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Journal:	*Environmental Engineering Science*
Manuscript ID:	EES-05-0048
Manuscript Type:	Original Article
Date Submitted by the Author:	18-Aug-2005
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Keyword:	anaerobic processes < Biological treatment processe, Reactor kinetics and design < Biological treatment processe

Evaluation of an UASB reactor under different operational conditions used to treat wastewaters from pulp and paper plants

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Key words: anaerobic process; black liquor; UASB reactor; pulp and paper wastewaters; wastewater treatment.

Running Title: UASB under several operational conditions

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ABSTRACT

The main purpose of this study was to evaluate the performance of an UASB reactor used to treat wastewater from an unbleached pulp plant under different operational conditions, including partial recycling of the effluent**.** The reactor's performance was evaluated from the standpoint of COD, pH, volatile acid concentration, alkalinity, concentration of methane in the biogas, and microbiological examinations of the sludge. Without recirculation the reduction of the HRT from 36 h to 30 h did not significantly affect the average COD removal efficiency. The parameter displaying the greatest variation was the average concentration of effluent volatile acids, which rose by 15.6%. With recirculation the reduction of the HRT from 30 h to 24 h increased the average COD removal efficiency from 75% to 78%. In this case, the average effluent alkalinity also showed an increase. During the course of the experiment, the predominating morphologies remained unaltered. The use of partial recirculation of effluent did not improve significantly the COD removal under the operational conditions tested in this work, however it was possible to lower the hydraulic retention time.

Key words: anaerobic process; black liquor; UASB reactor; pulp and paper wastewaters; wastewater treatment.

INTRODUCTION

The pulp and paper industry is responsible for large discharges of highly polluted effluents, whose main characteristics are their high toxicity and low biodegradability of their tannins, lignins, resins and chlorophenolic compounds. The composition of these effluents, which has a great influence on its treatability, may vary considerably, depending on the raw material and manufacturing process utilized (Sierra-Alvarez, 1990; Kortekaas *et al.*, 1998; Vidal *et al.*, 2001; Thompson *et al.*, 2001). It should also be mentioned that the liquors from alkaline pulping (Kortekaas *et al.*, 1998), wood resins (McCarthy *et al.*, 1990; Sierra-Alvarez, 1990) and tannins (Sierra-Alvarez, 1990) are potentially toxic to methanogenic Archaea. Moreover, the degradation of lignin by an anaerobic consortium is limited to the low molecular weight fraction (Sierra-Alvarez, 1990; Kortekaas *et al.*, 1998; Vidal *et al.*, 2001). At present aerobic treatment systems are the most used method to treat effluents from pulp plants. However due to lower operational costs and its potential to degrade some recalcitrant compounds, the anaerobic treatment process is an alternative that must be considered.

The anaerobic process has been successfully applied in the treatment of nontoxic and easily biodegradable wastewaters from pulp and paper plants, such as the effluents from mechanical pulping, from paper recycling and from evaporator condensates. However, the high toxicity of the effluents from chemical, semi-chemical and chemo-thermomechanical pulping have restricted the application of the anaerobic process for the treatment of these effluents (Kortekaas *et al.*, 1998).

The advances in the application of UASB (Upflow Anaerobic Sludge Blanket) reactors in the treatment of industrial wastewaters and domestic sewage, allied to exhaustive research on the system, have consolidated the technology (Lettinga *et al.*, 1991). Nonetheless, the scientific and technological studies, developed over the years, have played a crucial role in identifying the negative points of this system and the adverse conditions for its practical application. As the result of this research it is known that the use of UASB reactors in the treatment of complex effluents often requires long hydraulic retention times (Speece, 1996). In UASB reactors the upward velocity is very low, hindering the mass transfer processes. Campos and Anderson (1992) found that the upward velocity exerts a considerable effect on the development of sludge in UASB reactors, acting as a selector in the development of the biomass. An increase in the flow velocity, that may improve the reactor performance, can be achieved by partial recirculation of the effluent.

The main objective of the work described here was to assess the performance an UASB reactor used for the removal of organic matter from diluted black liquor using different operational conditions, including partial recirculation of the effluent to increase the upward velocity.

According to Mansilla *et al.* (1993), black liquor can closely represent the constituent of the effluent of a Kraft pulping plant. Black liquor is a by-product generated in the decomposition of the constituents of wood during the chemical pulping process. In a later stage, this liquor is concentrated by evaporation and then burned in order to recover energy and inorganic compounds for reuse in the industrial process. It must be kept in mind, however, that part of the liquor is released in the wastewater as the pulp is washed.

METHODS

Reactor design

A UASB-type reactor with a total volume of 15.0 *l* was used. This reactor was made of plexiglass and the gas-solid-liquid separator of ANSI 316 stainless steel. The development of the sludge blanket occurs in the reaction zone, which is 45 cm high with a circular section of 15 cm in diameter. The gas-solid-liquid separator is located 60 cm from the reactor's entrance (Figure 1).

The reactor, kept in a chamber acclimatized at a temperature of 30±3°C to prevent any possible interference caused by variations in temperature, was fed using a dosing pump (Prominent, model Gamma G/4b) with a maximum outflow of 2.3 l h^{-1}. A similar pump was used for recirculating the effluent.

Inoculum

The reactor was inoculated with granulated sludge from a UASB reactor treating effluent from a poultry slaughterhouse (Avícola Dacar S.A., Tietê, state of São Paulo, Brazil). The amount of sludge added to the reactor was 4.5 l, i.e., about one third of the total volume.

Wastewater characteristics

Black liquor has been used by other researchers as the basis for the composition of synthetic wastewater and has proved suitable for bench scale studies on the treatment of effluent from integrated pulp and paper plants or simply from cellulose pulp plants (Sierra-Alvarez, 1990; Springer *et al.*, 1996; Buzzini and Pires, 2002). Because it contains the chemical reagents used in the process and the by-products generated in the decomposition of the constituents of wood, black liquor can satisfactorily simulate this type of effluent. Its use is also very convenient for fundamental studies since the synthetic wastewater can be prepared within a very narrow range of variability, thus eliminating one cause of uncertainty in these experiments, namely variations in input load. Yeast extract (120 mg.l $^{-1}$), ammonium chloride (70 mg.l $^{-1}$), monobasic sodium phosphate (35 mg.l $^{-1}$), and ethanol (0.025%) were added to the diluted black liquor. These compounds were used to supply the amounts of nitrogen and phosphorus recommended for the process. Ethanol was used as an additional source of carbon. Table 1 lists the main characteristics of the wastewater used in this study.

It should be noted that not all the micronutrients specifically required for the growth of anaerobic microorganisms were added because this study aimed to keep the effluent as similar as possible to the wastewater from the Kraft cellulose pulping plant. Acetic acid (0.01% final concentration) was added to the diluted black liquor to lower the pH to a suitable value for the anaerobic process.

Reactor operation

Operation of the reactor began with the effluent described in Table 1, with a mean COD (Chemical Oxygen Demand) of 800 mg l $^{-1}$. The reactor was batch operated from day 1 to day 4, after which it was continually operated at a 36 h hydraulic retention time. After 25 days of operation, the mean COD was increased to 1,000 mg l $^{-1}$ and, 10 days later, to 1,200 mg l $^{-1}$. From day 56 on, the COD was increased to 1,400 mg l $^{-1}$ and then, on day 75, the hydraulic retention time was reduced to 30 h.

The changes in organic loading and hydraulic retention time were made according to the reactor's performance in response to the COD removal efficiencies and the other monitored parameters (Table 2). The partial recirculation of the effluent began on the 90th day of operation. Table 2 lists the recirculation rates used.

Analytical methods

The COD, pH, volatile acid concentration and alkalinity were determined according to the methodology described in the Standard Methods (APHA, 1998). The composition of the biogas was monitored by gas chromatography, using a Gow Mac chromatographer equipped with a thermal conductivity detector (TCD) and a Porapak Q (2 m X ¼" – 80 a 100 mesh) column. Hydrogen was used as a carrier gas at 60 ml min^{-1}.

The volatile acid composition was assessed by gas chromatography, using a HP 6890/FID chromatographer (flame ionization). A HP Innowax column was employed (30 m X 0.25 mm X 0.25µm film thickness). Hydrogen was used as a carrier gas at 2.0 ml min^{-1}. All the physicochemical analyses were carried out in duplicate.

Microscopic examination

The development of the anaerobic consortium was evaluated by optical microscopic examination of phase contrast and fluorescence and by scanning electron microscopy (SEM). The optical microscopy was performed with an Olympus model BH2 microscope and the SEM analysis was performed using a digital Zeiss DSM-960 scanning electron microscope.

Statistical analyses

Analysis of variance (ANOVA) was applied to the data obtained from the different operational conditions in order to assess whether the applied changes in the hydrodynamics of the reactor caused significant effects on the performance of the COD removal efficiency. The analyses were done using Microsoft Excel.

Fluctuations in the COD removal efficiency were evaluated based on the coefficient of variability (CV), which is the standard deviation expressed as a percentage of the mean.

RESULTS AND DISCUSSION

Figure 2 and Table 3 show the variation in influent and effluent COD and the COD removal efficiency. Figures 3, 4 and 5 illustrate the variation in methane concentration in the biogas, the organic loading rate (OLR), alkalinity and volatile acid concentration between day 4 and 180 of continuous operation.

Between the 5^{th} and 25^{th} day, the COD effluent and methane concentration in the biogas remained relatively constant, while the COD removal efficiency remained between 83% and 88% (Figure 2). The alkalinity and volatile acid concentration also remained stable (Figures 4 and 5).

The influent COD was gradually increased from day 26 on, i.e., the hydraulic loading rate (HLR) was kept constant and the organic loading rate (OLR) was increased. The COD removal efficiency dropped from 87% to 83% from day 26 to day 36, and the methane concentration in the biogas remained in the range of 33.1 $\mu mol\ ml^{-1}$ and 38.2 $\mu mol\ ml^{-1}$.

The influent COD was increased to 1,200 mg l^{-1} between the 37th and 55th days. As can be seen (Figure 2), the influent COD increased and the removal efficiency dropped from 85% to 78%. The methane concentration in the biogas diminished to 30.7 µmol ml^{-1} and then gradually rose to 39.1 µmol ml^{-1}. From the 56th day of operation on, the influent COD was increased to 1,400 mg l^{-1} and the average COD removal efficiency was 81±1%. The concentration of methane in the biogas displayed a behavior similar to that of the previous period (Figure 3).

From days 75 to 89 the influent COD was kept constant (1,400 mg l^{-1}) and the HRT was reduced from 36 h to 30 h, which resulted in an increase in hydraulic loading rate (HLR) from 0.67 d^{-1} to 0.80 d^{-1} and an increase from 0.93 kg_{COD} d^{-1} m^{-3} to 1.12 kg_{COD} d^{-1} m^{-3} in the organic loading rate (OLR). The statistical analyses showed that there is no difference between the COD removal efficiencies when the reactor operated with HRT of 36 h (period IV) and 30 h (period V), as seen in Table 3 and Table 4 (line 1).

As can be seen (Figure 4), the effluent alkalinity remained higher than the influent, indicating that the anaerobic metabolism generated alkalinity. The concentration of volatile acids in the effluent was lower than in the influent (Figure 5), even with the increased loading rate. The influent volatile acid concentration rose as the OLR increased owing to the higher concentration of black liquor and the consequent need to increase the dosage of acetic acid to adjust the pH suitable for the anaerobic process. The influent pH was kept within 7.1 and 7.6 during the 180 days of operation.

The analysis of variance showed that there were significant differences (α=0.05) for COD removal efficiencies when the OLR increased from 0.53 kg_{COD} day^{-1} m^{-3} (period I) to 0.93 kg_{COD} day^{-1} m^{-3} (period IV) as shown in Table 4 (line 2), since the obtained F is greater than the F_{crit} and the P_{value} is less than alpha. The COD removal efficiency was found to decrease approximately six percentage points when the OLR was increased from 0.53 kg_{COD} day^{-1} m^{-3} to 1.12 kg_{COD} day^{-1} m^{-3} (close to 110% increase). This result can be considered satisfactory, considering that the inoculum was not preadapted to the wastewater and that black liquor is considered potentially toxic for methanogenic Archaea.

Grover *et al.* (1999) reported higher loadings but with similar results. These authors found that increasing the concentration of black liquor caused the COD removal efficiency to decrease. Their reactor became unstable at high loading rates (6.0 kg_{COD} day^{-1} m^{-3}), leading to wash out of the biomass. At a COD of 4,000 mg l^{-1} and HRT of 48 h, the removal efficiency remained at about 68% and the percentage of methane in the biogas was about 59%. It should be kept in mind, however, that a preadapted inoculum was used, the black liquor derived from a different source (cereal chaff) and that those conditions were held for a very short period (10 days). The results obtained by the aforementioned authors must be evaluated with caution for, after a longer operating period, the system could have become unstable. Ali and Sreekrishnam (2000) confirmed the inhibition imposed on methanogenic Archaea by black liquor, and found that the addition of glucose (a co-substrate) significantly increased the production of methane (from 33% to 80%) and the COD removal efficiency (from 43% to 71%). One must take into account that these results were achieved in batch reactors and that the black liquor came from a mixture of bagasse, rice and wheat residues.

The increase in the OLR from 0.53 kg_{COD} day^{-1} m^{-3} to 1.12 kg_{COD} day^{-1} m^{-3} (Periods I and IV) changed significantly (ANOVA with α=0.05) the effluent volatile acids concentration (F=23.69; F_{crit}=5.32; P_{value}=0.001), from 33 mg l^{-1} (Period I) to 27 mg l^{-1} (Period IV).

Partial recirculation of the effluent began on the 90th day of operation. With the beginning of recirculation, the mean COD removal rate dropped from 81±1 % to 75±2 %. The analysis of variance (Table 4, line 3) showed that there were significant differences (α=0.05) for COD removal efficiencies with recirculation (period VI) and without (period V) recirculation for the same HRT of 30 h. However, as seen in Figure 2, after a gradual drop the COD removal efficiency recovered and stabilized as the experiments proceeded and the recirculation rate rose. The alkalinity and volatile acid concentration of the effluent remained relatively constant with the increased recirculation rate, Table 5.

From days 112 and 133 (Period VII) the recirculation flow rate increased 50% while the HRT was kept at 30 h. For this operational condition the COD removal efficiency remained in the 75% to 78% range, a difference that although small was statistically significant as demonstrated by ANOVA (Table 4, line 4).

Starting on the 134th day (Period VIII), the recirculation/feed rate was kept at 0.6, which for comparison purposes is close to the 0.5 used during Period VI, while the HRT was reduced from 30 h to 24 h. With these new operational conditions the average COD removal efficiency increased from 75% to 78%, a difference that although small was statistically significant as demonstrated by the ANOVA (Table 4, line 5).

The recirculation rate was again increased on the 156th day of operation (Period IX), while the HRT of 24 h was maintained. The COD removal efficiency showed a slight reduction at first, but then stabilized. Although the statistical analysis indicates that the COD removal efficiency changed significantly when the recirculation flow rate increased (Periods VIII and IX, Table 4, line 6), this difference is not meaningful from an operational point of view as it is too small to have an influence downstream of the UASB reactor. The average effluent volatile acids concentration increased from 30±1 mg l^{-1} to 32±1 mg l^{-1} (Table 6, Periods VIII and XIX). Although small the ANOVA confirmed that this is a significant difference (α=0.05): F=7.76; F_{crit}=4.96; P_{value}=0.019. The remaining parameters continued stable (Figs. 3 to 5).

Table 3 presents the average values, the standard deviation and the coefficient of variation (CV) of the COD removal efficiency for each experimental period. The coefficient of variation remained within 0.5% to 3.7%, an indication that the fluctuation of the reactor performance was limited and its behavior controlled. It should be pointed out that even though the number of experimental points for each operational condition is small the whole experimental set shows the same behavior and this conclusion would not change as the resulting CV is close to 1.9%. Tables 5 and 6 show the average values monitored between days 1 and 89 (without recirculation) and days 90 and 180 (with recirculation) for all the parameters presented in this paper.

It should be pointed out that recirculation increases upward velocity and improves mixing conditions, which should induce an increase in efficiency, but on the other hand may favor the concentration of recalcitrant compounds in the reactor's base, that is, in the sludge blanket. According to Speece (1996) the recycle may have an adverse effect on system performance because it reduces the effective influent COD concentration and promotes the recirculation of short-chain fatty acids and COD that leak through the sludge bed.

In the experiments reported in this paper, the system's removal efficiency was not highly changed by recycle. It is also important to note the effect of the substrate's characteristics; black liquor, for instance, possesses wood-derived compounds that are not easily biodegraded. Higher concentration of these compounds may cause not only the effluent COD to increase but also results in greater toxicity to methanogenic Archaea in the long run. However in the case of less complex effluents, the reactor may display a significantly different behavior.

Due to the low biodegradability of the wood derivatives the COD removal efficiency did not increased in the operational conditions tested in this work, although the partial effluent recirculation improved mixing between the substrate and the microorganisms. This result is an indication that with wastewater of this kind the mass transfer may not be a limiting factor.

Another issue that needs attention in the treatment of toxic wastewaters is the loss of solids and the resulting solids retention time. At the beginning of the operation a common occurrence was the flotation of granules. As the experiment proceeded, with better adaptation of the microorganisms, this loss diminished and the solids retention time (SRT) increased by 37% between periods I and IV (Table 2). The decrease in HRT from 36 h to 30 h (17%) resulted in a SRT decrease close to 24%, probably due to the increase in the flow velocity (around 20%) that intensifies the drag of solids to the outflow.

With the beginning of the recirculation (period VI) the solids retention time decreased from 53 days to 48 days, a 9% decrease. Again, this was probably caused by the increase in the up flow velocity, a 50% increment, and the consequent drag of finer solids. The decrease in the HRT from 30 h to 24 h (20% decrease) also reduced the SRT by 23%. It was noted that the increase in the recirculation rate (periods VII and IX) did not influence the solids retention time in the conditions evaluated in this paper (Table 2).

Without recirculation the reduction of the HRT from 36 h to 30 h (Periods IV and V), which corresponds to a decrease of 16.7% in the treatment time, did not substantially alter the mean COD removal efficiency (Table 4, line 1) and the mean concentration of methane in the biogas (Figures 2 and 3; Tables 3 and 5). The parameter displaying the greatest variation was the average concentration of effluent volatile acids, which rose by 15.6%. The ANOVA confirmed that there is a significant difference (α=0.05) in the effluent concentration of volatile acids in periods IV and V (F=6.82; F_{crit}=5.32; P_{value}=0.031). The effluent alkalinity was not significantly changed with the decrease in HRT from 36 h to 30 h (F=1.43; F_{crit}=5.32; P_{value}=0.267).

With recirculation the isolated effect of the reduction in the hydraulic retention time is less clear, as it depends also on the recirculation/feed rate. When the recirculation/feed rate was set around 0.5 (Periods VI and VIII) the decrease in the HRT resulted in an increase in the COD removal efficiency from 75% to 78%, however when the recirculation/feed rate was set to 1.0 (Periods VII and IX) with the decrese in HRT the efficiency also decreased, from 78% to 77%. In both cases the changes were small, no more than three percentage points for a COD removal efficiency varying from 75% to 78%, although the change in the HRT represented a 20% difference. The same observation is valid when the recirculation/feed rate is considered as, from an operational point of view, doubling this parameter does not improve or hinder the COD removal efficiency. It should be pointed out that, even though small, some of the observed changes are statistically significant. However one always needs to take into consideration the usual fluctuations that appear in wastewater treatment operations and consider only the differences that may impose actual changes in the overall behavior of the treatment system being studied.

Concerning effluent alkalinity a clear increase in its value occurs when recirculation is imposed. At first its value rose from 183 mg l^{-1} (without recirculation and HRT of 30 h) to 212 mg l^{-1} (with recirculation/feed ratio of 0.5 and the same HRT), which corresponds to a

16% gain. The ANOVA confirmed that the effluent alkalinity was significantly (α=0.05) distinctive in periods V and VI (F=8.40; F_{crit}=5.11; P_{value}=0.018).

However, after the HRT was reduced to 24 h the average effluent alkalinity dropped to 207 mg l^{-1}. This value is still significantly higher than the values observed without recirculation. If the whole period of monitoring is considered, with and without recirculation, the increase in the average effluent alkalinity with recirculation is 8.1% higher than without recirculation. The ANOVA showed that there were significant differences (α=0.05) for effluent alkalinity in the periods V and VIII (F=15.33; F_{crit}=4.96; P_{value}=0.003). According to Speece (1996) effluent recycle does not reduce the alkalinity required for neutralization of carbonic acid, but it does dilute the incoming COD, thus reducing the maximum potential VFA excursion and supplying inherent alkalinity.

The increase in the influent VFA that occurred during periods VII, VIII and IX of the experiments, due to the strategy used to control the influent pH with acetic acid, was not sufficient to cause any noticeable disturbance in the reactor performance. The additional amount of acetic acid was metabolized and the effluent VFA remained within the same range observed in the previous periods.

Figure 6 indicates the composition of the volatile acids in the reactor's effluent, as determined by gas chromatography. It can be seen that, after recirculation began, the concentrations of acetic and propionic acid increased in relation to the period without recirculation, but showed no marked behavioral tendency. With recirculation an amount of the acids, proportional to the recirculation rate, returns to the reactor, instead of being discarded with the effluent, thus increasing the concentration of VFA in the reactor. The "zero" values on the graph indicate that the concentration was below the method's detection limit. The maximum concentration of acetic acid in the reactor's effluent was 27 mg l^{-1} and that of propionic acid was 7 mg l^{-1}. Elevated propionate concentrations may indicate difficulties in any one or more metabolic steps of anaerobic treatment (Speece, 1996). In this work the propionic acid concentration in the effluent can be considered low, which indicates that the anaerobic digestion reached the last phase of the process.

The concentration of acetic acid in the reactor influent varied from 52 mg.l^{-1} to 119 mg.l^{-1} and that of propionic acid varied from 10 mg l^{-1} to 33 mg l^{-1}. The remaining acids were detected in lower concentrations in the influent. The highest acid concentrations obtained in this stream were: isobutyric (8 mg l^{-1}), butyric (9 mg l^{-1}), isovaleric (9 mg l^{-1}), valeric (5 mg l^{-1}) and caproic acid (5 mg l^{-1}).

The microscopic examination of the inoculum revealed the presence of diversified cellular morphologies with a predominance of sarcina-shaped cysts and cells related to the genus *Methanosarcina* sp., and cocobacilli-shaped cells and nonfluorescent filaments related to the genus *Methanosaeta* sp. After 47 days of operation, the material showed a predominance of rods, cysts and sarcina cells related to the genus *Methanosarcina* sp. and of filaments related to the genus *Methanosaeta* sp. During the course of the experiment, the predominating morphologies remained unaltered. Microscopic examinations of the sludge indicated that the use of the partial recirculation of the effluent did not change significantly the predominant morphologies in the reactor under the tested conditions. The detailed evaluation of the microbial diversity in the UASB reactor during this experiment was studied using the denaturing gradient gel electrophoresis (DGGE) technique and is described in Buzzini *et al.* (2005).

After a few days of continuous operation, the granules began to float. The SEM analyses performed after 83 days of continuous operation revealed that the floating granules were "hollow" in the central region, and a difference was found in the cross section of the sludge blanket granules (Figure 7a) in relation to the cross section of the floating granules (Figure 7c). Figure 7b shows the surface of the granules in the reactor. A greater concentration of microorganisms was found on the surface than inside the granule.

Possibly due to the low upward velocities applied, the granules were quite dense at the bottom of the reactor. In this case, according to Kosaric *et al.* (1990), a nutrient deficiency may occur in the central region of the granule owing to densification, since the substrate can only penetrate the granule by diffusion. When the size of the granule exceeds a certain limit or when densification occurs, the concentration of substrate in the central region becomes very low, leading to starvation of the microbial population and resulting in autolysis. This is a stressful condition for the cell caused by the scarcity of substrate. The process of flotation may also be related to the age of the sludge.

With the beginning of partial recirculation of the effluent (day 90), the flotation of granules diminished considerably. Kosaric *et al.* (1990) suggested to recirculate the effluent to reduce the flotation process. Besides increasing the transfer of mass, this procedure may cause the "hollow" granules to break, releasing the gas they contain (which causes flotation) and, hence, leading to sedimentation of the remaining biomass (minimizing the wash out). After 180 days of operation, the presence of "hollow" granules in the sludge blanket and on the surface of the reactor was no longer detected in the samples evaluated (Figure 7d, f). Figure 7e shows the surface of the granule after 180 days of operation.

The cross sections of the granule from the reactor, before and after recirculation, revealed a large amount of polymeric material with crystal-like structures that suggest the presence of extra cellular or polymeric material and of filaments related to the genus *Methanosaeta* sp. The surface of the granule showed the presence of cocci and bacilli. However, it was not possible to identify significant morphological differences between the surface and that of the central region of the granule, or layered structures such as those observed by several authors (McLeod *et al*., 1990; Grotenhuis *et al*., 1991; Fang *et al*., 1994; Fang, 2000). According to these authors, easily degradable substrates show multilayered microstructures. On the other hand, substrates in which the initial degradation stage is limiting result in microbial structures with more uniform distributions. According to Fang (2000), the microbial distribution in a granule depends on the nature of the degradation, as both the thermodynamics of the reaction and their kinetics have a role in establishing which microorganisms will predominate.

CONCLUSIONS

Even though the inoculum was not preadapted to the black liquor, the mean removal efficiency during the reactor start-up, between the 5^{th} and 25^{th} day, was 86%.

Without recirculation the increase in the OLR from 0.53 kg_{COD} day^{-1} m^{-3} to 1.12 kg_{COD} day^{-1} m^{-3} led to approximately six percentage points reduction in the mean COD removal rate and an increase of 13% in the mean concentration of methane in the biogas. The average effluent volatile acids concentration decreased from 33±2 mg l^{-1} to 27±2 mg l^{-1}.

The increase in OLR from 0.93 kg_{COD} day^{-1} m^{-3} to 1.12 kg_{COD} day^{-1} m^{-3}, which represents a decrease in HRT from 36 h to 30 h, did not significantly change the average COD removal efficiency, the average methane concentration in the biogas and the effluent alkalinity. The average volatile acids concentration in the effluent increase 15.6%.

The average COD removal efficiency was 81% to 85% (without recirculation) and 75% to 78% with recirculation, but in this latter case the hydraulic retention time was 30 h and 24 h, while without recirculation the HRT was 36 h. Thus it can be considered that under the tested conditions applied in this work the partial recycle of the effluent did not improve the COD removal efficiency. However it allowed to operate the reactor with lower hydraulic retention time.

For the wastewater used in this research and under operational conditions of this work the partial recirculation of the effluent caused the average COD removal efficiency to drop from 81±1 % to 75±2 % for a HRT equal to 30 h. However when the HRT was reduced from 30 h to 24 h the average COD removal efficiency increased from 75±2 % to 78±0.4 %.

The effluent alkalinity was always higher than the influent one, even though the loading rate increased, which indicates that the anaerobic metabolism produces alkalinity. When the recirculation was imposed the average effluent alkalinity showed an increase. The concentration of volatile acids in the effluent was lower than the influent concentration in all situations.

The reactor presented stable operation conditions even when some granule flotation appeared. After 83 days of operation it was observed that the floated granules were hollow in the center, however after 180 days these hollow granules were no longer observed either in the sludge blanket or in the few granules that reached the reactor surface.

It was not possible to notice remarkable differences between the surface and the center of the granules or to identify a layered structure.

Partial recirculation of the effluent caused no alteration in the predominance of cellular morphologies in the reactor under the conditions studied.

ACKNOWLEDGEMENTS

The post-doctoral grant awarded to Dr. Andréa Paula Buzzini (Process 01/06244-1) and financing of this research (Process 01/05489-0) by FAPESP (Brazil) is gratefully acknowledged, as is Votorantim Celulose e Papel for supplying the black liquor. To the CNPq (Brazil) for the research grant awarded to Prof. Eduardo Cleto Pires.

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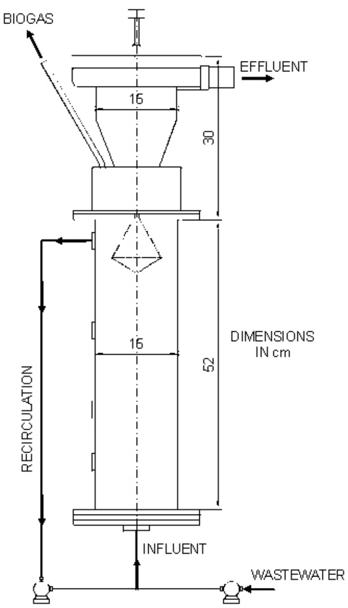

Figura 1. Diagram of the UASB reactor.

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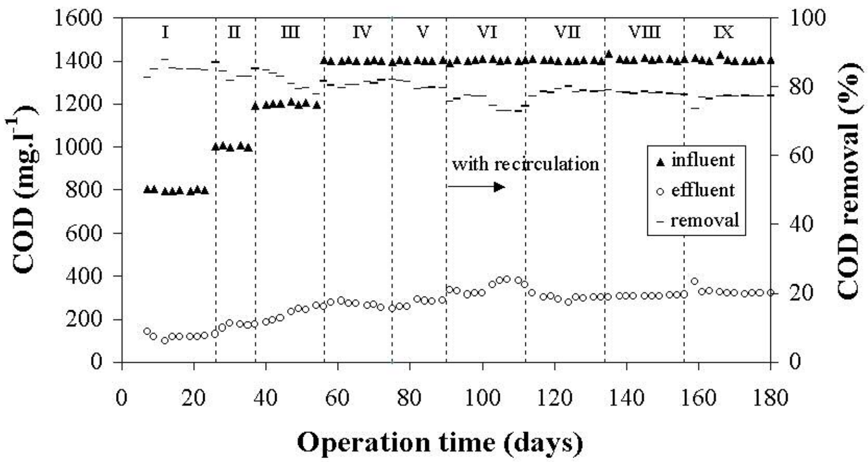

Figure 2. Variation of influent and effluent COD and COD removal efficiency. Roman numerals indicate the experimental period (Table 2).

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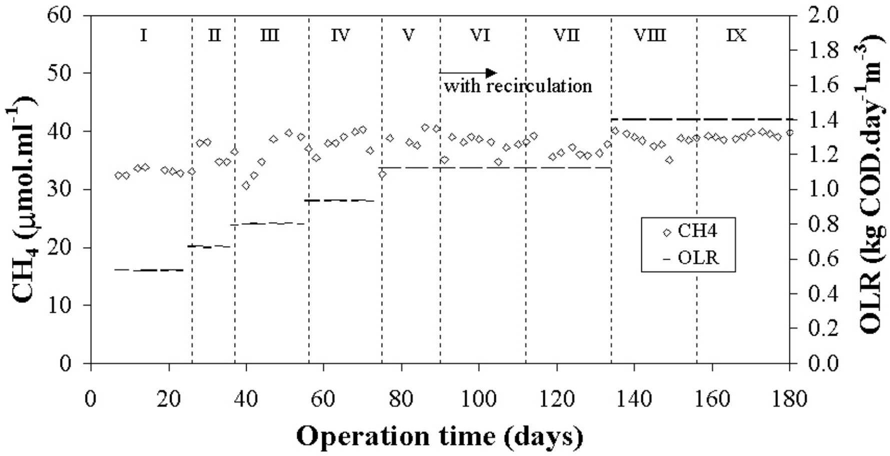

Figure 3. Variation of methane concentration and organic loading rate. Roman numerals indicate the experimental period (Table 2).

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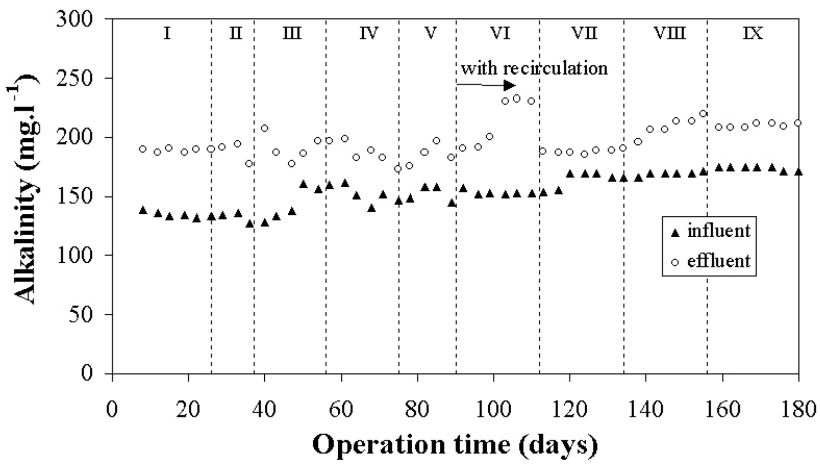

Figure 4. Variation of alkalinity influent and effluent. Roman numerals indicate the experimental period (Table 2).

254x190mm (96 x 96 DPI)

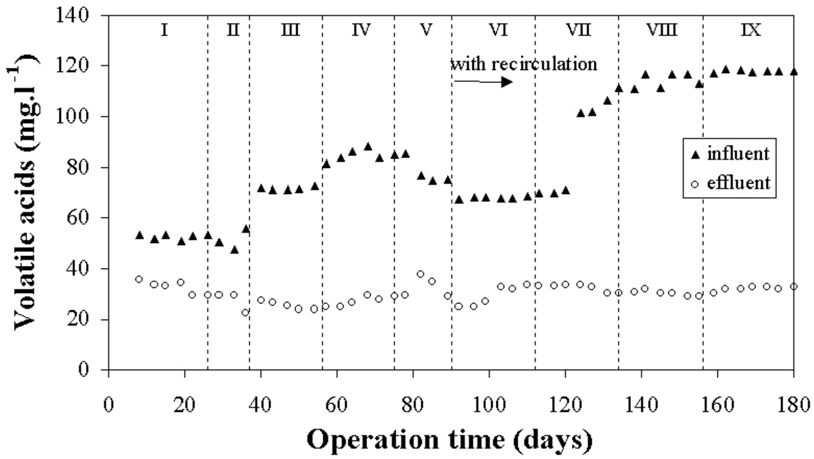

Figure 5. Variation of volatile acids concentrations influent and effluent. Roman numerals indicate the experimental period (Table 2).

254x190mm (96 x 96 DPI)

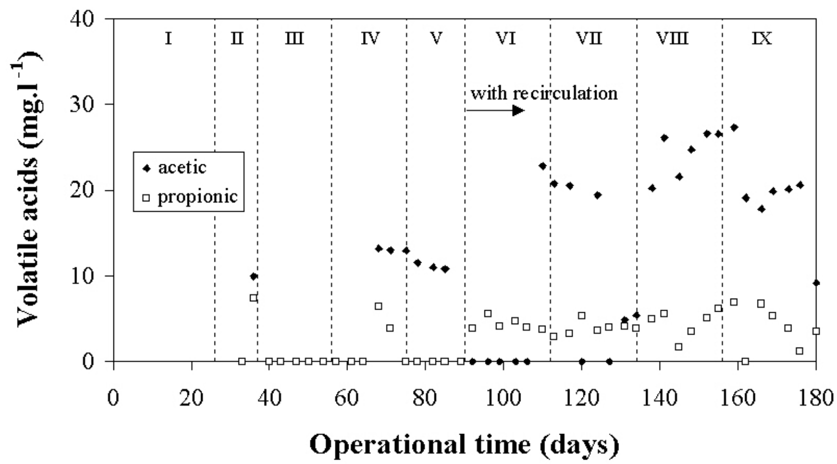

Figure 6. Composition of volatile acids concentrations in the effluent. Roman numerals indicate the experimental period (Table 2).

254x190mm (96 x 96 DPI)

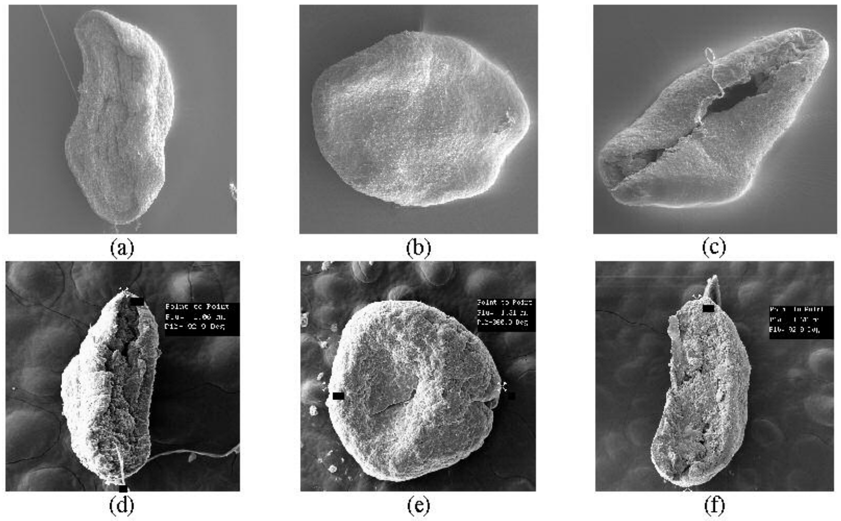

Figure 7. Photomicrografs of SEM analyses of granules after 83 days of operation: (a) cross section of reactor granule (50X); (b) reactor granule surface (40X); (c) cross section of floated granule (74X); after 180 days: (d) cross section of reactor granule (200X); (e) reactor granule surface (150X); (f) cross section of floated granule (120X).

254x190mm (96 x 96 DPI)

Table 1. Main characteristics of the synthetic wastewater

Parameter	Value
pH	6.8 – 7.2
COD (mg l^{-1})	1,400
BOD (mg l^{-1})	660
N_{total} (mg l^{-1})	24.0
sulfide (mg l^{-1})	7.0
P_{total} (mg l^{-1})	4.0

254x190mm (96 x 96 DPI)

Table 2. Hydraulic and organic loading rate, hydraulic retention time, recirculation feed ratio and sludge retention time.

	Period	Days	HLR (d^{-1})	OLR ($kg_{COD}\ d^{-1}\ m^{-3}$)	HRT (h)	R/F	SRT* (d)
	___	1 to 4	___	___	batch	___	___
without recirculation	I	5 to 25	0.67	0.53	36	___	51
without recirculation	II	26 to 36	0.67	0.67	36	___	63
without recirculation	III	37 to 55	0.67	0.80	36	___	69
without recirculation	IV	56 to 74	0.67	0.93	36	___	70
without recirculation	V	75 to 89	0.80	1.12	30	___	53
with recirculation	VI	90 to 111	0.80	1.12	30	0.5	48
with recirculation	VII	112 to 133	0.80	1.12	30	1.0	48
with recirculation	VIII	134 to 155	1.00	1.40	24	0.6	37
with recirculation	IX	156 to 180	1.00	1.40	24	1.0	37

* calculated according to Cavalcanti (2003).
HLR: hydraulic loading rate; OLR: organic loading rate; HRT: hydraulic retention time; SRT: sludge retention time; R/F : recirculation/feed ratio.

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Table 3. COD removal efficiency

Period	Days	Sample number (n)	Mean (%)	Standard deviation (%)	CV (%)	Min. (%)	Max. (%)
I	5 to 25	8	85	1	1.2	83	88
II	26 to 36	5	84	2	2.4	82	87
III	37 to 55	8	82	3	3.7	78	85
IV	56 to 74	8	81	1	1.2	80	82
V	75 to 89	7	81	1	1.2	79	82
VI	90 to 111	9	75	2	2.7	73	77
VII	112 to 133	10	78	2	2.7	74	80
VIII	134 to 155	9	78	0.4	0.5	78	79
IX	156 to 180	11	77	1	1.3	74	78

254x190mm (96 x 96 DPI)

Table 4. One-way ANOVA to assess whether operational conditions cause distinct effects on the COD removal efficiency ($\alpha = 0.05$).

Line	Experimental period	Operational condition changed COD removal efficiency (%)	F^1	F_{crit}^2	F/F_{crit}	P_{value}^3
1	IV and V	↓ HRT: 36 to 30 h 81 – 81	0.29	4.67	0.06	0.60
2	I and IV	↑ OLR 85 – 81	56.47	4.60	12.28	0.000003
3	V and VI	Recirculation 81 – 75	40.57	4.60	8.82	0.00002
4	VI and VII	↑ R/F; HRT: 30h 75 – 78	12.49	4.45	2.80	0.002
5	VI and VIII	↓ HRT: 30 to 24h 75 – 78	19.57	4.49	4.36	0.0004
6	VIII and IX	↑ R/F; HRT: 24h 78 – 77	11.06	4.41	2.51	0.0006

[1]: ratio of mean square (MS) between groups and within groups; [2]: associated with the selected value of α; [3]: probability associated with the obtained value of F.

254x190mm (96 x 96 DPI)

Table 5. Summary of results obtained before recirculation (mean ± standard deviation).

Parameter	Days				
	5 to 25 (I)	26 to 36 (II)	37 to 55 (III)	56 to 74 (IV)	75 to 89 (V)
	HRT: 36 h				HRT: 30 h
COD_i (mg l^{-1})	801±5	1,005±6	1,200±7	1,401±3	1,401±4
COD_e (mg l^{-1})	120±12	163±21	219±34	268±11	273±17
CH_4 (µmol ml^{-1})	33.0±0.6	35.8±2.2	36.0±3.3	38.0±1.7	38.0±2.9
$Alkalinity_i$ (mg l^{-1})	135±2	132±4	143±14	153±9	151±6
$Alkalinity_e$ (mg l^{-1})	189±1	188±7	191±11	190±8	183±10
VFA_i (mg l^{-1})	52±1	52±4	72±1	85±3	79±5
VFA_e (mg l^{-1})	33±2	28±4	25±1	27±2	32±4

i: influent; e: effluent

254x190mm (96 x 96 DPI)

Table 6. Summary of results obtained after recirculation (mean ± standard deviation).

Parameter	Days			
	90 to 111 (VI)	112 to 133 (VII)	134 to 155 (VIII)	156 to 180 (XIX)
	HRT: 30 h		HRT: 24 h	
	(R/F:0.5)	(R/F: 1.0)	(R/F:0.6)	(R/F: 1.0)
COD_i (mg l^{-1})	1,402±6	1,402±3	1,410±10	1,406±9
COD_e (mg l^{-1})	348±28	306±22	307±3	325±17
CH_4 (µmol ml^{-1})	37.5±1.6	37.0±1.2	38.3±1.5	39.2±0.4
$Alkalinity_i$ (mg l^{-1})	153±2	164±7	168±2	173±2
$Alkalinity_e$ (mg l^{-1})	212±21	187±1	206±9	209±4
VFA_i (mg l^{-1})	68±0.4	87±18	114±3	118±0.5
VFA_e (mg l^{-1})	29±4	33±1	30±1	32±1

i: influent; e: effluent

251x188mm (97 x 97 DPI)