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Performance of an anaerobic baffled reactor with an aerobic chamber treating low-strength wastewater

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ABSTRACT

The objective of this study was to evaluate the performance of an anaerobic baffled reactor (ABR) with an aerobic chamber (AC) in the treatment of domestic wastewater with a low organic load (0.10 ± 0.02 to 0.51 ± 0.10 kg COD·m^{-3}·d^{-1}). The entire system consisted of three anaerobic chambers (C1, C2, C3), one aerobic chamber (AC) and one laminar settling tank (LST), operated for 30 weeks (203 d) with different total hydraulic retention times (HRT) of 33, 22, 16.5, and 8.25 h. During the operation of the system, the values of COD of the influent varied between 105 and 381 mg·L^{-1} and the effluent varied between 12 and 147 mg·L^{-1}, with average concentrations of 214 ± 63 mg·L^{-1} in the influent and 48 ± 25 mg·L^{-1} in the effluent. Considering the entire system (ABR + AC + LST), the values of total removal efficiency for COD varied between 49 and 92%, with an average removal of 78 ± 9%. No accumulation of volatile fatty acids (VFA) was found, as the VFA concentration remained between 32 and 76 mg HAc·L^{-1} at the influent and between 21 and 53 mg HAc·L^{-1} at C3. Bio molecular analyses showed a great variety of bacterial communities established in all phases of monitoring and low archaeal community diversity. The combined configuration (ABR + AC) has shown great potential for the treatment of domestic wastewater, thereby being considered as a promising alternative for decentralized treatment.

Keywords: Anaerobic baffled reactor; Aerobic chamber; Volatile fatty acids; PCR/DGGE

1. Introduction

It is quite evident that attention must be given to studies involving wastewater treatment because of its connection with sanitation. Sanitation in Brazil has been facing enormous challenges in recent years: in 2010, approximately 1,915,292 Brazilian residences did not have proper water supply, and approximately 7,218,079 Brazilian residences discharged their wastewater into the environment without proper treatment, according to the Brazilian Institute of Geography and Statistics (IBGE) [1].

Besides this deficit in meeting sanitation demands, Brazil has been experiencing an economic crisis, so it is essential to develop technologies that are inexpensive, simple in design, efficient, and able to reduce the pollution in bodies of water. Most or all of the wastewater treatment plants in Brazil use conventional or centralized systems that are not the most cost-effective or appropriate options for all situ-

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ations because they rely on sophisticated technologies, on their operation by highly skilled personnel [2], and on large areas for their construction. A decentralized system, rather than a centralized system, might be especially beneficial in developing countries and it allows local residents to handle their situation when there is a lack of action or capacity by the central governing body [3].

One favorable technology that fits into this scenario is the anaerobic baffled reactor (ABR). The ABR is a wastewater treatment system initially developed at Stanford University by McCarty and co-workers in 1981 [4], which originated from UASB reactors (up flow anaerobic sludge blanket), but with the structural modification of multiple vertical baffles or chambers, in series and individually, which imposes a downward and an upward flow of the liquid through the chambers, ensuring greater contact of the effluent with the biomass present in the lower part of the reactor [5,6]. The ABR is able to retain large amounts of biomass for long periods, and it has low excess sludge production and a low operating and capital cost [7,8]. The most significant advantage of the ABR is its natural ability to longitudinally separate acido genesis and methano genesis down the chambers [9,10]. The ABR can also be effectively used to integrate other treatment units. Therefore, the modernization and modification of ABRs using combinations of processes (anaerobic/aerobic) has shown promising results [11,12]. Several studies have been carried out in recent years, showing the potential of ABRs to treat different types of wastewaters [7,11,14–16]. However, few of them have studied the performance of ABRs towards domestic wastewater. Moreover, although anaerobic wastewater treatment system is a consolidated technology, it removes approximately 70% of organic matter in terms of COD [17], which might be improved with the addition of an aerobic post-treatment unit leading to effluent of a better quality [11].

Thus, the aim of this study was to investigate the performance of an ABR on a pilot scale with an aerobic chamber in place at start-up, feeding it with low-strength wastewater. In addition, an inventory of microbial diversity using molecular techniques was further associated to contextualize the experimental data in order to suggest potential metabolic pathways under different operating conditions.

2. Materials and methods

2.1. Experimental setup

This study was carried out on a pilot scale wastewater treatment system constructed at the campus of the São Paulo State University (Bauru, São Paulo, Brazil). It consisted of the following components: a metal screen placed at a 45° angle relative to the horizontal, with a height of 100 cm and a width of 18 cm, composed of 9 iron bars 1 cm thick, with spaces of approximately 1 cm between them; settling and equalization tanks with capacities of 5,000 L and 2,200 L, respectively and a HRT of 24 h; a storage tank (200 L); an anaerobic baffled reactor (ABR), the features of which are described in Table 1, with three cylindrical anaerobic chambers (C1, C2 and C3) built of polyvinyl chloride (PVC); followed by a tertiary system, with an additional aerobic chamber (AC) and an 86 L laminar settling tank (LST). The inner plates of the LST consisted of 9 polypropylene blades arranged at a 60° angle relative to the horizontal, 40 cm in length by 30 cm in width and spaced approximately 5 cm apart. The dimensions of the LST were 55 × 32 × 45 cm (length × width × height). The highest hydraulic load on the LST was 20 $m^3 \cdot m^{-2} \cdot d^{-1}$. The scheme of the experimental setup is presented in Fig. 1. In the AC, two conical-shaped microporous air diffusers (10 µm) are placed on the bottom of the chamber (75 mm in diameter and 70 mm in height) and connected to an air compressor supplying the air. The air flow was controlled by a flow meter. The AC was filled with bamboo rings (*Bambusa vulgaris)* as an inert support for biomass immobilization. They were placed 50 cm below the top of the chamber, which had a height of 0.6 m and a diameter of 0.4 m. The total area used to construct the reactor was 2.0 m × 3.0 m.

Table 1
Features of the ABR chambers

Chamber	Specifications		
	Height (m)	Diameter (m)	Volume (L)
C1	0.90	0.6	405
C2	0.90	0.3	96
C3	0.90	0.3	96
AC	1.70	0.4	220

The first chamber was larger than the others, because this structural modification promotes greater organic matter removal, as well as the assimilation of hydraulic and organic shocks when compared to ABRs with chambers of the same size [18].

The reactor was fed with domestic wastewater from the campus, with a flow that varied from 576 $L \cdot d^{-1}$ to 2.304 $L \cdot d^{-1}$ during the experimental period. The total initial HRT was 33 h, for sludge acclimation during the start-up period. After reaching steady-state at 8 weeks (56 d), the HRT was gradually decreased to 22, 16.5, and 8.25 h (h), according to the stability of the system. The operating conditions of the reactor are described in Table 2. The reactor was inoculated with sludge from an upflow anaerobic sludge blanket reactor located at the municipal wastewater treatment plant (WWTP) of Bauru, Sao Paulo, Brazil.

2.2. Analysis

2.2.1. Physical and chemical

For the physical and chemical analyses, the top samplings points were used, located 50 cm above the top of each chamber. Chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), and pH were measured following the methodologies described in Standard Methods for the Examination of Water and Wastewater, 22nd ed. [19]. Total alkalinity (TA) was measured following the methodology described in Converse, Improved alkalimetric monitoring for anaerobic digestion of high-416 strength wastes. Journal Water Pollution Control Federation [20] for samples collected at the following points: influent (I), chambers 1, 2, and 3 (C1, C2, C3), the

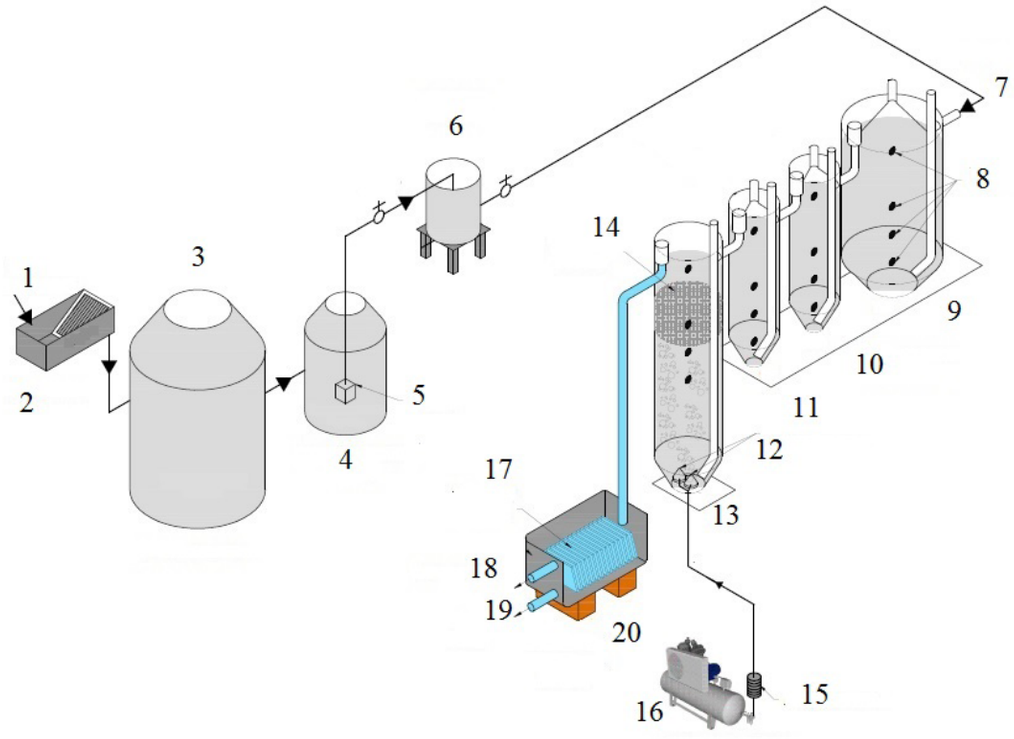

Fig. 1. Profile of the system: 1-Raw wastewater; 2-Screen; 3-Settling tank; 4-Equalization tank; 5-Pump; 6-Storage tank; 7-Influent (I); 8-Chamber sampling points (for this study, the higher points were used); Anaerobic chambers: 9-Chamber 1; 10-Chamber 2; 11-Chamber 3; 12-Air diffusers; 13-Aerobic chamber; 14-Bamboo rings; 15-Air flow meter; 16-Air compressor; 17-Plastic plates; 18-Effluent (E); 19-Sludge output; 20-Laminar settling tank.

Table 2
Operational conditions of the reactor

Phase	Operation period (wk/d)	Flow ($L \cdot h^{-1}$)	Organic loading rate (kg $COD \cdot m^{-3} \cdot d^{-1}$) (Average ± standard deviation)	HRT (h)				
				C1	C2	C3	AC	Total
1	8/56	24	0.10 ± 0.02	12	6	6	9	33
2	7/49	36	0.16 ± 0.05	8	4	4	6	22
3	7/49	48	0.32 ± 0.07	6	3	3	4.5	16.5
4	7/49	96	0.51 ± 0.10	3	1.5	1.5	2.25	8.25

aerobic chamber (AC), and effluent (E). Volatile fatty acids (VFA) was measured according to Development and validation of two methods to quantify 418 volatile Acids (C2-C6) by GC/FID: Headspace (Automatic and manual) and liquid-liquid extraction 419 (LLE). American Journal of Analytical Chemistry [21] for samples collected at the points I, C1, C2, and C3. The analyses of BOD were done at points I, C3, and E. An important fact related to the wastewater source is that the Physical Education Department has an Anatomy Lab. Students and employees should not discharge waste containing formaldehyde to the wastewater collection network. However, it is possible that illegal dumping might occasionally have occurred. Given this situation, the samples from point I were analyzed for formaldehyde according to Water Analysis Handbook, 2a ed., Hach Co.: USA, Loveland [22], using the MBTH Method 8110.

2.2.2. Biomolecular

The analyses of the microbial structure were performed for samples of the inoculation sludge and sludge collected at the bottoms of the anaerobic chambers C1, C2, and C3 (Fig. 1) of the ABR in all HRT changes (Table 2). The samples (Table 3) were washed in phosphate-buffered saline (PBS) and centrifuged at 6000 rpm for 10 min.

Nucleic acid was extracted following the procedure according to Rapid Method for Coextraction of DNA and RNA from Natural 423 Environments for Analysis of Ribosomal DNA and rRNA-Based Microbial Community Composition [23]. The quality of the DNA was confirmed employing an ND-2000 spectrophotometer (Nanodrop Inc., Wilmington, DE, USA).

A segment of the 16S rRNA gene fragments was amplified using the polymerase chain reaction (PCR) with

Table 3
Classification of each sample collected from the ABR during the experimental period for biomolecular analyses

Phase	Sample	Designation			
		Inoculum	Chamber 1	Chamber 2	Chamber 3
Start-up	Inoculum	*i*	–	–	–
1	Sludge	–	C1F1	C2F1	C3F1
2	Sludge	–	C1F2	C2F2	C3F2
3	Sludge	–	C1F3	C2F3	C3F3
4	Sludge	–	C1F4	C2F4	C3F4

primers for the Bacteria Domain [24] and for the Archaea Domain [25]. The PCR for *Bacteria Domain* [24] consisted of 35 cycles as follows: initial denaturation to 94°C for 2 min, 10 cycles of denaturation at 94°C for 1 min, annealing at 69°C for 3 min, extension at 72°C for 3 min, final extension at 94°C for 10 min and cooling to 4°C. For *primers* of Archaea Domain [25] the PCR consisted of 35 cycles as follows: initial denaturation to 94°C for 7 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, final extension at 72°C for 7 min, and cooling to 4°C. The products of the PCR were purified using an Ultraclean PCR Clean-up kit (MoBioLaboratories, Inc.) according to the manufacturer's instructions.

The analyses of the microbial community were carried from the amplification of 16S rRNA fragments by PCR and denaturing gradient gel electrophoresis (DGGE) of the samples (Table 3) were performed using PCR with set primers for the Bacteria domain [26] and the Archaea domain [27]. They were separated on polyacrylamide gel with DGGE containing a linear gradient varying from 40% to 60% of the denaturant. The run was carried out at 60°C and 75 V (6 V cm^{-1}) for 16 h. The gel was cured with ethidium bromide for 20 min. To read the banding patterns obtained in the DGGE, a TMIII Eagle Eye (Stratagene) was used under exposure to UV at 254 nm, coupled to the computer running Eagle Sight software.

The evaluation of the DGGE band patterns was done considering presence or absence and intensity if present. Multivariate DGGE analysis of the band profiles were performed by BioNumeric 2.5 software (Applied Maths, Belgium), which analyzed them using Pearson correlation. Dendrograms were constructed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and diversity indices were calculated using Shannon & Wiener.

3. Results and discussion

3.1. Organic matter and removal of suspended solids

Table 4 shows the average values (A) and standard deviation (SD) for COD, BOD, TSS, and Volatile Suspended Solids (VSS) obtained operating with the total HRT (ABR + AC + LST). Figs. 2, 3, and 4 show the values of removal efficiencies for COD, BOD, and TSS for the anaerobic and aerobic systems (ABR and ABR + AC). Most of the raw influent fed to the system was from public toilets, washbasins, pool extravasations, floor washing, dilutions, etc. Accordingly,

Table 4
Average values for COD, BOD, TSS, and VSS in the influent and effluent

Parameters ($mg \cdot L^{-1}$)	HRT (h)			
	1 (33 h)	2 (22 h)	3 (16.5 h)	4 (8.25 h)
	Influent (I)			
	A±*sd*	*A*±*sd*	*A*±*sd*	*A*±*sd*
COD	75 ± 43	104 ± 24	127 ± 28	154 ± 51
BOD	55 ± 17	106 ± 40	114 ± 25	69 ± 22
TSS	38 ± 21	36 ± 23	71 ± 35	27 ± 14
VSS	28 ± 13	23 ± 10	46 ± 33	24 ± 14
	Effluent (E)			
	A±*sd*	*A*±*sd*	*A*±*sd*	*A*±*sd*
COD	40 ± 19	39 ± 14	64 ± 40	48 ± 14
BOD	23 ± 15	21 ± 9	24 ± 14	23 ± 6
TSS	4 ± 1	3 ± 2	3 ± 1	6 ± 6
VSS	3 ± 4	2 ± 2	2 ± 1	4 ± 5

the average organic load varied between 0.10 ± 0.02 and 0.51 ± 0.10 kg $COD \cdot m^{-3} \cdot d^{-1}$ and the average concentration of COD at point I was 214 ± 63 $mg \cdot L^{-1}$. The wastewater could be categorized as low-strength wastewater, according to Metcalf and Eddy [28].

The value of COD at point I varied between 105 and 381 $mg \cdot L^{-1}$, and at point E it varied between 12 and 147 mg^{-1}. The values of COD total removal efficiency, considering the entire system (ABR + AC + LST), varied between 49 and 92%, with an average removal of 78 ± 9%. Regarding the contribution of each chamber, of the 55% removal of the average value of COD reached in the anaerobic system, 33% was already removed in chamber C1, considered as essential in the removal efficiency of the system. Chamber C2 removed 18%, and chamber C3 removed 16%. The additional aerobic basin and the laminar settling tank were responsible for removal of the residual organic matter, removing an average of 28% and 27%, respectively. The AC and LST were important as a polishing step for the effluent.

Sarathai et al. [7] and Bae et al. [29] have found similar values for COD removal efficiency with ABRs operated with low-strength synthetic wastewaters. Lee et al. [30] has had values for COD efficiency removal of 84%, but the authors have related this high value to the secondary polishing system, which consisted of an anaerobic fluidized-membrane bioreactor. The removal of the ABR was lower (60%) than in this study.

The values for the average removal of COD obtained in each of the HRT used were similar: HRT_1 = 77 ± 10%, HRT_2 = 79 ± 6%, HRT_3 = 76 ± 14%, HRT_4 = 78 ± 6%. A statistical test [31] was used with a significance level of 0.05 to determine if there was a significant difference between the averages of COD removals, and the averages showed no significant difference among each other. This demonstrates that the removal of organic matter occurred in each operational step, even with the reduction in the HRT.

After the system (ABR + AC + LST) reached steady-state (phase 1), the HRT was changed to 22 h, then to 16.5 h, and

finally to 8.25 h. After each change in the HRT, the performance of the system in removing the target parameters was reduced, but it improved over time to a steady-state level. Based on Figs. 2–4, the performance first dropped when the HRT was switched from 33 h to 22 h; however, it recovered to higher values of removals. The same behavior was observed by Aqaneghad et al. [32]. This demonstrates that the system rapidly adapted to the HRT changes.

The average values of BOD at point I varied between 36 and 162 mg·L^{-1} and at point E they varied between 4 and 39 mg·L^{-1} with an average removal efficiency of 70 ± 18%.

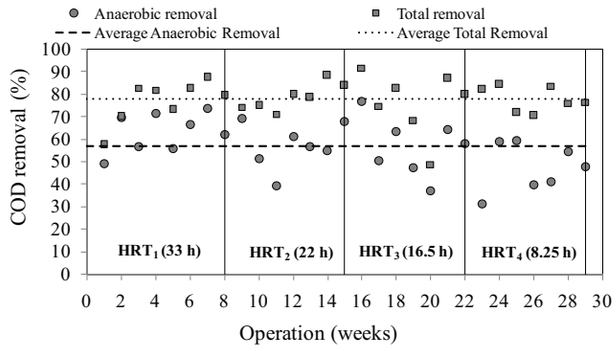

Fig. 2. Values of total and anaerobic COD removal efficiency.

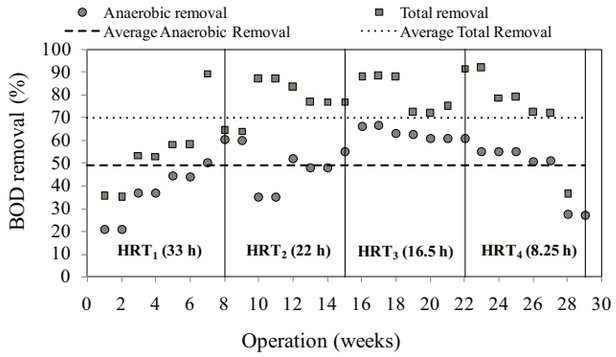

Fig. 3. Values of total and anaerobic BOD removal efficiency.

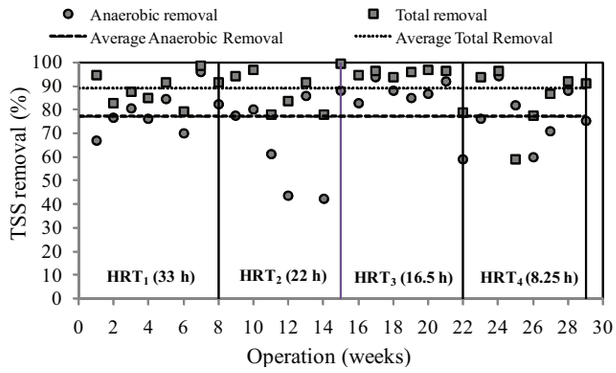

Fig. 4. Values of total and anaerobic TSS removal efficiency.

Gopala Krisna et al. [5] have obtained values of removal efficiency above 90% with an ABR treating low-strength synthetic wastewater (1.5 to 1.2 kg COD·m^{-3}·d) operated with HRTs of 8–10 h. The authors have concluded that biological treatment is viable in this type of reactor even with low-strength wastewater. Regarding the removal of total suspended solids (TSS), removal efficiencies above 90% were obtained during the operational period. The values of TSS in the effluent were between 6 and 130 mg L^{-1}.

The MBTH test revealed the presence of formaldehyde in the wastewater. Formaldehyde is commonly found in wastewaters from industries and laboratories. It is considered as a germicidal agent and inhibits microbial activity at concentrations higher than 250 mg CH_2O·L^{-1} [33] in wastewater treatment systems, thus reducing the ability to remove organic matter. In this study, an average value of 304 µg CH_2O·L^{-1} of formaldehyde was found at point I. This is a low concentration, so it probably did not influence microorganism activity.

To illustrate the efficiency of the reactor used in this study, Table 5 shows the performance of the ABR in the treatment of municipal wastewater given in the literature. Comparing the results from the literature with the results from the ABR + AC + LST used in this research, it is possible to infer that the ABR + AC + LST was effective even when being fed with low-strength wastewater and operated with HRTs as low as 8.25 h. The ABR was shown to be a robust and efficient anaerobic reactor configuration.

According to Reynaud and Buckley [34] the separation of the reactor into chambers is a strongly stabilizing factor, with feed fluctuations being evened out across reactor chambers or peaks shifting treatment to the rear chambers without affecting the overall effluent quality. In addition, significant COD reduction occurs almost exclusively in the first three chambers, whereas the reduction observed in the ABR of the present study occurred mostly in the first and second chambers.

The authors also mention that performance data on full-scale ABR implementations are extremely scarce, having not been sufficiently understood.

3.2. Stability of reactor operation

The average pH values at point I varied between 7.2 and 7.3, at point C1, C2, and C3 they varied between 7.1 and 7.3, and at point AC they varied between 7.1 and 7.3. The average pH values obtained during the operation were in the neutral range, considered optimal for microbial activity. One important fact related to the pH values is that the settling and equalization tank could have promoted an initial anaerobic digestion during the primary treatment, but did not acidify the influent, which is proven by these neutral and stable pH and low volatile fatty acid values and consequently did not impact the performance. The average values of total alkalinity (TA) at each sampling point were: 337 ± 93 mg $CaCO_3$·L^{-1} (I), 325 ± 83 mg $CaCO_3$·L^{-1} (C1), 327 ± 86 mg $CaCO_3$·L^{-1} (C2), 328 ± 88 mg $CaCO_3$·L^{-1} (C3), 252 ± 93 mg $CaCO_3$·L^{-1} (AC), and 256 ± 91 mg $CaCO_3$·L^{-1} (E). The average values of volatile fatty acids (VFA) at each sampling point were: 56 ± 22 mg HAc·L^{-1} (I), 71 ± 26 mg HAc·L^{-1} (C1), 59 ± 29 mg HAc·L^{-1} (C2), and 42 ± 23 mg HAc·L^{-1} (C3).

Table 5
Performances of different ABRs in the treatment of municipal wastewater in the literature and the ABR used in this study

Reactor	Average influent COD ($mg \cdot L^{-1}$)	HRT (h)	Organic loading rate (kg $COD \cdot m^{-3} \cdot d^{-1}$)	Average COD removal (%)	Reference
ABR (phase 4)	220	6	0.51	55	This study
ABR + AC (phase 4)	220	8.25	0.51	78	This study
Combined ABR	305	48	0.15	79	[12]
Modified ABR	300	8	0.9	79	[15]
Modified ABR	400	6	1.6	84	[14]
ABR	550	12	1.69	89	[35]
ABR	716	22	0.78	73	[36]

Alkalinity is directly related to pH and VFA. The TA generated between points C1, C2, and C3 was considered low, with the consumption of TA at point AC, providing a neutral pH. The reduced TA generated can be explained by the low generation and consumption of VFA in the anaerobic chambers, related to the low organic load during operation. The consumption of TA at point AC, which was responsible for reducing the average value from 328 ± 88 mg $CaCO_3 \cdot L^{-1}$ at point C3 to 252 ± 93 mg $CaCO_3 \cdot L^{-1}$ at point AC, can be related to the nitrification process; on the other hand, the average value of nitrate increased from 2 ± 1 mg· L^{-1} at point C3 to 18 ± 24 mg. L^{-1} at point AC, and ammonia nitrogen decreased from 55 ± 19 $mg \cdot L^{-1}$ at point C3 to 43 ± 15 mg. L^{-1} at point AC.

The results presented for pH and TA show that the pH was practically stable, with values between 7.1 and 7.3 for the total system. Together with the values of TA, this indicates the presence of alkaline constituents in sufficient quantity to ensure the buffering of the system. In this case, there is agreement with the low values of VFA obtained.

The balance of VFA is important when studying anaerobic reactors, because high concentrations of VFA may affect the biochemical process and eventually disturb the anaerobic digestion, which may cause the reactor to the collapse [37]. The average values of VFA at each sampling point are presented in Fig. 5.

No accumulation of VFA was observed during the operation of the ABR. The values of VFA varied between 32 and 76 mg $HAc \cdot L^{-1}$ at point I, 37 and 80 mg $HAc \cdot L^{-1}$ at point C1, 46 and 71 mg $HAc \cdot L^{-1}$ at point C2, and between 21 and 53 mg $HAc \cdot L^{-1}$ at point C3. According to Pereira et al. [36], the ABR studied in their research have always operated with values of total acidity below 100 mg HAc L^{-1} and they have concluded that the system worked well below the instability threshold, indicating an optimal performance. Fig. 5 shows that there was a discrete increase in the concentration of VFA throughout the chambers, rising in chamber C1 or C2, depending on the HRT in operation, after which there was a reduction in these concentrations. This fact may be showing a subtle phase separation of the digestion of the organic matter, with the higher values of VFA (production of acids) obtained in the chambers being related to acidogenesis and similarly, consumption being related to methanogenesis. In HRT1 (30 h) the concentration of VFA decreased in C1, demonstrating the consumption of VFA. Due to the start-up period of 56 d the microorganisms most likely were adapting to the new conditions, proved by the lower efficiency of organic removal shown in this phase. Along the operation and stabilization of the process (C1, C2 and C3), it may be noticed that the VFA decreased in C2, especially in HRT2 (22 h) and HRT3 (16.5 h), that is, the reduced HRT favored the process of acidogenesis and methanogenesis with the consumption of VFA. The role of the microorganisms is related to the VFA activity. It is possible that in C1, the digestion of carbohydrates and sugars through fermentation generated acids that were used in C2 and C3 by the acetogenesis and methanogenesis phases. Within biological reactors the microorganisms act in a syntrophic relationship, where one depends on the other. In the present paper, the biomolecular analyses were not performed to prove consistently the organic matter digestion in phase separation, but there were differences in the diversity of bacteria and archaea communities according to the mode of operation, such as the TDH applied to the reactor configuration itself, favoring both aerobic and anaerobic processes in the consumption of organic matter through the acid concentrations found.

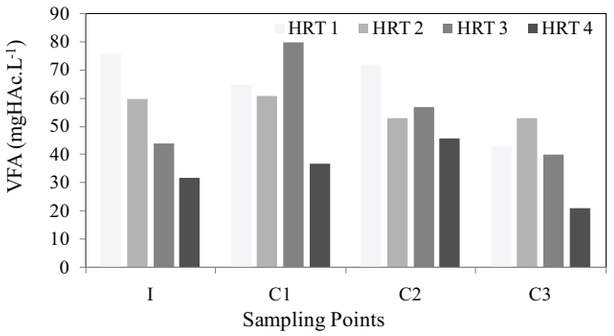

Fig. 5. Values of VFA in each sampling point, along the monitoring of the ABR.

Similar behavior was observed by Gopala Krishna, et al. [5] in the treatment of low-strength soluble wastewater (COD of approximately 500 $mg \cdot L^{-1}$), using an eight-chambered ABR. The formation of VFA (53–85 $mg \cdot L^{-1}$) was observed in the first chamber, because of acidogenesis and acetogenesis, and the concentration of VFA decreased longitudinally down the reactor, according to the authors. They also note that observations from scanning electron micrographs suggest that distinct phase separation occurs in an

ABR. The authors Feng et al. [38] have investigated the performance of an ABR in the treatment of low-strength domestic wastewater (with COD in the effluent of 165 mg· L^{-1}), and they have concluded that hydrolysis occurred in the first chamber, while methano genesis occurred in all five of them.

The acids found in the ABR were acetic, butyric, isobutyric, propionic, valeric, isovaleric and caproic. The highest concentrations found were for acetic acid (50 mg·L^{-1}), the main intermediate in the anaerobic degradation of organic matter, with smaller amounts of butyric and propionic acids. Jurgensen et al. [39], using a bench-scale ABR with four chambers and fed with high-strength synthetic wastewater (4.000 mg·L^{-1}), have reported that acetic acid (2.700 mg·L^{-1}) was formed as the main intermediate, in addition to lower concentrations of butyric and propionic acids. Comparing the results of Jurgensen et al. [40] with this study, it is possible to tell that the low concentration of acids produced is related to the organic loading rate at which the system operates.

3.3. Microbiological results

3.3.1. Bacterial consortium

The results of the similarity coefficients of the DGGE band patterns for the Bacteria domain of samples from Chamber 1 (C1), Chamber 2 (C2), and Chamber 3 (C3) throughout the different phases operated [Phase 1 (F1), Phase 2 (F2), Phase (F3),and Phase 4 (F4)] in the ABR + AC, are presented in Fig. 6 (A, B, and C). The results showed a great variety in the bacterial communities among the samples.

The inoculum (i) showed high similarity among samples C1F1 (75%), C2F1 (78%), and C3F1 (79%), corresponding to phase 1. In other words, the bacterial consortia from the inoculum was maintained in all chambers in phase 1 (C1F1, C2F1, and C3F1). From F2 onwards, the operating conditions changed, with an increase in organic loading (Table 2). Thus, the development of different communities can be noticed, especially in phases 3 and 4 (F3 and F4) with the establishment of the anaerobic bacteria consortia along the chambers. The inoculum had greater influence on the community structure in phase 1, which agrees with [39], which says that the start-up phase of an anaerobic reactor is considered to be a critical point. The inoculation and start-up phase will help the development of an active microbial biomass.

Analyzing each chamber, for C1 (Fig. 6A) the consortia of anaerobic bacteria began to differentiate from the inoculum in C1F2, reaching a high similarity between C1F3 and C1F4, with a value of 92%. A new consortium developed and remained in F3 and F4 probably because of the changes in operating conditions. For C2 (Fig. 6B), the same pattern was observed; however, the highest similarities were

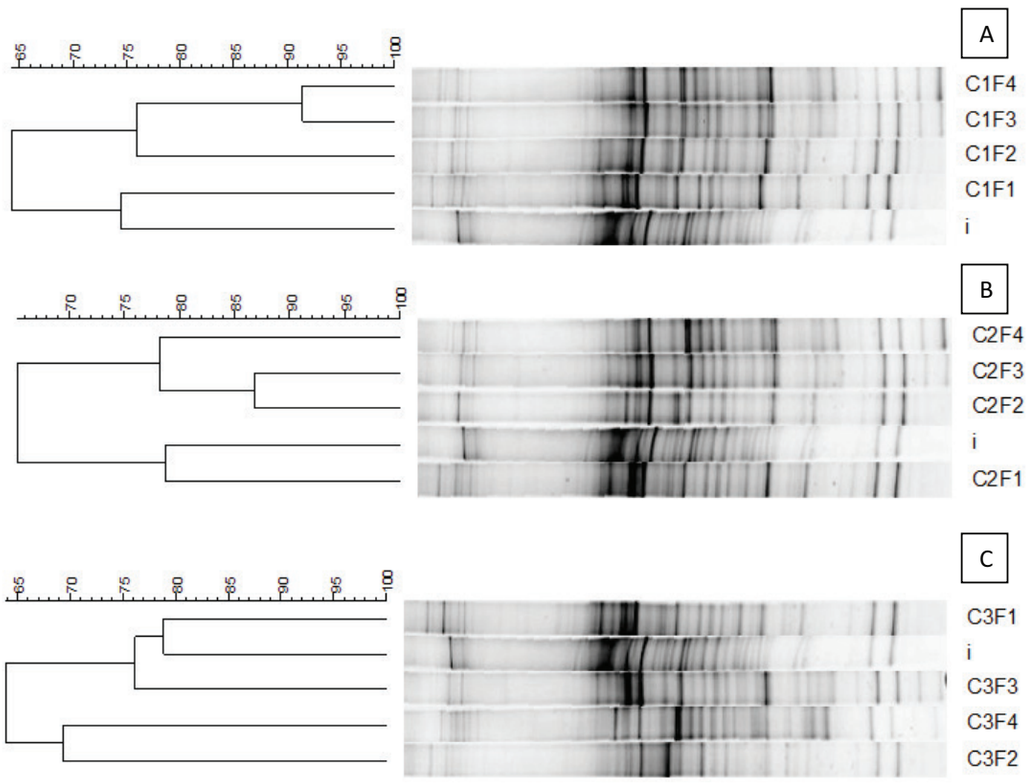

Fig. 6. Similarity coefficients of the DGGE band patterns for the Bacteria domain of samples from the ABR + AC, in the different phases operated. Fig. 6A corresponds to samples collected in Chamber 1 (C1), Phases 1,2,3, and 4 (F1, F2, F3, and F4), and inoculum (i). Fig. 6B corresponds to Chamber 2 (C2), Phases 1,2,3, and 4 (F1, F2, F3, and F4), and inoculum (i). Fig. 6C corresponds to Chamber 3 (C3), Phases 1,2,3, and 4 (F1, F2, F3, and F4), and inoculum (i).

between C2F2 and C2F3, with a value of 87%. Under condition C, similarity was observed between C3F2 and C3F4, with a value of 69%.

The conditions for F3 and F4 are less similar in C3 (Fig. 6C) than in C1 and C2. The development of a different community between the phases may have occurred, and the community did not endure because of the different physico chemical and hydraulic conditions of the chambers.

3.3.2. Archaeal consortium

The results of the similarity coefficients of the DGGE band patterns for the Archaea domain of samples from Chamber 1(C1), Chamber 2 (C2), and Chamber 3 (C3) throughout the different phases operated [Phase 1 (F1), Phase 2 (F2), Phase (F3), Phase 4 (F4)] in the ABR + AC are presented in Figs. 7A, B, and C. The results show little variety in the archaeal communities among the samples. The inoculum (i) showed higher similarity to the samples from phase 1 in conditions A and B, with 71.5% of similarity for C1F1 and 70% for C2F1, respectively. Archaea are very sensitive to operation changes and one of the conditions that changed the most during the inoculation process was the concentration of organic matter in the feeding wastewater.

4. Conclusions

The average concentrations of COD were 214 ± 63 mg.L^{-1} at point I and 48 ± 25 mg·L^{-1} at point E, with an average removal value of 78 ± 9%. The values for the average removal of COD obtained in each operated HRT were: HRT1 = 77 ± 10%, HRT2 = 79 ± 6%, HRT3 = 76 ± 14%, and HRT4 = 78 ± 6%, with no significant difference between them. The average concentrations of BOD were 85 ± 36 mg· L^{-1} at point I and 23 ± 11 mg·L^{-1} at point E with an average efficiency removal of 70 ± 18%.

The qualitative analysis of VFA revealed that acetic acid had the highest concentration, which usually occurs as acetic acid is the major precursor of anaerobic digestion. The low-strength wastewater provided a reduced formation of VFA, even though it did not negatively influence the efficiency. A discrete phase separation of the organic matter digestion was observed, with the generation and consumption of VFA.

The DGGE profiling was useful to diagnose the presence and relative abundance of microorganisms in the ABR + AC system, showing that the microbial populations changed between the different phases of operation.

The results obtained from this reactor confirmed the viability of the combination of aerobic and anaerobic processes for low strength wastewater and for municipal/domestic sewage treatment with a good capability of achieving a

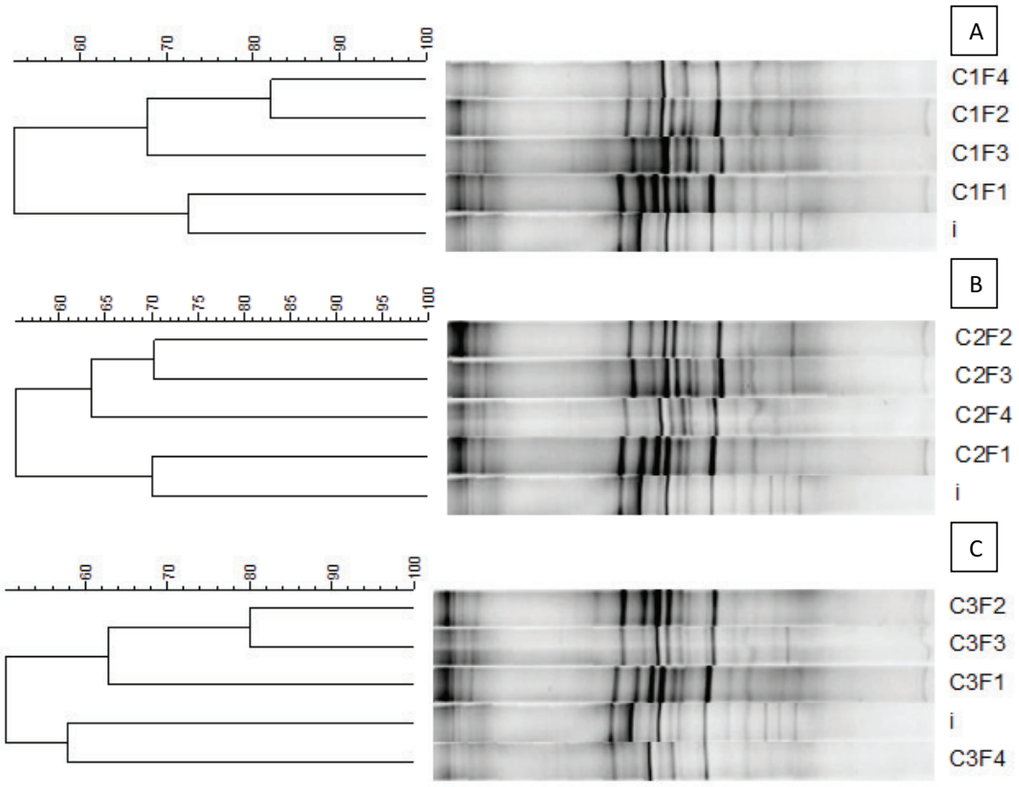

Fig. 7. Similarity coefficients of the DGGE band patterns for the Archaea domain of samples from the ABR + AC, in the different phases operated. Fig. 7A corresponds to samples collected in Chamber 1 (C1), Phases 1,2,3, and 4 (F1, F2, F3, and F4), and inoculum (i). Fig. 7B corresponds to Chamber 2 (C2), Phases 1,2,3, and 4 (F1, F2, F3, and F4), and inoculum (i). Fig. 7C corresponds to Chamber 3 (C3), Phases 1, 2, 3, and 4 (F1, F2, F3, and F4), and inoculum (i).

high quality of final effluent. This is encouraging for full-scale development as a decentralized option for wastewater treatment.

The additional aerobic chamber and LST were applied successfully as a polishing-stage system, improving the removal of organic matter.

The HRT reduction did not significantly affect the performance of organic matter and suspended solids removal. This may imply reducing the size of the units in the case of full scale application. Studies focused in the application of the ABR at full scale are very important and still demand well-monitored long-term full-scale reactor investigations and encouraging results that support the use of ABR as one of the solutions that attend the global necessity for low-maintenance, robust treatment systems.

Other recommendations and adjustments to scaling up these units can be addressed to additional tests to remove nitrogen with the introduction of a bypass of the raw sewage or another organic source such as ethanol, for example, in the last chamber (AC) to promote the denitrification. Another strategy would be to aerate the third chamber and change the process of the AC to anaerobic or even to add a fifth anaerobic chamber after the AC.

Additionally, such systems using a combination of aerobic and anaerobic processes in chambers are expected to produce better results with higher-organic-load wastewater.

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