Energy recovery in high rate algal pond used for domestic wastewater treatment
Paula Peixoto Assemany, Maria Lúcia Calijuri, Eduardo de Aguiar do Couto, Fernanda Pereira da Silva and Mauro Henrique Batalha de Souza

ABSTRACT
High rate algal pond (HRAP) was evaluated according to its energy potential and productivity by two rates, net energy ratio (NER) and specific biomass productivity. All energy inputs were calculated according to one HRAP with pre-ultraviolet disinfection treating anaerobic domestic sewage. The outputs were calculated for two energetic pathways: lipid and biogas production for the raw biomass (RB) and biomass after lipid extraction. The non-polar lipid content in dry biomass was 7.6%, reaching a daily lipid productivity of 0.2 g/m²·day and the biogas production potential was 0.20 m³/kg solids. For the biomass after lipid extraction, the biogas production reached 2.6 m³/kg solids. NER values of 10°C03 for the RB were similar for lipids and biogas routes. The specific biomass productivity was 0.7 mg/kJ. For the residual biomass, after lipid extraction, NER value was 10°C2 for the integrated route (lipids + biogas) and the specific biomass productivity of the extracted biomass was 0.4 mg/kJ. The best energetic pathway was to integrate both lipids and biogas route.

Key words | bioenergy, biogas, high rate algal pond, lipids, microalgae

INTRODUCTION
The search for renewable energy sources is a world reality across the depletion of fossil fuels, and also due to the environmental impacts caused by them. Among renewable energy sources like sun, wind and hydroelectric, biomass is a promising source. Energy from biomass is regarded as one of the most important future renewable energy sources. It can provide a continuous power generation, compared with solar and wind energies, and it plays an important role in the current CO₂ mitigation policy (Appels et al. 2011). Non-food alternatives and alternatives more efficient than vegetable crops used as biomass for biofuels production have been studied, and among them, microalgae come with a great potential.

As a way to make microalgae production process sustainable from both economic and environmental aspects, the use of effluents and exploration of the concept of biorefinery are viable alternatives. Within this context, the high rate algal ponds (HRAPs) are one of the main alternatives for wastewater treatment and algae biomass production. Despite the greater area requirement and lower productivity yields compared with closed reactors, they have low installation costs and energy consumption, which reduces operating costs (de Godos et al. 2010).

Even though there is a lot of research dealing with microalgae bacteria systems for wastewater bioremediation, the investigation devoted to the use of this generated biomass is scarce (González-Fernández et al. 2015). Nowadays, the most targeted energy use for algal biomass is the production of biodiesel, due to large capacity of these microorganisms to accumulate lipids. However, microalgae with low lipid content are common in effluent cultivation (Mehrabadi et al. 2015). Faced with effluent cultivation and low lipid accumulation, anaerobic digestion of algae biomass could be a potentially attractive alternative for energetic purposes, besides the advantage of using humid biomass, minimizing costs of harvesting and concentration of this biomass. Biogas can be used as a primary source of energy in a great part of the algal biomass production process, thus reducing the costs of biodiesel production and other products with high aggregate value (Chisti 2007). More than that, Sialve et al. (2009) estimated that the anaerobic digestion of microalgae biomass after
lipid extraction for methane generation can generate as much energy as that obtained with the extraction of lipids.

Collet et al. (2011) have concluded that the combination of lipid extraction with biogas production would be optimum from both environmental and economic points of view. In contrast, Quinn et al. (2014) illustrated the importance of considering the effects of the removal of lipids from microalgae for anaerobic digestion performance in a life cycle analysis of microalgae biofuels, since these components are energy rich and extracted biomass (EB) would provide lower methane yield than raw biomass (RB). If, on one hand, the previous lipid extraction can be considered as pre-treatment, increasing the bioavailability of microalgae intracellular content; on the other hand, such process includes dehydration, destruction of the cell wall and use of solvents, steps that influence the quality of the biomass for digestion.

Therefore, in this study, the potential for biogas production and the anaerobic biodegradability of the biomass, before and after lipid extraction, were assessed. The main objective was to apply energy analysis in an integrated context of biorefinery, in order to define the best use of biomass cultivated in an HRAP using domestic sewage as culture medium.

**MATERIAL AND METHODS**

**Biomass production unit**

The experiment was carried out outdoors in the city of Viçosa, Minas Gerais, Brazil (20°45′14″ S and 42°52′54″ W). Viçosa is located at approximately 648 m above sea level; the annual average precipitation is 1,221 mm and the annual average temperature ranges between 19°C and 20°C. The average relative humidity is 81%. The local climate is characterized as tropical altitude, with hot and rainy summers, and cold and dry winters.

The experimental HRAP was operated with domestic sewage pre-treated by a full-scale upflow anaerobic sludge blanket (UASB) reactor and pre-disinfected by ultra-violet (UV) disinfection. The HRAP was operated in batch mode (four batch operations: July and September 2014; and February and March 2015), until the decay phase of algal growth was reached, measured every day by the variable chlorophyll-α (according to NEN 6520; 1981; APHA 2005). The duration of batch operations was on average 8 days. Chemical and physical composition of HRAP input and output is presented in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Input</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>–</td>
<td>7.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>25.5</td>
<td>25.9</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>mg L⁻¹</td>
<td>0.3</td>
<td>11.9</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>mg L⁻¹</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>Volatile suspended solids</td>
<td>mg L⁻¹</td>
<td>56</td>
<td>62</td>
</tr>
<tr>
<td>Ammoniacal nitrogen</td>
<td>mg L⁻¹</td>
<td>50.3</td>
<td>29.6</td>
</tr>
<tr>
<td>Nitrate</td>
<td>mg L⁻¹</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>TKN</td>
<td>mg L⁻¹</td>
<td>55.8</td>
<td>37.5</td>
</tr>
<tr>
<td>Dissolved phosphorus</td>
<td>mg L⁻¹</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>mg L⁻¹</td>
<td>142.0</td>
<td>67.9</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>mg L⁻¹</td>
<td>32.8</td>
<td>9.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>MPN</td>
<td>5.81 × 10⁵</td>
<td>1.78 × 10³</td>
</tr>
</tbody>
</table>

The HRAP had the following characteristics: width = 1.28 m, length = 2.86 m, total depth = 0.5 m, culture depth = 0.4 m, surface area = 3.3 m², culture volume = 1.32 m³. The disinfection system was designed to achieve a final concentration of 10⁵ MPN (100 mL)⁻¹ of *Escherichia coli*, with an adopted effective dose of 21 mJ/cm² and absorbance of 42%. The disinfection phase had the objective of removal microalgae predator’s organisms and competitors for nutrients, helping the algae biomass growth, as demonstrated by Santiago et al. (2013).

**Biomass characterization**

After production, the biomass was separated and concentrated by sedimentation for posterior energy analysis. A proportion of the biomass was subjected to the processes of lipid extraction and anaerobic digestion, whereas another part of the biomass was submitted to anaerobic digestion only. The biomass obtained after lipid extraction and submitted to anaerobic tests was named EB. Similarly, the biomass sent directly for anaerobic digestion was named RB.

Both biomasses, RB and EB, were characterized in terms of chemical oxygen demand (COD), total solids (TS), total volatile solids (TVS), water content, pH, total Kjeldahl nitrogen (TKN) and total phosphorus (TP), according to APHA (2005). All analysis were done in duplicate. The phytoplankton community was also assessed at the end of each batch operation. For qualitative analysis, the samples were preserved with formaldehyde (4%). The identification and cell counting was performed with an inverted optical...
microscope (Olympus CK2). The morphologic and morphometric characteristics of the vegetative and reproductive cycles of significant taxonomic value for the species were analyzed according to specialized literature (Bourrelly 1970; Parra et al. 1982). The cells were counted by using 2 mL sedimentation chambers.

**Lipids**

The lipid content was assessed by solvent extraction, as described by Assemany et al. (2015). The biomass was oven dried at 50 °C for 12 h and the cells disrupted with hydrochloric acid (HCl) 5 M. For 0.2 g of dry biomass, 5 mL of HCl were added under stirring during 10 min. Then, 25 mL of methanol and 5 mL of petroleum ether were added under heat at 30 °C during 15 min. After disruption, petroleum ether was added to the dry biomass, totalizing three cycles of extraction (10 mL in the first and third cycles, 20 mL in the second cycle). The non-polar lipid content (% of lipids in the dry biomass) was determined gravimetrically after the evaporation of the solvent for 2 h at 105 °C.

**Biogas**

The Biogas Production Potential (BPP) tests followed the procedures described by Jawed & Tare (1999), with modifications described by Viana et al. (2012). Tests were carried out using 250 mL Erlenmeyer’s reagent (200 mL of useful volume), filled with sludge from an UASB reactor (12.5 mL) as anaerobic inoculum, substrate (RB and EB) (100 mL), buffer solution (1 g of sodium bicarbonate) and distilled water (67.5 mL), with a food to microorganism (F/M) ratio of eight to one (v/v). The best F/M was previously chosen after vary specific methanogenic activity (SMA) tests, during which others’ ratios were tested (four and six) and eight achieved the best performance. Micro- and macronutrients were added (20 mL) to prevent deficiency during the tests, according Viana et al. (2012). The test was also performed on the control without the addition of substrate to measure the endogenous respiration of the microorganisms. After closure of the bottles, N2 was injected for 4 min to purge the oxygen. The tests were performed at 35 ± 2 °C under continuous agitation (120 rpm) in an incubator (TECNAL, TE-420). The volume of biogas was monitored daily in a Mariotte bottle filled with 25 g NaCl/L (pH = 2) solution for biogas measurement. The calculation of the BPP was based on the cumulative production of biogas after 50 days of incubation, and on the mass of substrate used in the tests. Anaerobic biodegradability was also assessed, converting the biogas production to organic load, considering that at 35 °C, 0.395 L of methane correspond to 1 g of COD and that the biogas is composed of 62.5% of methane (mean value of the interval reported by European Commission 2005).

**Energy analysis**

The energy performance of the production system was evaluated in terms of specific productivity of biomass, \( P_B/E_C \) (mg/kJ), defined as the productivity of biomass (\( P_B \), mg/L·d) per input energy (\( E_C \), kJ/L·d) (Pegallapati et al. 2014) and the net energy ratio (NER), which relates the total energy produced and that consumed by the system. Equation (1) presents NER calculation:

\[
NER = \frac{\sum \text{Energy produced}}{\sum \text{Energy consumed}} \tag{1}
\]

The produced energy can be with respect to its lipid and/or BPP. The energy consumption for the agitation of the culture medium by the paddlewheels was considered, and also for the UV lamps in the disinfection unit.

**Quantification of the energy consumption**

The energy consumption of each operation (COP, kWh/day) was determined with Equation (2):

\[
C_{OP} = \frac{24 \times P_0 t}{1000} \tag{2}
\]

where Pot is the power in W. The annual energy consumption (\( C_T \), kJ/year) can be determined by Equation (3):

\[
C_T = C_{OP} \times \text{days of operation in the year} \times 3600 \tag{3}
\]

Considering the continuous operation of the HRAP during the year, Equation (3) can be substituted by Equation (4):

\[
C_T = C_{OP} \times 1, 314, 000 \tag{4}
\]

**Quantification of the energy produced from lipids and biogas production**

The annual energy production from the anaerobic digestion of the biomass (\( E_G \), kJ/year) and lipids (\( E_L \), kJ/year) was...
determined from the annual lipid and biogas productions, considering that: (1) the energy content of 1 L of lipids is equivalent to 35,133.33 kJ (Jorquera et al. 2010), (2) lipid density is approximately 0.9 kg/L (Jorquera et al. 2010), (3) 1 m³ of biogas equals 23,400 kJ (mean value of the interval reported by Chisti 2007), and (4) the duration of each operation as being equal to 8 days, according to Equations (5) and (6):

$$E_L = \frac{P_{L,\text{annual}}}{0.9} \times 35,133.33$$  \hspace{1cm} (5)

where $P_{L,\text{annual}}$ is the annual lipid production (kg/year) and

$$E_G = P_{G,\text{annual}} \times 23400$$  \hspace{1cm} (6)

where $P_{G,\text{annual}}$ is the annual biogas production (m³/year).

RESULTS AND DISCUSSION

Biomass characterization

The phytoplanktonic community was dominated by Chlorophyceae during all the batch operations. The algae Chlorella vulgaris was the predominant specie, with an average of $9.4 \times 10^5$ individuals/mL, followed by the gender Scenedesmus sp. with $6.5 \times 10^5$ individuals/mL.

Table 2 presents the physical and chemical characterization of the biomass used in the energetic tests.

The pH of RB presented a value close to neutrality; on the other hand, EB had an acid pH. The average value below 2, can be explained by the addition of hydrochloric acid for cell wall disruption during lipid extraction. COD concentration of EB was two times higher than RB. This may be related to the use of solvents, such as methanol and petroleum ether, in the extraction procedure, with the possibility of incorporation of the carbon of such reagents into the biomass. For phosphorus and nitrogen (NTK), we highlighted lower concentrations in EB compared to RB, caused by the extraction of phosphorus elements and complex lipids, such as phospholipids, that have besides phosphorus, nitrogen on its composition. Specifically, for nitrogen reduction in EB, membrane proteins could also be extracted due to cell wall disruption.

We highlight greater concentration of solids and lower value of water content in RB, due to the dilution of EB. Specifically, for EB the high water content, 99%, was due to the addition of water after the separation of the solvents and hydrochloric acid. The separation, carried out by sedimentation of the biomass, was necessary since the reagents used in the extraction are extremely toxic to the anaerobic microorganisms, potentially inhibiting anaerobic digestion. This inhibition was confirmed in BPP preliminary tests, during which no methanogenic activity was detected in the bottles with biomass, additional to the reagents after extraction.

Moreover, the high content of ash in both biomasses (45 and 45% of TS) should be pointed out as an intrinsic characteristic of the culture medium used to grow the biomass. In this study, the domestic sewage was used in the HRAP after a prior treatment in an UASB reactor. However, the UASB reactor is not properly operated, since anaerobic sludge is discarded with no time control, consequently with a large proportion of material already stabilized (inorganic) to the detriment of volatile material.

Energetic output

Lipids

The biomass presented a non-polar lipid content of $7.6 \pm 0.6\%$, reaching an average lipid productivity of $0.2 \text{ g/m}^2\cdot\text{d}$. The cultivation in effluents under less appropriate conditions, and in competition with other microorganisms, may have been the cause of the low lipid accumulation. According to Mehrabadi et al. (2013), the main reason for the low lipid content of biomass grown in HRAPs with wastewater is the composition of the biomass, since biomass contains a mixture of algae and bacteria. As the lipid content of bacteria is typically below 10% (Brown et al. 1996), this

<table>
<thead>
<tr>
<th>Biomass</th>
<th>pH</th>
<th>Water content (%)</th>
<th>TS (g/L)</th>
<th>Ash (%TS)</th>
<th>Volatile (%TS)</th>
<th>TKN (g/L)</th>
<th>N-NH₄ (g/L)</th>
<th>TP (g/L)</th>
<th>COD (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>7.5 (1)</td>
<td>98 (1)</td>
<td>21 (7)</td>
<td>45</td>
<td>55</td>
<td>3 (3)</td>
<td>ND</td>
<td>0.65 (0.1)</td>
<td>53 (27)</td>
</tr>
<tr>
<td>EB</td>
<td>&lt;2 (1)</td>
<td>99 (1)</td>
<td>7 (2)</td>
<td>45</td>
<td>57</td>
<td>0.1 (0.06)</td>
<td>–</td>
<td>0.3 (0.1)</td>
<td>100 (25)</td>
</tr>
</tbody>
</table>

ND: not detected; –: not performed.
reduces the overall lipid content and hence energy content of the biomass.

**Biogas**

Figure 1 presents the cumulative biogas production for the biomass before (RB) and after (EB) lipid extraction.

The incubation time of EB was over the 30 days. The objective was to maintain incubation until the stabilization of biogas production, which was observed next to the 70th day. Despite the high biogas production, reaching 2,114 mL at the end of incubation, during the first 10 days of incubation there was no production observed. The exponential phase of production was only observed after the 20th day of incubation of EB. This time lag was probably due to the adaptation of the anaerobic microorganisms to the substrate EB. A factor that may have contributed to this adaptation was that the pH in the EB was extremely acid (lower than 2), as discussed earlier (Table 2). Thus, an alternative to decrease the adaptation time would be the correction of the pH of the biomass prior to incubation, following a pH target of 6.8–7.2 or an adoption of a period of acclimation before the anaerobic digestion. A long lag period of more than 18 days was also observed by Yun et al. (2014) when digesting microalgal biomass after lipid extraction with a solvent concentration of 200 mL chloroform/L.

The results of the anaerobic biodegradability and BPP tests for the biomass, before and after lipid extraction, are presented in Table 3.

We can observe the low anaerobic biodegradability of the RB. The cellular wall is considered the main characteristic of the difficult biodegradability of algal biomass (Zamalloa et al. 2012). According to Golueke et al. (1957), microscopic analysis showed that a great amount of algal cells were intact, but not viable, after digestion, probably indicating that the cellular wall inhibited contact of the anaerobic microorganisms with substrate. The ultrastructure of the cellular wall of the genera found in this study, *Chlorella* and *Scenedesmus* (*Chlorococcales*), consists of two shells, the internal one consisting of microfibers of cellulose and amorphous matrix; and the external, which in its trilaminar shape can contain substances such as carotenoids and lignin (sporopollenin) (Weng & Chapple 2010). Therefore, components such as cellulose and lignin could be the cause of the low biodegradability indices observed for RB.

We highlight the high BPP value for EB, reaching 2.6 m³ biogas/kg TVS, a yield approximately 10 times higher than the results of the RB and those reported in the literature. Studies for pre-treated algal biomass also cultivated in HRAPs with wastewater presented results of: ~0.3 m³ biogas/kg TVS after 45 days of incubation and application of microwaves (Passos et al. 2013) and 0.155 m³ CH₄/kg TVS for pre-treatment of freezing and thawing and 0.136 m³ CH₄/kg TVS for thermal pre-treatment (Kinnunen et al. 2014).

This result reinforces the use of lipid extraction as a pre-treatment of algal biomass. The process of lipid release using solvents probably increased the bioavailability of the intracellular content of the microalgae, making the substrate...
more available for the anaerobic microorganisms to perform digestion. Also, it is possible to affirm that the best yield of the digestion using EB was due to the shortening of the hydrolysis phase, leaving the hydrolytic bacteria very little to do, since they received the substrate almost or completely hydrolyzed. As already mentioned by González-Fernández et al. (2015), when working with particulate substrates, as microalgae biomass, the hydrolysis step determines the successful production of methane. Otherwise, in easily degradable substrates, methanogenesis is considered the limiting phase.

Besides the pre-treatment effect, lipid extraction also increased by two times the COD content in the biomass (see Table 2), contributing for better biogas performance of EB, compared with RB. An external source of organic carbon from the solvents used in the extraction was incorporated into the EB, consequently increasing biogas production.

### Energetic Analysis

In order to operate paddlewheels and UV disinfection, the HRAP production system had an annual energy consumption of 5.4 GJ. Its biomass productivity of RB was 7.75 mg/L·d, with a volatile suspended solids (VSS) concentration of 62 mg/L after 8 days of batch operation (Table 4). Productivities reported for HRAP treating domestic sewage are usually greater than those obtained in this study (Assemany et al. 2015; Mehrabadi et al. 2015). Batch mode operation and the 40 cm of HRAP depth could be considered the main factors for low biomass yield. For the EB, the productivity was 62.7% of RB, discounting lipid content of 7.6% and 29.7% of losses in the lipid extraction process, totaling 4.86 mg/L·d. Due to the small scale of the extraction process, loss was potentiated, since part of the biomass was retained in the walls of the glass used for the process, and during reagents separation. However, such weaknesses can be minimized by increasing the scale of the process. The low values of the specific productivity of biomass (PB/EC) of both biomasses (Figure 2) are also a consequence of the low HRAP biomass productivity.

Annual energy outputs of the EB were 0.01 GJ for lipids and 0.02 GJ for biogas routes. The EB presented a biogas energy gain of 0.14 GJ/year, contributing in large proportion

### Table 3 | Anaerobic biodegradability, biogas production potential (BPP) tests and energetic output for the biomasses from the HRAP, before and after lipid extraction (n = 4 for RB and n = 2 for EB, mean and standard deviation values)

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Anaerobic biodegradability (%)</th>
<th>BPP (m³ biogas/kg TVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>10.9 (7.8)</td>
<td>0.20 (0.1)</td>
</tr>
<tr>
<td>EB</td>
<td>28.5 (15.4)</td>
<td>2.6 (0.5)</td>
</tr>
</tbody>
</table>

### Table 4 | Energy analysis data

#### Energy input

<table>
<thead>
<tr>
<th>Energy Consumption</th>
<th>Power (W)</th>
<th>KW</th>
<th>KWh/day</th>
<th>Daily energy consumption (KJ/day)</th>
<th>Daily energy consumption (GJ/day)</th>
<th>Annual energy consumption (GJ/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRAP</td>
<td>170</td>
<td>0.17</td>
<td>4.08</td>
<td>14,688</td>
<td>0.014688</td>
<td>5.36112</td>
</tr>
</tbody>
</table>

#### Biomass productivity

<table>
<thead>
<tr>
<th>HRAP VSS (mg/L)</th>
<th>Operation days</th>
<th>Volume (L)</th>
<th>Productivity (mg/L·d)</th>
<th>Productivity mg/day</th>
<th>Productivity kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>62</td>
<td>8</td>
<td>1320</td>
<td>7.75</td>
<td>10,230</td>
</tr>
<tr>
<td>EB</td>
<td>38.9</td>
<td>8</td>
<td>1320</td>
<td>4.86</td>
<td>6,419</td>
</tr>
</tbody>
</table>

#### Energy Output

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Content (%)</th>
<th>Lipid productivity (mg/day)</th>
<th>Daily energy yield (KJ/day)</th>
<th>Daily energy yield (GJ/day)</th>
<th>Annual energy yield (GJ/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.6</td>
<td>0.00077748</td>
<td>30.35</td>
<td>0.000030</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biogas</th>
<th>BPP (m³/kg SV)</th>
<th>Biogas productivity (m³/day)</th>
<th>Daily energy yield (KJ/day)</th>
<th>Daily energy yield (GJ/day)</th>
<th>Annual energy yield (GJ/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>0.2</td>
<td>0.002046</td>
<td>47.8764</td>
<td>0.000048</td>
<td>0.02</td>
</tr>
<tr>
<td>EB</td>
<td>2.6</td>
<td>0.016690</td>
<td>390.552</td>
<td>0.000391</td>
<td>0.14</td>
</tr>
</tbody>
</table>
to the energy output of the integrated (lipids + biogas) route of 0.15 GJ/year.

NER values were below one for all the energetic routes (Figure 2), indicating that energy gain was not high enough to compensate the energetic input. For RB, both lipids and biogas presented similar NER values of 10⁻³, associated with low lipid content and low BPP of this biomass. For the EB, NER value of biogas route was 10⁻², indicating the improvement of energy efficiency due to the greater value of BPP, although the lower EB productivity. Because of the RB NER value, the integrated route presented NER of 2.9 × 10⁻², demonstrating that energy gain can be obtained through route integration.

Although a favorable result was obtained in the present study for the integrated route, a same energy gain could be obtained through a biomass treatment prior the anaerobic digestion in order to disrupt microalgae cell wall. Data of energy consumption for concentration and dewatering of the biomass for lipid extraction, as well for the extraction process itself, could be determinant for choosing either a pre-treatment of the whole biomass for anaerobic digestion or the biogas production after lipid extraction.

According to Sialve et al. (2009), lipid extraction of biomass containing less than 40% of lipids combined with anaerobic digestion of the residual biomass is not effective in terms of energy nor in terms of costs. For these authors, the anaerobic digestion of the whole biomass appeared to be the optimal strategy on an energy balance basis.

The scarcity of energy analyses on cultivation using effluent makes comparison difficult; however, these results show the necessity of intervention in the productive system in order to improve its energy performance, by using low-energy equipment and improve biomass productivity.

**CONCLUSIONS**

Lipid extraction could be considered as a biomass pre-treatment for anaerobic digestion tests. According to NER values, the best energetic pathway was the integrated route (lipids + biogas), having the biogas production of the EB major contribution for this result. However, for all the studied energetic routes, the energy input was higher than the output, indicating that the HRAP needs intervention in order to improve its energy performance and increase its biomass productivity.

Moreover, other stages of the production system should be improved in order to scale-up the technology and consequently get more precision in measuring energy efficiency. Biomass harvesting and concentration, lipids extraction and a specific anaerobic reactor for algal biomass digestion are processes that should be improved. For instance, in a larger scale, chemicals should be included in the economic balance, and also pumping energy demand in the energetic analysis.

The integration between energetic, economic and environmental spheres should be included in a major context analysis, as a life cycle analysis, for example.

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