PERFORMANCE OF THE THERMOPHILIC ANAEROBIC REACTOR IN THE START-UP AND ADAPTATION PHASES TREATING THE CANE ALCOHOL DISTILLERY WASTEWATER

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Abstract:

An alternative treatment strategy for cane vinasse was investigated in an anaerobic sequencing batch biofilm reactor (ASBBR). The operational mobility of this system is the greatest advantage for application in the cattle ranching and agricultural activities. The reactor was operated with mechanical agitation (300-rpm) at 55 °C and with initial operational cycles adjusted for each phase. The results of the adaptation phase showed that after the addition of minerals and bicarbonate (31st cycle), the mesophylic poultry slaughterhouse sludge can be used as inoculum for the anaerobic reactor for thermophylic conditions.

Key-words: batch reactor, thermophylic, adaptation, start-up, sugar cane.

1. Introduction

The sugar and alcohol production in Brazil comes from the sugar cane (Saccharum officinarum) processing. The industrialization of the sugar cane results in a generation of great quantities of by-products as bagasse, ashes, vinasse, gaseous emissions, etc. [1] and most of them has a high organic load. In this context, the vinasse one of the residues of great concern, due to the quantities that it is generated – for each liter of alcohol produced, 12 - 14 liters of vinasse is generated – and its polluter potential, that can exceed 100g.l⁻¹, expressed as chemical oxygen demand (COD) [2]. It is extremely saline and the potassium concentration can be responsible for 0,8% of the total solids [3]. Besides, it contains phenolic compounds [4], that difficult the biological treatment.

During the harvest of 2004/2005 it was produced 15 billions liters of alcohol [5]. Based on the values cited by Wilkie et al. [2], it could be supposed that the vinasse generated in this last harvest would have a pollution potential equivalent to 7,5 billions of habitants discharging raw wastewater in the environment, concentrated in a small part of Brazil.

The use of vinasse as fertilizer for sugar cane cultivation is normal in Brazil, but due to, State of Sao Paulo Ambient Sanitation Technology Company (CETESB), recommendation, the water should be adequately treated for organic matter and minerals removal, especially, potassium, due to its high reductive potential [6], that for long term assessment can contaminate the soil, aquifers and water bodies.

The effects can be minimized with previous treatment for removal of the organic matter. Some of the possible alternatives for dealing with vinasse wastewater are wetlands treatment [7], aerobics processes like pond systems [3], activated sludge, sequencing bath reactor [8] and anaerobic digestion.

Nowadays, considerable attention has been paid towards the development of anaerobic reactors to the conversion of organic matter into biogas. Several authors evaluated the upflow anaerobic sludge blanket for vinasse thermophilic anaerobic treatment [9, 10, 11, 12 and 13].
Some other configurations of reactors studied for vinasse wastewater treatment were [14, 15, 16 and 17].

Nowadays, a lot of attention has been directed to high rate anaerobic reactors, aiming the methane production. Thus, this biogas can be utilized by the industry.

For this work, a technology that is getting more attention due to its operational flexibility has been used [18]: the anaerobic sequencing batch reactor that has feeding, reaction, sedimentation and effluent discharge phase. This reactor can be used by industries that have an intermittent liquid residues production, as milk processing plants, piggery manure, winery, sugar cane industries and others. It can be applied to treat toxic compounds and recalcitrant liquids, because of the possibility of retaining the treated liquid until achieve the desired treatment level.

Besides, these reactors have a simple operation and control that allows it to be used in fundamental research to elucidate some specific aspects of the residues degradation [19] and to obtain kinetic parameters.

By these means, the main objectives of this work were: (i) evaluate the startup of an anaerobic sequencing batch reactor inoculated with UASB (upflow anaerobic sludge blanket) sludge treating aviculture residues in mesophylic conditions (35 °C) and (ii) evaluate the biomass adaptation to vinasse and to the thermophylic conditions (55 °C).

2. Material and Methods

The raw vinasse was collected in da Serra industry in Ibaté city, São Paulo state, Brazil and stored in a refrigerator at 4 °C.

The bench scale ASBBR was built in acrylic, composed of two concentric tubes 25 cm height, 19 and 22 cm internal diameters, total volume of 7.2 l and reaction volume of 3.5 l (Fig. 1). Between the tubes, there was water circulation to keep the reactor temperature in 55 °C – thermophylic condition. There was a stainless steel 18 cm height and 18 cm diameter (0.5 cm mesh) basket installed inside the reactor to keep the support material. The basket had a central cylinder space where the stirrer could be allocated. The agitation speed was set up for 300 rpm. The basket was filled with cubic polyurethane foam matrices, with 1 cm side, density of 23 kg.m⁻³ and porosity of 95%. The reactor fill and discharge were accomplished by diaphragm pumps, timed controlled.

Fig. 1. ASBBR reactor scheme: (1) Anaerobic sequencing batch biofilm reactor, (2) warm water circulation, (3) stainless steel basket, (4) Engine of the mechanical propeller, (5) propeller, (6) feeding pump, (7) discharge pump, (8) heat exchangers, (9) sampler, (10) bench. Source[20].
The ASBBR was inoculated with the UASB reactor treating poultry slaughterhouse sludge from Dacar industrial S/A. For sludge immobilization in the polyurethane foam matrices, first the granular sludge was triturated and then sifted, after that the foam matrices and the sludge were put in contact during 24 h. After this step, the adhered cells were put in contact with wastewater with an organic matter concentration of 0.3 g.l⁻¹ measured as COD (chemical oxygen demand), in order to washout the solids not adhered. The adhered biomass was approximately quantified using total volatile solids analysis. The biomass was also examined by optical microscopy (Olympus BH2) and by SEM (scanning electronic microscopy). The samples were taken from the reactor surface, middle and bottom.

The monitoring physical-chemical analyses as COD, total solids and pH were made according to the Standard Methods for the Examination of Water and Wastewater [21] and bicarbonate alkalinity (BA) by [22] with modifications [23] and total volatile acids (TVA) by [22]. The steps for biomass adaptation are presented in Table 1.

Table 1 – Experimental steps during the sludge adaptation phase.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Duration (d)</th>
<th>Temperature (°C)</th>
<th>t_cycle(nc)* (d)</th>
<th>COD Affluent (g/L)</th>
<th>Alkalinity supplementation Affluent (g HCO₃/g COD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>35</td>
<td>1 (14)</td>
<td>1,0</td>
<td>0,00</td>
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<tr>
<td>II</td>
<td>5</td>
<td>35</td>
<td>1 (5)</td>
<td>1,0</td>
<td>0,36</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>35</td>
<td>1 (2)</td>
<td>1,0</td>
<td>0,73</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>55</td>
<td>1 (7)</td>
<td>0,3</td>
<td>1,20</td>
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<tr>
<td>V</td>
<td>4</td>
<td>55</td>
<td>2 (2)</td>
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<tr>
<td>VI</td>
<td>15</td>
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<td>2 (7)</td>
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<td>VII</td>
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*t_cycle = cycle time (number of cycles).

3. Results and discussion

The reactor adaptation phase took approximately 63 days. The beginning of the operation was not monitored due to the time needed for discharge of the non adhered biomass that corresponded to approximately 18 g.l⁻¹, as volatile suspended solids. The beginning of analyses in the reactor effluent was started just after detection of volatile suspended solids decrease (approximately 7g.l⁻¹). The mesophylic (35 °C) inoculum adaptation to thermophylic (55 °C) conditions analysis results are presented in Fig. 2.

After the 22nd cycle, the applied organic load was 1.0 g.l⁻¹ as COD and the reactor was operated with a temperature of 35 °C (Fig. 1a). From this cycle, the temperature was raised instantly to 55 °C as recommended.

Aiming to develop a better biomass adaptation to the new temperature and avoid the reactor failure due to organic acids production, the organic loading rate (OLR) was reduced to 0.3 g.l⁻¹ (Fig. 1a), because, with high temperature, high rate reactions are expected. After verify the COD removal approximately 30% higher, the OLR was raised to 0.5 g.l⁻¹ as COD (it corresponds to 1.0 g.l⁻¹.d⁻¹ for cycles of 2 days) in the 31st cycle. Immediately an efficiency reduction was observed in the 33rd cycle, presenting a COD removal efficiency of 2%. Concurrently, the mineral salts addition [24], in the 31st cycle, promotes the system recovery, reaching 55% of COD removal in the 38th cycle.
Fig. 2. Mesophylic to thermophylic biomass adaptation phase: (a) COD values and COD removal percentage; (b) pH and effluent intermediate and partial alkalinity ratio (IA/IP) and (c) Bicarbonate alkalinity and total volatile acids.
After the temperature change, it was not observed any pH decrease (Fig. 1b), there was even a progressive increase in its values, due to the alkalinity supplementation strategy. It was supplemented 0.36 g of HCO$_3$ for each gram of COD in the reactor until the 20$^{th}$ cycle, and 0.73 g of HCO$_3$ for each gram of COD in the 21$^{st}$ cycle and 1.20 g of HCO$_3$ for each gram of COD from the 22$^{nd}$ cycle, until the end of operation. A small difference in the pH values was detected, from 7.1 to 6.7 during the 29$^{th}$ cycle and after the OLR increase, some oscillations were detected, from 7.1 until 8.2, but all of them were in the anaerobic process limits.

The ratio IA/IP (Fig. 1b), may be an indicator of occurrence of process disturbs, as instability in volatile acids production. This ratio varies for different reactors, but the literature recommends that it should be close to 0.3 for stable process [23]. After the 1.2 g of HCO$_3$ supplementation, addition of minerals and increase of OLR, this ratio increased until 0.6 (IA/IP).

With the temperature increase to 55 °C and the bicarbonate / COD ratio increase to 1.20, it was observed a decrease in volatile acids values and a production of bicarbonate alkalinity in the effluent from the 22$^{nd}$ cycle until the 30$^{th}$ (Fig. 1c). When the organic matter load was increased to 1.0 g.l$^{-1}$ in the 30$^{th}$ cycle, an increase in the bicarbonate alkalinity values and volatile acids consumption in the effluent was detected. This was an indication that the reactor was adapting to the organic load of 0.5 g.l$^{-1}$.d$^{-1}$, as COD.

The morphologic aspects found in the inoculum and the biomass, after the adaptation phase to 55 °C, are presented in Fig. 3, 4 and 5.

It was observed that the UASB reactor, where the inoculum was taken from presented great microorganism diversity. This is a very important characteristic for a sludge that will be used as inoculum. This inoculum presented much different morphologies, as several forms of bacillus (rod, curve, fine, coccus, grouped coccus), filamentous similar to Archeal, *Methanosaeta* spp., long filaments and fines, structures similar to fluorescent *Methanosarcine* spp. (Fig. 3).

![Fig. 3. Observed morphologies in the inoculum in optical microscopy: (a) diversity of bacillus, (b) Similar to *Methanosarcine* spp., (c) *Methanosaeta* spp. similar and coccus bacillus.](image)

In the start-up phase in mesophylic conditions (stage III) at 35 °C, a reduction in the morphologic diversity was observed and in the reactor surface sample there was a predominance of small bacillus as coccus and rods. The same morphologies were observed in the reactor middle and bottom, some filamentous with bigger size and yeast similar structure, probably remaining from the fermentation process of alcohol (Fig. 4).

In the stage V, thermophylic 55 °C conditions, after 9 cycles, bacillus, filaments, and some *Methanosaeta* spp. were observed in the reactor. The morphologies diversity reduction can be attributed to the lack of minerals in the vinasse. The nutrients in the vinasse weren’t enough for the development of anaerobic biomass until this adaptation phase.
Fig. 4. Observed morphologies in the biomass during the final mesophylic operation of the reactor in optical microscopy: (a) bacillus from the reactor's top, (b) round bacillus and coccus, (c) Bigger morphologies and probable yeast (arrow).

Starting in the stage VI, it was decided to supplement the reactor inflow with essential macro and micro nutrients for anaerobic biomass development and vinasse treatment.

After 14 cycles with minerals supplementation (final of stage VII) it was observed, in reactor top, that the yeast quantity, *Methanosaeta* spp. similar morphologies (some with vacuoles) had increased. In the middle of the reactor, besides the morphologies observed in the top, there was a predominance of methanogenic archea similar to fluorescents *Methanosarcine* spp. and the presence of an inert fluorescent precipitated material, probably sulfur, potassium or other element crystals. It is known that these kind of materials shine when they are under the incidence of ultra fluorescent light. In the reactor's bottom it was observed fluorescent bacillus, fine bacillus, yeast, similar to *Methanosaeta* spp., curve bacillus similar to sulfate reducing bacteria (Fig. 5).

Fig. 5. Observed morphologies in the biomass during the final thermophylic (55 ºC) adaptation of the reactor in optical microscopy: (a) yeast germination, (b) crystals, (c) Morphology similar to *Methanosaeta* spp. (arrow), (d) similar to *Methanosarcine* spp., (e) possible a *Methanosaeta* spp. and sulfate reducing bacteria and (f) *Methanosaeta* spp. e bacillus.

The presence of sulfate reducing bacteria was stimulated due to the quantity of sulfate affluent (~100 mg.l⁻¹ measured as sulfur). The sulfate is originated from the addition of sulfuric acid, in the cane sugar industries, to adjust the pH to the *Saccharomyces cereavise* yeast.

In this phase, it could be observed a great microorganisms diversity. This was attributed to the supplementation of minerals and the increase of working temperature, indicating that the
system could be considered as adapted at 55 ºC and that the microorganisms were adapted to vinasse degradation.

4. Conclusions

Observing the results obtained during the monitoring of the biomass adaptation period to the thermophylic conditions, it was concluded that it is possible to use the mesophylic poultry slaughterhouse sludge as inoculum to the ASBBR and that it presented a good adaptation to the thermophylic conditions, as soon as the minerals and bicarbonate addition was started better operation conditions were achieved. The minerals addition (31st cycle) was essential due to the lack of trace metals, essentials for the good biological reactor performance, especially methanogenic reactors.

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