Energy recovery in high rate algal pond used for domestic wastewater treatment

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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 domestic sewage. The outputs were calculated for three energetic pathways: biomass burning, lipid and biogas production for the raw biomass and biomass after lipid extraction. The total lipid in dry biomass was 7.37% reaching a daily productivity of 4.6 mg/L and the biogas production potential was 0.22 m³/kg solids. For the biomass after lipid extraction, the biogas production reached 2.55 m³/kg solids, 10 times higher than the biogas potential production of the raw biomass. NER values for the raw biomass were 0.134, 0.0122 and 0.0141 for burning, lipids and biogas, respectively. The specific biomass productivity was 4.26 mg/kJ. For the residual biomass, after lipid extraction, NER value was 0.0173 for the integrated route (lipids + biogas). The biogas generation from the raw biomass was the most favorable energy route.

KEYWORDS
Bioenergy; biogas; biorefinery; lipids; microalgae.

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

The ability of microalgae to adapt and survive in a wide variety of environments is enormous and its cultivation can be combined with wastewater treatment and energy generation. 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 (Chen et al., 2014). In effluents, there are the presence of suspended solids that prevent solar radiation penetration, toxic substances, and other microorganisms that require nutrients and space, turning difficult the development of algal biomass, compared with cultivation in a synthetic medium. Therefore, the anaerobic digestion of the biomass could be a potentially attractive alternative for energetic purposes.

Regardless of the production system and of the final energetic product, for the viable large-scale production of biofuels, the energy used for cultivation must be minimized in order to maximize the energy yield. The net energy analysis, which uses concepts of the lifecycle analysis, is one of the most widely accepted methods for assessing the energy potential of a system in general (Poldy, 2008). Another coefficient for assessing the efficiency of the cultivation system is biomass specific productivity, defined as the ratio between biomass productivity and the energy input, as proposed by Pegallapati et al. (2014).

The proposal in the current paper was to apply energy analysis in an integrated context of biorefinery, with the main objective of defining the best use of biomass, in addition to determining the energy efficiency of the production system. Therefore, we assessed the energy potential, in terms of lipids and biogas, of the biomass, prior and after lipid extraction, cultivated in a high rate algal pond (HRAP) using domestic sewage as culture medium.

**MATERIALS AND METHODS**

**Biomass production unit**

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-a (according to APHA, 2005 and NEN 6520, 1981).

The HRAP had the following characteristics: width = 1.28 m, length = 2.86 m, total depth = 0.5 m, culture depth = 0.3 m, surface area = 3.3 m², culture volume = 1 m³. The disinfection system was designed to achieve a final concentration of $10^3$ MPN (100 mL)$^{-1}$ of *Escherichia coli*, with an adopted effective dose of 21 mL/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.

**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 residual biomass (RB). Similarly, the biomass sent directly for anaerobic digestion was named entire biomass (EB).

Both biomasses, EB and RB, were characterized in terms of chemical oxygen demand (COD), total solids (TS), total volatile solids (TVS), humidity, pH, total kjeldhal nitrogen (TKN) and total
phosphorus (TP), according to APHA (2005). 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 (Bourrely, 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 Assemamy et al. (2015). The biomass was oven dried at 50°C for 12 h and the cells disrupted with hydrochloric acid 3M. After disruption, petroleum ether and methanol were added to the dry biomass for lipid extraction, totaling three cycles of extraction. The lipid content (% of lipids in the dry biomass) was determined gravimetrically after the evaporation of the solvent for two hours at 105°C.

**Biogas**

The biogas production potential (BPP) tests followed the procedures described by Jawed and Tare (1999), with modifications described by Viana et al. (2012). Tests were carried out using 250 mL erlenmeyers (200 mL of useful volume), filled with anaerobic sludge from an UASB reactor, substrate (EB and RB), buffer solution and distilled water, 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 ratio were tested (four and six) and eight achieved the best performance. Micro- and macronutrients were added to prevent deficiency during the tests. 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 four minutes 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 25g NaCl/L (pH=2) solution for biogas measurement. The calculation of the BPP was based on the cumulative production of biogas after 30 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 liters of methane correspond to 1 g of COD.

**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:

$$NER = \frac{\sum_{i} \text{Energy produced}}{\sum_{i} \text{Energy consumed}}$$

The produced energy can be measured in terms of the total energy content of the biomass or only with respect to its lipid and/or biogas production potential. The energy consumption for the agitation of the culture medium was considered, i.e., the energy consumed by the paddlewheels and also for the UV lamps in the disinfection unit.
Quantification of the energy consumption
The energy consumption of each operation (C_{OP}, kWh/day) was determined with Equation 2:

\[ C_{OP} = \frac{24 \times P_{ot}}{1000} \]  

(2)

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

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

(3)

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

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

(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) the total 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) hydraulic retention time of the HRAP was considered to be 4 days, according to Equations 5 and 6:

\[ E_L = \frac{P_{L,\text{annual}}}{0.9} \times 35,133.33 \]  

(5)

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

\[ E_G = P_{G,\text{annual}} \times 23400 \]  

(6)

where P_{G,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 dominant specie, with an average of 9.4x10^5 individuals/mL, followed by the gender *Scenedesmus sp.* with 6.5x10^5 individuals/mL. Table 1 presents the physical and chemical characterization of the biomass used in the energetic tests.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unit</th>
<th>Average</th>
<th>SD</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>7.5</td>
<td>1</td>
<td>&lt; 2</td>
<td>---</td>
</tr>
<tr>
<td>Humidity</td>
<td>%</td>
<td>98</td>
<td>1</td>
<td>99</td>
<td>---</td>
</tr>
</tbody>
</table>

Table 1. Characterization of the biomasses used as substrate for energetic tests.

Energy Recovery in high rate algal ponds
<table>
<thead>
<tr>
<th></th>
<th>mg/L</th>
<th>7E10^3</th>
<th>21E10^3</th>
<th>TS</th>
<th>mg/L</th>
<th>2E10^3</th>
<th>4E10^3</th>
<th>12E10^3</th>
<th>TVS</th>
<th>mg/L</th>
<th>1E10^2</th>
<th>6 E10^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN</td>
<td>mg/L</td>
<td>3E10^3</td>
<td>1E10^2</td>
<td>4E10^3</td>
<td>organic nitrogen</td>
<td>3E10^3</td>
<td>---</td>
<td>3E10^3</td>
<td>---</td>
<td>3E10^3</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>mg/L</td>
<td>6.5 E10^2</td>
<td>100E10^2</td>
<td>3E10^2</td>
<td>1E10^2</td>
<td>53E10^3</td>
<td>27E10^3</td>
<td>100E10^3</td>
<td>25E10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD=standard deviation; --- = non-realized; n = number of samples.

The pH of EB presented a value close to neutrality; on the other hand, RB 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 RB was 2 times higher than EB. 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. The low nitrogen (TKN) concentration in RB, indicated that together with lipids, proteins were also extracted. The high COD concentration and the low nitrogen concentration, leaded RB to present a carbon/nitrogen ratio, C:N, of 26.1:1, and for EB this ratio was 16:1. For phosphorus, we highlighted the lower concentration in RB compared to EB, caused by the extraction of phosphorous elements, such as complex lipids and phospholipids, during lipid extraction.

**Energetic Output**

The energetic output of the biomass, in terms of lipids and biogas, is presented in Table 2.

<table>
<thead>
<tr>
<th>Lipids (%)</th>
<th>Lipid Productivity (mg/L.day)</th>
<th>Anaerobic biodegradability (%)</th>
<th>BPP (m³biogas/kg TVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB</td>
<td>7.4</td>
<td>4.6</td>
<td>20.5</td>
</tr>
<tr>
<td>RB</td>
<td>16.7</td>
<td>11.0</td>
<td>16.7</td>
</tr>
</tbody>
</table>

The biomass presented a lipid content of 7.4%, reaching a lipid productivity of 4.6 mg/L.day. The cultivation in effluents under less appropriate conditions, and in competition with other microorganisms, may have been the cause of the low lipid accumulation. Another probable reason is the composition of the biomass, since it contains a mixture of algae and bacteria, and lipid content of bacteria is usually very low.

A low anaerobic biodegradability of the EB was presented, with an average value of 20.5%. For the biomass after lipid extraction, this value was even lower, 16.7%. Cell wall is considered as the main characteristic of the difficult digestibility of algal biomass (Zamalloa *et al.*, 2012). The difficult degradability was reflected in BPP, with an average value of biogas production of 0.22 m³biogas/kg TVS. Passos *et al.* (2013) using raw algal biomass produced in domestic sewage for anaerobic digestion achieved a biogas production of 0.172 m³/kg TVS. For RB, the BPP reached 2.55 m³/kg TVS, 10 times higher than the EB results. Lipids extraction by solvents addition probably increased the biological availability of the microalgae intracellular content, facilitating
the digestion by the anaerobic microorganisms (Alzate et al., 2014). Moreover, the best performance of digestion using the RB was due to the shortening of the hydrolysis step, i.e. the acidogenesis phase was largely completed in the lipid extraction step, leaving the little bacteria hydrolytic to do, since they have received almost or completely hydrolyzed substrate.

**Energetic analysis**

For the large-scale production of microalgal biofuel, the energy consumption of the cultivation must be minimized in order to maximize the net energy production. In order to sustain the production of energy in a system, the NER must be > 1, and as high as possible. Figure 1 shows NER data and the specific productivities of biomass for all the energetic routes.

![NER and specific biomass productivity for different energetic routes.](image)

For all the studied energetic routes, NER values were below 1. The higher value was 0.134 for EB burning route. Lipids and biogas routes for EB presented similar NER values, varying from 0.0122 to 0.0141, for lipids and biogas, respectively. For RB, biogas route presented the lowest NER value; therefore, its contribution for the integrated route – lipids and biogas – was reduced. The integrated route presented a NER value slightly higher than the separate routes; however, it was not high enough to compensate the energetic input. System efficiency in terms of biomass productivity per energy input (P_B/E_C) was also assessed. Results showed that for EB, the biomass production have compensated the energy input, presenting a specific productivity (P_B/E_C) value of 4.26 mg/kJ. RB presented a very low P_B/E_C value, indicating that the energetic input for its production was too high. This fact is related with the required concentration and dewatering of the biomass for lipids extraction.

Comparing NER value from the integrated route with NER value of the EB biogas route is possible to observe the low energetic gain of the integrated route. In this study, this may be due to the following factors: (i) low NER of the RB biogas route, because of the low biomass productivity after dewatering for lipids extraction, and (ii) low lipid content of the EB. As in NER calculation
was not considered the energy input for the biomass separation stage, neither its drying process for lipid extraction; we can state that lipid extraction is not an energetic feasible process for the studied biomass. Therefore, biogas production of the EB could be considered as the most favorable energy route. 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 the 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. The increase of the productive unit with greater capacity of biomass production and lower energy use through low-energy equipment would be options to increase the NER and $P_B/E_C$ values. Another possible cause of the low energy performance is the use of effluent for cultivation, which makes algal production less efficient because of competition with other organisms for space and nutrients, leading to low lipid accumulation.

CONCLUSIONS
For all the studied energetic routes, the energy input was higher than the output. The integrated route (lipids + biogas) presented a NER value slightly higher than the separate routes; however, it was not high enough to compensate the energetic input. The low energetic gain of the integrated route compared with biogas route of the raw biomass, besides the low lipid content of the biomass and the energetic input associated with biomass dewatering, turned lipid extraction as an energetic unfeasible process for the studied HRAP biomass. Biogas production of the raw biomass could be considered as the most favorable energetic route.

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