TREATMENT OF WASTEWATER WITH HIGH FAT CONTENT EMPLOYING AN ENZYME POOL AND BIOSURFACTANT: TECHNICAL AND ECONOMIC FEASIBILITY

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Abstract - The combination of 0.5% weight per volume (w/v) crude enzyme consortia produced by solid state fermentation with *Penicillium brevicompactum* and 27 milligrams per liter (mg/L) of rhamnolipid biosurfactant produced by submerged fermentation with *Pseudomonas aeruginosa* was employed to pretreat wastewater from a poultry slaughterhouse in bench upflow anaerobic sludge blanket (UASB) bioreactors. One bioreactor was fed with untreated wastewater (Control) and another with pretreated wastewater (Test). Under an organic loading rate of 11 kg COD/m^3.d, the Test bioreactor showed higher specific methane production (67.8 mL CH$_4$ (STP)/g COD removed), higher chemical oxygen demand (COD) (91.2%), and oil and grease (O&G) (95.8%) removal than the Control bioreactor, which produced 36.1 - 16.9 mL CH$_4$ (STP)/g COD removed with 72.5% COD removal and 48% O&G removal. The Control bioreactor experienced five clogging episodes due to the accumulation of fat on the surface, while the Test bioreactor operated without operational problems. The scum of the Control bioreactor contained more than 10 times higher quantities of fat than the Test bioreactor. The economic analysis indicated that such alternative technology has lower costs than conventional technology.

Keywords: Enzymes, anaerobic processes, bioreactors, wastewater treatment, fat, rhamnolipid.

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INTRODUCTION

Due to the high energy potential of lipids present in wastewater from poultry slaughtering plants, the anaerobic biological treatment is highly attractive for generating large amounts of methane, which can be used as an energy source by the poultry industry (Angelidaki and Sanders, 2004; Alves et al., 2009).

Although efficient, anaerobic biological processes may present problems during the treatment of wastewater with high levels of fat, such as the development of sludge with low activity, with inadequate physical characteristics and high tendency for flotation, production of scum, clogging of sludge bed, and drag of biomass, which leads to lost efficiency and even the collapse of the reactor. Moreover, fat adsorption occurs at the surface of the sludge, hindering the assimilation of nutrients and decreasing the rate of substrate conversion (Pereira et al., 2004; Jeganathan et al., 2007; Alves et al., 2009).

Fat accumulation in biomass and the operational problems mentioned above occur because the substrate feeding rate becomes faster than the substrate degradation rate. For this reason, the anaerobic biological treatment of wastewater from poultry processing industries is almost always preceded by one or more pretreatment steps to reduce fat concentration (Del Nery et al., 2007; Nardi et al., 2008). Induced and dissolved air flotation, chemically aided or not, is often used to remove oil and grease from wastewater before biological processes. However, the cost of the air and reagents (chemically assisted flotation) is high, the dissolved and / or emulsified material removal efficiency is low, and sometimes sludges that are extremely difficult to treat are produced (Al-Mutairi et al., 2008).

Few studies are available in the literature on the use of enzymes to pretreat effluents from slaughterhouses. When enzymatic pretreatment is employed, the triglycerides are hydrolyzed to fatty acids and glycerol, which improve the efficiency of biodegradation by microorganisms during biological treatment. In a series of studies, Masse et al. (2001; 2003) described the use of a commercial pancreatic lipase (PL-250 Genecor International) for the hydrolysis of fat particles from slaughterhouse wastewater. The efficiency of the hydrolysis was confirmed by reducing the particle diameter as well as verifying the production of long chain fatty acids. However, no significant differences were found between reactors fed with pre-hydrolyzed wastewater and reactors fed with unhydrolyzed wastewater in the treatment of a slaughterhouse

wastewater containing 550 mg/L of fat. Dors et al. (2013) studied the addition of commercial pancreatic lipases on biodegradability of wastewater from a poultry processing industry. Enzymatic treatments were performed by varying the enzyme concentrations from 1 to 3 g/L. When the wastewater was biodegraded for approximately 30 d at 35ºC/100 rpm, the COD removal achieved was 3 times higher than the removal obtained with the raw wastewater. The enzyme concentration did not interfere significantly in the rate of removal of organic material.

Valladão et al. (2009) reported that enzymatic pretreatment (0.1% solid enzymatic preparation - SEP, for 4 h) of slaughterhouse wastewater in a UASB bioreactor resulted in improved production of methane and higher COD removal (882 mL CH_4, 93.5 %) than the values obtained when the bioreactor was fed with raw wastewater (642 mL CH_4, 91.0%).

The application of biosurfactants in industrial wastewater treatment has been reported in the literature (Nakhla et al., 2003; Daverey and Pakshirajan, 2011). The biosurfactant facilitates the biodegradation by dissolving fats and oils, which can be incorporated directly in the biological process, thus eliminating additional pretreatment processes to remove fat and resulting in lower capital and operating costs (Nakhla et al., 2003).

However, the combined use of enzyme and biosurfactant is a novel approach that could improve the wastewater treatment from food industries with high fat content. It is envisaged that this approach will reduce operational problems, treatment costs, and disposal of waste, while at the same time it could increase the production of methane from anaerobic processes. The objective of this study was to evaluate the technical and economic feasibility of the combined application of rhamnolipid type biosurfactant produced by *Pseudomonas aeruginosa* PA1 and crude enzyme consortia produced by the fungus *Penicillium brevicompactum* in anaerobic treatment of wastewater from poultry processing industry with high-fat content.

MATERIALS AND METHODS

Crude enzyme consortia production

The crude enzyme consortia was produced using solid-state fermentation of the fungus *Penicillium brevicompactuum*, which was isolated from an industrial *Orbignya oleifera* (babassu) oil extraction waste (Freire et al., 1997). The agro-industrial waste generated in the babassu seed oil extraction, known

as babassu cake, was used as the culture medium. Fermentations were conducted in 600 ml beakers containing 15 g of babassu cake with 65% initial moisture using procedures described in Gutarra et al. (2005). The beakers were autoclaved at 121ºC/15 min and, after cooling, they were inoculated with 10^7 spores/g of dry cake and incubated for 48 h in a fermentation chamber at 30ºC and 95% humidity. At the end of the fermentation process, part of the fermented cake was sampled to determine lipase (average value of 48.6 ± 5.2 U/g) and protease (average value of 3.4 ± 0.5 U/g) activities. One lipase unit was defined as the amount of enzyme that catalyzes the release of one μmole of fatty acids per minute, under assay conditions. One protease activity unit was defined as the unit difference in absorbance between the reaction blank and the sample per minute under the assay conditions. The remainder of the fermented cake was vacuum-packed and stored in a freezer (-20ºC) until use.

Biosurfactant production

Biosurfactant was produced using *Pseudomonas aeruginosa* PA1 isolated from oil production water at Sergipe, Brazil (Santa Anna et al., 2002), which was grown in optimized medium described by Santos et al. (2002). After seven days, fermentation was stopped and the cell-free crude fermented broth (after centrifugation and sterilization at 121ºC/15 min) was characterized for rhamnolipid concentration (10.2 g/L), surface tension (29 mN/m), critical micelle concentration (CMC = 53.3 mg/L), emulsification index using n-hexadecane (63%), and chemical oxygen demand (COD = 26,609 mg/L). The cell-free fermented medium was stored at -20ºC until use. Sterilization of the cell-free medium denatured all proteins with catalytic activity that could influence the hydrolysis of oil and grease and/or proteins from the wastewater. The only molecule with interfacial activity in this medium was rhamnolipid (Santa Anna et al., 2002).

Wastewater and sludge characterization

The wastewater used was collected in a local poultry slaughterhouse, between the equalization and flotation steps, analyzed using standard methodologies (Greenberg et al., 2005), and kept at -20ºC until use. The granular anaerobic sludge was collected in the UASB reactor operated at the same slaughterhouse and presented a concentration of volatile suspended solids (VSS) of 14.7 g/L and specific methanogenic activity (SMA) of 0.173 g COD_{CH4}/gVSS.d.

Pretreatment of the wastewater with crude enzyme consortia and biosurfactant

The conditions of this pretreatment were determined in experiments in which different combinations of enzyme consortia, biosurfactant, time, and temperature were evaluated on solubilization/hydrolysis of fats and subsequent anaerobic treatment conducted in sequencing batches (Damasceno et al., 2012). Wastewater from a poultry slaughterhouse, containing around 1300 mg O&G/L, was pretreated with 0.5% (w/v) of the solid enzymatic preparation (SEP) and 27 mg/L of biosurfactant for 4 h at 30ºC and 150 rpm. This condition produced the best synergistic effect for the availability/hydrolysis of fat through the combined action of biosurfactant and enzyme consortia, with promising results for COD removal, methane production, and low fat accumulation. After hydrolysis, the babassu cake was separated from the wastewater by screening (mesh 14), and the filtrate (hydrolyzed wastewater) was maintained at 4ºC until used in the bioreactor feed.

Experimental Equipment

The anaerobic treatment was carried out in two UASB reactors that were built using acrylic fiber (1.08 L working volume). The dimensions of the bioreactors were 8.5 cm in diameter and 26.1 cm in height. Porcelain beads with a mean diameter of 0.7 cm were distributed on the base of the bioreactors to allow better distribution of feed and to prevent formation of preferential channels. Three sampling points were located at 4 cm, 8 cm, and 13.5 cm from the base of the bioreactors. The biogas was vented in the upper portion of the UASB reactors immediately after passing through a three-phase separation system, consisting of one inverted funnel with a diameter of 6.8 cm. A deflector ring, with 45º angle, was installed at a height of 19.35 cm, at 0.5 cm away from the three-phase separator. The gas outlet was connected to a Ritter biogas meter (Milligascounter). After inoculation with 360 mL of anaerobic sludge, the continuous feed (prepared two times per day to prevent deterioration and under agitation to prevent solid material settling) started with hydraulic retention times (HRT) of 20 h. One UASB bioreactor was fed with pretreated wastewater (called the Test Reactor), while the other was fed with raw wastewater (untreated, called the Control Reactor). The bioreactors operated at 30ºC for 187 days. The anaerobic sludge was adapted to the poultry processing wastewater in the initial 44 days, during which there was a progressive increase

in the COD removal efficiency. During operation, two regimes were evaluated according to the organic load: 1st (with HRT of 20h and 6 kg COD/m³.d for 68 days) and 2nd (with HRT of 12h and 11 kg COD/m³.d for 75 days). At the end of each regime, the three phase separators were cleaned and samples were collected from the scum to establish the O&G percentage. Sludge samples were also collected at different heights of the bioreactors to determine the VSS concentration and the percentage of O&G adhered to the biomass.

The performance of both bioreactors was monitored by analysis of pH, flux, total suspended solids, fixed and volatile, COD, volatile acidity, alkalinity, O&G, methane and biogas production. The parameters used to monitor the UASB bioreactors were analyzed through the Student t test with significance level of 5% using the Statistica software 5.0.

Analytical Methods

The lipase activity of the fermented cake (SEP) was determined for each solid-state fermentation, which was conducted according to Gombert et al. (1999). After fermentation, phosphate buffer (100 mM, pH 7, 5 mL/g) was added to each beaker containing the fermented solids. The enzyme extraction was carried out in a rotary shaker at 35ºC and 200 rpm for 20 min. Later solid-liquid separation was performed by pressing, followed by centrifugation at 2000g for 5 min (Gombert et al., 1999). The supernatant was used to determine lipase and protease activities as previously described (Freire et al., 1997). In the cell-free crude fermented broth containing the biosurfactant, the rhamnolipid concentration was determined as rhamnose using the method described by Pham et al. (2004). The emulsification index was determined according to the method described by Cooper and Goldenberg (1987), and the surface tension was analyzed in a tensiometer Aqua-Pi (Kibron Inc., Helsinki, Fimland) at 25ºC, based on the Du Noüy (1925) method using small probes. The critical micelle concentration (CMC) was determined using the method described by Cooper et al. (1979).

Biogas composition was determined in a Varian Micro GC 4900 gas chromatograph using a 10 m x 0.32 mm PPQ column, column temperature of 50ºC, thermal conductivity detector (TCD) at 250ºC, injector temperature of 80ºC, and helium as carrier gas. The other parameters used in the wastewater and sludge characterization and to monitor anaerobic bioreactors (pH, COD, O&G, volatile acidity, total alkalinity, suspended solids, fixed and volatile, total nitrogen,

and total phosphorus) were determined according to the Standard methods (Greenberg et al., 2005).

Techno-economic evaluation

A comparative preliminary techno-economic analysis was carried out to evaluate if the new process that employs the biosurfactant and the crude enzyme consortia for poultry wastewater treatment (conditions described in topics Pretreatment of the wastewater with crude enzyme consortia and biosurfactant, and Experimental Equipment) is cost-competitive in relation to the conventional process that employs floater and chemicals.

The SuperPro Designer software (version 8.0, full license, Intelligen, Inc.) was used to construct process flowsheets as well as perform mass and energy balances of the two processes of wastewater pretreatment. The plant operation of both processes was 24 h per day (3 shifts) and 312 days (6 working days x 52 weeks) per year. The flow rate of wastewater was 750 m³/day. The two processing scenarios were compared based on the total capital investment and the operating cost required for each flowsheet.

The estimate of the fixed capital investment was based on the cost of the installed equipment multiplied by a factor of 1.2 for existing plants (Turton et al., 2012). The working capital was estimated by taking into consideration three months of raw materials and one month of labor (Turton et al., 2012). The total capital investment was calculated as the sum of fixed capital investment and working capital.

The costs to purchase equipment were obtained via vendor quotes except for the equalization tanks that were calculated based on literature data. Sizing of equalization tanks was based on average volume per hour according to recommendations of specialized companies and/or were calculated based on the Brazilian Technical Standards (NBR 7229 - Project, construction and operation of septic tank systems - procedure; NBR 6118 - Design of structural concrete - procedure) (ABNT, 1993; 2004) and procedures described in Peters et al. (2003). All the costs to purchase equipment were updated to April, 2015, using the market prices general index (IGP-M), available through the Brazilian Central Bank. The estimation of costs for installed equipment was carried out according to Equation 1 (Turton et al., 2012):

$$C_{BM} = C_p F_{BM} \qquad (1)$$

where C_{BM} = installed equipment cost; C_p = cost to purchase equipment; and F_{BM} = Bare module factor.

The operating cost was calculated using Equation 2 including the cost of raw materials (C_{RM}), utilities (C_{UT}), labor (operating labor and direct supervision) (C_{OL}), waste treatment (C_{WT}), supplies, laboratory charges, and costs related to fixed capital (equipment maintenance and depreciation) (F_{CI}) (Turton et al., 2012):

$$C_{OM} = 0.243\,F_{CI} + 2.215\,C_{OL} + C_{RM} + C_{UT} + C_{WT} \quad (2)$$

The plant life cycle was set as 10 years (Turton et al., 2012). Costs for raw materials were obtained via quotes from chemical industries and specialized websites. The adopted costs were: coagulant ferric chloride with density 1.42 kg/L (US$ 0.58/kg, YIXING BLUWAT CHEMICALS), anionic flocculant polyacrylamide for wastewater (US$2.25/kg, CHANGZHOU KEWEI TIANSHI ENVIRONMENTAL TECHNOLOGY), rhamnolipid biosurfactant (US$ 16.12/L, VICTEX CHEMICAL INDUSTRIES), and lipase enzyme 20,000 U/g (US$ 2.36/kg, AODIER GROUP). To calculate the cost of labor, a monthly salary and additional charges of US$ 272.48 per operator were considered, which is based on Brazilian law. The plant operates with 3 shifts per day and 6 days per week. The conventional plant requires 5 working stations per shift, whereas the novel plant requires 3 working stations per shift. The labor requirements were calculated according to the methodology proposed by Ulrich and Vasudevan (2004) using a fraction of operator per equipment, while the sum of operators for all equipment was multiplied by 3.9.The cost of the waste disposal (US$ 66.00/ton) was calculated according to the weight allocated to the landfill, considering humidity 40% to 70%, and data provided by the company responsible for the disposal of solid waste from a poultry processing industry. The quantities of utilities required were estimated by the software SuperPro Designer, according to the specifications of the equipment and the volume of treated wastewater. The utilities required for the conventional plant were electricity (US$ 0.12/kWh - motor pumps, agitators, and floater) and steam 15 kgf/cm^2 (US$ 44.38/ton - for heating the tri-decanter, equipment used to remove excess moisture from the fat that was separated in flotation). The alternative plant required only electricity (motor pumps for filling and emptying of equalization tanks and agitators). The costs of utilities are values experienced in Brazilian industries and were updated to April, 2015, using the market prices general index (IGP-M), available through the Brazilian Central Bank.

RESULTS AND DISCUSSION

Performance of UASB reactors fed with pretreated wastewater (Test) and raw wastewater (Control)

Table 1 summarizes the results obtained during the operation of the Control and Test bioreactors in both feeding regimes: in the 1st regime, the organic loading rate (OLR) was maintained at around 6 kg COD/m^3.d, while in the 2nd regime, it was increased to 11 kg COD/m^3.d. The OLR values of the Control and Test bioreactors were similar, which is important to rule out any influence of the applied organic load on the operating efficiency of the bioreactors. The variation over time in both bioreactors was due to the use of industrial wastewater, which introduced variability and made it difficult to control every collection.

In both raw and pretreated wastewater, the pH of the feed was adjusted to 7.0. During operation, there was an increasing trend in pH values for the outputs of both bioreactors. However, the values remained within the range described as ideal for the operation of UASB reactors (Table 1). During the 1st regime, there was a similar behavior for total alkalinity in both bioreactors, which remained relatively constant. In the 2nd regime, although the observed averages remained statistically the same (Table 1), total alkalinity in the two bioreactors showed a downward trend, which was probably related to a greater accumulation of acids in the reactors. The volatile fatty acids (VFA) present in the bioreactor effluent, such as acetic, propionic, and butyric acids, which were derived from decomposition of organic compounds during digestion, were almost constant in the first regime in the two bioreactors. In the second regime, there was a slight variation in the VFA concentrations in both bioreactors. However, despite this variation, the average VFA concentrations remained statistically equal. In the first and second regimes, the relationship between VFA/Alkalinity remained low for both Control and Test bioreactors, and within the limits recommended in the literature for anaerobic digestion (between 0.1 and 0.5 mg acetic acid/mg CaCO$_3$). The values obtained were similar in statistical analysis between bioreactors and regimes (Table 1).

Note that there were variations in the total influent COD for the two bioreactors, reflecting the variability of the wastewater, as previously mentioned. It is noteworthy that the values of the influent COD were close in both bioreactors (5304 ± 103 mg/L - average of the two regimes for the Control bioreactor and

Table 1. Summary of the results obtained during the operation of the Control and Test bioreactors.

Parameter	Control		Test	
	1st regime	2nd regime	1st regime	2nd regime
OLR (kg COD/m³.d)	6.3±0.6[AC]	10.8±1.2[BC]	6.0±1.0[AC]	11.1±1.3[BC]
$pH_{influent}$	6.6±0.2[AC]	6.7±0.4[AC]	6.5±0.3[AC]	6.7±0.4[BC]
$pH_{effluent}$	7.6±0.4[AC]	7.4±0.4[BC]	7.6±0.3[AC]	7.4±0.3[BC]
VFA (mg HAc/L)	196±42[AC]	268±96[AC]	181±25[AC]	222±79[AC]
Alkalinity (mg CaCO₃/L)	914±68[AC]	983±243[AC]	998±160[AC]	976±247[AC]
VFA/Alkalinity	0.21±0.03[AC]	0.28±0.08[AC]	0.18±0.02[AC]	0.23±0.10[AC]
Total $COD_{influent}$(mg/L)	5231±520[AC]	5377±612[AC]	5000±829[AC]	5537±668[AC]
Total $COD_{effluent}$(mg/L)	546±206[AC]	1470±806[BC]	480±223[AC]	489±238[AD]
Total COD removal (%)	89.1±4.4[AC]	72.5±15.6[BC]	90.2±5.0[AC]	91.2±4.0[AD]
Soluble $COD_{influent}$(mg/L)	1415±147[AC]	1487±194[AC]	1612±200[AD]	1580±209[AC]
Soluble $COD_{effluent}$(mg/L)	190±55[AC]	269±87[BC]	166±61[AC]	154±51[AD]
Soluble COD removal (%)	86.1±4.4[AC]	82.0±6.6[BC]	89.5±4.0[AD]	90.4±4.0[AD]
$O\&G_{influent}$(mg/L)	1243±191[AC]	1240±119[AC]	1229±185[AC]	1185±97[AC]
$O\&G_{effluent}$(mg/L)	164±37[AC]	626±337[BD]	49±31[AC]	50±32[AC]
O&G removal (%)	87.0±3.0[AC]	48.0±29.0[BC]	96.0±3.0[AD]	95.8±3.0[AD]
Scum O&G (mg/L)	1642±2095[AC]	10548±2773[BC]	155±8[AD]	904±564[BD]
CH_4(%)	78±4[AC]	70±5[BC]	79±3[AD]	80±2[BD]
Biogas production (STP mL/d)*	436.3	709.5 (143.9)	598.5	662.2
SMP (STP mL CH₄/g COD_removed)	59.2	36.1 (16.9)	71.6	67.8
$VSS_{reactor}$(g/L)	20.2	3.2	23.7	21.4

*During the operation of the 2nd regime, biogas production in the Control reactor remained at a higher value than that obtained in the 1st regime (until the 95th day). Thereafter, biogas production declined and maintained a much lower biogas production value (in parentheses). Test t – α = 0.05, identical letters mean statistical similarity. A and B = comparison between the 1st and 2nd regimes of each bioreactor; C and D = comparison between average values obtained in Control and Test bioreactors in the 1st regime or 2nd regimes.

5269 ± 380 mg/L - average of the two regimes for the Test bioreactor), which could rule out the influence of different applied load values on the behavior of the treatment systems. The Control bioreactor presented a total average COD effluent 2.7 times higher in the 2nd operating regime, while the Test bioreactor maintained statistically equal values in the 1st and 2nd regimes (Table 1). In the 2nd regime of the Control bioreactor, increased OLR caused greater variability of the total COD effluent. In this regime, the outlet piping of the effluent clogged five times because of the accumulation of solids on the top of the bioreactor, probably due to adsorption of fat onto sludge particles, followed by flotation of these particles to the surface. Several authors mention the flotation and subsequent washout of the sludge as disadvantages of the treatment of effluents with high fat content in granular sludge reactors (Jeganathan et al., 2006; Alves et al., 2009).

The results of influent soluble COD show values for the Test bioreactor that are 12% and 6% higher in the 1st and 2nd regimes, respectively. This can be explained by the application of biosurfactant and enzyme consortia in the wastewater pretreatment. With respect to the soluble COD effluent, the values obtained in both bioreactors were very close, although the Control bioreactor displayed an increase of 29% in the 2nd regime (Table 1).

Variations in the COD removal efficiencies in the anaerobic bioreactors throughout the operational period and under the two feeding conditions are shown in Table 1. The total and soluble average COD removal efficiencies achieved indicate that the Test bioreactor remained stable even with increasing organic load from the 1st to the 2nd regime, with values statistically equal to the total and soluble COD removal in both regimes (Table 1). The Control bioreactor showed a small reduction of soluble COD removal and a 19% decrease in total COD removal from one regime to the other (Table 1). This decrease in efficiency can be attributed to the particulate matter present in the wastewater without pretreatment, particularly insoluble fat.

By increasing the OLR and consequently decreasing the hydraulic retention time, the Control bioreactor reduced its O&G removal efficiency by 45% (87% in the 1st regime to 48% in the 2nd regime), which may be attributed to the increased total COD output provided by the Control bioreactor. On the other hand, in the Test bioreactor, the effluent O&G concentrations were statistically equal in the 1st and 2nd regimes, maintaining efficiencies of close to 96%. It is noteworthy that the influent O&G concentrations were statistically similar to avoid any relationship between these and the removal efficiencies obtained (Table 1).

During the study period, there were five obstructions in the outlet pipe of the Control bioreactor. These obstructions were caused by the buildup of solids (scum) in the three-phase separator. When each obstruction occurred, the top cover of the bioreactor was disconnected to remove the scum. Although the Test bioreactor did not become obstructed because the presence of solid material on its surface was less frequent, the same cleaning procedure was also applied to this reactor. The removed material was analyzed in terms of O&G and total solids. The O&G concentration and the percentage of O&G in the dry residue removed at each cleaning procedure are presented in Table 1 and Figure 1A, respectively. The accumulation of fatty material in the Control bioreactor was at least 4.6 times greater than in the Test bioreactor (Figure 1A). Other authors report sludge flotation as one of the most frequent operational problems in wastewater treatment with high fat content (Miranda et al., 2005; Jeganathan et al., 2006). The amount of O&G adhered to the biomass was also determined after each operation regime (Figure 1B). There was an increase of fatty material adhered to the anaerobic sludge granules in both bioreactors. However, in the bioreactor fed with pretreated wastewater, accumulation was much lower, with 4.4% in the 1st regime and 7.8% in the 2nd regime. In the Control bioreactor, the average percentage of O&G accumulated in the sludge was 18.2% and 47.2% in the 1st and 2nd regimes, respectively.

In the 1st regime, the average biogas production in the Control bioreactor was 436.3 mL/d (STP). In the 2nd regime, biogas production increased to 709.5 mL/d (STP) until the 95th day of operation, and subsequently was reduced to 143.9 mL/d (STP) (Table 1). This sharp decline in biogas production can be associated with accumulation of scum on the surface of the reactor, which made release of the produced biogas difficult, and fat accumulation in the biomass, which reduce the rates of mass transfer (organic matter - anaerobic sludge - biogas). In the Test bioreactor, the biogas
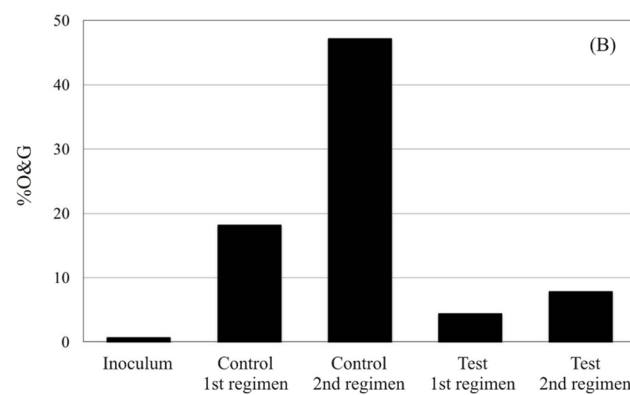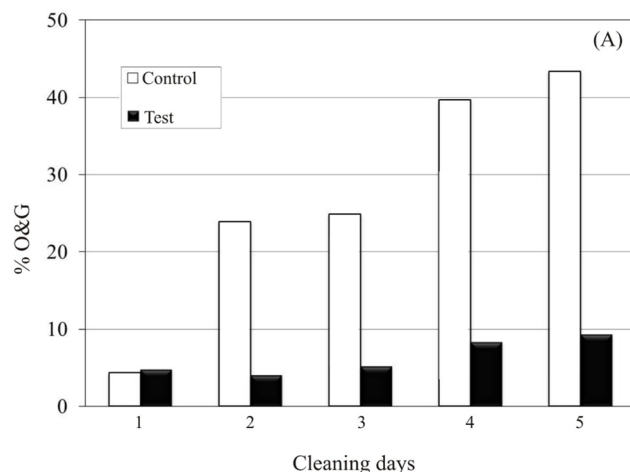

Figure 1. Accumulation of O&G (%, dry basis) in the scum (A) and in the anaerobic sludge (B) of Control and Test reactors. At the end of each regime, the three phase separators were cleaned and samples were collected from the scum to establish the O&G percentage. Sludge samples were also collected at different heights of the bioreactors to determine the percentage of O&G that adhered to the biomass.

production was almost constant, 598.5 and 662.2 mL/d (STP) in the 1st and 2nd regimes, respectively, confirming the stability of biogas production under different organic loads.

The average specific methane production (SMP) was calculated for each regime in the two bioreactors through graphs of cumulative biogas production versus cumulative removed COD. Both bioreactors showed values well below the theoretical limits reported (350 mL CH_4 (STP)/ g COD removed). The values obtained in the Test bioreactor were higher than those obtained in the Control bioreactor in the 1st and 2nd regimes (Table 1). One hypothesis is that the HRT of UASB reactors (12 to 20 h) was not sufficient to permit complete assimilation of the adsorbed COD, which resulted in high removed COD values and low methane production, leading to a lower SMP.

At the end of each regime, the biomass within the bioreactors was evaluated and quantified as volatile suspended solids (VSS) (Table 1). The initial

concentration of sludge in the reactor was 14.7 g VSS/L. In the 1st regime, the biomass concentration increased by 37.4% and 61.2% in the Control and Test bioreactors, respectively. In the 2nd regime, the biomass concentration decreased in both bioreactors. This decrease was significant in the Control bioreactor due to substantial formation of scum and sludge flotation with subsequent loss of biomass in the bioreactor output. In the Test bioreactor, the loss of biomass from the 1st to the 2nd regime was low, remaining within the range of optimal concentration recommended for UASB reactors (20 - 40 g VSS/L) (Stronach et al., 1986). Some authors have reported that wastewaters with high fat and protein contents are not appropriate for granulation of the biomass in UASB reactors. Fat can impair the reactor performance, envolving the granule, which decreases the mass transfer and causes washing of cells or inhibition of the activity (Alves et al., 2009). The anaerobic sludge structure could be important for mineralization of long-chain fatty acids (LCFA), and suspended sludge could be more advantageous over granular sludge due to its higher capacity of LCFA adsorption and degradation (Pereira et al. 2002; 2004). However, other authors state that suspended and flocculent sludges, which have a higher specific surface area, could suffer much more inhibition than granular sludges (Hwu, 1997). That author recommended the use of granular sludges as appropriate inocula for reactors treating lipid (fats, oils, and greases) wastewaters, to decrease the toxic impact of their hydrolysis products-LCFA. Disintegration of the granules of sludge was not observed in either bioreactors of the present study, but some authors state that at neutral pH LCFA act as surfactants, lowering the surface tension of the medium and causing sloughing-off from granular sludge and the selective washout of the acetogenic microorganisms (Alves et al., 2009).

In the bioreactor fed with the pretreated wastewater, the addition of biosurfactant and enzymes improved fat assimilation by microorganisms, allowing the VSS concentration to remain high. The statistical analysis conducted with average values obtained from evaluating both UASB reactors (Table 1) found significant differences that were always favorable to the operation of the Test bioreactor for COD removal, O&G removal, O&G in the scum, and percentage of methane in the 1st and 2nd regimes. These results demonstrate the benefit of the biosurfactant and enzyme consortia pretreatment as a preliminary step for the treatment of wastewaters containing high fat content in UASB reactors.

Techno-economic evaluation

A cost analysis was carried out for two process scenarios. The first process scenario was related to the conventional treatment of wastewaters from poultry slaughterhouses, similar to that adopted by the industry that provided the wastewater used in this study (Figure 2A). The usual wastewater treatment was divided into four operations: equalization, chemically assisted (ferric chloride and anionic flocculant) dissolved air flotation (DAF), tri-decanter (to separate the float, which is bagged in 20 liter plastic bags and destined for the landfill), and anaerobic reactor (which receives the effluent streams of the FAD and tri-decanter).

In the second process scenario, the plant was set up to implement the alternative technology proposed in this study (addition of biosurfactant and enzyme consortia) employing the optimum conditions found (Figure 2B). The duration of each batch cycle was 4 h. This was achieved by assuming the simultaneous operation of three identical equalization tanks. In each batch cycle, one tank is always loaded with wastewater, enzyme consortia and biosurfactant, one tank is always treating wastewater, and the third tank is always uploading the treated wastewater. Three equalization tanks are required in order to ensure a continuous flow of treated wastewater at the same flow rate (31.25 m³/h). The total number of batch cycles will be 1872 batches per year.

Table 2 presents the cost of the installed equipment, the fixed capital investments, the working capital, and total capital investment for implementation of the conventional and the proposed wastewater pretreatment plants. The fixed capital investment for the proposed process was about 60% lower than the conventional plant, representing a cheaper capital investment. The higher total installed equipment cost of the conventional plant is due to the equipments (dissolved air flotation; tri-decanter centrifuge; and mixing tank) used to remove insoluble material in this type of wastewater treatment, especially in the form of fats (Table 2). The fatty materials can cause operational problems in biological treatment systems when they are not removed or properly pretreated. However, with appropriate technology for solubilization and hydrolysis, microbial communities can effectively convert lipids into methane, which can be used as an alternative energy source (Alves et al., 2009). The energy efficiency of the anaerobic processes depends on the organic matter in the wastewater and can be especially high when dealing with lipids and long-chain fatty acids (LCFA), which are often found in industrial
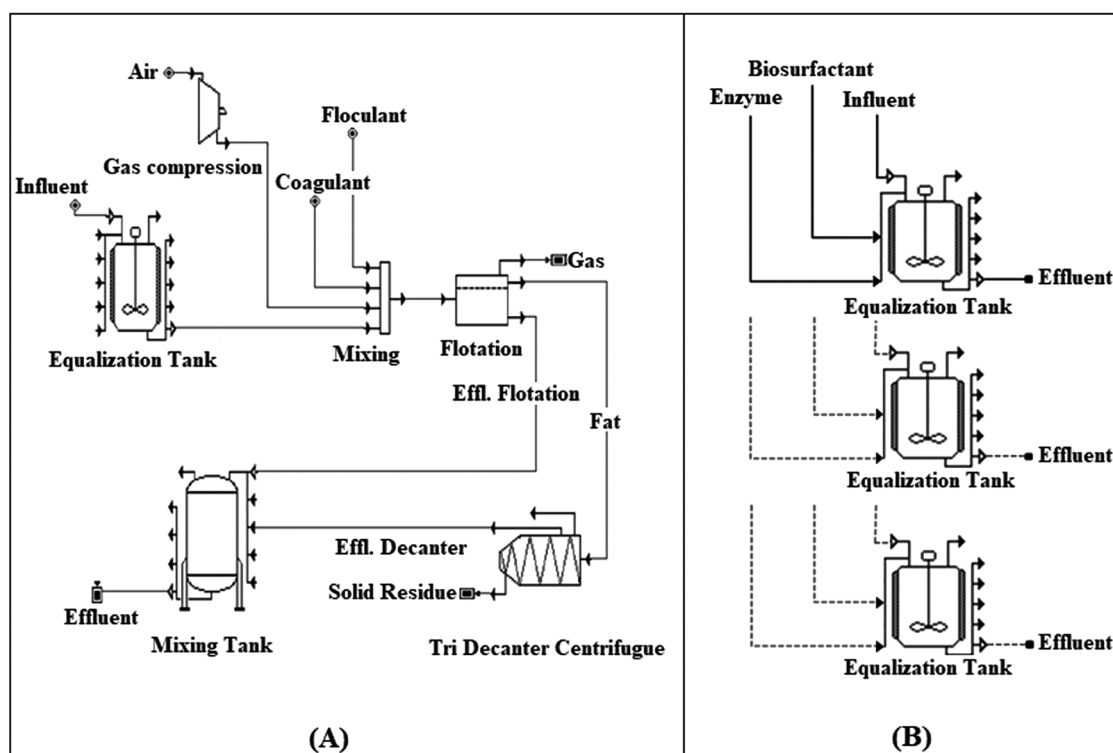
Figure 2. Flow chart for the treatment of wastewaters from poultry processing using the conventional technology (A), and the alternative technology proposed in this study (B). In the conventional technology, the treatment is divided into four operations: equalization, chemically assisted dissolved air flotation (DAF), tri-decanter, and anaerobic reactor (mixing tank). In the alternative technology, three equalization tanks are required in order to ensure a continuous flow of treated wastewater.

effluents as the major hydrolysis product (Sousa et al., 2007). Their removal by physical-chemical processes before the anaerobic treatment is a common practice. However, LCFA have high energy potential and could be used to generate profits.

Table 3 shows the operating costs for the conventional and the proposed processing scenarios. The costs related to labor requirements, fixed capital related expenses, disposal of solid waste, and utilities are higher for the conventional technology. However, the alternative plant requires a greater expense with raw material than the conventional plant. Overall, the operating cost of the novel treatment plant is 6% lower than the operating cost of the conventional plant. The waste disposal cost (Table 3) refers to the cost of disposal in landfill. This solid residue is composed practically of fats, separated from the effluent by physicochemical process, which could be converted into methane, a source of bioenergy. Its use would lower operating cost by substituting some of the electricity supplied by the grid with cogeneration from the methane produced. Depending on the method used in industrial plants, O&G removals from 50% to 99% (Nardi et al., 2008; Del Nery et al., 2007; Del Nery et al., 2013) can be obtained. Considering the flow and

O&G concentration in the effluent of the industry in question (31.25 m^3/h and 1200 mg/L) and an average removal of 75%, about 675 kg/d of fatty material could be removed from DAF. Based on the theoretical potential of methane production of the lipids - 0.99 L CH_4/g (Alves et al., 2009), up to 668 m^3 CH_4/d could be generated from this residue.

In the proposed process, the cost of solid waste disposal is zero, since the fatty material is solubilized by the addition of biosurfactant and can thus be hydrolyzed by the action of enzymes. This, however, contributes to a higher expenditure for raw materials that is approximately 41.4% of the operating cost of the flow sheet for the proposed process. The use of this alternative technology also enables an increase in biogas production and longer maintenance of the secondary treatment unit that receives the pretreated effluent, because all the fatty materials are pre-hydrolyzed, which will facilitate action of anaerobic microorganisms, thereby reducing the accumulation of such material and operational problems.

For a plant treating 234,000 m^3 of wastewater per year, the cost of treatment per m^3 of treated effluent will be 3.07 US$/$m^3$ for the conventional process and 2.88 US$/$m^3$ for the novel process. This means that

Table 2. Estimation of fixed capital investment for the conventional and the proposed processes for pretreatment of poultry slaughterhouse wastewater.

Equipment	Quantity	Volume/capacity	Material	Fob Cost (US$)[a]	Bare Module Factor	Installed Equipment Cost (US$)
Conventional Technology						
Equalization tank	1	30 m³	Concrete	4,709.00	4.07	19,165.63
Dissolved air flotation	1	30-35 m³/h	Fiberglass	55,310.00	4.07	225,111.70
Tri-Decanter centrifuge	1	10-15 m³/h	Stainless steel	64,962.00	2.60	168,901.20
Mixing tank	1	30 m³	Fiberglass	2,784.00	4.07	11,330.88
Subtotal						424,509.41
Fixed Capital Investment (installed equipment * 1.2)						509,411.29
Working Capital						12,051.50
Total Capital Investment						**521,462.79**
Alternative Technology						
Equalization tank	3	158 m³	Concrete	13,686.00	4.07	55,702.02
Subtotal						167,106.06
Fixed Capital Investment (installed equipment * 1.2)						200,527.27
Working Capital						151,867.50
Total Capital Investment						**352,394.77**

[a]Estimate of specialized companies, except for the Equalization tank whose cost was calculated using the Brazilian technical standards (ABNT, 1993; 2004) and procedures described in Peter et al. (2003).

Table 3. Annual operating cost (US$) required for wastewater pretreatment employing the conventional and the proposed process flowsheets.

Item	Operating Cost (US$)	
	Conventional Technology	Alternative Technology
Raw material (C_{RM})	42,756	604,204
Labor (C_{OL})	16,350	9,810
Treatment / disposal of waste (C_{WT})	297,229	0
Utilities (C_{UT})	217,851	427
Annual operating cost (C_{OM})[a]	717,838	675,088

[a]calculated using Equation 2.

the process proposed in this study could replace the conventional process as it requires both less capital investment and operating cost per m³ of treated effluent (6.0 % less operating cost).

CONCLUSIONS

This study showed a technically and economically viable alternative process to pretreat poultry slaughterhouse wastewater. The monitoring of UASB reactors proved the technical feasibility of pretreatment using enzymes and rhamnolipid for 4 h at 30ºC, to obtain higher removal rates of COD and O&G as well as higher methane production without frequent operational problems. The economic analysis of the novel plant (without considering methane production) revealed that this alternative technology to pretreat wastewater with high content of fat has lower installation and operational costs than the traditional technology (using floaters/chemicals) with rates of 60% and 6%, respectively.

NOMENCLATURE

Acronyms
CH_4 - methane
CMC - critical micelle concentration (mg/L)
COD - chemical oxygen demand (mg/L)
DAF - dissolved air flotation
HRT - hydraulic retention time (h)
LCFA - long-chain fatty acids
O&G - oil and grease

OLR - organic loading rate (kg COD/m^3.d)

SMA - specific methanogenic activity (g COD_{CH4}/ gVSS.d)

SMP - specific methane production (mL CH_4 (STP)/ g COD removed)

SEP - solid enzymatic preparation

STP - standard temperature and pressure

UASB - upflow anaerobic sludge blanket

VFA - volatile fatty acids concentration (mg Acetid acid/L)

VSS - volatile suspended solids (mg/L)

Symbols

C_{BM} - installed equipment cost

C_{OL} - cost of labor (operating labor and direct supervision)

C_p - cost to purchase equipment

C_{RM} - cost of raw materials

C_{UT} - cost of utilities

C_{WT} - cost of waste treatment

F_{BM} - Bare module factor

F_{CI} - cost of supplies, laboratory charges and fixed capital related costs (equipment maintenance and depreciation)

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