mg/L)</td>
<td>245.3</td>
<td>132.1</td>
</tr>
<tr>
<td>N/P*</td>
<td>4.8</td>
<td>16.1</td>
</tr>
</tbody>
</table>

*N/P: nitrogen was considered in the forms of N-NH₄⁺, N-NO₃⁻, N-NO₂⁻, and phosphorus in the form of P₈ in the medium.

UD – undetected values.
As expected, due to the higher depuration provided by the activated sludge system, there was a lower concentration of organic material, phosphorus, and suspended solids in the SE in relation to the PE. The soluble fraction of organic matter was represented by the concentration of DOC and filtered COD. It was observed that the DOC fraction in the TDC was lower in the SE (35–46%), vs. 78–99% in the PE) due to the degradation of the organic matter in the treatment of the activated sludge. The high concentration of organic carbon is particularly important in the absence of light. Genera such as \textit{Chlorella} sp. and \textit{Scenedesmus} sp., which are recognized as preferentially photoautotrophic, can survive in heterotrophic conditions using some forms of organic carbon in the absence of light. This fact can result from the microorganism’s respiration and the absence of photosynthesis during the night.

From the pH behavior, it is possible to note the relationship between the carbon addition cycles and the photosynthetic activity, which is illustrated by the difference between the higher frequency of gas addition during the day compared with that during the night. This behavior was also observed by Arbib \textit{et al.} (2013) and Valdés \textit{et al.} (2012) who operated a tubular airlift and a bubble column PBR, respectively. These authors stated that during the day, microalgae use light and nutrients to assimilate CO$_2$, which causes the pH of the medium to increase. In a period of intense solar radiation, the balance of photosynthesis in relation to respiration is great and the withdrawal of carbon dioxide causes a rise of pH. During the night, the photosynthetic activity is null, and due to microorganism’s respiration, CO$_2$ is released. However, the pH still increases slightly during the night. According to Valdés \textit{et al.} (2012), this fact is attributed to the release of CO$_2$ from the liquid medium to the air, which is promoted by the continuous injection of compressed air into the reactor.

During the initial culture period, the pH of the PE took a longer time to reach 8 (t $>$ 50 hours) in contrast with the SE, which took a maximum of 4 minutes. The slow increase in the pH represents an adaptation period of the inoculum to the cultivation conditions. This fact can be associated with factors such as the lower initial value of pH (5.8 ± 0.5) and the higher concentration of VSS and organic matter (Table 2) that were observed in the PE in relation to the SE. Initially, because there was a higher concentration of other microorganisms in the PE, the respiration process may have been more intense than photosynthesis during the first 50 hours. Moreover, due to high availability of
organic matter, CO₂ from heterotrophic bacteria respiration may have been enough to allow algae growth, resulting in a slow increase in pH. Similarly, early in the mornings, the pH of the PE also took a longer time to reach 8, which is also attributed to the release of CO₂ as a result from respiration and organic matter degradation. This fact highlights the symbiotic relationship that is established between algae and heterotrophic microorganisms in the PE.

Characterization of the biomass and phytoplankton community

It is important to note that both the inocula and the post-PBR effluents were not fully known cultures. Identifying the genera and, primarily, determining their counts were made difficult due to the high concentration of microorganisms, such as yeasts and bacteria, present in the samples.
The genus *Scenedesmus* sp. was dominant in the phytoplankton community with densities of $6.6 \times 10^5$ to $5.5 \times 10^6$ individuals/mL (inoculum) and $2.1 \times 10^6$ to $3.7 \times 10^6$ individuals/mL (final effluents, without considerable differences regarding the origin of the culture medium). Table 3 shows the characteristics of the phytoplankton community.

The dominance of the genus *Scenedesmus* (mixotrophic) in all the operations showed the ability of that genus to survive in extreme environments. As expected, in conditions of restricted availability of nutrients and consisting of DOC and $P_S$, which occurred in OPR 5 and 6, there was a limited growth of heterotrophic organisms, such as bacteria and yeasts. However, when the objective is to exploit the energy of the total biomass, characteristics of the effluents that favor the presence of other microorganisms, apart from microalgae, can be advantageous in certain cases. Cheirsilp et al. (2011) evaluated the lipid productivity of mixed cultures of yeasts (*Rhodotorula glutinis*) and microalgae (*Chlorella vulgaris*) in industrial effluents from the processing of seafood and sugarcane molasses. These authors reported that lipid productivity was higher in the mixed culture than in pure cultures, which was possibly due to the symbiotic nutritional circuit that is established between algae and yeast – similar to that between algae and bacteria.

The concentrations of VSS and chlorophyll-$a$ recorded during the microalgal cultivation in the evaluated effluents are shown in Figures 3 and 4, respectively. Wang et al. (2010) evaluated the cultivation of *Chlorella* sp. in raw, primary, and secondary municipal effluents as well as concentrated effluent after centrifugation. Although studies such as that by Martin et al. (1985) have suggested that the optimum N/P ratio for the growth of algae in fresh water is 10/1, the concentrate evaluated by Wang et al. (2010) (N/P of 0.56) showed best performance, which was assessed in terms of specific growth rate. According to the authors, despite the low N/P of the concentrate, the growth of microalgae was higher in this effluent due to the higher concentration of nitrogen, phosphorus, and DOC (131.5 mg/L of NT, 201.5 mg/L of Pt, and 2250.0 mg/L of DOC) in relation to the other effluents. This may explain the higher growth of biomass in the PE that was evaluated in the present study. The highest concentration of VSS (1,160.0 mg VSS/L, after 101 hours of operation) and the highest concentration of chlorophyll-$a$ (10.9 mg chlorophyll $a$/L, after 53 hours of operation) were observed in the cultivation in PE (OPR 2). The cultivation of biomass in SE reached concentrations of 225 to 371 mg VSS/L in OPR 6 and 4, respectively, and from 2.3 to 3.5 mg/L of chlorophyll-$a$ in OPR 4 and 5, respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Genus/species</th>
<th>Total density (individuals/mL)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPR 1</td>
<td><em>Scenedesmus</em> sp.</td>
<td>–</td>
<td>High density of yeast and bacteria</td>
</tr>
<tr>
<td>OPR 2</td>
<td><em>Scenedesmus</em> sp.</td>
<td>–</td>
<td>High density of yeast and bacteria</td>
</tr>
<tr>
<td>OPR 3</td>
<td><em>Scenedesmus</em> sp.</td>
<td>$2.1 \times 10^6$</td>
<td>Presence of yeasts and bacteria</td>
</tr>
<tr>
<td>OPR 4</td>
<td><em>Scenedesmus</em> sp.</td>
<td>–</td>
<td>High density of yeast and bacteria</td>
</tr>
<tr>
<td>OPR 5</td>
<td><em>Scenedesmus Staurastrum</em></td>
<td>$2.3 \times 10^6$</td>
<td>Presence of bacteria</td>
</tr>
<tr>
<td>OPR 6</td>
<td><em>Scenedesmus Staurastrum</em></td>
<td>$3.7 \times 10^6$</td>
<td>Absence of bacteria</td>
</tr>
</tbody>
</table>

Figure 3 | Concentrations of VSS recorded during microalgal cultivation in the evaluated effluents (a) PE and (b) SE.
When the objective is the utilization of biomass, lower performances resulting from cultivation in secondary or very diluted effluents are frequently reported in the literature. Wang et al. (2010) observed that the lower production of microalgae in the SE was attributed primarily to the recalcitrant nature of the organic matter. Xin et al. (2010), using autoclaved secondary municipal effluent as the culture medium (0.5 mg/L of Pt, similar to the concentration observed in the present study for the SE, of 1.6 ± 0.31 mg/L), observed a production of 0.11 g Scenedesmus sp. LX1 per liter. These authors attributed the limited production of algal biomass to the reduced concentration of nutrients, primarily phosphorus, in the culture medium. In the present study, the lower performance of SE in terms of algae biomass production is related both to the recalcitrant nature of the existing organic matter and the lower concentration of phosphorus.

### Removal of pollutants

#### Organic matter

Table 4 shows the removal of organic matter by the cultivation of biomass in the evaluated effluents.

A lower removal of filtered COD was expected in the SE due to the higher recalcitrance of the organic matter. However, there was a removal of 50% (OPR 6) – similar to the PE (OPR 1 and 2) – a removal of 10% (OPR 5), and an increase of 36.3% (OPR 4) of filtered COD. These results reflect the wide variation in the effluent composition. The increase of CODs in OPR 4 can be related to the release of organic compounds by the microalgae. According to Wang et al. (2014), a high nutrient load and highly unbalanced N/P ratio may cause unfavorable conditions for algae, which can lead to the production of a large amount of exocellular protein. OPR 4 was the operation in which the PBR worked with the higher concentration of ammoniacal nitrogen, and consequently, a higher N/P ratio (Table 2).

During all of the operations, TDC was removed. However, the DOC behavior was different between the effluents. While a fast removal of DOC was observed at the start of cultivation in the PE, there was an increase in DOC during the cultivation in the SE. This behavior can be related to the recalcitrance of the existing organic matter in the SE.

#### Nitrogen and phosphorus

Figure 5 shows the concentrations of N-NH₄⁺ and Pᵢ recorded during the cultivation in the primary and SE.

The removal of N-NH₄⁺ in all the operations was higher than those obtained by Wang et al. (2010) through the cultivation of Chlorella sp. in raw municipal effluent (82.4%), primary municipal effluent (74.7%), and concentrated municipal effluent after centrifugation (78.3%). The required
time for the maximum removal of N-NH₄⁺ was similar only between OPR 2 and 3 (50 hours) and OPR 5 and 6 (75.5 hours). As also reported by Ruiz-Martínez et al. (2012), the pH control allowed the assumption that much of the removal of N-NH₄⁺ is due to its assimilation by the biomass because values that would favor volatilization were not achieved.

After achieving complete removal of N-NH₄⁺, N-NO₃⁻/C₀ was expected to be the preferably consumed form of nitrogen. However, there was a wide variation in the concentration of N-NO₃⁻ during all operations, which even occurred in increments of this component in the final effluent (OPR 2, 5, and 6). Among the effluents, higher removal of N-NO₃⁻ was observed in OPR 1 (63.9%) and OPR 4 (66.7%). The increases in N-NO₃⁻ were possible due to the characteristics of the culture medium, such as the presence of N-NH₄⁺ and DO. Evaluating the consortium cultivation of mixotrophic algae in the primary municipal effluent that had been previously sterilized and filtered, Mahapatra et al. (2014) attributed the increase in the nitrate concentration (from 1.07 ± 0.15 to 1.54 ± 0.28 mg/L) to nitrification, a two-stage process performed by nitrifying bacteria – autotrophic organisms that are extremely common in mixotrophic cultivation systems. Furthermore, the permanence of N-NO₃⁻ in the culture medium can be associated with the absence of another nutrient, such as phosphorus, which can limit the biomass growth and subsequently the assimilation of this nutrient.

With the PE, a significant presence of N-NO₂⁻ was not identified, whereas the reduced concentration of N-NO₂⁻ in the SE was completely removed in the first 75 hours (OPR 5 and 6) and in the first 120 hours (OPR 4). The removal of N-NO₂⁻ recorded during the cultivation in SE is possibly associated with the occurrence of nitrification because the presence of oxidizing bacteria of N-NH₄⁺ and N-NO₂⁻ occurs frequently in activated sludge systems.

As shown in Figure 5(b), different from the PE, the behavior of the SE regarding the removal of Pₛ was similar between OPR 4, 5, and 6, which resulted in complete removal in up to 75 hours. A similar performance was obtained by Ruiz-Martínez et al. (2012), who identified removal of 97.8% of P-PO₄ through the treatment of secondary domestic effluent by the cultivation of microalgae and cyanobacteria. Both in the PE and in the SE, the Pₛ removal was attributed to the assimilation by the biomass because the pH was controlled between 6 and 8.

With the exception of OPR 3, which showed a high variation in the Pₛ concentration in the medium, alternating between increases and complete removal of this nutrient, all of the other PE operations showed high removal of Pₛ, i.e., 95% (OPR 1, after 192 hours) and 100% (OPR 2, after 53 hours), which are higher than what was reported by...
Wang et al. (2010) in the cultivation of *Chlorella* sp. in PE (91%, in terms of P-PO$_4$).

Similarly to OPR 3, fluctuations of P-PO$_4$ were reported by Kumar et al. (2010) in all the feeding frequencies that were evaluated for the cultivation of *Chlorella* sp. in pig-farming effluent. The authors stated that the alterations of P-PO$_4$ in the culture medium can be associated with various factors, such as the concentration of organic particles and sediments. In certain cases, the removal of P-PO$_4$ can be the result of its adsorption by the sediments and organic particles that exist in the culture medium. When saturation of the sediments is reached, the phosphorus content is released, which increases its concentration in the medium.

According to Wang et al. (2010), a limitation that hinders the spread of effluent treatment technologies based on algae is their long hydraulic retention time (HRT) in relation to conventional treatment systems, such as activated sludge. However, the full removal of N-NH$_4$ and P$_5$ in the first 53 hours of OPR 2 allowed the recovery of nutrients and a high production of biomass with a wide range of application, which again indicates the potential use of the system at the secondary level of treatment.

**Quantification of the accumulated lipid and lipid productivity**

Table 5 shows the results of the total lipid content, total biomass productivity, and lipid productivity that were determined for each operation.

Both the evaluated effluents had a lipid content of less than 10%. Therefore, the highest lipid productivity observed in the PE, i.e., 3.7 g/m$^2$·d (considering PBR surface area) or 15.6 mg/L·day (considering its volume), was attributed to its higher biomass productivity in relation to the SE due to its nutritional condition, which is more favorable for the growth of biomass.

In this study, the nutritional condition influenced only the production of biomass, showing no difference in the accumulation of lipids by the algal cell, which is primarily due to the abundant availability of (ammoniacal) nitrogen in both effluents. This fact may explain the low lipid content of the cultivated biomass, which did not need to store fat at any moment but needed to grow. Although, OPR1 and OPR3 were exceptions, with a low N/P ratio and indicating nitrogen limitation, lipid content for these operations was also low. The biomass cultivation during a longer period of time could result in a larger accumulation of lipids because the culture would be subjected to longer periods of nutritional restriction. However, under strict nutrient conditions, despite the larger accumulation of lipids, a loss of algal biomass can occur.

**Table 5** Total lipid content (% in the dry biomass), total biomass productivity (g/m$^2$·d), and lipid productivity (g/m$^2$·d) for each evaluated effluent

<table>
<thead>
<tr>
<th>Unit</th>
<th>PE</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPR 1</td>
<td>OPR 2</td>
</tr>
<tr>
<td>Total lipids</td>
<td>%</td>
<td>7.2</td>
</tr>
<tr>
<td>Biomass productivity$^a$</td>
<td>g/m$^2$·d</td>
<td>32.2</td>
</tr>
<tr>
<td>Lipid productivity$^a$</td>
<td>g/m$^2$·d</td>
<td>2.3</td>
</tr>
</tbody>
</table>

$^a$Calculations based on the PBR surface area.

Even with low lipid content, we can estimate a biodiesel productivity of 12,333.33 L/ha-year. This performance is similar to that observed by Woertz et al. (2009) with 9,000 L/ha-year, which is 25 times greater than the productivity of soybean oil (490 L/ha-year) (USDA 2005). However, it must be highlighted that biodiesel from microalgae, up to date, is economically unfeasible, due to the overall cost of downstream processing.

Therefore, the accumulated energy in the biomass could alternatively be used in other energy routes. According to Sialve et al. (2009), a more energetically favorable use of the biomass with a lipid content of less than 40% is anaerobic digestion. For these authors, the anaerobic digestion of this substrate for methane generation, considering the control of the several parameters involved, can generate as much energy as that obtained with the use of lipids. Moreover, the digestate after anaerobic digestion can be reused for microalgae culture medium as it still contains considerable amounts of nutrients. Besides energetic purposes, microalgae biomass can be used as an organic fertilizer in the agriculture. In the present study, the complete phosphorus and nitrogen assimilation into the biomass has to be outlined and microalgae cultivation can be pointed out as a system for nutrient recovery from water and effluents and use as fertilizer.
CONCLUSIONS

The results demonstrate the potential of cultivating microalgae in agro-industrial effluents previously treated at the primary and secondary levels. Distinct nutritional conditions differently affected the production of biomass, which reached the highest value in the PE, mainly due to its higher availability of phosphorus, nitrogen and organic matter. Complete removal of ammoniacal nitrogen and soluble phosphorus were observed in both effluents. The nutritional condition, although crucial for the growth of biomass, did not influence lipid accumulation. Moreover, it is important to highlight the potential of the proposed microalgae system to recover valuable products from wastewater, whether lipids or nutrients, which can be valued for several purposes.

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