Microbiological Characterization of Biofilm From Different Immobilization Structures Used in Submersed Aerobic Biofilters in Domestic Effluent Treatment at the City of Joinville, Brazil

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Research Article

Keywords: biological treatment of domestic sewage, microbiological diversity, non-potable reuse of effluents, polymers, genetic sequencing, Shannon index, Simpson Dominance Index

Posted Date: June 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2831075/v1

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Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on November 15th, 2023. See the published version at https://doi.org/10.1007/s11356-023-30856-3.
Abstract

The objective of this work was to develop a structure for microbiological immobilization, in polymeric material of low environmental impact associated with its production, being used as support material in a biofiltration unit of domestic effluents, capable of promoting the efficient removal of pollutants, to meet with Brazilian legislation and/or regulations for the disposal and/or reuse of effluents. Four different structures were tested, namely: polypropylene casings without filling material (TF1); polypropylene casings, filled with expanded polystyrene grains (TF2); polypropylene casings, filled with polyurethane foam (TF3); polypropylene casings, filled with polyvinyl chloride pellets (TF4). A flow of 0.216 m3.d-1 was applied to the system, and the biofilters operated in sequential batches with a hydraulic retention time of 6 hours. The efficiency potential of the four immobilization structures was verified regarding the parameters biochemical oxygen demand, chemical oxygen demand, total ammoniacal nitrogen and total phosphorus. The microbiological analysis of the formed biofilm, performed with the 16S library sequencing method, with amplification of the 16S rRNA V3 and V3-V4 genomic regions, showed a high diversity of microbiological colonization in the four immobilization structures, linking better results and consequently greater Community stability in TF2. It is recommended to use the filter bed made up of unfilled casings, followed by the filter bed made up of casings filled with expanded polystyrene grains.

Introduction

According to NBR 9,648 (ABNT 1986), domestic sewage is the effluent resulting from the use of water for hygiene and human physiological needs. It mainly consists of bath water, excrement, soap, detergent and washing water in general. These effluents contain high concentrations of organic and inorganic matter, which can be harmful to human health and the environment if disposed of without proper treatment (Bassin et al. 2012).

The usual domestic sewage treatment systems in Brazil were designed to remove only organic matter. However, most of them result in effluents with concentrations of certain nutrients, such as nitrogen and phosphorus, close to those of raw sewage (Mota and Von Sperling 2009). The increase in nitrogen and phosphorus, promoted by the release of these effluents into water bodies, markedly accelerates the eutrophication process, deteriorating the quality of the water, making it unavailable for various uses and considerably increasing the costs of the treatment process for drinking purposes.

A technically and economically viable alternative to obtain adequate efficiency in the removal of complex pollutants (such as nitrogen and phosphorus) is the so-called biological treatment (Reismann et al. 2019). This type of treatment uses biological and / or biochemical activities of microorganisms present in the environment, a situation similar to what happens in nature (Metcalf and Eddy 2014). Thus, through processes such as respiration and fermentation, complex substances are reduced to more stable compounds, such as mineral salts, nitrogen gas, methane and others (Costa et al. 2008).
Metcalf and Eddy (2014) emphasize that to ensure the functioning of the biological treatment system, it is necessary to understand the dynamics of the microorganisms involved in the process, as they play a fundamental role in the decomposition of organic matter. The main groups of microorganisms responsible for the degradation of matter in wastewater treatment systems are the prokaryotes belonging to the Bacteria and Archaea domains. These microorganisms agglomerate in organized structures, usually composing a biofilm, or in flakes dispersed in the liquid medium (IST 2008).

Species diversity is one of the most studied attributes in ecology, with several indices to measure it. The most useful indices are those that combine two attributes of a community: species richness and its uniformity (Melo 2008).

Local population diversity indices, also called α (alpha) diversity measures, are obtained from mathematical expressions. The most used in microbial ecology studies are the Shannon and Simpson indices (Lima et al. 2016).

The Shannon index (H') assumes that, during random sampling for an infinite community, all species are sampled (Lima et al. 2016). This index usually varies between the ranges of 1.5 to 3.5, reaching values above 4.0 when the sample size is high. The lower the Shannon index value, the lower the sample diversity is (Magurran 2011).

The Simpson Dominance Index (D), in turn, measures the probability that two individuals, randomly selected from the sample, belong to the same species (Brower and Zarr 1984). However, in practice, the Simpson's diversity index (D') is applied, which is the conversion of Simpson's probability into a measure of diversity. The latter (D') varies between 0 and 1, the closer to 1, the lower the dominance and the greater the local biological diversity.

Another crucial factor in determining an efficient biological treatment system is the correct understanding of the cycles of chemical elements and substances involved in the degradation process of organic matter. The distribution and concentration of elements and substances in the medium depends on the fixation and active concentration of macronutrients (carbon, hydrogen, nitrogen, phosphorus and sulfur) and micronutrients (manganese, iron, copper and zinc) (Tundisi and Matsumura-Tundisi 2008), as well as the composition of the established microbiological community. Thus, the design of biological reactors is based on biochemical cycles existing in nature, mainly the elements nitrogen, carbon, sulfur and phosphorus (Lima et al. 2007).

In domestic sewage, nitrogen (N) is present in the form of ammonia (NH3 or NH4+), being biologically removed through two steps, nitrification and denitrification.

Nitrification occurs under aerobic conditions and can be divided into two phases: the oxidation of ammonia (NH4+) to nitrite (N-NO2) and the oxidation of nitrite to nitrate (N-NO3). Equations 1 and 2 describe the process (Jordão and Pessoa 2005).
2\(NH_4(aq) + 3O_2(aq) \rightarrow 2NO_2(aq) + 4H(aq) + 2H_2O(l)\) (Eq. 1)

2\(NO_2(aq) + O_2(aq) \rightarrow 2NO_3(aq)\) (Eq. 2)

To complete the cycle, there must be a complete reduction of nitrogen oxides to nitrogen gas (\(N_2\)), that is, after the occurrence of nitrification, denitrification must occur (Zoppas et al. 2016). In the denitrification process, bacteria, under limited oxygen (\(O_2\)) conditions, reduce \(NO_3\) to nitrogen gas \(N_2\), which returns to the atmosphere, through the action of bacteria and/or some fungi (Tundisi and Matsumura-Tundisi 2008). Therefore, the concentration of dissolved oxygen (DO) must be kept relatively low in certain locations of the treatment units, for denitrification to occur (Metcalf and Eddy 2014). In Eq. 3, the denitrification reaction is presented.

\[
5C_6H_{12}O_6(aq) + 24NO_3^-(aq) + 24H^+(aq) \rightarrow 30CO_2(g) + 42H_2O(l) + 12N_2(g) \quad (Eq. 3)
\]

Most denitrifying bacteria are facultative anaerobes, which can use a wide variety of organics as electron donors for energy and as a carbon source. Under anoxic conditions (low oxygen concentration and high nitrogen concentration), the enzymes necessary for the use of \(NO_3^-\) or \(NO_2^-\) are rapidly formed (Battistelli et al. 2018).

The biological treatment processes that aim at the complete removal in the same reactor, allow the coexistence of environments with and without available oxygen in the same treatment unit. These units, the nitrifying bacteria, settle in regions where there is high availability of oxygen and denitrifying bacteria where the oxygen concentration is low (Bueno et al. 2017).

Biological phosphorus removal occurs simultaneously with the nitrification and denitrification processes (Bassin 2012). In sanitary effluents, phosphorus is present in the form of orthophosphates (inorganic and soluble), polyphosphates (complex inorganic) and organic forms of phosphate (Metcalf and Eddy 2014). Polyphosphates, which are polymers of orthophosphates, are naturally transformed into orthophosphate by hydrolysis. Finally, as organic forms are degraded, they can yield orthophosphates or polyphosphates which, when hydrolyzed, are converted to orthophosphates. In sewage, orthophosphate is a predominant form of phosphorus (Loures et al. 2006).

Considering the variety of metabolic processes of microorganisms existing in domestic effluents, it is considered that the simultaneous occurrence of the anaerobic and anoxic phases is essential to maximize the efficiency of the treatment in the unit.

Thus, a major challenge to improve the efficiency of biological filters is the use of filling materials with specific characteristics that allow the coexistence of different oxygenation conditions, to promote an effective microbiological colonization for the degradation of pollutants.
As main characteristics, the characteristics of porosity, lightness, high surface area, mechanical, chemical and biological resistance are evaluated in the filling material of biological filters.

According to Wolff et al. (2010) and Baettker et al. (2018) in different immobilization structures, the removal of pollutants in filters can present considerably varied results.

The objective of the research was to characterize, in terms of biological diversity, the composition of different immobilization structures used in submerged aerobic biofilters in the treatment of domestic effluents. Furthermore, to correlate this characterization with the efficiency of the biological treatment, mainly in the removal of organic matter, nitrogen and phosphorus.

This experiment was carried out in Joinville, Santa Catarina, Brazil, from April 4th to October 29th, 2019.

**Materials and methods**

An experimental prototype was used that simulated the technical performance of an individual domestic effluent treatment system, consisting of a combination of a septic tank and anaerobic filter (FAN), followed by a reaction tank (aeration) and filtration tanks (TF).

The experimental prototype, which ran uninterruptedly for 209 days, was supplied with sewage from the restrooms and cafeterias of a company in the Parque Fabril de Joinville (SC), Brazil. The period for carrying out the experiment was defined in order to obtain sample data from the period with the lowest temperature in the region (period in which microbiological development naturally appears to be less expressive, that is, more unfavorable to treatment).

The discharges were made with a submersible centrifugal motor BCS-255, automatically regulated, using the Rain Bird EPS RZX timer, to turn on every six hours (4:00, 10:00, 16:00 and 22:00). Each discharge had a volume of 0.054 m³, totaling the average flow (Q) of 0.216 m³.d⁻¹.

Four different immobilization structures were tested, each one composed of a filter bed with 150 polypropylene (PP) casings. Each housing had 80 1.0 mm openings distributed along the outer perimeter. In one of the filtration tanks, the enclosures contained in the filter bed had no material inside, in the other TFS they were filled with other materials.

The four different immobilization structures tested were: (a) unfilled PP casings - TF1; (b) PP casings filled with expanded polystyrene (EPS) grains - TF2; (c) PP casings filled with polyurethane (PU) foam - TF3; and (d) PP casings filled with Polyvinyl Chloride (PVC) pellets - TF4.

During the entire experimental period, the efficiency of the system was monitored considering the biochemical oxygen demand (BOD), chemical oxygen demand (COD), total ammonia nitrogen and total phosphorus. The methodology used in the analysis is described in Chart 1.

**Chart 1** Efficiency monitoring parameters and methodology used in the analysis.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>SMWW, 22ª Edição, Método 5210 B</td>
</tr>
<tr>
<td>COD</td>
<td>SMWW, 22ª Edição, Método 5220 D</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>Method HACH 10127 / 8190–2ª Edition</td>
</tr>
<tr>
<td>Total ammoniacal nitrogen</td>
<td>Method HACH 10072–2ª Edition</td>
</tr>
</tbody>
</table>

The parameters dissolved oxygen (DO), pH and temperature were monitored to correct any problems in the operation of the prototype. The equipment used was the portable multiparameter meter, model AK88.

After 195 days of system operation, samples were collected to perform qualitative and quantitative analyzes of biological species that were present inside the casings.

Six casings were removed from each of the TFs, two from the lower portion (position 1), two from the middle (position 2) and two from the upper part of the filter bed (position 3) (Fig. 1). In this configuration, oxygenation conditions are expected to be higher in the lower portion of the filter bed (close to the aeration system) and lower in the upper portion.

The casings were opened and sampling was performed on the inner edge in contact with the surface (position a), at 3/4 (position b) and at the center (position c) (Fig. 2).

The sampling procedure was carried out using a sterile tube swab (sterilization by ethylene dioxide), without a growing medium, of brand Absorve®.

The samples were submitted to the DNA extraction process, using the MagMAX ™ CORE Nucleic Acid Purification Kit. The quality of the extracted DNA was verified by agarose gel electrophoresis and quantified in a spectrophotometer. The KingFisher equipment was used for extraction.

The extracted DNA was amplified using locus-specific primers that access the V3-V4 region of the 16S ribosomal RNA (rRNA).

The data were analyzed according to the procedure defined by Callahan et al. (2016), comprising raw readings and community analysis. First, readings were assigned to biological samples using the DADA2 program for modeling and error correction. Subsequently, taxonomies were assigned to each ASV (Amplicon Sequencing Variants) using the same program. The Silva 132 database was used as a reference. The taxonomic identifications were then imported into the Phyloseq program, in which diversity analyzes were performed. ASVs that were not classified in at least the family level were filtered, and ASVs marked as belonging to the same species were agglomerated. ASVs that were not present in at least 5% of the samples were also filtered.

**Results and discussion**
The experimental procedure made it possible to evaluate the operational efficiency of the four different microbiological immobilization structures tested, in addition to characterizing the microbiological community immobilized in biofilms.

The average monthly feeding temperature of the TFs varied between 30.18 °C (April / 19) and 19.03 °C (July/19). The average concentration, for raw sewage, of BOD was 396.66 mg.L\(^{-1}\) and DO was 1.61 mg.L\(^{-1}\).

In the reaction chamber, 20 L.min\(^{-1}\) oxygen was continuously fed with an air compressor (Boyu / ACQ-001), which gave the effluent (in the TFs) an average DO concentration of 3.68 mg. L\(^{-1}\), with a minimum of 3.12 mg.L\(^{-1}\) and a maximum of 4.25 mg.L\(^{-1}\) over the experimental period.

The results related to the BOD parameter indicate that the most restrictive release limits in watercourses are met, which is 60 mg.L\(^{-1}\) (State Law No. 14,675 / 2009), after 4 months of operation of the system, in tanks called TF2, TF3 and TF4. In TF1, despite the effluent having reached the required efficiency in the 4th month of operation, the values obtained in the 5th and 6th month of operation were non-compliant.

For the total ammoniacal nitrogen parameter, the results of the effluent quality indicated that the TF2, TF3 and TF4 achieved adequate efficiency in the treatment of effluents to comply with CONAMA Resolution nº. 430/2011 (20 mg.L\(^{-1}\)) from the fifth month. TF1 achieved the desired efficiency, however after the sixth month of operation of the system.

For the parameter total phosphorus, the results indicated that the four TFs reached, from the second month of operation of the system, adequate efficiency in the treatment of effluents to comply with the Santa Catarina legislation, defined in the State Law nº. 14,675 (Santa Catarina 2009), which determines the limit of 4.0 mg.L\(^{-1}\) for launching in stretches of lagoons, lagoons and estuaries. There is no legal or normative provision, at the federal level (Brazil), for the limit for launching the phosphorus parameter.

The statistical analysis of the results of efficiency carried out through the model of analysis of variance (ANOVA), did not point out a significant difference of the results for the four TFs if the parameters BOD, COD and total ammonia nitrogen are considered, admitting 5% of significance. For the total phosphorus parameter, TF2 and TF3 were more efficient.

Regarding the composition analysis of the microbiological community present in the biofilm, the results pointed to the existence of microorganisms representing the Bacteria and Archaea domains. A set composed of 332 ASV sequences was obtained which were compared to a set of reference sequences with known taxonomy.

The abundance analysis shows an average dominance of microorganisms in the Bacteria Domain (304 ASV / 91.6%) related to Archaea (28 ASV / 8.4%) in the four BASs studied, which can be seen in Fig. 3.
Considering the microorganisms belonging to the Archaea domain, there was an expressive predominance of 6 genera, namely: Methanosaeta, Methanobacterium, Methanosporillum, Methanomassiliicoccaceae, Methanosarcina and Methanoregula.

The predominant genera in the samples are archaea with methanogenic metabolism, which promotes the degradation of organic matter in anaerobic situations, being found exclusively in microorganisms belonging to that kingdom. This metabolic pathway is important in the final transition of the carbon cycle, as it promotes a means for the carbon to return to the atmosphere as carbon dioxide and methane (Pazinato 2007).

Shannon's diversity indices for microorganisms belonging to the Archaea Domain showed very low values, which ranged from 0.80 to 1.24. The sample mean for the index was 1.028 and the median was 1.026 (Fig. 4).

Statistical analysis, using the ANOVA method, with a significance level of $\alpha = 0.05$, indicated that there were no differences for the Shannon diversity index, in individuals from the Archaea Domain, between the TFs, nor between the heights and collection positions.

The low index of diversity among the community of individuals belonging to the Archaea Domain was already expected, since it is known that the levels of mutation in these organisms are low, due to the stable bonds of their DNA molecules (Jacobs and Grogan 1997).

For microorganisms belonging to the Bacteria Domain, the amplified sequences (ASV) allowed the individuals to be classified into 36 phyla, 67 classes, 112 orders and 304 distinct genera, according to the Silva 123 database.

The phyla that presented the highest relative abundance in the four TFs studied were Bacteroidetes, Firmicutes, Proteobacteria, Chloroflexi, Caldiserica and Verrucomicrobia. The Phylum Actinobacteria, despite appearing in all TFs, heights and positions analyzed, showed greater abundance only in TF1, specifically in position 1.

Normally, there is a predominance in the raw sewage of microorganisms belonging to the phyla Bacteroidetes and Firmicutes (Mac Connell et al. 2015). It is worth mentioning that these organisms are representatives of the main phyla found in the human intestinal microbiota (Eckburg et al. 2005). Therefore, its abundance is consistent with the context of the study.

Microorganisms belonging to the phylum Proteobacteria are also commonly found in the intestinal biota, although they are not normally dominant. It includes many nitrogen-fixing pathogenic organisms, being composed of organisms with metabolism mostly anaerobic (Mac Connell et al. 2015).

Filos Chloroflexi and Caldiserica are found in sanitary sewage treatment systems. Both of which are predominantly characterized by bacteria involved in anaerobic digestion (Mori et al. 2009; Mac Connell et al. 2015).
The predominance of the phylum Verrucomicrobia in TFs, which are organisms rarely found in the human microbiota, suggests that the initial bacterial community present in the raw sewage has been modified throughout the collection and treatment system. The phylum Verrucomicrobia is characterized by aerobic bacteria (Mac Connell et al. 2015).

The Phylum Actinobacteria, showed greater abundance only in TF1, height 01. This phylum is composed of gram-positive and, in its majority, aerobic bacteria (Almeida 2019). Its greater abundance in TF1, which has no filling in the casing, and at height 1 (lowest TF position, close to the tank inflow, with a higher concentration of oxygen), shows that the amount of oxygen available inside the casings present at this time is superior when compared to other heights and other TFs. Filling inside the envelopes in TF2, TF3 and TF4, therefore, provides environments with a lower oxygenation rate compared to TF1, which showed greater colonization of aerobic bacteria, especially in position 1.

Among the genera identified in the samples of the 4 TFs, 46 clusters were classified as uncultured bacterium (UBA). They are genera of organisms that do not have a single cultivable representative, knowing their existence only from genetic sequencing directly from samples of the environment. Little is known about its ecology, metabolism, reproduction and other specific characteristics. The genera that showed the greatest relative abundance were: UBA 6082 (aerobic), UBA4810 (anaerobic), Thioalkalivibrio A (anoxic) and Caldisericaceae (anaerobic).

Considering the diversity of the main genera present in the samples, as well as their relative abundance, it can be said that the four TFs provided the coexistence of aerobic, anoxic and anaerobic environments. In other words, the four tested microbiological immobilization structures were suitable for use in aerobic filtration units for the treatment of sanitary effluents.

The different filling materials of the enclosures and, in the case of TF1, the absence of filling material, led to differences in the distribution of oxygen inside the casings, in addition to different attachment surfaces for the microorganisms, favoring or not (as the case may be) the permanence inside the enclosures of species that favored the treatment process.

The results related to the Shannon diversity index demonstrate that the samples present a great local diversity for microorganisms belonging to the Bacteria domain, with high indexes ranging from 3.45 to 4.48 among the evaluated biofilters.

The simple weighted means of these indices were higher in TF2 and TF3. The lowest average of biodiversity was observed in TF4. Figure 5 shows that in TF2 most samples found indices with values clustered above the median. In TF3, most samples found indices with values grouped below the median, which can be better visualized in Fig. 5.

The Simpson diversity index showed values for the four TFs very close to the maximum value of the index (1), indicating that the local bacterial population does not have predominant dominant species (prevalence of one or more species), being an indicator of the great diversity of the species of the
sampled bacterial community. The values obtained were higher in TF1 and TF2, while TF4 presented the lowest value, that is, the lowest biodiversity.

Figure 6 shows that in TF2, most samples had indices with values grouped above the median. In TF3, most samples found indexes with values grouped below the median. In TF1, the samples were homogeneously grouped around the median.

For both indexes, the higher the value, the greater the diversity. The greater the diversity, the greater the stability of the biological system.

The ANOVA hypothesis test for the Bacteria Domain indicated that there is evidence that the differences in Shannon's diversity indexes are statistically significant (P-value < 0.05) for TF factors height of the sample inside the filter bed and position of the sample inside of the enclosure. That is, the different materials used in the filter bed of the TFs and the position of the sample inside the enclosure, influence non-randomly the composition and diversity of the bacterial population present in the biofilm. The result of the multifactorial ANOVA is described in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-value</th>
<th>Parameter</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>4.00x10^{-9}</td>
<td>Height x TF</td>
<td>0.005370</td>
</tr>
<tr>
<td>Height</td>
<td>0.090104</td>
<td>Height x Position</td>
<td>0.300571</td>
</tr>
<tr>
<td>Housing position</td>
<td>0.010315</td>
<td>TF x Position</td>
<td>0.004822</td>
</tr>
</tbody>
</table>

For the interactions that showed significant values in ANOVA (height x TF and TF x position), the Tukey test was performed, which compares all interactions between 2 to 2, to present the difference in the confidence interval.

The results of the Tukey test for comparison between height x TF interactions indicated that for TF1, TF3 and TF4 there are no statistically significant differences between the 3 heights (all interactions for these biofilters and their heights were not statistically significant). Therefore, it can be said that bacterial diversity, within TF 01, 03 and 04, is not influenced by the collection height. For TF 02, there is a statistically significant difference in just one interaction (2: 1 interaction).

Thus, as there were no statistical differences between interactions at different heights for the same biofilter (except for TF2, height 1), there is evidence that height influences diversity, albeit insignificantly. Figure 7 illustrates the results obtained by the Tukey test for the TF x Height interactions.

Analyzing Fig. 7, it can be concluded that for the set of interactions between the factors TF and height, the structure that best benefits conferred to biological treatment (facilitated microbiological colonization),
was the structure contained in TF2, given its superiority of bacterial diversity of the biofilm (especially at height 1 of this biofilter).

The results of the Tukey test for comparison between the position x TF interactions showed that for TF1, TF2 and TF4 there are no statistically significant differences between the three collection positions inside the casings. Therefore, it can be said that bacterial diversity, within TF1, TF2 and TF4 does not suffer significant influence from the collection position. For TF3, there is a statistically significant difference between interactions A: C and A: B. However, the B: C interaction was not significant. Therefore, it is concluded that the PU foam (TF 03) gave the interior of the enclosures heterogeneous environmental conditions in the ¾ portions of the enclosure and center.

However, the average diversity of TF2 proved to be higher than the average diversity of TF3, in all 3 sample positions. Figure 8 illustrates the average diversity of the samples, considering the position inside the envelopes.

Analyzing Fig. 8, it can be concluded that for the set of interactions between the factors TF and position, the structure that best gave biological treatment was the structure contained in TF2 (EPS grains), regarding the superiority of diversity of the biofilm, present in positions A, B and C, when compared to the other TFs.

**Conclusions**

Through this study, it was possible to evaluate the performance of four different microbiological immobilization structures used in the biofilter filter bed in biological treatment systems for domestic effluents.

The tested structures were: (a) PP casings; (b) PP casings filled with granular EPS; (c) PP casings filled with PU foam; (d) PP casings filled with PVC pellets.

The experimental treatment system comprised two distinct and sequential units, UND 01 (septic tank + anaerobic filter) and UND 02 (aeration tank and filtration tank). The operation occurred uninterruptedly for 209 days, with a flow rate of 0.216 m³.d⁻¹, average ambient temperature ranging from a minimum of 14.6 °C to a maximum of 27.2 °C throughout the day, an average temperature of the effluent ranging from a minimum of 19.0 °C to a maximum of 30.2 °C, and a volumetric organic load of 0.40 kgBOD.m⁻³.d⁻¹ and 1.06 kgCOD.m⁻³.d⁻¹ (COD / BOD ratio = 2.87).

Through the analysis of the efficiency results for the different parameters observed, namely BOD, COD, total phosphorus and total ammoniacal nitrogen, it was possible to verify that the four structures studied had the potential to meet the parameters allowed in Brazilian legislation and/or standardization for disposal in watercourses and / or non-potable reuse.

It was possible to verify a tendency for better performance of the structures with filling of the casings (EPS grains, PU foam and PVC pellets), when compared to the structure with the empty casings,
regarding the removal of total phosphorus. The results of the other parameters did not show different statistics that would indicate better efficiency between the tested structures, considering a significance level of 5%.

Through the method of sequencing 16S libraries, by amplifying the genomic regions V3, and V3-V4 of the ribosomal RNA (rRNA) 16S, it was observed that the four tested structures present expressive diversity and richness of the immobilized microbial population, especially individuals from the Archaea and Bacteria domains.

The diversity of genres existing in the sampling, which have strategic metabolic differences, showed that the use of different structures led to the formation of gradients inside (varying according to the height of the filter bed and the position of the sample inside the envelopes) with oxygenation conditions, different nutrition, temperature and pH, to meet the diverse demands of microorganisms.

The analysis of the contents of the envelopes pointed to greater diversity in the colonization/maintenance of microorganisms in the structure composed of PP casings filled with EPS grains (TF2), when compared with the samples of the other tested structures (TF1, TF3 and TF4). According to the literature, the greater the biological diversity, the more stabilized the ecosystem will be.

Combining the result of the microbiological characterization and the efficiency of the units, it is possible to affirm that the superiority of the bacterial diversity present in the biofilm of the filter beds composed by the casings filled with other materials (EPS grains, PU foam and PVC pellets), influenced the efficiency treatment unit. The use of EPS grains inside the casings gave the system greater stability of colonization since it favored not only immobilization by encapsulation, but also by adhesion. However, the use of unfilled casings also showed satisfactory results and close to the values obtained in the other structures, in addition to the less environmental impact associated with the production of the structure.

Therefore, it is suggested to use the unfilled casings or the casings filled with EPS grains as an immobilization structure in biological filters for domestic effluent treatment.

**Declarations**

**Competing Interests**

The authors have no relevant financial or non-financial interests to disclose and declare no competing interests.

**Acknowledgements**

The authors gratefully acknowledge the Santa Catarina State University (UDESC, Brazil) and the company TIGRE Group. (Brazil) by the infrastructure support.

**Ethical Approval**
The authors have unanimously approved the submission of this paper.

Consent to Participate

Not applicable.

Consent to Publish

The paper is submitted with the consent of all listed authors.

Authors Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Daniela Damasceno, Larice Armstrong and Fábio Wegbecher. The first draft of the manuscript was written by Daniela Damasceno. Fernando Lafratta, Luiz Valentina, Larice Armstrong and Fábio Wegbecher commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

Not applicable.

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Figures

Figure 1
Location of the sampling point, by height in the TFs

Figure 2
Location of the sampling point, by position inside the casing
Figure 3

Plenty of kingdoms

Figure 4

Shannon's Alpha Diversity Index, by BAS, for the Archaea Domain
Figure 5

Shannon's Alpha Diversity Index, by TF, for the Bacteria Domain

Figure 6

Simpson's Alpha Diversity Index, by TF, for the Bacteria Domain

Figure 7

Average diversity by TFs and sample height in the filter bed
Figure 8

Average diversity by TFs and position of the sample inside the enclosure