Characterization of the Biomass of a Hybrid Anaerobic Reactor (HAR) with Two Types of Support Material During the Treatment of the Coffee Wastewater

Vivian Galdino da Silva[1]**, Cláudio Milton Montenegro Campos**[2]**, Erlon Lopes Pereira**[3*] **and Júlia Ferreira da Silva**[4]

[1]*Unidade de Ciências Exatas e Tecnológicas; Universidade Estadual de Goiás; CP.:459; Goiânia - GO - Brasil.*
[2]*Departamento de Engenharia; Setor Recursos Hídricos e Meio Ambiente; Universidade Federal de Lavras; CP.: 3037; Lavras - MG - Brasil .*[3]*Departamento de Biotecnologia Industrial; Escola de Engenharia de Lorena; Universidade de São Paulo; Lorena - SP - Brasil.* [4]*Instituto de Ciências Ambientais e Desenvolvimento Sustentável; Universidade Federal da Bahia; Barreiras - BA – Brasil*

ABSTRACT

This study investigated the microbiology of a hybrid anaerobic reactor (HAR) in the removal of pollutant loads. This reactor had the same physical structure of an UASB reactor, however with minifilters inside containing two types of support material: expanded clay and gravel. Two hydraulic retention times (HRT) of 24h and 18h were evaluated at steady-state conditions, resulting in organic loading rates (OLR) of 0.032 and 0.018 $kgDBO_5m^{-3}d^{-1}$ and biological organic loading rates (BOLR) of 0,0015 and 0.001 $kgDBO_5kgSVT^{-1}d^{-1}$, respectively. The decrease in concentration of organic matter in the influent resulted an endogenous state of the biomass in the reactor. The expanded clay was the best support material for biofilm attachment.

Key words: Agricultural microbiology, endogenous, anaerobic process, agro-industrial wastewater treatment, rural sanitation

INTRODUCTION

The wet processing of coffee produces a better quality of coffee. The process is being currently explored for improvement to avoid the negative effects on product quality and reduce energy consumption (Campos et al. 2010). A large amount of water is used during the washing and peeling of the fruits. The resulting wastewater is quite rich in organic and inorganic materials, and therefore, must be treated before being released into water bodies (Prado and Campos 2008).

The effluent treatment processes can be classified as physical, chemical and biological. Biological processes are mainly used to remove the biodegradable organic substances, which become a food source for the microorganism (Torres et al. 2003). According to Pereira et al. (2009), Pereira et al. (2010a), Pereira et al. (2010b) and, Pereira et al. (2011), the essence of biological wastewater treatment lies in the ability of the microorganisms involved to use the biodegradable organic compounds, transforming them into products, which can be removed in the treatment system. The efficiency of the anaerobic process depends on the interactions between the species of microorganisms with different capabilities to degrade various substrates. This also depends on their ability to acclimatize and develop a high

*Author for correspondence: erlonlopes@gmail.com

microbial activity and capacity to bear the hydraulic and biological shocks (Pereira et al. 2009). Therefore, an evaluation of the specific methanogenic activity (SMA) of an anaerobic sludge becomes an important analysis for monitoring the process (Chaiprasert et al. 2003).

The upflow anaerobic sludge blanket reactor (UASB) has difficulties in producing an effluent that accomplishes within the standards set by the environmental legislation. Thus, it becomes important to apply some form of post-treatment to its effluent, in order to make the treated wastewater meet legal requirements and adequately protect the receiving water bodies (Pereira et al. 2010a). Anaerobic filters are among the alternatives of post-treatments. These filters are characterized by the presence of a stationary packing material, to which the bacteria can stick to form the biofilm and also, in the interstices, the biomass can grow loosely but with a good capacity of removing additional organic matter (Fia et al. 2010; Silva et al. 2010; Silva et al. 2011b).

Among the anaerobic filters, the hybrid anaerobic reactors (HAR) have the structure of a UASB reactor; however, their bed is composed of composite material in order to support the biofilm formation. This technology is still developing rapidly and its various configurations allow great flexibility in design and construction with regard to the shape, direction of flow and support material (Chaiprasert et al. 2003). The purpose of the support material is to retain the bacterial biofilm within the reactor, serving also as a device for separating the solids from the gases, helping to promote a uniform flow in the reactor, improving the contact between the organic constituents of the influent and microorganisms in the biofilm and interstitial forms. This process allows the accumulation of large amounts of biomass, with the consequent increase in cellular retention time, and also acting as a physical barrier, preventing the solids to be washed out from the treatment system (Omil et al. 2003).

As explained before, the retention of biomass around the support material forms a biologically active structure, called biofilm, and the microorganisms that are not able to stay in the biofilm, form a type of interstitial flocculent or granular sludge inside the interstices. This interstitial sludge is biologically active and if withdrawn from the system, can affect the efficiency negatively.

As coffee is an annual crop, usually at the end of the harvest, there is reduction in the effluent concentration due to reduced discharge. This fact does reduce the concentration of biomass due to inbreeding occurred in the system. This study aimed at evaluating the process of endogenous in a hybrid anaerobic reactor (HAR) treating the wastewater from coffee wet process (WCWP) using two types of support material. The study included the effect of operating parameters on the endogenous process by assessing the microbiological response using a scanning electron microscopy and epi-fluorescence microscopy and followed the process parameters as described by Silva et al. (2011b).

MATERIALS AND METHODS

The Hybrid Anaerobic Reactor

The hybrid anaerobic reactor (HAR) was made using the polyester with catalyst orthophthalic resin and, subsequently, capped with roving fiber reinforced with steel rings and covered with orthophthalic gel-coat (Fig. 1b). The unit had a diameter of 1.0 m, height of 4.0 m and volume of 3,107 L (Fig. 1a). Also, there was a three-phase separator (TPS) with a height of 1.37 m, built of fiberglass (Figures 1c and 1d). A nylon screen was adapted to the TPS in order to avoid the minifilters to occupy its inner space (Fig. 1d).

Inside the HAR, 54 minifilters, constructed of Vinyl polychloride (CPV- 1 m long and 0.1 m in diameter) were placed. This quantity was determined according to the diameter of the reactor. These minifilters contained expanded clay and rolled pebble. Four days after the end of the harvest, the minifilters were placed in the HAR, after being defined by means of experimental tests, the ratio between the resources expanded clay and rolled pebble (Silva et al. 2010).
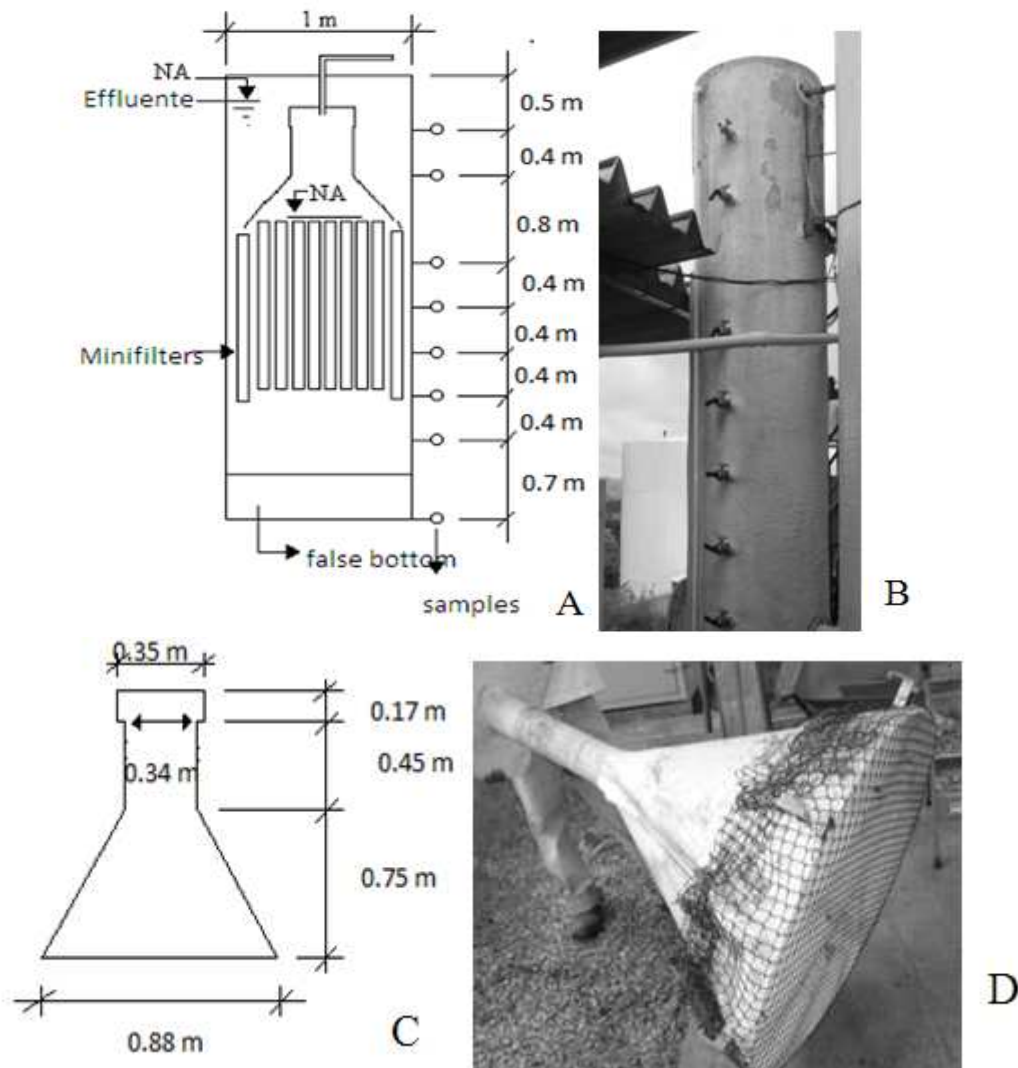
Figure 1 - Hybrid Anaerobic Reactor (HAR) located in CCRCC / UFLA. (A) Longitudinal section. (B) Partial view. (C) Longitudinal section of TPS. (D) TPS with a nylon screen.

Start up of the System and Steady State: Operational Control System

The experiment was conducted at the pilot sewer treatment system located at Coffee Center for Research on Coffee Culture (CCRCC), in the Agriculture Department, Federal University of Lavras (UFLA). The system was composed with preliminary treatment, with screen, sand sedimentation tank, secondary treatment composed by a stabilization pond (SP), two concentric UASB reactors (R1 UASB and R2 UASB) working in series with an equalization pressure tank, an HAR, three filters for H_2S removing, three gas-meters, two aerobic batch reactors (ABR), a drainage bed sludge (DBS), six reservoirs ($T_{1, 2, 3 ...}$) and a pumping system with six pumps (Fig. 2).

The COD and BOD_5 of the effluent of stabilization pond and also the profile of solids digestion compartments of R1 and the RAH were analysed, in order to determine the load for start-up according to the HRT. On the 24th day of the experiment, several adjustments in the *Leder* program of the PLC were made and also exchanged some solenoid valves. It was further monitored for the pH, conductivity, salinity, total dissolved solids, and also COD and BOD_5. More details concerning the flow control, pH, alkalinity, temperature and other control parameters can be found in Silva et al. (2010). The system was monitored daily for the influent and effluent temperature, ambient temperature, relative humidity, pH, electric conductivity (EC), salinity

and total dissolved solids (TDS). At pre-determined intervals, COD, $BOD_5^{20°C}$, total solids (TS), total fixed solids (TFS), total volatile solids (TVS), and total alkalinity (TA), partial alkalinity (PA) and intermediate alkalinity (IA) were

analyzed. Periodically, the samples were collected from the profile of the reactor to determine the concentrations of total, fixed and volatile solids. The analysis were conducted following the APHA (2005).
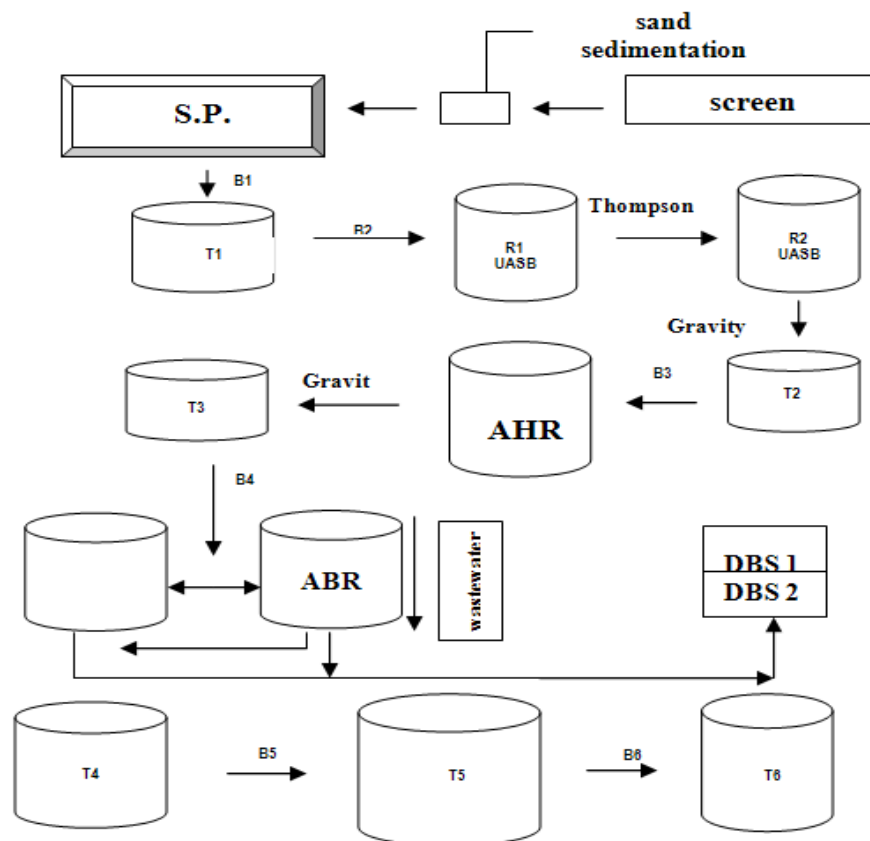
Figure 2 - Operational flowchart of the pilot system treating coffee wastewater.

Epi-Fluorescence Microscopy

To investigate the fluorescence of *methanogenic archaeas*, sludge samples of HAR's seventh port, counted from the top to the bottom, were diluted by 1/5 with distilled water, pipetted on slides and taken for observation under epi-fluorescence microscope, Olympus, Model BX60.

Scanning Electron Microscopy (SEM)

Sludge samples of HAR's seventh port and also from the middle bracket (expanded clay, rolled pebble) were processed using a scanning electron microscopy (SEM), applying the following methodologies:

Desiccation method

This method was used to analyze only the samples of the seventh compartment digestion. Initially, the

methodology used was as described by Pereira et al. (2009). The samples were immersed in liquid nitrogen at -196°C and, once frozen, cut with a scalpel and mounted on the stubs coated with aluminum foil using double-sided tape (Fig. 4a). At room temperature, the sample returned to a liquid state and thereafter, the methodology was changed. The samples were dried in an oven at 70°C for two hours and then taken to a desiccator for 24 h..

Coverslip method

Samples (± 0.5 mL) of the seventh compartment digestion were deposited on the glass slides with 1 mm thickness housed within a small Petri dish (Fig. 4b) and immersed in modified Karnovisky fixative (2.5% glutaraldehyde, 2% paraformaldehyde in 0.05 M cacodylate buffer at

pH 7.2) for one hour. They were then washed in cacodylate buffer solution three times for 10 minutes in order to remove the residual glutaraldehyde solution. In a protected environment (hood) six drops of osmium tetroxide were added for post-fixation. Petri dishes were wrapped with parafilm to keep the osmium gas. After four hours, the samples were washed three times in distilled water and then dehydrated in gradient of acetone three times for ten minutes (25, 50, 75, 90 and 100%) and then placed in open desiccator for 24 h for drying.

After drying, the samples were mounted on the stubs and transferred for the coverage of gold (sputter coater) in apparel brand BAL-TEC SCD050 model. The specimens were observed under the scanning electron microscope LEO brand, model EVO 40.

Support material

Samples of the support material, expanded clay and rolled pebble, were obtained by breaking up the pebbles in a conventional vise, while the expanded clay pellets were fragmented using a table vise. This procedure was necessary in order to be able to insert the samples into glass penicillin bottles with a modified Karnovsky's fixation solution. This was followed by the standard procedures for SEM (excluding the post-fixation with osmium tetroxide), and dehydrated in acetone, critical point drying, gold cover and observation.

RESULTS AND DISCUSSION

Start up of the System and Steady State

The parameters of the HAR in the study can be observed in the inoculation and in three matches during the monitoring in Table 1. Evidently the values were close in three start-ups for most of the observed parameters, except for $BOD_5^{20°C}$, which showed an increase, probably by the occurrence of solids washout out from the reactor. There was a reduction in the biological organic load rate (BOL) as a result of lower concentrations of BOD_5, in the first start-up, about 310mg L^{-1} and in the second start-up, dropped to 280mg L^{-1}. In the third start-up, there was an increase of 18% at BOL, provided by the addition of coffee hulls in the pond in order to increase the organic matter present in it. The values of salinity, involving total dissolved solids (TDS) and electrical conductivity (EC) were increased around 70%.

Table 1 - Parameters observed in the inoculation and the three matches held in the pilot system treating wastewater from wet coffee proccess, located in CCRCC / UFLA.

Parameter	Inoculation	1st start-up	2nd start-up	3rd start-up
COD inf (mg L^{-1})	-	828	665	645
COD eff (mg L^{-1})	-	310	280	380
BOL (kgBOD$_5$.kgTVS^{-1}.d^{-1})	-	0.0156	0,0103	0.01213
Xvt (KgTVS)	-	51.926	71.25	82.09
HRT$_{HAR}$ (hour)	-	28.5	28.5	28.5
Q$_{infl.}$ (m^{3}d^{-1})	-	2.62	2.62	2.62
OLR $_{inf}$ (Kg BOD$_5$m^{-3} d^{-1})	-	0.26	0.236	0.32
OLR $_{inf}$ (Kg COD$_5$m^{-3} d^{-1})	-	0.70	0.56	0.54
pH $_{inf}$	5,34	7.34	7.06	6.73
Temperature $_{inf}$ (°C)	19,05	19.06	22.3	22.6
EC $_{inf}$ (dS m^{-1})	0,63	0.83	0.82	1.15
TDS $_{inf}$ (mg L^{-1})	507	645	640	903

inf – Influent of HAR; eff- Effluent of HAR; Xvt- total volatile solids in each start-up.

Evaluation System At Steady-State Conditions

After three successive matches and after the transient period, the system was monitored daily to maintain the steady-state conditions. Aiming at increasing the biomass concentration in the reactor, it was decided to increase the volumetric organic loading rate (VOLR), increasing the flow and consequently decreasing the hydraulic retention time. Thus, in the 79th day of operation, the rotations of the pumps 2 and 3 were increased from 1,000 rpm (2.67 m^{3}d^{-1} and 2.6 m^3 d^{-1}, respectively) to 1,060 rpm (3.140 m^{3}d^{-1}) and 1,200 rpm (3.156 m^{3}d^{-1}), respectively, at 99th day to 1,960 (5.65 m^{3}d^{-1}) and 1,600 rpm (4.13 m^{3}d^{-1}),

respectively, affecting the volumetric organic loading rate (VOLR), which remained at this level until the end of experiment. These values are presented in Table 2.

Table 2 - Parameters analyzed at 79 and 99 days of monitoring pilot system treating wastewater from the coffee, located in CCRCC/ UFLA.

Parameters	79° Day	99° day
Rotation B3 (rpm)	1200	1600
Q_{B3} (m^3 d^{-1})	3.14	4,13
$BOD_{5\,inf}$ (mg L^{-1})	31	14
COD_{inf} (mg L^{-1})	195	58
BOL_{inf} (kg BOD_5 kgTVS^{-1} d^{-1})	0.0014587	0.00099
HRT_{inf} (hours)	23.73	18.03
COV_{inf} (kgBOD_5 m^{-3}d^{-1})	0.032	0.018
COV_{inf} (kgCOD m^{-3}d^{-1})	0.199	0.077
$X_{vt\,inf}$ (kgSVT)	66.77	58.3963

inf – Influent of HAR; eff- Effluent of HAR; Xvt- total volatile solids in each start-up.

EPI-Fluorescence Microscopy

At the end of the experiment, only the last three compartments presented sludge: the number 6, 7, 8 measuring 1.5 m from the base, representing 57% of the initial volume inoculated in the HAR. The samples were analyzed in epi-fluorescence microscopy. The analysis showed fewer bacteria in the material (dispersed biomass), confirming the low concentration of biomass at the end of the experiment. Bacteria could not be counted, as by Campos and Chernicharo (1991) in which, after the dilution, samples were placed in a haemocytometer chamber under fluorescent light for counting the methanogenic non-fluorescent and non-methanogenic. This situation was resulted as there was no ideal fit focus, due to the difficulty of adjustment between the camera and lens. In Figure 3 there are some images showing the auto-fluorescence of *methanogenic archaea*. This auto-fluorescence was due to the existence of specific coenzymes and cofactors such as coenzyme F420, coenzyme F430, coenzyme M, and methanoplerina methanofurano (Pereira et al. 2009).
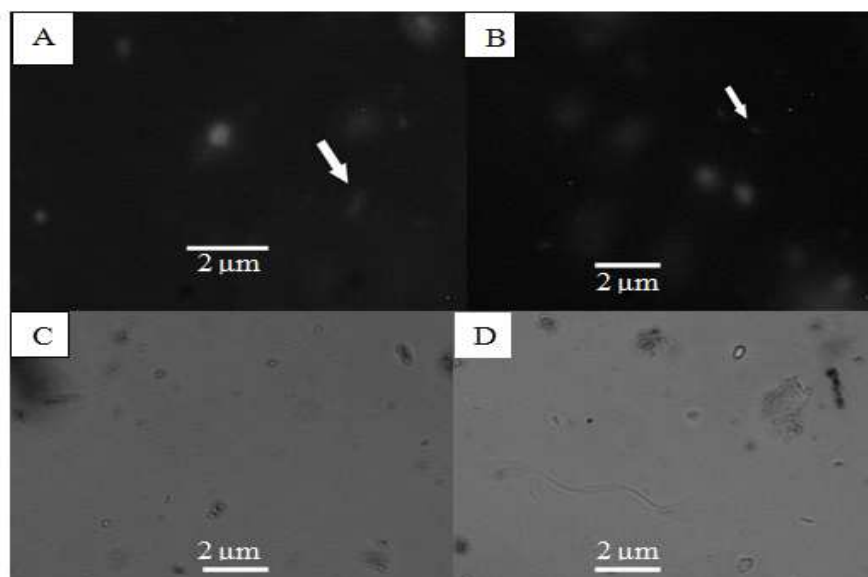
Figure 3 - Epifluorescence photomicrographs of samples of dispersed sludge digestion compartment 7 of the AHR, under fluorescent light (A,B) and under white light (C,D). Arrows indicate possible auto-fluorescent methanogenic archaea.

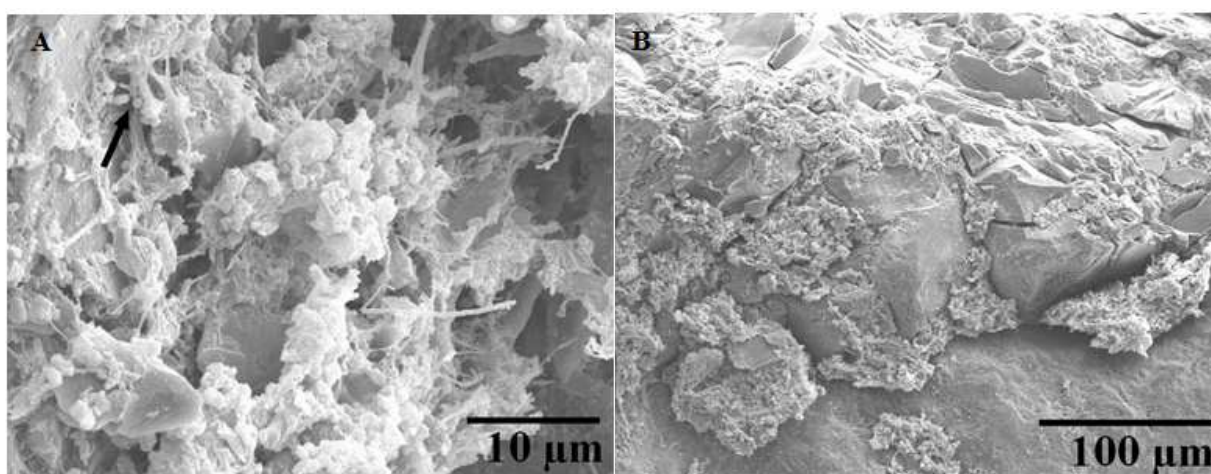## Scanning Electron Microscopy (SEM)

The scanning electron microscopy was applied also to the support materials in order to verify the sludge adhered (biofilm), namely the attached biomass. The samples of 7th digestion compartment were also analyzed for the dispersed biomass (sludge blanket). The samples had fewer bacteria, confirming the low concentration of biomass at the end of the experiment. The images produced using the technique of drying and methodology of mounting coverslips are shown in Figures 4, 5, 6 and 7. Some images showed cavities that could be considered the outputs of the bubbles of biogas. They also had fewer bacteria in the biofilm, many fragments of inert material (because of the support defragmentation) and wastes from the bark of coffee.
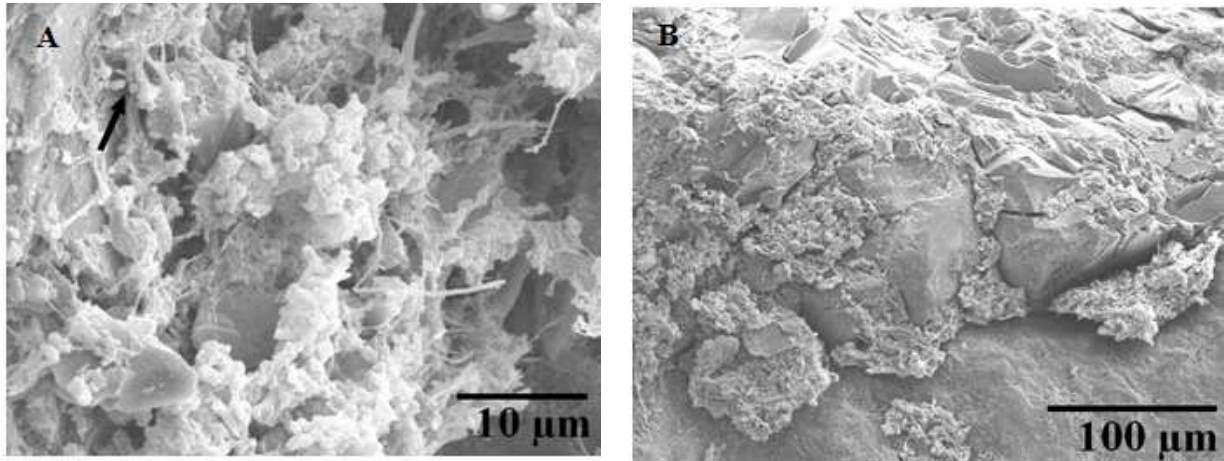
The scanning electron microscopy images were obtained for the support materials using the technique of drying. Figure 6 showed that the gravel, due to its structure, presented, as a support material, less favorable to aggregation of bacteria and other elements. Expanded clay, on the other hand, being more porous, facilitated the adhesion of biomass and other organic and inorganic materials (Fig. 7). The sludge adhered to the support material also showed the growth of bacteria.
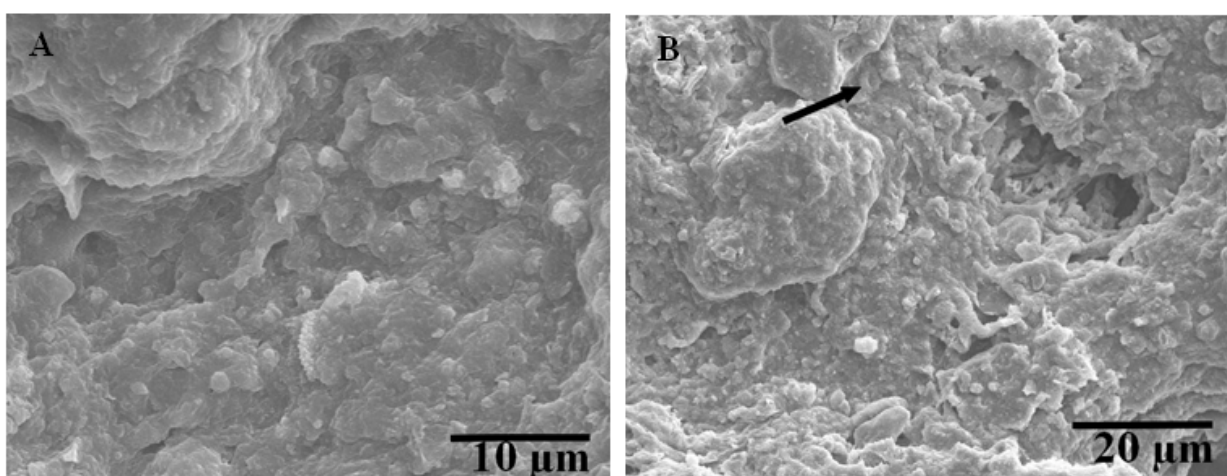

Figure 4 - Electron scanning of samples of the sludge spread of the 7th the digestion compartment of the HAR pilot system treating wastewater from coffee, located in CCRCC using the technique of drying (A) aspect of biomass in endogen process (B) presence of bacteria in the sludge dispersed. Arrow indicates bacteria.

Figure 5 - Scanning Electron Microscope (SEM) photograph of samples of dispersed sludge from digestion compartment 7 of the HAR, using the technique of drying: (A) arrow indicates the residue of coffee pods scattered on the sludge, (B) arrow indicates possible release of bubbles of biogas.

Figure 6 - Scanning Electron Microscope (SEM) photograph of sludge adhered to pebbles used as a medium of HAR: (A) presence of bacteria in the wells of gravel, (B) material that adheres to the surface of the pebble.
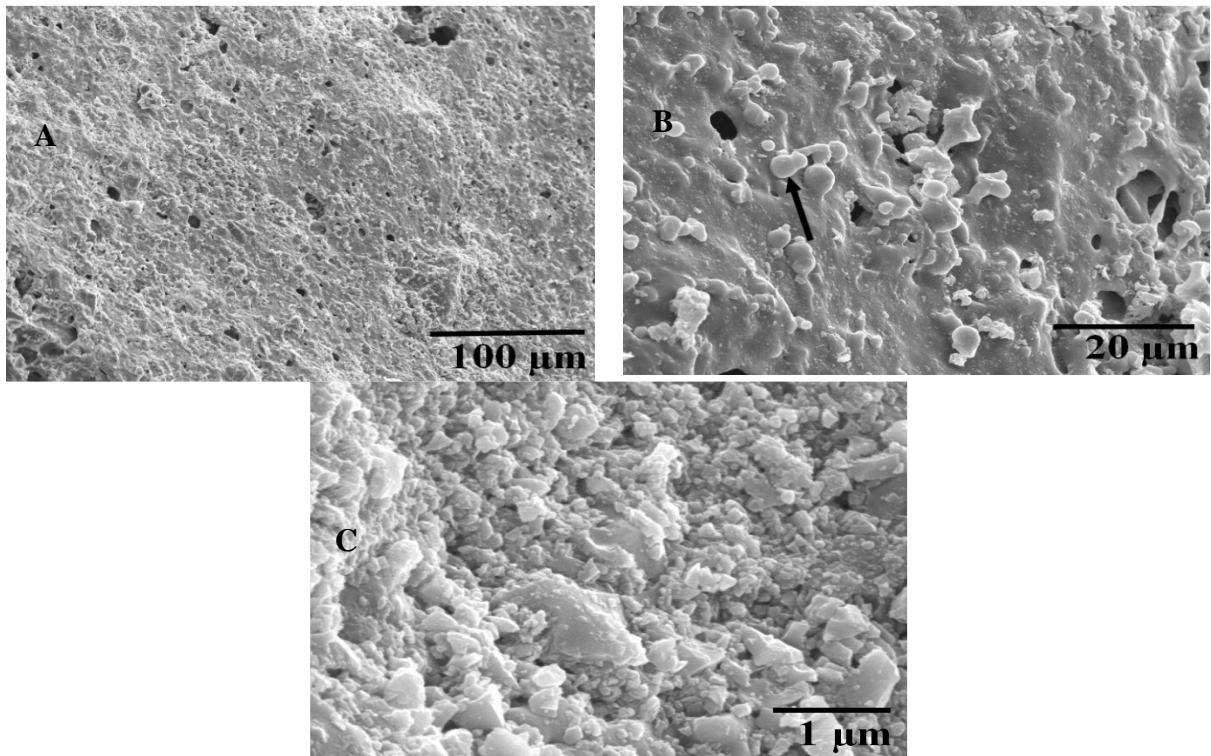
Figure 7 - Scanning Electron sludge adhered to the expanded clay used as a material of HAR: (A) surface of the expanded clay, (B) budding bacteria, (C) material that adheres to the surface of expanded clay.

The SEM micrographs showed that the outer surface of the expanded clay was smoother than the inner surface of the sample. The internal surface showed a higher amount of pores, without causing the interconnectedness of them. The higher porosity of the inner surface was related to the appearance of gas bubbles generated in the manufacturing process of expanded clay.

Fia et al. (2010) evaluated the influence of media type support on the efficiency of fixed bed

anaerobic reactors (FBAR) treating coffee wastewater, with COD concentration of organic matter ranging between 812 and 5,320 mg L^{-1}. The media used to immobilize the biomass were blast furnace slag, polyurethane foam and stone nº 2, with voids of 53, 48 and 95%, respectively. The biofilm formed on all the supports showed the presence of different bacterial morphologies such as the bacilli, coconuts and filamentous. The bacilli were present in all the conditions studied; these were not fluorescent and had rounded contours, being similar to those of the genus *Methanosaeta sp*. There were also the cells similar to the genus *Methanosarcina sp*. in the reactor in which the slag was used as media support. The study used the reactors at high loading rates and did not evaluate the endogenia conditions, which justified the greatest diversity and quantity of morphologies observed when compared to this work. Fia et al. (2010) reported that the polyurethane foam presented the best COD efficiency of about 80%. The authors attributed this result to the voids, which was greater than other medias, which also provided greater biomass retention quantified in terms of TVS (about 1,301 mg g^{-1}). In the present work, the expanded clay had higher porosity and showed higher aggregation of biomass and more morphological diversity. Fia et al. (2010) characterized the morphological diversity found along the height of the reactors (base, middle and top) for all the media under high loading rate, and therefore, had higher amount of morphological types of bacteria. Based on this, in this work the biomass of the 7° compartment, at the top of the reactor, presented some bacteria of different morphologies and methanogenic, as shown in Figure 3. It could be possible that the HAR, due to the minifilters at the top, retained even under endogenia, large quantity of biomass within the reactor, enough to operate at low loading rate, and therefore, achieve high efficiencies as described by Silva et al. (2011). Probably, if there were low loading rate conditions on minifilters studied, no biomass would have been observed in the SEM and epi-fluorescence analysis.

CONCLUSIONS

The treatment units in the HAR resulted a low-organic loading causing in a decreased efficiency of the reactor. The expanded clay used as the support material in the internal minifilters proved to be more appropriate than the pebbles for biofilm attachment due to its higher roughness as well as because of its lower weight, which could help to reduce the cost of construction.

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