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II-527 - EVALUATION OF THE MICROBIOLOGY OF ACTIVATED SLUDGE IN SEWAGE TREATMENT PLANTS IN THE METROPOLITAN REGION OF RIO DE JANEIRO

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Abstract: The activated sludge system, widely used worldwide, consists of an aerobic biological treatment of sewage. Activated sludge microfauna components play an important role in effluent clarification and can be used as process indicators. The determination of the Sludge Biotic Index - IBL is a model used to evaluate the effectiveness of treatment processes. Biological floc and filament diameter measurements help in the evaluation of sludge sedimentability. The objective of the study was to evaluate the microbiology of activated sludge from five Sewage Treatment Plants operated by activated sludge in the Metropolitan Region of Rio de Janeiro, aiming to correlate population dynamics with operational conditions. One of the ETEs performs co-treatment of leachate from a sanitary landfill and this influence was also evaluated. For the study, field measurements, physical-chemical analyses, qualitative and quantitative analyzes of the microfauna, measurements of floc diameter and length of filamentous bacteria were carried out. The results were correlated with physical-chemical and operational parameters and the IBL was calculated. It was verified the occurrence of all the morphofunctional groups of the microfauna. Density and species richness analyzes showed diversified microfauna and dominance of flake predatory ciliates, sessile ciliates and thecamebas that indicated good clearance and high quality effluent for most of the studied period. Statistical tests showed the following correlations: naked amoebas indicated a high food/microorganism ratio and BOD removal; small flagellates indicated poor clearance; flake predator ciliates, good clearance and *Aspidisca cicada* occurrence of nitrification; micrometazoans indicated high sludge age and BOD removal. The IBL proved to be applicable to the evaluation of treatment efficiency. Floc diameter analyzes related small flocs to low organic load and shear caused by mechanical aeration. The volume of landfill leachate contributed to the increase in floc diameter and interfered with the species richness and population of thecamebas. The proliferation of filamentous bacteria influenced the sedimentability of the sludge and was caused by changes in the following factors: pH and dissolved oxygen concentration. Filamentous bacteria also contributed to the efficiency of removal of BOD and total suspended solids in the effluent.

Keywords: Microfauna, Activated Sludge, Sewage Treatment, Biological Floc, Filamentous Bacteria.

INTRODUCTION

Most large-scale Sewage Treatment Plants (ETEs) in the Metropolitan Region of Rio de Janeiro use the activated sludge system in sewage treatment, which is a system widely used worldwide for domestic waste (Von Sperling. 2002).

The ETEs operated by the activated sludge system can also act as an alternative for the treatment of landfill leachate. Several authors cite combined treatment with domestic sewage as an effective and widely used alternative worldwide for the treatment of landfill leachate (Franco, 2009; Nascentes *et al,* 2015; Santos Jr, 2017).

The quality of the effluent obtained in an ETE operated by the activated sludge system depends on the biotic quality present in the sludge. Several microbiological groups participate in this process, such as: suspended bacteria, floc-forming bacteria, filamentous bacteria, protozoa and micrometazoans (Sant'Anna Jr., 2013). The microfauna plays an important role in the activated sludge system, limiting the density of bacterial populations by predation and contributing to the flocculation process, being responsible for improving the quality of the effluent. The microfauna is

very sensitive to environmental conditions, variations in the affluent, in operational conditions and in the community can affect the entire food chain of these ecosystems, the biological performance of the ETE and the quality of the final effluent. The structure of the microfauna community is, therefore, an indicator of the operational conditions of the ETEs and the characteristics of the effluent (Madoni, 1994; Bento *et al.* 2005; Silva, 2019).

In this context, protozoa and micrometazoans are a target of interest within research centers because they are excellent bioindicators of the activated sludge process (Santos, 2012). The main groups of protozoa found in activated sludge systems are amoebas, flagellates and ciliates (Madoni, 1994). Other authors in their studies have correlated the presence of the organism and the characteristics of the process or operational condition, with results presented in tables such as Madoni (1994), Vazollér (1989) and Foissner (2016).

For a good performance of an activated sludge system, an adequate separation between the sludge and the liquid phase, which occurs in the secondary decanter, is essential and that this is fast and efficient (Figueiredo, 2012). Therefore, the measurement of the diameter of the biological flocs of the activated sludge and the density of filamentous bacteria are important parameters to be monitored, as they allow us to assess whether the biological floc has adequate characteristics for adequate sedimentation and helps to predict possible trends in sedimentation. occurrence of filamentous *bulking.*

Monitoring the treatment efficiency of ETEs is commonly performed by physical-chemical and microbiological parameters in the influent and effluent to the ETE. However, according to Saar (2015), activated sludge microscopy, when systematically implemented in the ETE, becomes a valuable ally in preventing operational problems, as it allows the observation of changes in the sludge micro ecosystem before eventual impacts cause collapse. system or change its balance to an undesired level.

GOALS

The main objective of this study was to monitor and evaluate the microbiology of activated sludge from five Sewage Treatment Plants operated by the activated sludge system in the Metropolitan Region of Rio de Janeiro, aiming at a possible correlation of population dynamics with operational conditions. Therefore, the work had the specific objective of classifying the organisms present in the microfauna into morphofunctional groups, quantifying them and correlating them with the operational conditions of the treatment systems; to evaluate the applicability of the Sludge Biotic Index proposed by Madoni (1994) as an indicator of the efficiency of activated sludge processes; monitor the diameters of the biological flocs present in the activated sludge and correlate them with the sedimentability of the sludge and with the treatment efficiency; and measure the total length of filamentous bacteria present in the activated sludge and correlate them with operational parameters and with the sedimentability of the sludge.

METHODOLOGY USED

The study was carried out from May to December 2017 in five Sewage Treatment Stations located in the Metropolitan Region of Rio de Janeiro and operated by the sludge treatment system activated by the State Water and Sewerage Company of Rio de Janeiro (CEDAE), they are Alegria, Pavuna, Sarapuí, Penha and Ilha do Governador.

The collection of liquid from the aeration tanks and final effluents for physical-chemical analysis were performed daily, according to

the ABNT/NBR 9898:1987 methodology. Activated sludge collections for microscopic analysis were performed at least once a week at the outlet of the aeration tanks.

Daily measurements of dissolved oxygen were performed using a field oximeter. The physical-chemical analyzes were performed according to APHA methodologies (2017). The calculations of the operational control parameters (A/M ratio, sludge volumetric index-IVL and sludge age-θ) were performed as described by Jordão & Pessôa (2014).

Microscopic analyzes were performed under an optical microscope with phase contrast. Qualitative and quantitative analyzes were performed on the microfauna and measurement of the diameter of the biological floc and of filamentous bacteria in the activated sludge of each ETE.

Qualitative analyzes were performed using a slide and coverslip, while quantitative analyzes were performed in a Sedgewick-Rafter chamber. The methodology was carried out according to the instructions of Figueiredo (2012). The identification of organisms was made according to the guide of Foissner & Berger (1996), the atlas of Berk & Gunderson (1993), Vazollér (1989) and Figueiredo (2012). Organisms were identified to species level, whenever possible, and classified according to the following morphological-functional groups suggested by Madoni (1994): flake-predating ciliates, fixed ciliates, free-swimming ciliates (<50μm and ≥50μm), thecamoebas, naked amoebas, small flagellates and micrometazoans.

The measurements of the floc diameter and its classification were carried out according to Figueiredo (2012), in each analysis 40 flocs were measured and their averages were calculated. The analyzes of measurements of filamentous bacteria were performed in a Sedgewick-Rafter chamber, according to the methodology described in Figueiredo (2012).

The results obtained were submitted to statistical analysis through the estimation of Pearson's correlation coefficients (r) and the significance of r was verified through Student's t test, at 5% probability ($p<0.05$).

RESULTS OBTAINED

Through qualitative analysis of the microfauna of the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha, it was possible to identify the organisms present in the liquid under aeration and classify them according to the following morphological-functional groups: gymnamebas or naked amoebas, thecamebas or amoebas thecated, flake-predating ciliates, sessile, free-swimming (<50μm and 50μm), large and small flagellates and micrometazoans (rotifers, annelids, nematodes, tardigrades and gastrotrichs). The taxa/species found in the liquids under aeration of the activated sludge from the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha are shown in table 1.

The quantitative analyzes of the microfauna allowed to verify: the density of organisms, the richness of species of the liquid under aeration and the dominance of the groups found in the activated sludge of the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha.

In the evaluation of the density of organisms, the averages of the total number of organisms in the activated sludge were: 1.35×10^7 organisms.L in the ETE Alegria; 5.21×10^6 organisms.L in ETE Pavuna; 3.46×10^6 organisms.L in ETE Sarapuí; 1.10×10^7 organisms.L in the ETE Penha; and ; 1.18×10^7 organisms.L in ETE Ilha.

By means of species richness, it was possible to evaluate the diversity of the microfauna of the activated sludge of the studied ETEs. The average number of taxa identified in the activated sludge of the five ETEs was as follows: Alegria 14.6; Pavuna 10.9; Sarapui 10.4; Penha 12.5; and Island 8.2. Figure 1 shows the graph

large groups	ETE Alegria	ETE Pavuna	ETE Sarapuí	ETE Penha	ETE Island
Tecamebas	*Arcella vulgaris roundabout euglypha Centropyxisaculeata Difflugia* sp.	*Arcella vulgaris, roundabout euglypha Centropyxisaculeata Difflugia* sp.	*Arcella vulgaris, roundabout euglypha Centropyxisaculeata Difflugia* sp.	*Arcella vulgaris roundabout euglypha Centropyxisaculeata, Difflugia* sp.	*Arcella vulgaris roundabout euglypha, Centropyxisaculeata, Difflugia* sp.
Flake Predator Ciliates	*cicada aspidis Euplotesaediculatus Chilodonella* sp.	*cicada aspidis Euplotesaediculatus*	*cicada aspidis Euplotesaediculatus*	*cicada aspidis Euplotesaediculatus Chilodonella* sp.	*cicada aspidis Euplotesaediculatus Chilodonella* sp.
Free-swimming ciliates	*Uronema* sp. *Blepharisma* sp. *Spirostomum teres,* parameciumaurelia *Trachelophyllum* sp.	*Trachelophyllum* sp.	*Trachelophyllum* sp.	*Spirostomum teres, Trachelophyllum* sp.	*Colpidium sp. Trachelophyllum* sp.
sessile ciliates	*Thruricolakellicottiana Vaginicola* sp. *Epistylisplicatilis vorticella microstoma Carchesium* sp. *Opercularia* sp. *Chaetospira* sp. *Vasicola* sp.	*Thruricolakellicottiana Vaginicola* sp. *Epistylisplicatilis vorticella microstoma Opercularia* sp. *Caetospira* sp. *Vasicola sp.*	*Thruricolakellicottiana Vaginicola* sp. *Epistylisplicatilis vorticella microstoma Opercularia* sp. *Caetospira* sp.	*Thruricolakellicottiana Vaginicola* sp. *Epistylisplicatilis, vorticella microstoma Charchesium* sp. *Opercularia* sp. *Caetospira* sp. *Vasicola* sp. *Zoothamnium* sp.	*Thruricolakellicottiana Vaginicola* sp. *vorticella microstoma Epistylisplicatilis, Epistylischrysemydi s Carchesium* sp. *Opercularia* sp. *Chaetospira* sp. *Zoothamnium* sp.
Carnivorous Ciliates	SUGGESTIONS	*Litonotus* sp. SUGGESTIONS	*Amphileptus* sp. *Coleps* sp. SUGGESTIONS	*Litonotus* sp. *Amphileptus* sp. SUGGESTIONS	*Litonotus* sp. SUGGESTIONS
Great Flagellates	*Peranema* sp.	*Peranema* sp.	*Peranema* sp.	*Peranema* sp.	*Peranema* sp.
micro metazoans	OLIGOCHETE ROTIFEROS, TARDIGRADE NEMATODES	OLIGOCHETE ROTIFEROS	ROTIFEROUS TARDIGRADE NEMATODE	OLIGOCHETE ROTIFEROS, TARDIGRADE NEMATODES GASTROTRICHA	OLIGOCHETE ROTIFEROS, TARDIGRADE NEMATODES GASTROTRICHA

Table 1: List of taxa/species identified in liquids under aeration of activated sludge from Alegria, Pavuna, Sarapuí, Penha and Ilha ETEs.

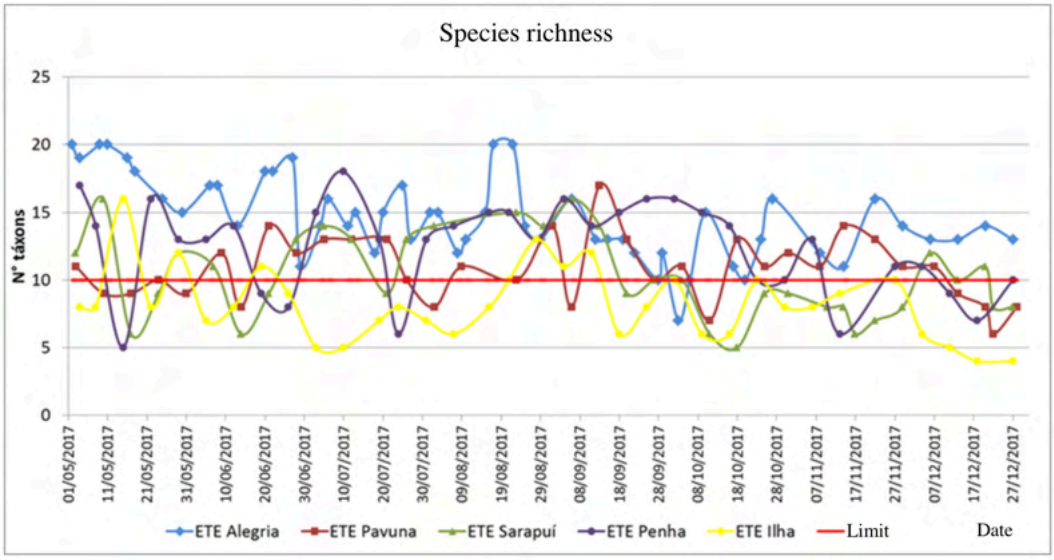

Figure 1: Graph of the species richness of the microfauna of the activated sludge from the Alegria, Pavuna, Sarapuí, Penha and Ilha ETEs expressed by the number of identified taxa per liter. The red line indicates the value beyond which species richness is adequate and indicative of good clearance performance.

of the species richness of the microfauna of the activated sludge of the ETEs during the entire period studied.

For the observation of the relative dominance of the morphofunctional groups present in the activated sludge of the studied ETEs, the results of the microfauna density were presented through the percentages of the groups. Over the period studied, the activated sludge of each ETE showed the relative dominance of the microfauna groups shown in figure 2.

The variation of the sludge biotic index in the ETEs Alegria Pavuna, Sarapuí, Penha and Ilha can be seen in figure 3.

The evaluation of the distribution of biological flakes from each ETE in the three diameter ranges was as follows: Alegria - 35% large flakes, 61% medium and 4% small; Pavuna - 3% medium and 97% small; Sarapuí - 6% medium and 94% small; Penha - 100% small; and Ilha – 6% medium and 94% small. At ETE Alegria, there was a predominance of medium flakes and at ETEs Pavuna, Sarapuí, Penha and Ilha, small flakes. The results are shown in figures 4 to 8. The mean diameters of the biological flocs of the studied ETEs were as follows: Alegria 407.6µm, Pavuna 87.6µm, Sarapuí 101.7µm, Penha 83.2µm and Ilha 86.4 µm.

Evaluation of filamentous bacteria - according to the measurements of the total length of filaments in the activated sludge of the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha, the following average filament lengths were found: Alegria 4.31 x 10^{8} µm.mL ; Pavuna 1.32 x 10^{8} µm.mL; Sarapui 2.33 x 10^{8} µm.mL; Penha 1.31 x 10^{8} µm.mL; and Island 3.50 x 10^{8} µm.mL. The variation of the measurements of the filament length of the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha are shown in Figure 9.

From the results obtained with the quantitative analyzes of the activated sludge microfauna of the five studied ETEs, the results of the physical-chemical analyzes and the statistical tests, the following correlations were found:

- Naked amoebas – in the activated sludge from the Pavuna and Sarapuí ETEs, statistical analyzes showed a significant positive correlation between the community of naked amoebas and the A/M ratio. In the Sarapuí and Penha ETEs, it was possible to observe a significant positive correlation between the presence of naked amoebas and the concentration of BOD in the effluent, indicating a loss of purification efficiency and an increase in BOD in the final effluent. In the ETE Penha, the presence of naked amoebas also indicated an increase in the effluent TSS, since the statistical analysis showed a significant positive correlation between the nude amoebas and the effluent TSS.
- Tecamebas – at ETE Alegria, the statistical evaluation showed a significant negative correlation between the tecameba population and the volume of slurry received. Figure 10 shows that thecamoebas were dominant at the beginning of monitoring and showed a sharp decrease with the increase in the volume of slurry received. of effluent BOD, evidencing these organisms as indicators of good depuration in the activated sludge of ETE Ilha.
- Flagellates - statistical analyzes show a significant negative correlation between small flagellates and BOD removal efficiency in the Penha and Ilha ETEs and SST in the Penha ETE effluent, confirming the presence of these organisms as indicators of low purification efficiency and decreased effluent quality.
- Flake predatory ciliates - in the activated sludge of the Ilha ETE, the statistical

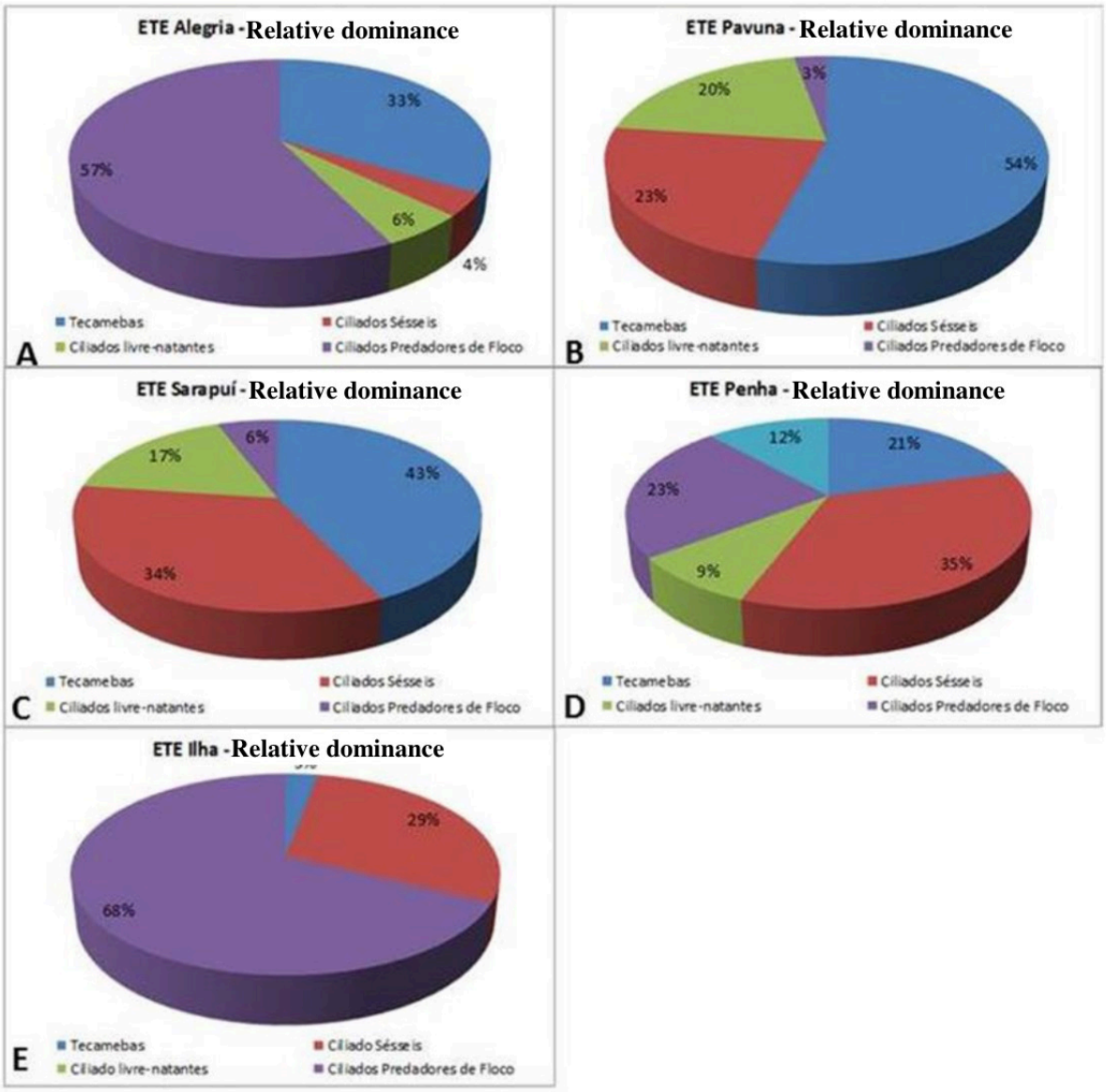

Figure 2: Graph of the relative dominance of activated sludge microfauna. A. ETE Alegria; B. ETE Pavuna; C. Sarapuí ETE; D. ETE Penha; E. ETE Island.

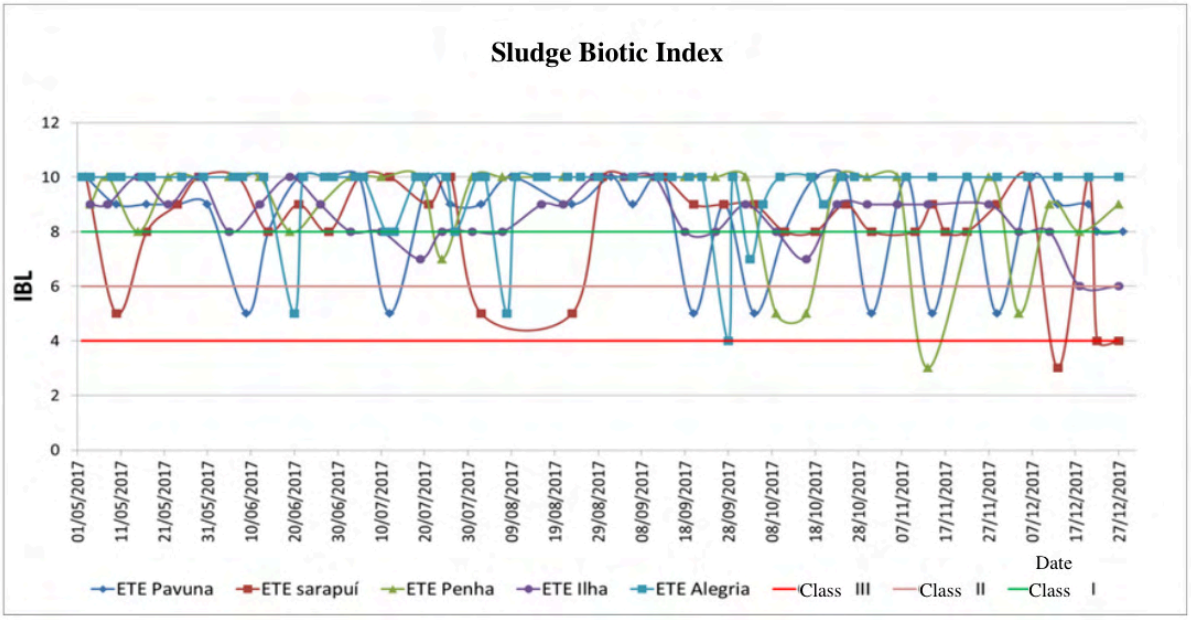

Figure 3: Graph of the variation of the Sludge Biotic Index of the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha in the studied period.

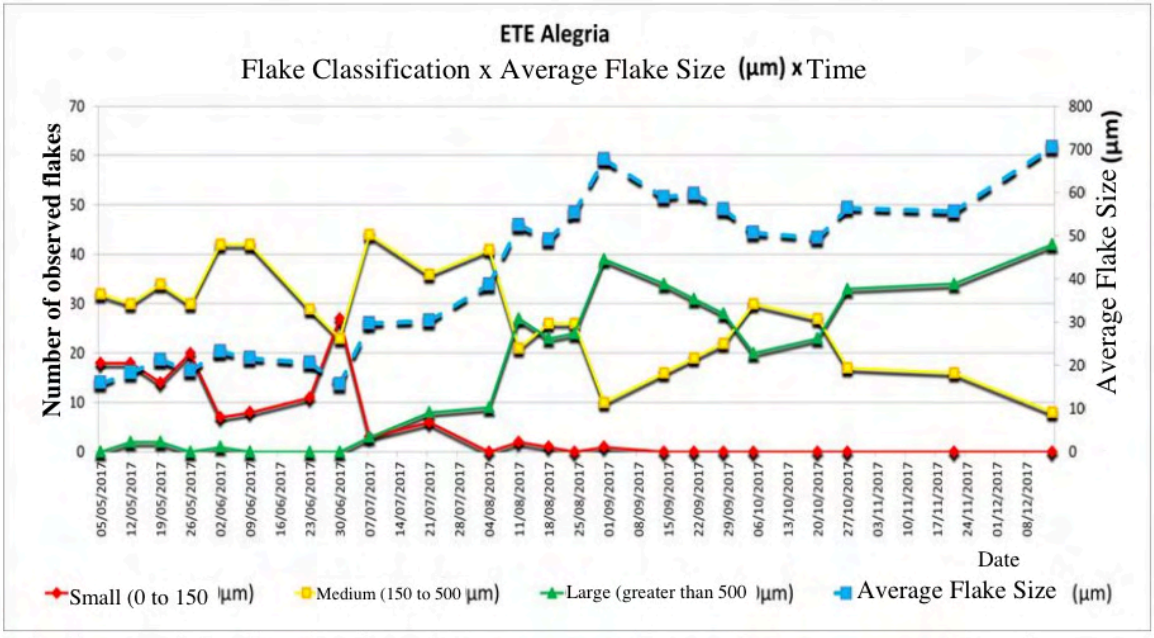

Figure 4: Graph of the distribution of the observed flocs and the average of the diameters of the biological flocs observed in the activated sludge from the ETE Alegria.

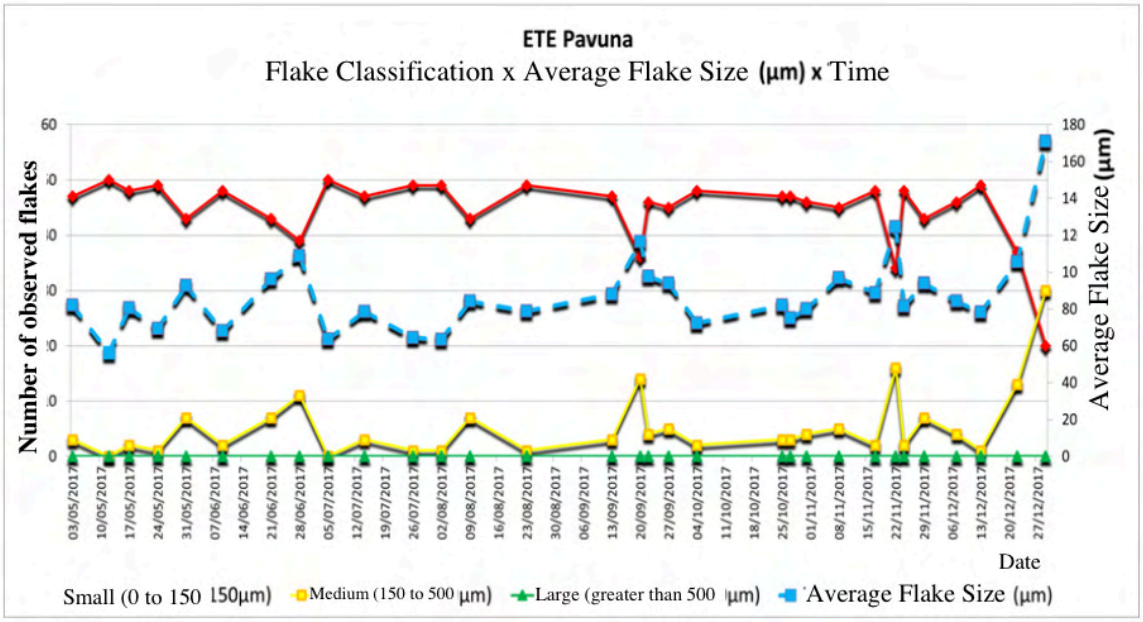

Figure 5: Graph of the distribution of the observed flocs and the average of the diameters of the biological flocs observed in the activated sludge of the ETE Pavuna.

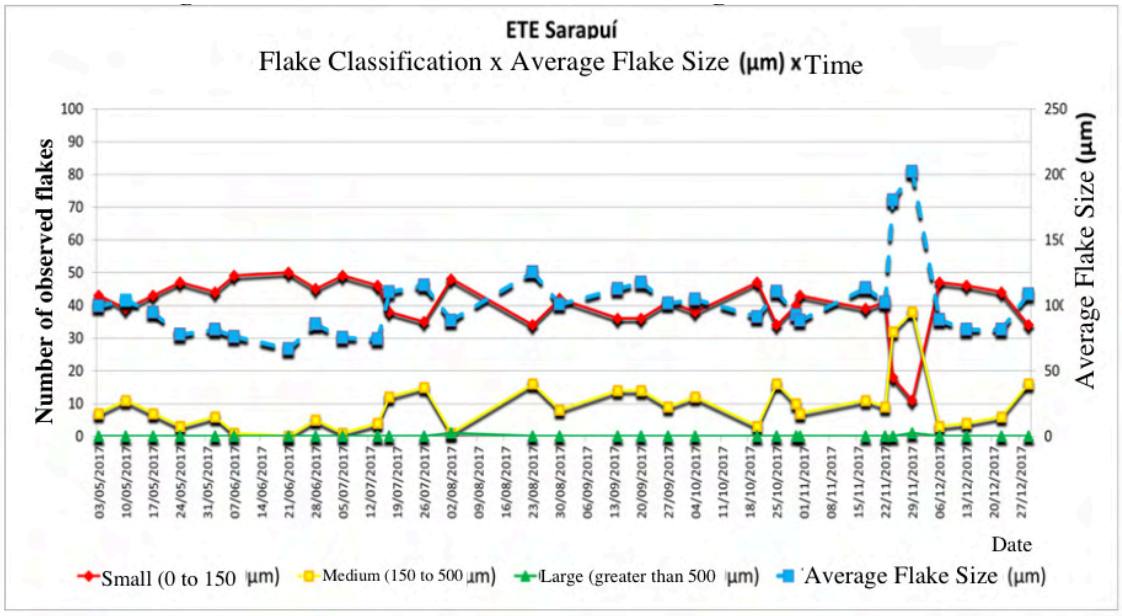

Figure 6: Graph of the distribution of the observed flocs and the average of the diameters of the biological flocs observed in the activated sludge from the Sarapuí ETE.

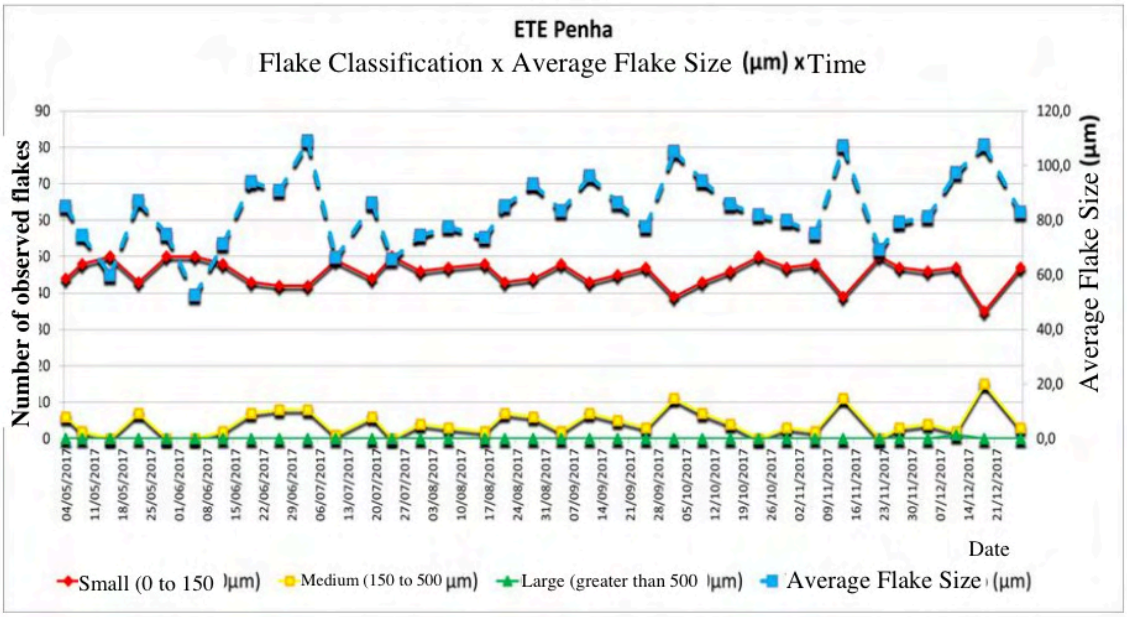

Figure 7: Graph of the distribution of the observed flocs and the average of the diameters of the biological flocs observed in the activated sludge from the ETE Penha.

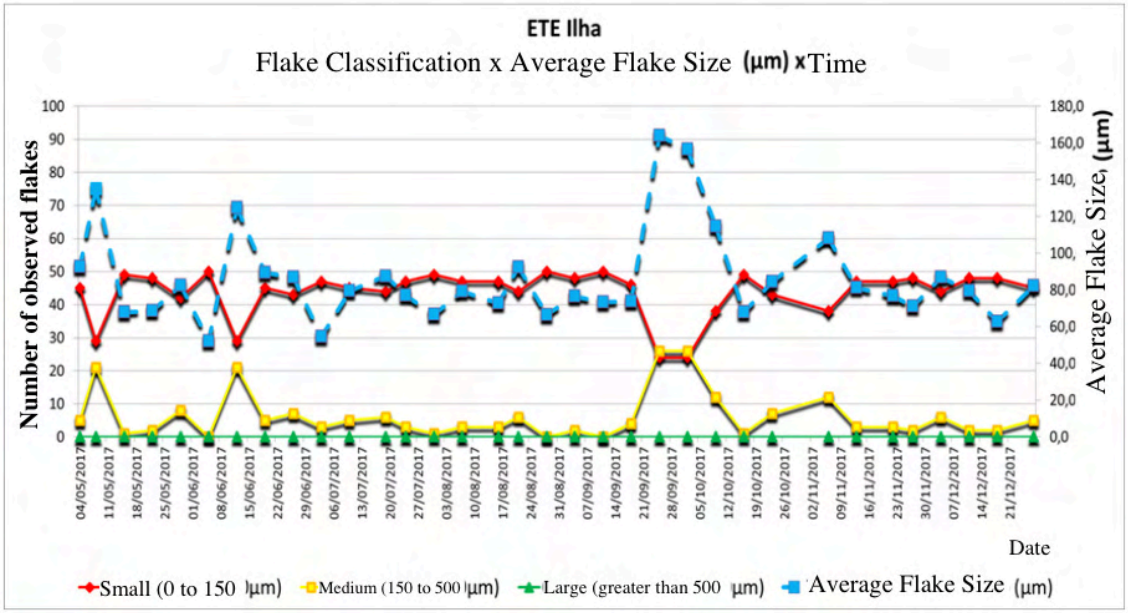

Figure 8: Graph of the distribution of the observed flocs and the average of the diameters of the biological flocs observed in the activated sludge of the ETE Ilha.

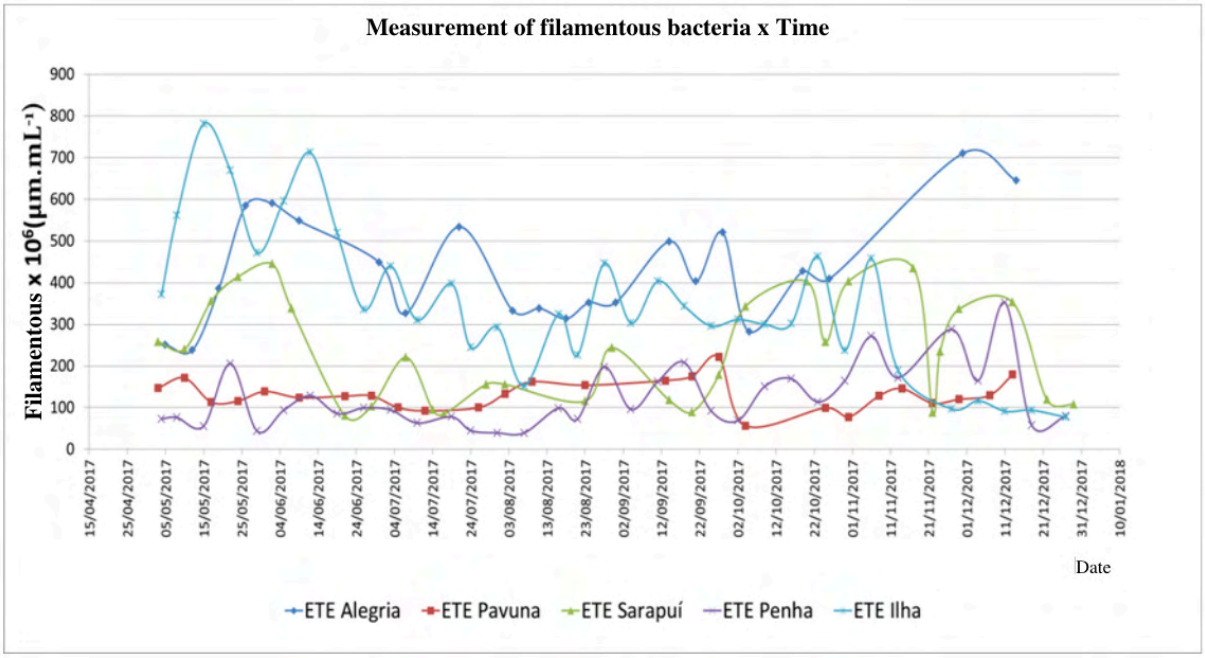

Figure 9: Graph of the variation in measurements of filament bacteria in the activated sludge of the Alegria, Pavuna, Sarapuí, Penha and llha ETEs

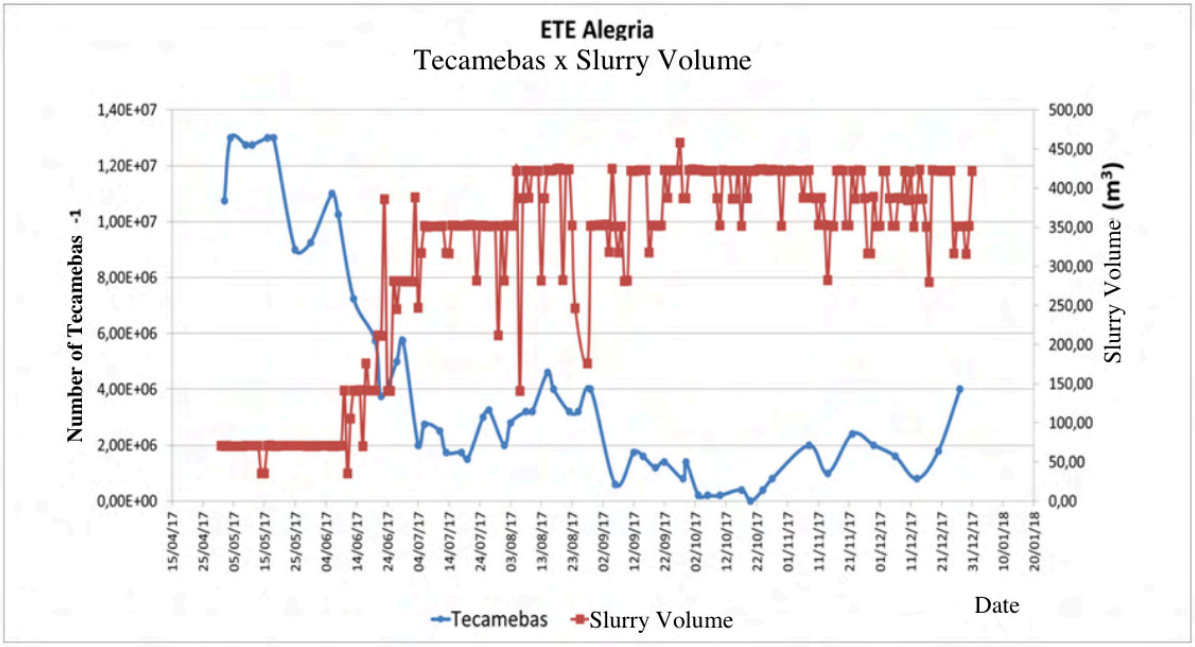

Figure 10: Graph of the distribution of the thecameba population in the activated sludge of ETE Penha and of the volume of slurry received for treatment.

analyzes showed a significant positive correlation between the population of flake predator ciliates and the efficiency of TSS removal and also a significant negative correlation with the SST of the effluent, the data show that the increase of the population of flake predator ciliates contributed to the removal of SST in the effluent. At ETE Penha, statistical analyzes showed a significant negative correlation between the population of flake predator ciliates and sedimentable solids and with pH, which can be explained by the abundant presence of the flake predator ciliate *Aspidisca* spp. Several authors have correlated *Aspidisca species* with nitrogen removal and nitrification (Bento *et al.,* 2002 and 2005; Hu *et al.*, 2013b; Vazollér, 1989). According to Figueiredo (2012), systems with strong nitrification activities generate acidity in the environment that can negatively influence protozoa. The nitrification process must be very well controlled, by increasing the supply of dissolved oxygen, so that the generated acidity does not harm the other organisms of the activated sludge microfauna.

- Micrometazoans - The results of the analysis of the density of organisms showed the presence of micrometazoans in 96% of the time in the sludge from the ETE Alegria and 91% in the sludge from the ETE Penha and 80% in the sludge from the ETE Pavuna, becoming the dominant group in the sludge of ETE Penha. This group encompasses all multicellular organisms with a characteristic of slow growth and according to Saar (2015) and Bento *et al.* (2002, 2005) are found in activated sludge systems of high age, which is the case of the mentioned ETEs, since they operate with a high age of the sludge (estimated data). Rotifers were the most frequent micrometazoans in the sludge of all ETEs, Saar (2015) mentions that tardigrades are affected by small concentrations of ammonia and indicate a fully nitrified effluent. The presence of these organisms in ETE Penha was observed in the months of May, June, July and September, months in which the increase in the population of flake predators ciliates, especially *Aspidisca* spp, indicated the possible occurrence of nitrification.

ANALYSIS AND DISCUSSION OF RESULTS

The evaluation of the microfauna composition in the aeration tanks of all ETEs showed the presence of representatives of all morphofunctional groups. Among the flake predators ciliates, the most frequent in all ETEs was *Aspidisca cicada.* Among the free-swimming ciliates, the most observed in all the ETEs was *Trachelophyllum* sp, whereas the *Spirostomun teres species* was only frequently observed in the sludge of the ETE Penha and less frequently in the ETE Alegria. The most common species among sessile ciliates were *Thruricolakellicotiana* and *Epistylisplicatilis.* Among thecamebas, *Arcella vulgaris, Euglypha rotunda, Centropyxisaculeata* and *Difflugia* sp. were the most frequent organisms. Among the micrometazoans, rotifers, oligochaete annelids of the genus *Aeolosoma* sp., nematodes of the genus *Rhabiditis* sp., tardigrades and gastrotrics were observed. Among the micrometazoans, rotifers were the most common.

In the quantitative analysis of the microfauna, through the evaluation of the density, it was possible to observe that the data of the total number of organisms per liter of the ETEs Alegria, Pavuna and Penha

remained above 10^6 throughout the studied period and indicate an adequate treatment, according to Madoni (1994), despite the BOD and TSS removal efficiency of each ETE having varied over the period. In the Sarapuí and Ilha ETEs, the total number of organisms per liter remained above 10^6 for most of the period studied, however in the periods when the total number of organisms was 10^5 organisms.$L^{-1,}$ statistical analyzes showed no significant correlations with the efficiency of BOD removal, indicating that this parameter is not the best to be used as an indicator of the efficiency of the treatment performed by the TEE studied. It was possible to observe for the ETE Alegria, a significant negative correlation between the total number of organisms and the volume of slurry received, indicating that the increase in the volume of slurry contributed to the decrease in the number of organisms in the sludge. For the Sarapuí ETE, it was possible to observe a significant positive correlation between the total number of organisms and the DO concentration. These data indicated the influence of DO concentration on the microfauna of the activated sludge from the ETE and the importance of maintaining appropriate concentrations of DO for the maintenance of the microfauna.

The evaluation of species richness in the activated sludge microfauna of the studied ETEs showed that the number of taxonomic units in the Alegria, Pavuna, Sarapuí and Penha ETEs remained above 10 for most of the monitored period.According to Madoni (1994), a microfauna diversified is essential for the biotic quality of the sludge and consequently for obtaining a quality effluent. The results presented indicate a good diversity of species within the bioreactor (Madoni, 2004). Statistical analyzes show the following corrections: in ETE Alegria, significant negative correlation between the number of taxa observed and the volume of leachate received, the A/ M, floc size and pH indicating that the increase in these parameters contributed to the decrease in the species richness of the activated sludge. In the ETE Pavuna, a significant negative correlation between the number of taxa observed and the SSTTA indicating that the operation of the aeration tank with a high concentration of total suspended solids negatively affected the species diversity of the activated sludge. In the ETE Sarapuí, a significant positive correlation between the number of taxa observed and the concentration of DO in the aeration tank, indicating that the increase in the concentration of DO in the aeration tank favored the increase in the richness of the species found. At Ilha ETE, significant positive correlations between these parameters SSTTA, SSVTA, SSed and SST removal efficiency and significant negative correlation with the effluent SST. The data indicate that in the aeration tank, which was being operated with SSTTA concentration in the appropriate range for the type of treatment, the loss of solids that occurred negatively affected the species diversity of the activated sludge.

According to the species dominance assessment performed by Bento *et al.* (2005), Madoni (1994) and Figueiredo *et al.* (2012), a good system performance is directly related to the dominant species in the process. In the present study, the dominance of the groups was alternated over time, as expected. According to Madoni (1994), a system that presents the dominance of flake predatory ciliates, sessile ciliates (except *Vorticellamicrostomae Opercularia* spp) or thecamebas is indicative of a good effluent treatment. Therefore, the results obtained indicate that the treatment carried out in the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha showed good clearance for most of the studied period.

The results of the Sludge Biotic Index presented show that the ETEs Alegria,

Pavuna, Sarapuí, Penha and Ilha had sludge classified as Class I, according to Madoni (1994) for most of the monitored period. This class indicates a very well colonized and stable sludge, excellent biological activity and high depurative efficiency. The statistical analyzes showed significant positive correlations between the IBL and the following groups: thecamebas in the ETE Pavuna sludge; total ciliates, flake predators ciliates and total number of organisms in the ETE Ilha sludge; and number of taxa observed in the sludges of the Alegria, Penha and Ilha ETEs. Significant negative correlations were found between the following groups: large flagellates in the activated sludges of the Alegria and Pavuna ETEs and free-swimming ciliates in the sludges of the Pavuna, Sarapuí and Penha ETEs. The results found are consistent with the studies carried out by Madoni (1994) who established as positive key groups the flake-predating ciliates, sessile ciliates and thecamoebs, and negative the small flagellates and free-swimming ciliates.

Evaluation of biological flakes - according to Vazollér (1989), the diameter of the flakes can vary between 10 and 800μm. The floc diameters of the monitored ETEs varied as described in the literature. Statistical analyzes showed a significant positive correlation between the diameter of the sludge flakes from the ETE Alegria and the volume of slurry received, indicating that the increase in the volume of slurry released for treatment at the ETE contributed to the increase in the diameter of the flakes (figure 11). A hypothesis that may explain the contribution of slurry to the increase in floc diameter in the activated sludge from the ETE Alegria, and which needs to be further investigated, is the higher concentration of divalent cations such as calcium (Ca^{+2}) and magnesium (Mg^{+2}) in the slurry. These ions are preponderant in the formation of the floc structure, as they act as bridges between negatively charged exopolymers (EPS) and bacteria, with calcium being the most important cation involved in the flocculation process of activated sludge (Zita & Hermansson, 1994; Jin *et al.*, 2004; Santos, 2012).

Figueiredo (2012) correlated floc size and organic load in the aeration tank. When a very low load is applied (A/M ratio <0.025KgBOD/KgSSVTA.d) the floc has a tendency to disintegrate into small particles, as there are no nutrients available to keep the floc-forming bacteria alive. In the ETEs Pavuna and Sarapuí, the statistical tests showed a correlation between the organic load and the floc diameter and the A/M ratio, the results indicated that the predominance of small biological flocs in these ETEs may be due to the low organic load influent to the aeration tank (represented by the A/M ratio).

At high loads (A/M ratio >0.4 to 0.6KgBOD/KgSSVTA.d) weak flakes are often found, as the bacteria are less inclined to form flakes, this may be the explanation for the predominance of small biological flakes in the Ilha ETE, since the average A/M ratio is 1.46KgBOD/Kg SSVTA.d.

In the middle range, mainly firm flakes are formed. If they are not found, there has probably been some disturbance in the treatment process. In the sludge from ETE Penha, whose average A/M ratio is 0.16KgBOD/Kg SSVTA.d, that is, in the intermediate range in which larger and well-formed flocs must predominate, no correlation was observed between floc diameter and the A/M ratio. The predominance of small flocs may be related to the shear caused by the turbulence in the bioreactors caused by the type of aeration (mechanical surface aerators).

Shear may be one of the factors that contributed to the predominance of small biological flocs in the activated sludge of the Ilha ETE, as the ETE also uses mechanical

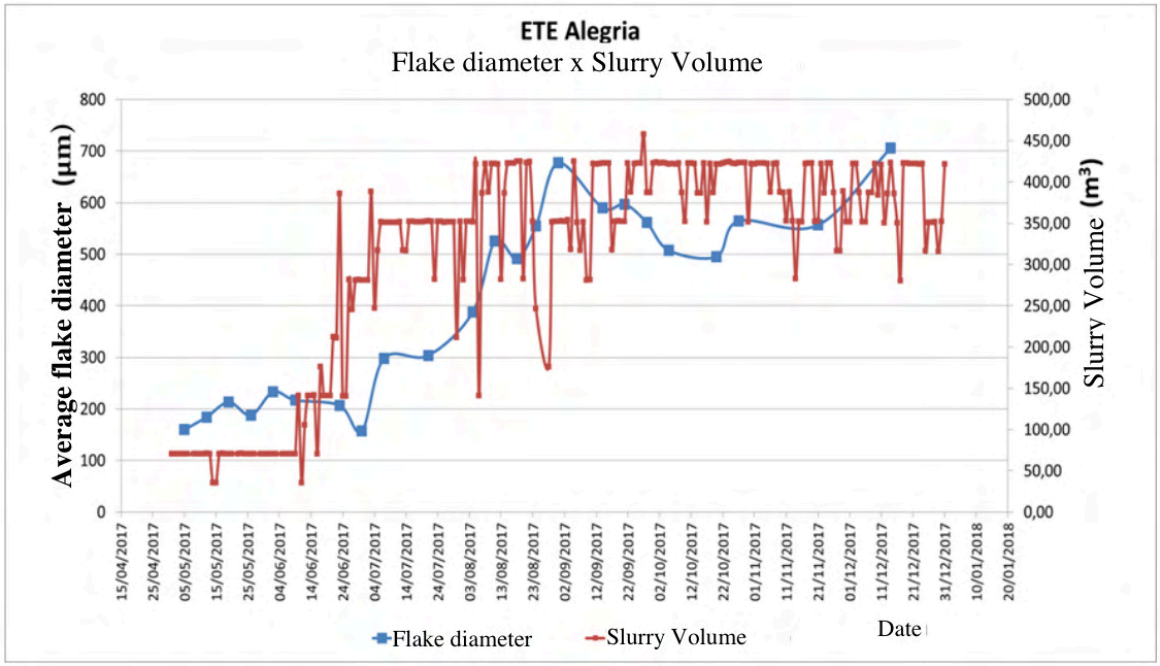

Figure 11: Graph of the flake diameter distribution of activated sludge from ETE Alegria and the volume of slurry received.

aeration in the aeration tanks. Through studies carried out by Wilén *et al.* (2003) it was possible to observe that when the biological flocs are exposed to a shear force (such as mechanical aeration) fragmentation occurs and the floc diameter is reduced.

Evaluation of filamentous bacteria - according to the results found, it was possible to observe that the values of the activated sludge filament lengths of all the studied ETEs remained above the value suggested by Jenkins *et al.* (2004), of 10^{7} µm.mL, throughout the entire study. However, the occurrence of filamentous *bulking* was not evidenced by microscopic analysis.

In ETEs Alegria, Pavuna and Sarapuí, the value of the length of filamentous bacteria may be related to the concentration of TSS adopted in the aeration tanks of these ETEs. The length of filamentous bacteria in the activated sludge from the Alegria, Pavuna, Penha and Ilha ETEs showed a significant positive correlation in the statistical tests with the IVL and with the sedimentable solids, indicating that the proliferation of filamentous bacteria in the activated sludge of these ETEs influenced the sedimentability of the sludge. Statistical tests showed significant correlations between some parameters and the length of filamentous bacteria in the activated sludge of the studied ETEs, they were: the concentration of DO that presented a significant negative correlation in the sludges of the Alegria and Sarapuí ETEs and the pH that presented a significant positive correlation in the sludge from ETEs Alegria and Penha. Von Sperling (2005) related, among other factors, the low concentration of DO and pH as factors that influence the proliferation of filamentous bacteria.

Statistical analyzes also showed significant negative correlations between the activated sludge from the Penha and Ilha ETEs and the A/M ratio in the aeration tanks, indicating that the amount of filamentous bacteria in the aeration tank of these ETEs contributed to the decrease in the A/M ratio in the aeration

tanks. aeration tanks. Through the statistical tests, it was possible to verify significant positive correlations between the filament length and the efficiency of removal of BOD in the ETE Penha, the efficiency of removal of SST in the ETEs Penha and Ilha and still showed a significant negative correlation with the BOD of the ETE Penha and with the SST of the effluent from the Penha and Ilha ETEs. Statistical data indicated that the concentration of filamentous bacteria was related to the efficiency of the treatment system.

From the results obtained with the quantitative analyzes of the microfauna of activated sludge, with the evaluation of the biological flocs, with the measurements of filamentous bacteria, with the results of the physical-chemical analyzes and statistical tests, table 2 was prepared containing a summary of the main correlations found between the activated sludge microbiology of the five ETEs studied and the operational and performance parameters of the ETEs treatment systems during the period studied.

CONCLUSIONS/ RECOMMENDATIONS

The activated sludge microfauna of the ETEs Alegria, Pavuna, Sarapuí, Penha and Ilha showed occurrence of all morphofunctional groups during the studied period.

The analysis of density and species richness showed the occurrence of microfauna in adequate and diversified amounts in most of the study and were parameters that indicated the performance of the treatment. The dominance of flake predator ciliates, sessile ciliates and thecamebas in most of the study indicated good clearance and good quality effluent. The eventual dominance of free swimming ciliates indicated loss of efficiency and median performance.

The evaluation of the Sludge Biotic Index showed the occurrence of Class I sludge in most of the studied period in all ETEs, indicating very well colonized and stable sludge, excellent biological activity and high depurative efficiency. In general, the IBL reflected the efficiency of the treatments performed by the studied ETEs and could be considered a practical parameter applicable to the evaluation of sludge quality and treatment efficiency.

At ETE Alegria, the increase in the volume of landfill leachate caused a decrease in species richness and the population of thecamebas and contributed to the increase in the diameter of the biological flakes of the activated sludge.

In the Pavuna and Sarapuí ETEs, the predominance of small flocs may be related to the low influent organic load and in the Penha and Ilha ETEs to the shear caused by the turbulence in the bioreactors caused by the mechanical surface aerators.

The proliferation of filamentous bacteria in the activated sludge of the studied ETEs affected the sedimentability of the sludge. The main factors that influenced the proliferation of filamentous bacteria were the decrease in pH and dissolved oxygen concentration. It was also possible to observe that in addition to the filamentous bacteria being related to the sedimentability of the sludge, they contributed to the efficiency of BOD and TSS removal.

The morphofunctional groups indicated the following operational conditions: naked amoebas, increased food/microorganism ratio, poor depuration and high concentration of BOD and TSS in the effluent; small flagellates low purification efficiency and poor quality of the final effluent; thecamebas and flake predators ciliates low influent organic load, good depuration and high effluent quality; *Aspidisca cicada* indicated the occurrence of nitrification; and micrometazoans, high sludge age and BOD removal.

Group / Parameter	observed at work	observed in the literature	authors
small flagellates	Occurrence indicated young sludge	They predominate at the beginning (start) of operation of the activated sludge system when the sludge is young and in the process of formation.	Madoni (1981); Vazoller (1989); Madoni (2004)
	Occurrence indicated loss of debug efficiency	A large concentration of flagellates in a not-so-young sludge is indicative of low clearance.	Curds and Cockburn (1970); Madoni (1981); Vazoller (1989); Madoni (2004)
Free-swimming ciliates (< 50 μm)	Dominance indicated average performance	Dominance indicates average performance, a young sludge in the process of formation, or a very low sludge age or low oxygen sludge.	Madoni (1981); Madoni (2004)
Flake predatory ciliates	Dominance together with sessile ciliates indicated good clearance and high quality in the final effluent.	Sludge dominated by flake-predating ciliates and fixed ciliates, and the number of flagellates and free-swimming ciliates is small (minimum), it can be affirmed that good depuration and high quality of final effluent are occurring.	Curds *et al.* (1969); Madoni and Guetti (1981); Salvador (1994); Madoni (1994); Bento *et al.* (2002 and 2005); Madoni (2004), Zhou *et al.* (2008)
sessile ciliates	Dominance together with flake predator ciliates indicated good clearance	The presence of these ciliates in codominance with flake-predating ciliates indicates good clearance and good SST removal.	Madoni (1981); Vazoller (1989); Madoni (1994); Hu *et al.* (2013a)
	Dominance over 80% indicated declining efficiency by observing excess sludge	Dominance at more than 80%, due to its rapid and massive growth indicates efficiency is declining; there is a transient phenomenon such as discontinuous loading, recent sludge extraction or slow recirculation time or a high sludge concentration (insufficient recycle)	Madoni (1994); Madoni (2004)
Tecamebas	There was dominance in ETEs with low loads and excess sludge. The increase in the A/M ratio (high load) and the increase in the volume of slurry released for treatment contributed to the decrease in thecamebas	Dominance/occurrence of thecamebas indicates low load	Madoni (1981); Madoni (2004); Bento *et al.* (2002 and 2005); Hu *et al.* (2013b); Madoni and Ghetti (1981); Zhou *et al.* (2006); Silva (2000); Cross (2014)
	Dominance indicated good clearance	Thecameba dominance indicates good clearance	Vazoller (1989); Madoni *et al.* (1993); Bento *et al.* (2002 and 2005); Zhou *et al.* (2008); Madoni (2004); Hu *et al.* (2013b)
naked amoebas	The occurrence indicated an increase in the concentration of BOD and SST in the effluent	Presence in abundance, associated with flagellates, is indicative of poor clearance with high BOD in the effluent.	Madoni (1981); Madoni (2004)

micrometazoans	Occurrence indicated high age of the sludge	They indicate a high age of the sludge.	Madoni (1981); Madoni (2004); Bento *et al.* (2002 and 2005)
	Occurrence indicated low concentration of BOD in the final effluent	The presence of many rotifers is associated with good debugging. There is a negative association between rotifers and BOD, COD and SST in the effluent.	Madoni (1981); Zhou *et al.* (2006)
Filamentous bacteria	*bulking* was not observed with a total length of filamentous bacteria of 10^{8} µm.mL $^{-1}$	Filament predominance indicates filamentous *bulking.* Values of 10^{7} µm.mL^{-1} indicate filamentous *bulking*	Vazoller (1989) Jenkins et *al.* (2004)
	The increase in the length of filamentous bacteria influenced the sedimentability of the sludge (IVL and sedimentable solids)	IVL increased with increasing filament length values	Jordan *et al.* (1997); Jenkins *et al.* (2004); Saar (2015)
	The OD and pH parameters influenced the proliferation of filamentous bacteria	The low concentration of DO and pH influence the proliferation of filamentous bacteria	Von Sperling (2005); Jenkins *et al.* (2004); Figueiredo (2012)
	The increase in the A/M ratio contributed to the proliferation of filamentous bacteria	The A/M ratio influences the proliferation of filamentous bacteria	Von Sperling (2005)
	The proliferation of filamentous bacteria contributed to the efficiency of removal of BOD and TSS	Filamentous bacteria contribute to the good efficiency of the process, as they actively participate in the degradation of soluble organic matter.	Saar (2015)
Total number of microfauna organisms	No correlation was observed between total number of organisms and treatment performance	When abundance is > 10^{6} /L good clearance; abundance <10^{4} /L poor clearance, 10^{4} –10^{6} /L average clearance	Madoni (1994); From Marco (1991)
	The increase in the volume of slurry contributed to the decrease in the total number of microfauna organisms	-	-
	Correlation with DO indicates the need to maintain the appropriate concentration of DO for an adequate number of organisms in the microfauna	-	-
species richness	There was no correlation between species richness and BOD removal efficiency. Species richness < 10 - indicated a decrease in the efficiency of TSS removal in the effluent	Species richness > 10 – good clearance	Madoni (1994)
	The increase in the volume of slurry and the A/M ratio (which represents the organic load) contributed to the reduction of species richness	The number of species decreases with increasing organic load.	Curds and Cockburn (1970)
	High concentration of solids in the aeration tank contributed to the decrease in species richness	-	-
	The increase in DO concentration in the aeration tank favored the increase in species richness	-	-

Diameter of the biological flake	Variation found between 52.2 to 706.2	Common range between 10 and 800um Flake diameter < 50um solids loss in final effluent	Vazoller (1989) Jordão and Pessoa (2014)
	The increase in the volume of slurry contributes to the increase in the diameter of the biological flake	-	-
	The increase in the floc diameter contributed to the SST removal efficiency	Large, well-formed flake is a requirement for good sedimentation	Figueiredo (2012)
	Predominance of small flakes in low organic load The increase in the A/M ratio contributed to the increase in the floc diameter	The floc size is dependent on the applied organic load – very low loads lead to the predominance of small flocs.	Figueiredo (2012)
	Predominance of small flakes with medium organic load, possibly caused by the shear of the flakes caused by turbulence (mechanical aeration)	In the middle range, mainly firm flakes are formed. If they are not found, there was probably some disturbance in the treatment process (turbulence in the aeration tank)	Figueiredo (2012); Wilén *et al.* (2003); Jordão and Pessoa (2014)
	Total ciliates and sessile ciliates contributed to the increase in biological floc diameter	Bacterivorous ciliates play important roles in the functioning of activated sludge, reducing dispersed bacteria and improving flocculation.	Madoni (1994); P ajdak- Stok ET *al.* (2017)

BOD- Biochemical Oxygen Demand; SST – Total Suspended Solids; IVL – Sludge Volumetric Index; DO – Dissolved Oxygen; A/M Ratio – Food-Microorganism Ratio.

Table 2: Summary of the main correlations between the microbiology of activated sludge from the Alegria, Pavuna, Sarapuí, Penha and Ilha ETEs and the operational and performance parameters of the treatment systems, observed in this study and the correlations observed in the literature by several authors.

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