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THE BEHAVIOUR OF AN ANAEROBIC BAFFLED REACTOR (ABR) AS THE FIRST STAGE IN THE BIOLOGICAL TREATMENT OF HOG FARMING EFFLUENTS

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Abstract - This present paper reports a study of the efficiency of an anaerobic Baffled Reactor (ABR) composed of three chambers working as the first stage of a biological treatment system for swine wastewater, over a period of 116 days. The average value of the volumetric organic loading rate (VOLR) was 17.8 kgCODtotal m^{-3} d^{-1}, the biological organic loading rates (BOLR) based on total and filtered COD influents of 14381 mg L^{-1} and 3610 mg L^{-1}, respectively, were: 1.3 kgCODtotal kgTVS d^{-1} and 0.98 kgCODfiltered kgTVS d^{-1}, respectively, and the hydraulic loading rate (HLR) was about 1.4 m^3 m^{-3} d^{-1}. The average removal efficiency for total COD was 80% at a hydraulic retention time (HRT) of about 18 hours. The average alkalinity in the effluent was 3801 mg$CaCO_3$ L^{-1}. The average removal efficiencies for oil and grease and total soluble solids were 41% and 78%, respectively. The sludge granulation and biogas production in the ABR were quite different between the first and third compartment, showing a distinct microbial consortium in each chamber. Through this research it was confirmed that this type of reactor can be employed as the first stage in a system treating swine wastewater.
Keywords: Wastewater; Anaerobic digestion; Methanogenic bacteria; Biogas; Methane.

INTRODUCTION

Over the past 30 years swine production has become increasingly intensive, with a larger number of animals confined. As a result, the wastes from this production system are produced in larger quantities, requiring a large amount of water for washing and resulting in a greater volume of wastewater. The swine effluents are composed of feces, urine and wash water from the confinement of animals, thus assuming a slurry form (Diesel *et al*., 2002). This type of wastewater is characterized by its high pollution potential and may reach concentrations of COD and BOD of 8057 mgO_2 L^{-1} and 2843 mgO_2 L^{-1}, respectively (Pereira *et al*., 2011), due to the high concentration of dissolved organic matter and suspended solids and also macronutrients, like organic nitrogen, ammonia, nitrites, nitrates and phosphorus (Cook *et al*., 2010). For these features the application of this sort of waste represents an additional risk to environmental resources, mainly by the excessive release of nutrients, salts and organic matter, among other pollutants; therefore, it is necessary to treat this wastewater in order to reduce

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the pollution potential. The anaerobic biodegradation of the effluent from hog farming is an alternative for treating this type of waste, due to the methane generated from the high content of organic matter digested by the biomass present in the reactor, which can supply energy input for other local activities. Other advantages of this process include: conservation of nutrients, solids degradation efficiency higher than 50%, reduction in odor and pathogen control (Angenent *et al*., 2002). The use of an anaerobic process into two stages, mainly in the presence of a high concentration of suspended solids in the influent, which may affect negatively the activity of the microbial consortium, can increase the development and maintenance of the granulated sludge in the second stage (Ferreira *et al*., 2003). In the separation of stages, the biomass of the first stage reactor is characterized by hydrolytic flocculent sludge, which partially absorb and break down the particulate organic matter in the influent into soluble compounds that are digested and absorbed in the second stage, allowing even the formation of granular sludge. According to Vossoughi *et al*. (2003), the anaerobic baffled reactor (ABR) combines the advantages of the anaerobic filter, which shows high stability and security for the subsequent treatment unit. The ABR is in the shape of a tank with several chambers arranged in series, each one separated by vertical walls, through which the liquid moves upward and downward along the reactor, allowing the wastewater to pass through regions with high concentration of active microorganisms formed in the sludge column along each chamber of the reactor (Barber and Stuckey, 1999). The hog wastewater contains a high proportion of suspended solids that resist biological degradation (Robinson *et al*., 1971). The non-biodegradable fraction of the influent becomes the major obstacle to the treatment and it is an essential part in the effluent. Studies conducted by ASAE (1990) characterized these animals diet, classifying the food according to the percentage of fixed solids, which varies from 15% to 30% of the ration. The biodegradable fraction is characterized by the fermentation of organic material resulting in the production of odors that are sources of pollution and can be a hindrance to the intensification of hog farming (Campos *et al*., 2005). Considering the fact that hydrolysis is a limiting step to anaerobic digestion of complex wastes, such as hog wastewater, anaerobic digestion has been proposed to be conducted in two stages, in which there is a separation of steps: partial hydrolysis of particulate organic compounds is carried out in the first reactor, and the stabilization of the soluble compounds resulting from the first reactor occurs in a second reactor, allowing, in this way, the production of methane gas. The ABR, studied under stationary conditions with high organic loading rate, is capable of supporting hydraulic shocks but presenting a high organic removal efficiency of organic matter, mainly at low HRT (Pereira *et al*., 2010b). Conversely, there are doubts about the performance of this reactor after shutdown of this unit for a period, and its later restart, a situation that often occurs in full scale systems due to maintenance and electricity cuts. As a result, this study describes operational parameters which ally microbiological characteristics of the biomass with the performance of the ABR, built as a pilot-scale for the first stage of a system treating hog wastewater.

MATERIAL AND METHODS

Experimental Apparatus

The ABR is one of the units that compose the system of hog wastewater treatment at the Federal University of Lavras (UFLA). This system consisted of two preliminary treatment units (solid retention box and a static sieve), installed in series before the primary treatment system. It consisted of an equalizing and acidification tank (EAT), where most of the solids were decanted. Subsequently, the effluent was directed to the secondary treatment system through a pump (brand NETZSCH, type NEMO) controlled by a frequency inverter (brand NM015BY01L06b, model WEG CFW08). The biological treatment consisted of two anaerobic units working in series; an anaerobic baffled reactor (ABR) and an upflow anaerobic sludge blanket reactor (UASB). The treated effluent was pumped to a greenhouse and used in fertirrigation experiments (Pereira *et al*., 2009; Pereira *et al*., 2010a; Pereira *et al*., 2010b; Moterani, 2010 and Pereira *et al*., 2011).

The Anaerobic Baffled Reactor - ABR

The ABR had three chambers, all operating with ascending flow. This reactor was built in solid brick masonry and sealed with fiberglass. The first compartment had a volume of 2.18 m^3, the second of 1.97 m^3 and the third of 1.91 m^3, corresponding to a total volume of 6.06 m^3 and the surface areas were 0.638 m^2, 0.787 m^2 and 0.832 m^2, respectively. The

upward flow in each compartment was equalized by triangular weirs, whose purpose was to provide a homogeneous flow rate in order to avoid dead zones and hydraulic short-circuit. The installation of the ABR prior to the UASB was expected to provide suitable conditions for the early stages of anaerobic reactions (hydrolysis and acidification), seeking thereby to accelerate and optimize the step of the methanogenic process in the UASB unit. Also, along each chamber, four valves (sampling points) were installed for monitoring the sludge profile. The first valve stood at 1.75 m from the bottom and the subsequent valves equally spaced, on a diagonal, every 0.30 m.

Monitoring of the ABR

The sludge in each compartment of the ABR was leveled up until the last sampler, at the beginning of the study. The sludge profile in the reactor compartment was evaluated to determine the volatile solids that remained during the shutdown period. After sampling, the pump system was turned on, submitting the reactor to the same hydraulic retention time (HRT), hydraulic loading rate (HLR) and volumetric organic loading rate (VOLR) as in the previously stage. The ABR was monitored by sampling the influent and effluent, using composite samples collected by mixing eight single samples of 250 mL: four samples collected in the morning and four in the afternoon. The samples were sent to the Water Analysis Laboratory of the Engineering Department and refrigerated at 4 °C. All samples were gathered in a two liter bottles to form a composite sample at the end of the day. Then physical-chemical analyses were carried out and the results presented in Table 1. The temperature and pH were measured at the time of collection. Periodically, the sludge excess was removed from the highest sampler in all ABR chambers in order to maintain a constant sludge volume inside the ABR and to allow the biological organic loading rate (BOLR) calculation. Analyses of total and filtered biochemical oxygen demand (BOD_5), total suspended solids (TSS) and oil and grease (O&G) were performed weekly according to APHA *et al*. (2005).Twice a week were analyzed: pH, total and filtered chemical oxygen demand (COD), settleable solids, total acidity and temperature according to APHA *et al*. (2005) and total alkalinity (TA), partial alkalinity (PA) and intermediated alkalinity (IA) according to Ripley *et al*. (1986) and Jenkins *et al*. (1983). Once a month were analyzed total, fixed and volatile sludge solids (APHA *et al*. (2005)), scanning electron microscopy of the sludge (Pereira *et al*., 2009; Moterani, 2010) and fungi, aerobic and facultative bacteria (Specific microbiological kits (BacTray®)).

Microscopy

For the observation of the granules composition, the biomass samples were collected in the profile along the reactor compartments and stored in sterile vials of 70 mL. Part of this sample was directed to a stereoscopic microscopy, fluorescence microscopy and scanning electron microscopy (SEM). For stereoscopic microscope the sludge was washed with distilled water leaving only the settling sludge to display. For fluorescence microscopy 20 μL of sludge were separated and placed in 1.5 mL Eppendorf vials with 100 μL of distilled water, obtaining thus a dilution of 1:5. These Eppendorf vials were homogenized and 20 μL of sample were placed on a smooth glass slide, covered with a cover slip and observed under ultraviolet light illumination at increases of 40X and 60X. No reagent or coloring was added to the samples, as described by Moterani (2010). For SEM analysis the sludge aliquots were prepared by following the methodology described by Moterani (2010), in which 0.5 mL of the sample was washed in distilled water and the granules were separated and deposited on glass slides 1 mm in diameter within Petri dishes. Subsequently, the granules were immersed in Karnosvisky solution, modified for 24 hours (drying time of the fastener). The Karnovisky solution consisted of 2.5% glutaraldehyde, 2.5% formaldehyde in 0.05 M cacodylate, buffered at pH 7.2 with 0.001 M $CaCl_2$. In a protected environment (laminar flow), 4 drops of 1% osmium tetroxide (OsO_4) solution were added and kept thus for 4 hours at room temperature. Then the samples were rinsed in distilled water and dried in an environment protected from humidity for 24 hours. After that, the samples were kept on metal stubs 12 mm in diameter and covered with gold sputtering. The samples were observed in a LEO scanning electron microscope, model EVO 40 and analyzed by LEOUIF software.

Microbiology *Fungi*

For microscopic analysis of fungi, the samples were collected by composite sample from each sampler of the ABR. The corresponding sludge volume of 5 mL was collected in sterile plastic bottles of 70 mL. The aliquots were sent to the

laboratory of Agricultural Research Company of Minas Gerais (EPAMIG) on the campus of UFLA, which were prepared for inoculation and cultivation. The sludge was cultivated in potato dextrose agar (PDA) as growth medium, to which was added 200 g of potato, 20 g dextrose, 20 g of bacteriological agar and one liter of distilled water. This growth medium was supplemented with chloramphenicol, in order to eliminate contamination by bacteria. After preparation, the growth medium was sterilized by autoclaving at 110 °C during 15 minutes. Later, they were placed in triplicate in Petri dishes of 90 mm and, under protection, in laminar flow. After solidification of the growth medium, the sludge samples were added and incubated in a chamber at 25 °C for 7 days. After this period, the fungi were isolated and identified.

Aerobic and Facultative Bacteria

The same methodology of collection for the fungi was applied to bacteriological tests. However, after the mixing of aliquots of sludge from the reactor, they were directly inoculated on eosinmethylene blue (EMB) solid medium, composed of eosin yellow, methylene blue, lactose, bacteriological peptone, dipotassium hydrogen phosphate, sucrose and agar for the isolation of enterobacteria, and cetrimide compound peptone agar, magnesium chloride, potassium sulphate, cetrimide and agar for isolation of bacteria of the genus *Pseudomonas*. Inocula were subjected to incubation in a chamber at 28 °C for 24 hours. The colonies were found and characterized, then subcultured into nutrient agar composed of meat extract, soy peptone, sodium chloride, sodium monohydrogen phosphate and subjected to agar and incubated for 24 hours at 28 °C, under the same conditions. After growth, Gram stain, catalase test, and oxidase and biochemical tests in the kit BacTray® were performed.

RESULTS AND DISCUSSION

Physical-Chemical Parameters

The biomass buffering conditions in the ABR are shown in Table 1. As can be seen, the values remained close to neutral pH, with low coefficients of variation, indicating that the ABR has not suffered pH shock. The total alkalinity in the effluent is higher than the total alkalinity in the influent; this may have occurred during the degradation of various biochemical compounds, mainly protein from the feed, present in the effluent. The alkalinity generation is quite important in the digestion process, since it allows the neutralization of the volatile acids produced, increasing the pH, which favors methane production and stabilization of the organic matter. The intermediate alkalinity (IA) is the buffering of volatile acids and presents a higher concentration in relation to the partial alkalinity (PA). This ratio indicates high generation of volatile acids during the partial hydrolysis and acidification of the organic matter present in the wastewater; however, the concentration of total acidity remained low due to excellent buffering of the system. The influent temperature favored mainly mesophilic bacteria.

Table 1: ABR influent and effluent buffering conditions and temperature

	PA	IA	TA				
Descriptive Statistics Influent	**($mgCaCO_3\ L^{-1}$)**			**IA/PA**	**Acidity ($mg\ L^{-1}$)**	**pH**	**Temp. (°C)**
Average±SD	1127±658	2239±1329	2716±1566				
Minimum	326	995	1032	0.3	1.5	6.4	18
Maximum	3630	6741	8416	0.6	64.2	8.9	23
CV	0.6	0.6	0.6	0.2	0.5	0.1	0.1
	PA	**IA**	**TA**				
Descriptive Statistics Effluent	**($mgCaCO_3\ L^{-1}$)**			**IA/PA**	**Acidity ($mg\ L^{-1}$)**	**pH**	**Temp. (°C)**
Average±SD	1889±412	2693±641	3801±816	0.7± 0.1	38±22	7.4±0.3	20± 1
Minimum	1279	2052	2916	0.5	1.7	6.8	18
Maximum	2741	4303	5508	0.9	94.8	7.9	23
CV	0.2	0.2	0.2	0.1	0.6	0.0	0.1

PA – partial alkalinity, IA – intermediate alkalinity, TA – total alkalinity, pH – hydrogenionic potential, Temp. – temperature, SD – standard deviation, CV – covariance

High concentrations of O&G are detrimental to granulation of the sludge in anaerobic reactors; these compounds evolve the grain by promoting density reduction and consequently flotation and washing out with the effluent. As can be seen in Table 2, the biomass of the ABR was subjected to a high influent concentration of O&G and proved effective in removing these compounds. This efficiency was due to the flow distribution of the baffles installed inside the unit, which helps the flotation of these compounds to the surface of the reactor, thus promoting more time for degradation of oils and greases. It can be noted that the ABR had high efficiency in removing settleable solids and suspended solids. As high concentrations of TSS affect the sludge methanogenic activity, the ABR proved to be adequate to prevent these TSS concentrations from reaching the second stage reactor.

Figure 1 shows the distribution of the removal efficiencies of COD and BOD in the reactors. As can be seen, in the first thirty days there was a low efficiency because the system stopped for a week, and also due to the removal of sludge, leveling the biomass in the reactor. It can be seen in the same figure that, even varying the global efficiency values, it tended to a more stable condition. However, the average values of COD removal were above 80% and BOD above 70%. The removal efficiency values were within the range described by Pereira *et al*. (2010).

Table 3 shows the observed operating parameters of the ABR during the research. According to Chernicharo (2007), for full-scale systems treating wastewater with VOLR below 15 $kgCOD_{total}\ m^{-3}\ d^{-1}$, it is possible to obtain an acceptable treatment efficiency. In this work higher efficiencies were obtained, as seen in Figures 2a and 2b. The ABR was submitted to an average VOLR of 17.8 $KgCOD_{total}\ m^{-3}\ d^{-1}$. However, Chernicharo (2007) says that efficiencies around 70% to 80% are found in anaerobic reactors with HRT relatively low (6 to 8 hours) treating domestic wastewater, with a maximum COD_{total} of 1000 mg L^{-1}. In this study almost two times the HRT was used for removing a COD_{total} of 26000 mg L^{-1}, getting average efficiencies of 80% due to the good conditions of the acclimatized sludge, even after the system shut-down. Another procedure that may have contributed to the biogas production was the VOLR applied based on filtered compounds, which facilitated the acidification and methanification processes (Equation (4)). Even keeping the rotation of the pump steady, flow changes were observed, which caused variations in the HRT and in the HLR, but these variations did not affect the efficiency of the process, since the covariance of the data obtained was low. Another positive factor was the low applied HLR when compared to those found in the literature for high concentration wastewater.

Table 2: O&G, ABR influent and effluent total settleable solids (TSS)

	O&G (mg L^{-1})			Settleable Solids (mg L^{-1})			TSS (mg L^{-1})		
Statistics	**Influent**	**Effl.**	**Effi. (%)**	**Influent**	**Effl.**	**Effi. (%)**	**Influent**	**Effl.**	**Effi. (%)**
Average ± SD	1028 ± 790	574 ± 354	41 ± 26	401 ± 337	79 ±67	77 ± 18	11973 ± 12324	1362 ± 1469	78 ± 24
Minimum	170	36	5,4	70	0.5	43	1403.3	507	21
Maximum	2239	1023	79	900	180	99	37495	5207	97
CV	0.8	0.6	0.6	0.8	0.8	0.2	1.0	1.1	0.3

SD – standard deviation; CV – covariance, O&G – oil and grease, Effl. – Effluent; Effi – Efficiency.

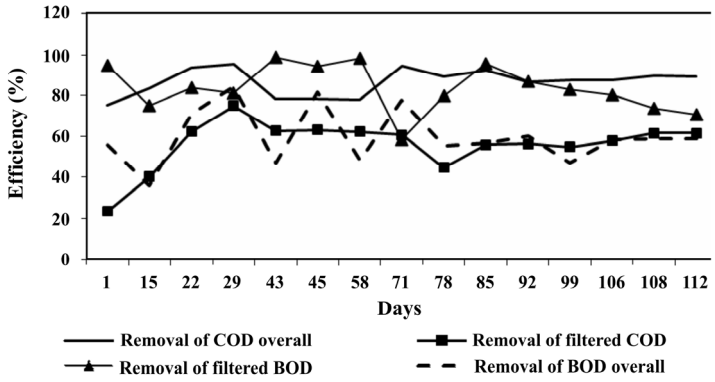

Figure 1: Removal of filtered COD, filtered BOD, COD global efficiency and BOD global efficiency

Table 3: Operational parameters applied to ABR: VOLR (in terms of COD and BOD), HRT and HLR.

Statistics	VOLR 1	VOLR 2	VOLR 3	VOLR 4	HRT	HLR
Average ± SD	17.8±5.1	4.5±2.4	1.7±1.1	4.6±2.1	17.7±1.3	1.4±0.1
Minimum	6.9	2.0	0.7	1.9	15.4	1.1
Maximum	30.3	10.2	4.3	8.3	21.5	1.6
CV	0.3	0.5	0.6	0.5	0.1	0.1

SD – Standard deviation; CV – covariance; VOLR – Volumetric organic loading rate; VOLR 1 – in terms of ($kgCOD_{total}$ m^{-3} d^{-1}); VOLR 2 – in terms of dissolved COD (kg m^{-3} d^{-1}); VOLR 3 – in terms of dissolved BOD (kg m^3 d^{-1}); VOLR 4 – in terms of total BOD (kg m^{-3} d^{-1}); HRT – (hour); HLR – (m^3 m^{-3} d^{-1}).

Correlating efficiency data obtained with the BOLR applied to the ABR during the research, it was found that the models can be described by the following polynomials:

Model 1:

$$E1 = -385\ BOLR^2 + 1025\ BOLR - 588.6 \quad (1)$$

Model 2:

$$E2 = -59.36\ BOLR^2 + 116.7\ BOLR + 3.384 \quad (2)$$

E1 represents the global efficiency of COD removal;

E2 represents the efficiency of dissolved compounds removal and BOLR (Biological Organic Loading Rate). For Models 1 and 2, equating the first derivative to zero, the BOLR that provides the maximum efficiency for the process under research can be obtained to.

Model 1:

$$\partial E1 = (-385\ BOLR^2 + 1025\ BOLR - 588.6)\partial\ BOLR \quad (3)$$

$$\frac{\partial E1}{\partial BOLR} = -770\ BOLR + 1025 \quad (3a)$$

$$\frac{\partial E1}{\partial BOLR} = 0 \quad \text{find} \quad BOLR = 1.3 \quad (3b)$$

Model 2:

$$\partial E2 = (-59.36\ BOLR^2 + 116.7\ BOLR - 3.384)\partial\ BOLR \quad (4)$$

$$\frac{\partial E2}{\partial BOLR} = -118.72\ BOLR + 116.7 \quad (4a)$$

$$\frac{\partial E2}{\partial BOLR} = 0 \quad \text{find} \quad BOLR = 0.98 \quad (4b)$$

As can be seen from the equations above, the maximum global efficiency, concerning the BOLR, is 1.3 COD_{total} $kgTVS^{-1}$ d^{-1}. In order to get the maximum efficiency for removing the COD, a BOLR of filtered COD about 0.98 COD_{total} $kgTVS^{-1}d^{-1}$ is necessary. It was also observed that the BOLR had a significant effect on methanogenic activity and the estimated biogas production. Therefore, by increasing the BOLR, the biogas production also increases in an exponential fashion, as shown in Equation (5).

$$AMA = 0.1834\ e^{0.798\ BOLR} \qquad (R^2 = 0.9955) \quad (5)$$

AMA: Apparent Methanogenic Activity (m^3Biogas $kgTVS^{-1}$ d^{-1})

BOLR: Biological Organic Loading Rate Applied ($kgCOD_{total}$ $kgTVS^{-1}$ d^{-1}).

In relation to biogas production, a strong relationship was found between this parameter and the global efficiency of the system, since the higher the overall efficiency of organic matter removal, higher the biogas production (Equation (6)).

$$E = 50.185\ BP^{0.1503} \qquad (R^2 = 0.9739) \quad (6)$$

E – Efficiency (%)

BP- Biogas Production (m^3 d^{-1}).

In this research, the average COD_{total} influent was 26000 mg L^{-1}. Compared to the data presented by Fernandes and Oliveira (2006), considering also the lower hydraulic retention times (HRT) applied, it can be concluded that the removal efficiencies of total COD were higher. When comparing with data reported by Abreu Neto and Oliveira (2009), it is

possible to observe that those authors, working with higher HRT and lower VOLR, obtained lower efficiencies. However, in both studies (Fernandes and Oliveira, 2006; Abreu Neto and Oliveira, 2009), high efficiency removal of dissolved material was observed, higher than those observed in this study. Also, it can be noted that the higher HRT applied by the authors (Fernandes and Oliveira, 2006; Abreu Neto and Oliveira, 2009), more significant was the dissolved material removal and, consequently, the observed efficiencies. On the other hand, the lower the values of HRT applied in this study, the higher the effectiveness in the degradation of total COD, showing that the ABR is efficient for the removal of total COD when working with high volumetric organic loading rates (VOLR) and with low hydraulic retention times (HRT).

Microbiology (Fungi)

Fungi were found in microbiological analysis of the sludge in all ABR compartments. They were mostly of the gender *Trichoderma sp.,* but gender such as *Monilia sp*., *Fusarium sp*., *Cladosporium sp*. and *Rhizoctonia sp*. were also observed. All these genders employ strict aerobic metabolism and are widely found naturally in soil and cultivars and can also colonize decomposing organic matter. These fungi have a growth temperature between 25 °C and 30 °C and do not grow above 35 °C. In this study the average temperature of the liquid in the reactor was 20 °C, which provided the latency of these microorganisms in the biomass (Abdel-Faftah *et al*., 2002). These results showed that, in addition to temperature, exposure to low concentrations of oxygen in the ABR did not hamper some species of fungi that may have been introduced into the reactor through the open channels, which carry the sewage to the system treatment. When these microorganisms are dormant, they can develop when exposed to suitable growth conditions, consuming organic matter and producing acids and other metabolites that can thus destabilize the anaerobic treatment unit. This behavior is also justified by the fact that most species of these genera of microorganisms can be spread through the air (airborne), which makes them very abundant in the environment, especially the genus *Cladosporium sp.*, which can be classified as a dominant and universal fungus, found in virtually any environment (Pelczar 2002).

Aerobic and Facultative Bacteria

According to Chen *et al*. (2007), the anaerobic digestion process can contribute significantly to the reduction of pathogenic microorganisms in wastewater treatment. There are several factors that influence the survival of these microorganisms in anaerobic digesters, including temperature, hydraulic retention time, pH of the sludge and wastewater, chemical interactions and also the type of feeding, continuous or batch (Smith *et al*., 2005). According to Ferreira *et al*. (2003) and Souza *et al*. (2004), an integrated system of anaerobic digesters can reduce the density of coliform bacteria and fecal streptococci. However, when the influent of the system presents a high load of enteric microorganisms, it can provide high levels of these microorganisms, especially *Escherichia coli*. The biomass in the three compartments of the ABR was identified by means of specific microbiological tests (BacTray®); and only species of pathogenic microorganisms, such as *Escherichia coli*, could be identified with 100% specificity. So, with these tests, it was evident that the biomass of the ABR has the prevalence of strict and facultative aerobic bacteria that can influence the stability of the biomass responsible for the degradation of organic matter, mainly in the hydrolysis and acidogenic stages, benefiting the production of methane by the *methanogenic archaea*.

Stereoscopic and Optical Microscope

Stereoscopic microscopy was employed to view the sludge macro-consortium of ABR. Figure 2 shows the diversity of materials found, which may or may not be part of the structure of the granules. The granule size ranged in diameter from 0.3 cm to 1.0 cm, which can be changed by the addition of minerals present in the effluent, such as sand or biological material, like plant residues and insects, as shown in Figures 2 (a, b, c, d). According to Schmidt & Ahring (1996), the granulation is a process by which dispersed biomass aggregates, forming well-defined pellets. It is a complex process involving different trophic groups of bacteria, as well as their physical-chemical and biological interactions. In more complex substrates, the bead can contain, on its surface, fermentative acidogenic bacteria and hydrogenotrophic *methanogenic archaea*, while *methanogenic archaea* and *acetogenic aceticlastic* that produce hydrogen (H_2) and carbon dioxide (CO_2) may occupy the innermost layers. The matrix of the granule can be a solid material such as stones, sand, soil and other waste, or be constituted by the agglomerated biomass (bead), as happened in the ABR, where the biomass grew in dispersed form in

liquid medium without a support structure, or in the attached form on a support (inert material) forming the biofilm, as shown in Figure 4 (a, b). The formation of these granules is crucial, since a specific biomass, mainly microorganisms involved in removing organic matter from sewage (Moterani, 2010), is regulated by the balance between the forces of cohesion and the shear stresses caused by the hydraulic parameters in the reactor and by the various chemical and biological conditions.

Optical microscopy using white light detected some species of protozoa in the ABR sludge, which may be due to the low oxygen concentration in the medium, or even due to the fact that these microorganisms are in the residues of the samplers, since the valves presented a more suitable condition for their survival, Figure 3 (a, b). Therefore, besides bacteria, fungi and protozoa were also found, and occasionally rotifers, nematodes and even insect larvae, depending on the treatment system and effluent to be treated, as cited by Branco (1978). There is evidence that the protozoa present in the effluent may secrete exopolysaccharides (EPS), similar to some species of bacteria that aid in the adherence of other microorganisms to the bead. However, the protozoa found in this study were not identified and more research is needed on this subject.

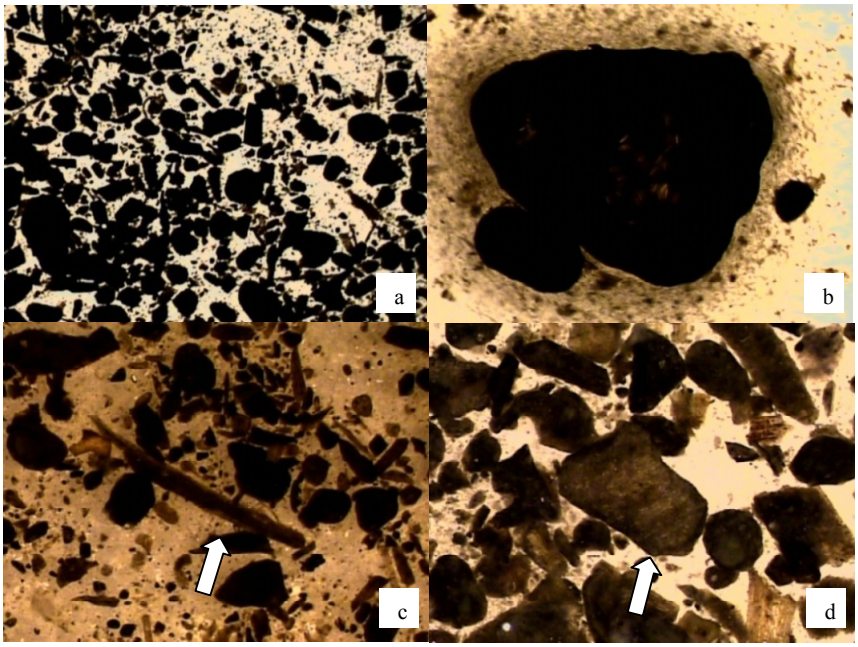

Figure 2: (a) Macroscopic structure of compartmentalized anaerobic granules. (b) Presence of organic residues. (c) and (d) Inert materials such as fragments and sand

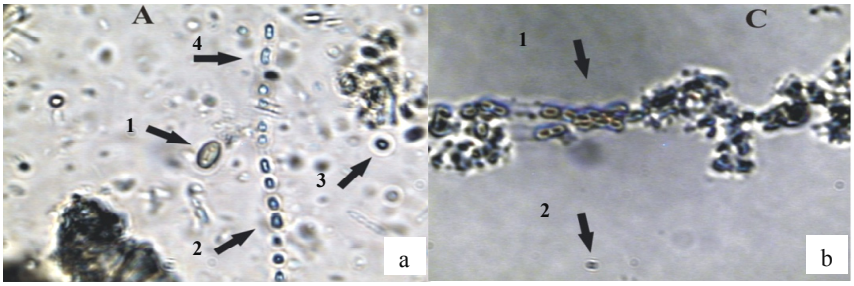

Figure 3: (a) View of anaerobic sludge (100X) using white light microscopy, showing the existence of protozoa (1 and 3) and their clusters (2 and 4). (b) Clusters of protozoa (1 and 2) in the ABR anaerobic sludge (100X) using optical microscopy with white light.

Scanning Electron Microscopy (SEM)

In the first chamber of the ABR, shapeless aggregates were observed, consisting of both: organic and inert matter, as well as more dispersed or less aggregated microorganisms, as shown in Figure 4 (a, b). A variety of microorganisms was observed a in the sludge in that chamber, including the presence of bacilli and the aggregation of these bacteria, together with the development of masses of microorganisms formed mainly by filamentous bacteria. The disaggregation and dispersed granules occur when bacteria lack cohesion, mainly because the organic loading applied in the chamber unit is very rich in complex substrates. This can occur even before a partial anaerobic digestion in the equalization and acidification tank (EAT). Micro-organisms that grow scattered in the ABR are normally carried out into the following chambers of the ABR, a process known as washout. However, the biomass can aggregate and form bonds between the microorganisms and other support material, and are then retained in the sludge in the first stage reactor.

In the second ABR compartment, more delimited granules were observed, when compared to the first compartment of the same reactor, as seen in Figure 5 (a, b). The presence of various groups of bacteria in the granules support material increased the density of the granules. In this second compartment, there was also the presence of a large number of acidogenic and acetogenic bacteria, which have different ecological functions and different metabolic rates.

In the third compartment of the ABR, more structured and denser granules were observed. Due to the consistency of these granules, many holes were found in them, used for output of the biogas produced by the bacteria. These holes were not observed in earlier compartments. Also noted was a better interaction between organisms and the surrounding supports, made of organic matter, inert materials and other organisms, as shown in Figure 6 (a, b). In Figure 6b a large number of filamentous bacteria are forming networks among themselves and with other structures of mud, thus constituting units that are more resistant to shear forces caused by hydraulic loads.

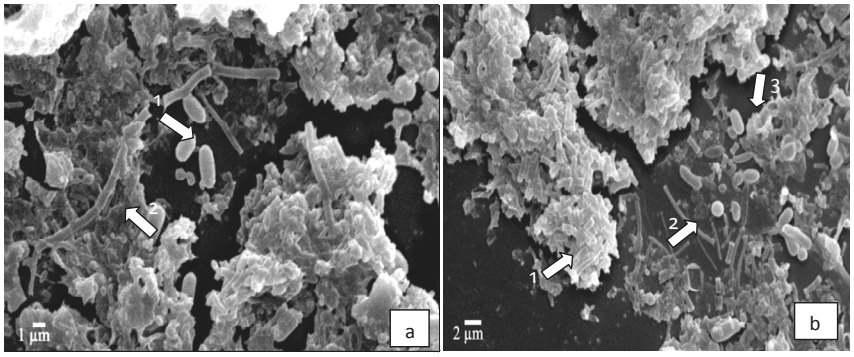

Figure 4: (a) In the first compartment of the ABR, groups of bacteria in the form of bacilli and filament (1 and 2). (b) (1) Microbial diversity and a training bead; (2) filamentous bacteria; (3) Bacilli

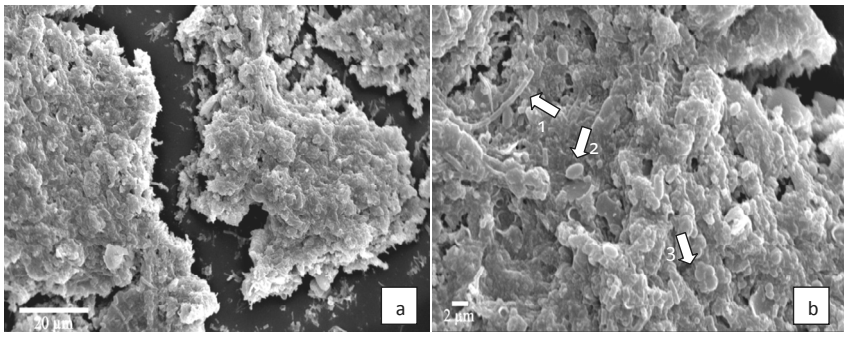

Figure 5: (a) Formation of more cohesive and defined granules in the second compartment of the ABR. (b) Presence of several bacteria on the surface of granules, as: (1) filamentous bacteria (2) bacilli and (3) coccus (Sarcina)

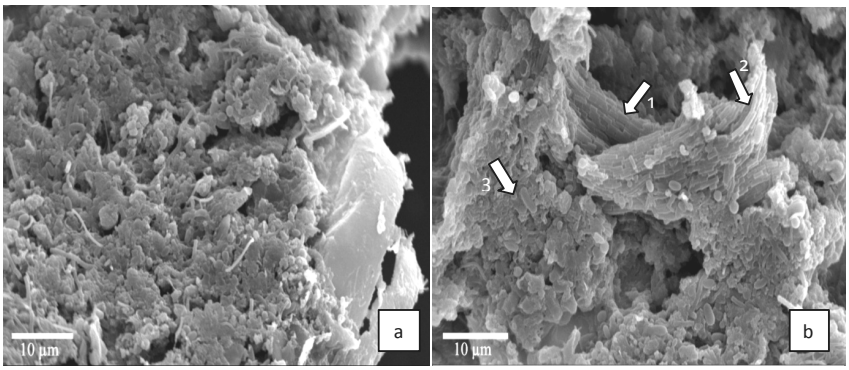

Figure 6: (a) Presence of inert material as a substrate for bacterial attachment in the formation of the granules in the third ABR chamber. (b) (1 and 2). Several bundles of filamentous bacteria in several layers of granules. (3) Association with other forms of these bacteria like bacilli.

Even for *methanogenic archaea*, which are more susceptible to large variations of the microenvironment, these crowded structures were beneficial. It is assumed that the predominance of filamentous bacteria occurs due to more selective substrates for this bacterial species, providing the necessary inputs and environmental conditions favorable for their growth in relation to other microbial groups. Among the metabolic products generated by acidogenic and acetogenic bacteria, only hydrogen and acetate can be used by *methanogenic archaea* as input for methane generation. However, there is interaction between these bacteria and other groups, as seen in Figure 6 (a, b).

CONCLUSION

The ABR (full-scale) required 116 days to recover the stability condition after a one month shut-down. The ABR showed an efficiency of removal of COD above 80%. The ABR was also effective in removing oil and grease and suspended solids. It qualifies as a suitable reactor to be used as a first stage, since these compounds affect granular sludge formation and, therefore, the methanification in the second stage reactor. The consortium of the biomass (sludge) present in the ABR is highly diversified, composed of several fungi, aerobic and facultative bacteria and protozoa. Granules were present from the first until the third chamber, where an even higher microbial diversity was observed, evidenced by cocci, bacilli and filaments. The largest aggregation of biomass occurred in the third compartment of the first chamber, where there were fewer granules and larger quantities of scattered biomass. There was a greater prevalence of coccus in the second, and more filamentous bacteria in the third chamber.

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