Structure and functional prediction of a microbial community involved in a full-scale biofilter treating wastewater odor

Data de entrada: 02/28/2023
Data de aprovação: 05/15/2023

Estrutura e predição funcional da comunidade microbiana presente em biofiltro de escala real para tratar o odor de águas residuais

Maria Joana Allievi[1*] | Maurício Egídio Cantão[2] | Tiago Palladino Delforno[3] | Luana Mattos de Oliveira Cruz[4] | Daniele Damasceno Silveira[1] | Paulo Belli Filho[1]

DOI: https://doi.org/10.36659/dae.2024.026

ORCID ID
Allievi MJ https://orcid.org/0000-0003-4814-1817
Cantão ME https://orcid.org/0000-0002-5988-6542
Delforno TP https://orcid.org/0000-0002-1705-0763
Cruz LMO https://orcid.org/0000-0003-3795-9111
Silveira DD https://orcid.org/0000-0002-8299-0030
Filho PB https://orcid.org/0000-0003-4338-2830

Abstract

The efficiency of biofilters depends on the microbial community, so it is crucial to understand the structural/functional dynamics and identify the key microbial genera associated with treating toxic gases such as hydrogen sulfide (H_2S). This study investigated the structural dynamics linked to the functional prediction of microbial communities in a full-scale biofilter installed near a sewage lift station. Sampling and analysis were conducted for 720 days. Illumina 16S rRNA sequencing was used to assess the taxonomic profile, followed by putative evaluation of functional genes. H_2S removal efficiency reached 90% of the compound concentration at the inlet halfway up the filter bed height. Spatial variability had a greater effect on the microbial community. Studies focused on bacterial behavior and structure in biofiltration systems are still scarce, and only through further research can we fully understand the dynamics.

Keywords: Wastewater odor. Biofiltration. Hydrogen sulfide. 16S rRNA metagenomic sequencing. Microbial dynamics structure. Microbial functional prediction.

Resumo

A eficiência dos biofiltros depende da comunidade microbiana, portanto é crucial conhecer a dinâmica estrutural/funcional e identificar os principais gêneros microbianos ligados ao tratamento de gases tóxicos, como o sulfeto de hidrogênio (H_2S). Este trabalho investigou a dinâmica estrutural ligada à predição funcional das comunidades microbianas de um biofiltro em escala real instalado próximo a uma estação elevatória de esgoto. A amostragem e a análise foram conduzidas por 720 dias. O sequenciamento Illumina 16S rRNA foi usado para avaliar o perfil taxonômico, seguido pela avaliação putativa de genes funcionais. A eficiência de remoção de H_2S atingiu 90% da concentração do composto na entrada já na metade da altura do leito filtrante. A variável espacial teve maior

[1] Universidade Federal de Santa Catarina – Florianópolis – Santa Catarina – Brazil
[2] Embrapa Suínos e Aves – Concórdia – Santa Catarina – Brazil
[3] Instituto Senai de Inovação em Biotecnologia – São Paulo – São Paulo – Brazil
[4] Universidade Estadual de Campinas – Campinas – São Paulo – Brazil
* **Corresponding author:** mjallievi@gmail.com

efeito sobre a comunidade microbiana. Estudos focados no comportamento e estrutura bacteriana em sistemas de biofiltração ainda são escassos, e somente com mais pesquisas poderemos entender a dinâmica comportamental.

Palavras-chave: *Odor de água residual. Biofiltração. Sulfeto de hidrogênio. Sequenciamento metagenômico 16S rRNA. Estrutura dinâmica microbiana. Predição funcional microbiana.*

1 INTRODUCTION

The emission of unpleasant gases by wastewater treatment plants (WWTP) and wastewater-pumping stations (WPS) is a major concern for neighboring communities. The health effects (Vikrant *et al.*, 2018) and the depreciation of property values directly affect communities near the odorant sources (Alfonsín *et al.*, 2015; Allievi *et al.*, 2018; Brancher *et al.*, 2017). The main odorant gases emitted by these systems are inorganic compounds, such as hydrogen sulfide (H_2S) and ammonia (NH_3), and volatile organic compounds (VOCs).

Traditionally, waste gases are treated using physicochemical processes. However, these methods are expensive and generate large amounts of byproducts. Moreover, operational problems can occur (Syed *et al.*, 2006; Vikrant *et al.*, 2018). In contrast, the biological processes used in odor treatment have become more common in recent decades. They are attractive alternatives for treating odors from wastewater treatment systems. Among these biological processes, biofilters can be highlighted.

Biofilters are well established both from a scientific point of view and in terms of their applicability in the treatment of gas streams from WWTP/WPS (Estrada *et al.*, 2012; Omri *et al.*, 2011, 2013; Vikrant *et al.*, 2018) and from sanitary landfill site (Li *et al.*, 2013). However, they are complex systems whose efficiency depends on many factors, such as temperature, pH, filter medium, filter bed humidity, and nutrient supply (Delhoménie; Heitz, 2005). The filter medium and biofilm are responsible for regulating complex phenomena such as microbial growth and activities, mass transfer, absorption, and adsorption (Vikrant *et al.*, 2018). In this layer, the adhered microorganisms biologically oxidize the polluting compounds, transforming them into less toxic compounds such as carbon dioxide, water, sulfate, nitrate, and other ecologically safe compounds (Barbusinski *et al.*, 2017; Cabeza *et al.*, 2013; Fulazzaky *et al.*, 2014). However, for these reactions to take place, the system must maintain constant performance despite fluctuating environmental and flow conditions. Thus, maintaining the stability of the system is of primary interest, but this is highly challenging because the mechanisms that drive the response of microbial communities to structural and behavioral disturbances have yet to be elucidated (Cabrol *et al.*, 2016).

Studies on microbial communities in biofilters have grown exponentially; however, few of them address full-scale biofiltration systems (Chouari *et al.*, 2015; Omri *et al.*, 2011; Ramírez *et al.*, 2011). In addition, few studies have used a multiphase approach to deepen knowledge of the microbiota present in the systems. Therefore, more studies are still needed to understand the dynamics of the microbial ecology involved in the H_2S removal process (Omri *et al.*, 2011; Li *et al.*, 2013) in order to help improve the system.

More specifically, in biofiltration systems, microorganisms are responsible for converting hydrogen sulfide into nontoxic compounds such as elemental sulfur and sulfate via the conversion reactions demonstrated in equations 1–4 and Figure 1. In addition, some intermediate products of H_2S biodegradation (such as HS^- and S^{2-}) are used as energy sources by microorganisms, contributing to an environmentally benign and self-reliant operation (Dumont; Andrès, 2010;

Wu *et al.*, 2018). The oxidation of inorganic compounds (such as sulfides) supplies energy to cells, a process that is responsible for the effectiveness of the biofilter bed (Vikrant *et al.*, 2018).

$$(1)\ H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$$

$$(2)\ H_2S + O.5\ O_2 \rightarrow S^0 + H_2O$$

$$(3)\ S^0 + H_2O + 1.5\ O_2 \rightarrow H_2SO_4^2$$

$$(4)\ 1.5\ S_2O_3^{2-} + 1.5\ H_2O + O_2 \rightarrow SO_4^{2-} + H^+$$

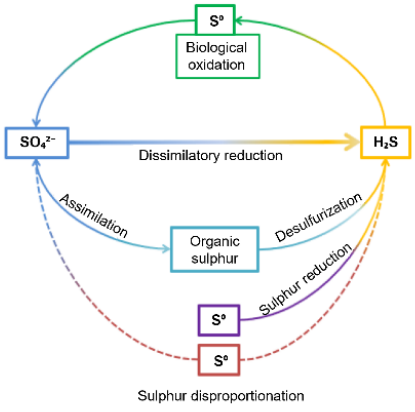

Figure 1 – Schematic representation of the microbial sulfur cycle.

Source: Adapted from Tang, Baskaran, and Nemati (2009).

Equation 1 shows that the dominant fraction of energy (ΔG^o= −145.5 kcal mol^{-1}) is released when microorganisms oxidize sulfides or other reduced sulfur compounds (e.g., thiosulfate). Equations 2 and 3 represent the sulfide oxidation pathway, which frequently involves elemental sulfur as an intermediate. Elemental sulfur is only produced by sulfide oxidation, and this reaction generates less energy than the conversion to sulfate under oxygen-limited conditions, probably due to the higher energy barriers (equations 3 and 4). The sulfur generated is deposited on the internal and external surfaces of the cell membrane. This oxidation reaction also produces energy from other reduced sulfur species, such as thiosulfate (Equation 4) (Vikrant *et al.*, 2018).

In summary, the treatment of malodorant gases in biofilters is closely linked to the microorganisms that convert the compounds. Thus, understanding the microbial communities and their activity, and the association of these communities with physicochemical and environmental factors is crucial to understanding the processes that occur within these systems (Ferrera; Sánchez, 2016). Information on the microbiota can improve the accumulation of desirable organisms, which, for example, can increase the degradation of specific pollutants. It may also help in the modeling, monitoring, and operation of full-scale systems (Cydzik-Kwiatkowska; Zielińska, 2016).

The microbiota of these biofilters have been investigated in some studies, but the empty bed retention time (EBRT) was high (60 seconds) and they were operated in a reduced-scale reactor and during a short period of time (Li *et al.*, 2013; Omri *et al.*, 2011, 2013). Hence, annual seasonal variation was not investigated.

In this framework, over two years, 16S rRNA sequencing was used to identify the structural dynamics (spatial and temporal) of the microbial community and to predict the functional potential in the biological removal of hydrogen sulfide in a full-scale biofilter. The EBRT was shorter (30 seconds) than that most commonly applied in other studies. In addition, lower loading rates (up to 1.2 $g/m^3.h$) were applied to understand how this affects microorganism variability and biofilter performance.

2 MATERIALS AND METHODS

2.1 Biofiltration system

A full-scale biofiltration system was set up at a WPS installed in a residential area in Florianopolis, Santa Catarina, Brazil (Figure 2). The odor emitted by the WPS was mainly characterized by the presence of hydrogen sulfide gas.

The biofilter had a closed rectangular structure with a working volume of 6 m^3, was operated in the up-flow

mode, and was equipped with sampling points before, after, and in the middle of the filter bed. Peat (virgin) was used as the filter bed material; the total height of the filter bed was 1 m. The empty bed retention time (EBRT) was approximately 30 seconds, and the air flow was about 720 $m^3.h^{-1}$. The sulfide loading rate varied from 1.2 $mg/m^3.h$ to 1.2 $g/m^3.h$. The gases were sucked in through PVC pipes by a centrifugal fan that directed them to the biofilter.

The biofiltration system was irrigated in the upper area made of peat. This process provided the necessary humidity for the growth of the microorganisms (40 to 60%), while the peat—organic matter—provided the essential nutrients for this growth. The peat had 60% porosity, a pH of 4–6, a moisture content of 60%–80%, and a bacterial concentration of 7×10^8 CFU/mL. A layer of wood chips supported the peat. The wood layer contained orifices to distribute the gas evenly throughout the filter layer. The physicochemical parameters analyzed in the biofiltration system were hydrogen sulfide concentration, temperature, pH, and humidity (Table 1).

Table 1 – Biofilter operational conditions.

Parameters	Range	Unit
Inlet H_2S concentration	0.01 - 10.17	$mg.m^{-3}$
Outlet H_2S concentration	0.00 - 0.35	$mg.m^{-3}$
Temperature	14 - 30	°C
pH	0.50 - 2.71	-
Humidity	45 - 61	%

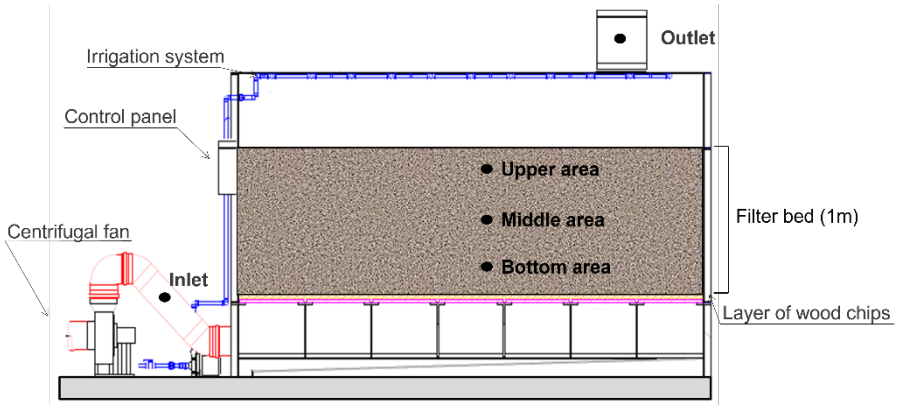

Figure 2 – Schematic representation of the biofiltration system.

2.2 H_2S concentration

H_2S gas was monitored for two years. H_2S concentration was analyzed at the inlet and outlet of the biofilter system, as well as over the filter bed, at three depths: 0.15 m, 0.45 m, and 0.75 m, on the upper, middle, and bottom areas, respectively. The gases were analyzed using the Jerome® Hydrogen Sulfide Analyzer (631-X – Arizona Instruments). This device is portable and measures the concentration of hydrogen sulfide at 0.003-50 ppm.

Sampling was carried out in triplicate, and the mean concentrations were considered when calculating the efficiency of the system. The removal efficiency (RE) was calculated using Equation 5.

$$(5)\quad RE = \frac{C_{inlet} - C_{outlet}}{C_{inlet}} \times 100$$

In which: RE: removal efficiency; C_{inlet}: inlet H_2S concentration; C_{outlet}: outlet H_2S concentration.

2.3 Microbial community analysis

2.3.1 Sampling

For the molecular analyses, homogeneous samples corresponding to temporal and spatial differences within the biofilter — depth from the filter bed (0.15 m [upper area] and 0.75 m [bottom area]) and seasonal variation over two years (winter and summer) — were collected. The seasons of the year were studied because the biofilter was located in a subtropical climate zone, where seasons are well defined, which can cause a considerable difference in the microbial communities. Samples were collected and identified as: Top Summer 1 (TS_1); Bottom Summer 1 (BS_1); Top Winter 1 (TW_1); Bottom Winter 1 (BW_1); Top Summer 2 (TS_2); Bottom Summer 2 (BS_2); Top Winter 2 (TW_2); Bottom Winter 2 (BW_2). The identification of the samples corresponds to the location in the system (Bottom/Top), season of the year (Summer/Winter), and year of analysis (1/2) (Table SM). The "summer" period refers to the months of January to April and October to January. The "winter" period runs from May to September.

The samples were collected in sterile plastic boxes and then sent to the laboratory, where they were stored at −20 °C until the time of the sequencing analysis. The analyses for this study were carried out two years after the biofilter started operating. The system was considered to be in normal operating conditions; thus, the bacterial community was considered stable during sampling.

2.3.2 16S rRNA sequencing and bioinformatic analysis

Metagenomic sequencing of the 16S rRNA was conducted on all samples to assess and determine the structure of the microbial community. The V3-V4 variable regions of the 16S ribosomal RNA gene (16S rRNA) were amplified from 5 ng/µL of DNA using the universal primers 341F 5´-CCTACGGGRSGCAGCAG-3´ (Wang; Qian, 2009) and 806R 5´-GGACTACHVGGGTWTCTAAT-3´ (Caporaso *et al.*, 2011) following the Illumina 16S Metagenomic Sequencing Library Preparation guide using the Miseq platform (Miseq™, Illumina Inc., USA).

The raw results from the paired-end read file were analyzed using mothur version 1.46.1(Schloss *et al.*, 2009), following the MiSeq standard operating procedure (SOP). The sequences were clustered into operational taxonomic units (OTUs) (99% similarity) and classified using SILVA database release 138.1 (Quast *et al.*, 2013). For the diversity analysis, the samples were normalized to the lowest number of sequences reads obtained. Chao1 and Shannon (- diversity) were calculated to represent the diversity and richness of the microbial communities.

Using the mean Euclidean distance, a heat map was created to assess the similarity patterns of the samples by Microbiome Analyst (https://www.microbiomeanalyst.ca/).

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict the metabolic dynamics of the communities (Langille *et al.*, 2013). The functional predictions were assigned to KEGG Ortholog, and the data were explored using KEGG modules.

Sequencing reads were deposited in the European Nucleotide Archive under project number PRJEB44572 with sample numbers ERS6336302 (TW_2), ERS6336303 (TW_1), ERS6336304 (BS_1), ERS6336305 (BS_2), ERS6336306 (BW_1), ERS6336307 (BW_2), ERS6336308 (TS_1), and ERS6336309 (TS_2).

3 RESULTS AND DISCUSSION

3.1 Biofilter performance

The concentration of H_2S was not a controllable parameter, and all the gas generated in the WPS was conducted to the biofiltration system. Figure 3 shows the inlet and outlet H_2S concentrations for the monitoring period. During this period, inlet concentrations ranged from 0.01 to 10.17 $mg.m^{-3}$ and outlet concentrations ranged from 0.00 to 0.35 $mg.m^{-3}$ (Figure 3). Thus, the removal efficiency was more than 99% for most of the run time, which was similar to other studies (Estrada *et al.*, 2012; Li *et al.*, 2013; Omri *et al.*, 2011, 2013; Vikrant *et al.*, 2018), although the sulfide loading rate was much lower (from 1.2 mg /m^3.h up to 1.2 g/m^3.h).

We also analyzed the removal efficiency (RE) in each part of the layer (bottom, middle, and upper) (Figure 4). The RE reached higher values in the lower part of the filter bed during the summer in the first year, when there was a higher density of values, close to 50%. In both years, the bottom and middle parts of the biofilter played a key role in the treatment system, removing more than half of H_2S concentration and leaving the upper part as a fine adjustment. This behavior of the filter (greater removal in the part closest to the inlet part) was also observed by other authors (Li *et al.*, 2013; Omri *et al.*, 2013), even when the flow was downward (Omri *et al.*, 2011). The removal rate capacity reached up to 1.2 g/m^3.h.

The physicochemical parameters of the biofiltration system varied: temperature ranged from 14 to 30 °C, pH varied from 0.5 to 2.7, and humidity ranged from 45 to 61%. The environmental temperature, pH, and humidity were measured at the same time as the H_2S sampling. These parameters are the backbone of an efficient biofilter system, as they support a stable microbial population (Feizi; Nasernejad; Zamir, 2016; Xue *et al.*, 2018). The temperature remained in the mesophilic range, and, according to Zhang, Li, and Jiang (2013), in a full-scale biofilter to treat H_2S, a temperature in the range of 24 °C to 32 °C is ideal for the growth of sulfur-oxidizing microbes. Increasing the temperature can favor an increase in the concentration of H_2S in sewage transport pipes due to the growth of microbial activity in an anaerobic environment. This behavior can be observed in Figure 3A, which shows that H_2S concentrations at the inlet were higher during the summer than during the winter.

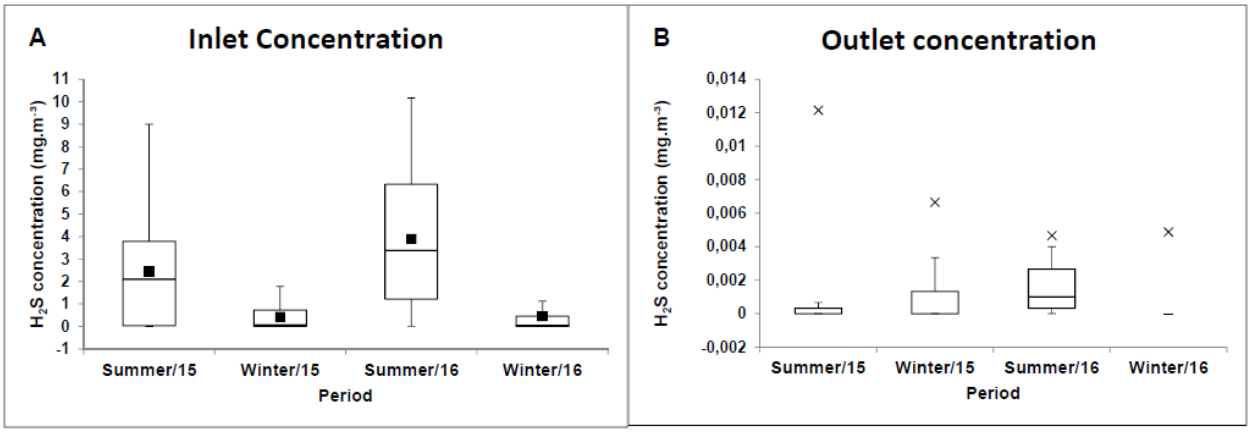

Figure 3 – Performance of the biofiltration system over two years; (A) inlet concentration in the box-plot graph; (B) outlet concentration in the box-plot graph.

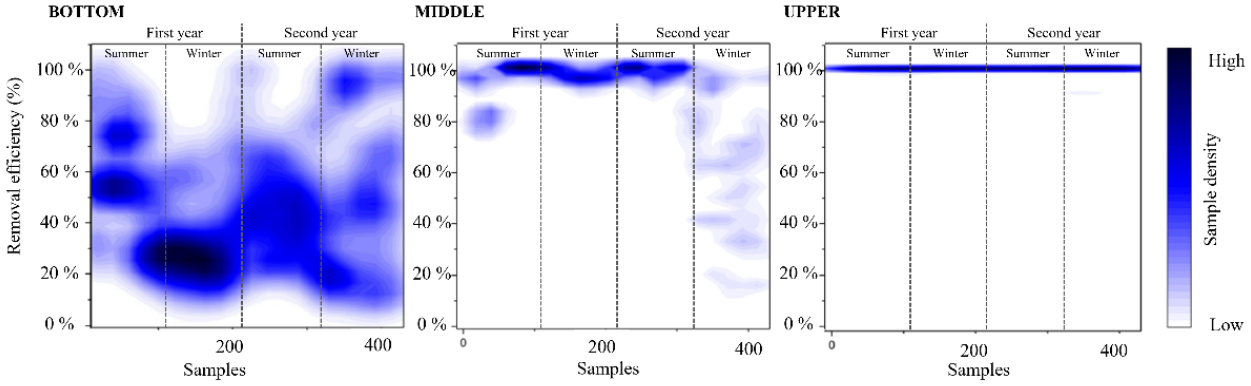

Figure 4 – Performance of the biofiltration system over two years: H_2S removal efficiency and sample density.

The pH of the percolate was extremely acidic, which suggests a degradation of the filter bed and the formation of sulfuric acid in the percolated effluent. However, acidic pH does not affect the efficient removal of H_2S (Kennes; Rene; Veiga, 2009; Shammay *et al.*, 2016). In fact, according to Omri *et al.* (2011), lower pH favors H_2S oxidation.

Humidity remained within the best range for biofilters: from 20 to 60% (McNevin; Barford, 2000; Omri *et al.*, 2013).

3.2 Diversity of the microbial community

A total of 269,359 sequences for eight samples were retrieved from the 16S rRNA Illumina MiSeq™ high-throughput sequencing platform. After quality control by mothur and chimera was carried out to remove low-quality reads (Phred < 24), a total of 258,590 high-quality sequences remained for further analysis. The Good's coverage estimator ≥ 99.5% suggested that most of the microbial richness in each sample had been accessed (Table 2).

Table 2 – Number of sequences analyzed; OTUs, Chao1, Shannon, and Good's coverage obtained.

Samples	Raw reads	Effective	Norm(*) Reads	OUTs	Norm(*) Chao1	Norm(*) Shannon	Norm(*) Good's (%)
TS_1	40825	24056	15304	410	412	4,89	99,92
BS_1	42739	25499	15304	422	423	**4,94**	99,94
TW_1	36239	22945	15304	418	420	4,89	99,92
BW_1	23806	15304	15304	393	395	4,86	99,87
TS_2	46446	27246	15304	**426**	**427**	4,92	99,95
BS_2	36162	22757	15304	354	356	4,57	99,92
TW_2	38115	25191	15304	393	397	4,69	99,9
BW_2	50270	31786	15304	**324**	340	**4,27**	**99,87**

*Normalized

3.3 Microbial community structure

To provide an insight into the microbial community involved in the H_2S removal process in biofilters, a deeper analysis of the bacterial community structure at different taxonomic levels was carried out to illustrate the spatial and temporal variations tested in the biofilter. Figure 5 shows the heat map with the overall relative abundance at the phylum level in response to H_2S (Figure 5). The heat map shows the behavior between the microbial groups throughout the duration of the experiment in all samples.

The heat map shows that each phylum had a positive and negative correlation with the analyzed samples and their variables, and the microorganisms' response to H_2S was observed along with their behavior throughout the process. Spatial variability was observed for most of the identified phyla in both years, indicating that the bacterial community was well established at the time of sampling. In general, there was a decrease in the relative abundance of almost all the phyla for the BW_2 (Bottom winter from the second year) sample (321 days after start-up), including the upper layer sample collected under the same environmental conditions. The similarity dendrogram also clustered this sample (BW 2) with a greater distance from the other samples. In contrast, this sample showed a predominance of the phyla Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Nitrospirae, and Verrucomicrobia, listed in descending order of predominance (Figure 5). Thus, it was possible to infer that the microorganisms belonging to these phyla were probably dominant in the bottom layer of the system.

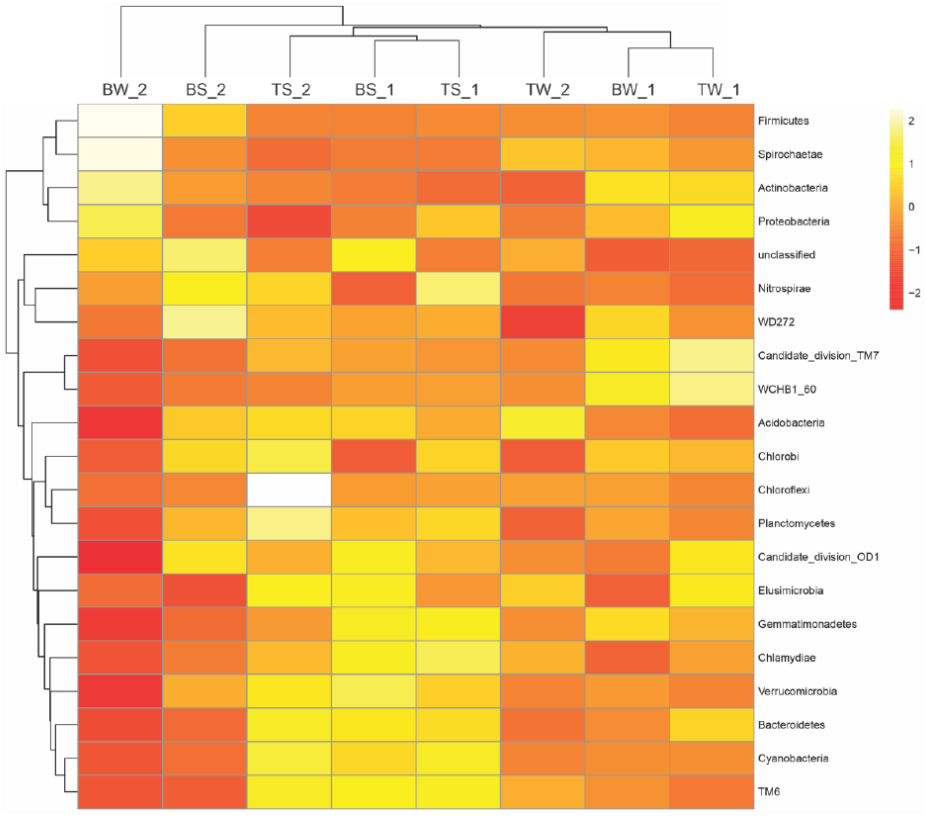

Figure 5 – Overall heat map for each sample. Each phylum was grouped based on the mean Euclidean distance. On the frequency scale, the more yellow the color, the more abundant the genus in the sample. The similarity dendrogram from the clustering analysis is at the top.

In addition, it was possible to observe that sample BS_1 (bottom layer; first year) showed a high relative abundance for most of the phyla identified (Figure 5). This result was similar to that found in the diversity indices. Similarity clustering showed that this sample was more similar to the other samples collected in the summer. Therefore, temperature influenced the structure of the bacterial community. However, the change in microbiota was observed over time, even in the summer (BS_1 to BS_2). These results show that this was probably due to competition for the energy source available in the inlet region of the system, reinforcing the previous finding that the spatial factor, i.e., greater contact with H_2S in the inlet region of the system, had the greatest influence on microbial structure.

At the genus level, the 20 most abundant genera were selected, as shown in Figure 6. The genus *Elsterales_ge* (phylum *Proteobacteria*) was the most abundant, predominating in the second year in the lower part of the system (Figure 6). The injection of H_2S increased the relative abundance of this genus, which reached values of up to 28%. It can therefore be inferred that this genus played a key role in the transformation of H_2S.

The genus *Acidothermus* (phylum *Actinobacteria*) was the second most abundant genus identified, predominating in the winter samples in the lower part of the system (Figure 6). The relative abundance increased over a range of 8% to 16% depending on seasonal and spatial distribution, although it did not predominate in a specific sample. This indicates stability over time.

Furthermore, the genus that stood out in the bottom region of the system was *Sulfobacillus* (phylum *Firmicutes*). Microorganisms of this genus are resistant in an acidic environment, as is the case with biofilters. The species are moderately thermophilic acidophiles and can oxidize Fe^{2+}, sulfides, or sulfur (Ding *et al.*, 2006). The genus *Sulfobacillus* was also found by Ding *et al.* (2006) in a biofilter used to treat H_2S in the presence of methanol. *Sulfobacillus* had an increase in relative abundance during the second year of the treatment, especially in the lower part of the biofilter, and was one of the most abundant genera in sample BW_2. In addition, other genera, such as *Occallatibacter,* were predominant in the bottom region of the system during the second year.

In contrast, some genera had a higher relative abundance in the upper region of the system, such as the bacteria of the *Acidobacteriaceae* family (phylum *Acidobacteria*). The phylum *Acidobacteria* is one of the dominant bacterial groups in soils, surpassed by Proteobacteria (Janssen, 2006). This predominance was also observed in this biofiltration system.

Among the samples in the upper part of the biofilter, the genus *Methylovirgula* (phylum *Proteobacteria*) showed little structural variation after the injection of sulfuric gas throughout the experiment, with a slightly decreasing relative abundance in the upper layer. The upper part of the biofilter showed stability throughout the experiment.

The genus *Bryobacter* (phylum *Acidobacteria*) showed little structural variation during the experiment, with a slight increase in relative abundance in the second year. Bacteria of this genus are described as slow-growing aerobic chemoheterotrophs, which use a narrow spectrum of carbon as an energy source and grow in low-nutrient media. They are also acid-tolerant, mesophilic, and can be found in different types of soil (Kulichevskaya *et al.*, 2010).

According to the results, in general, it seems that the biofilter was a stable environment for the microbial community and structure during the two years of analysis. The genera found in the system showed a small structural variation according to the season and the injection of the gas. The biofilter provided a suitable environment for the microorganisms from the Acidobacteria and Proteobacteria phyla, which are aerobic microorganisms. In the bottom layer,

the favored microorganisms were the facultative Actinobacteria and Firmicutes. The other most abundant phyla showed no trend. These results advance what is understood about bacterial ecological succession in bioremediation in this type of treatment, especially in relation to H_2S removal.

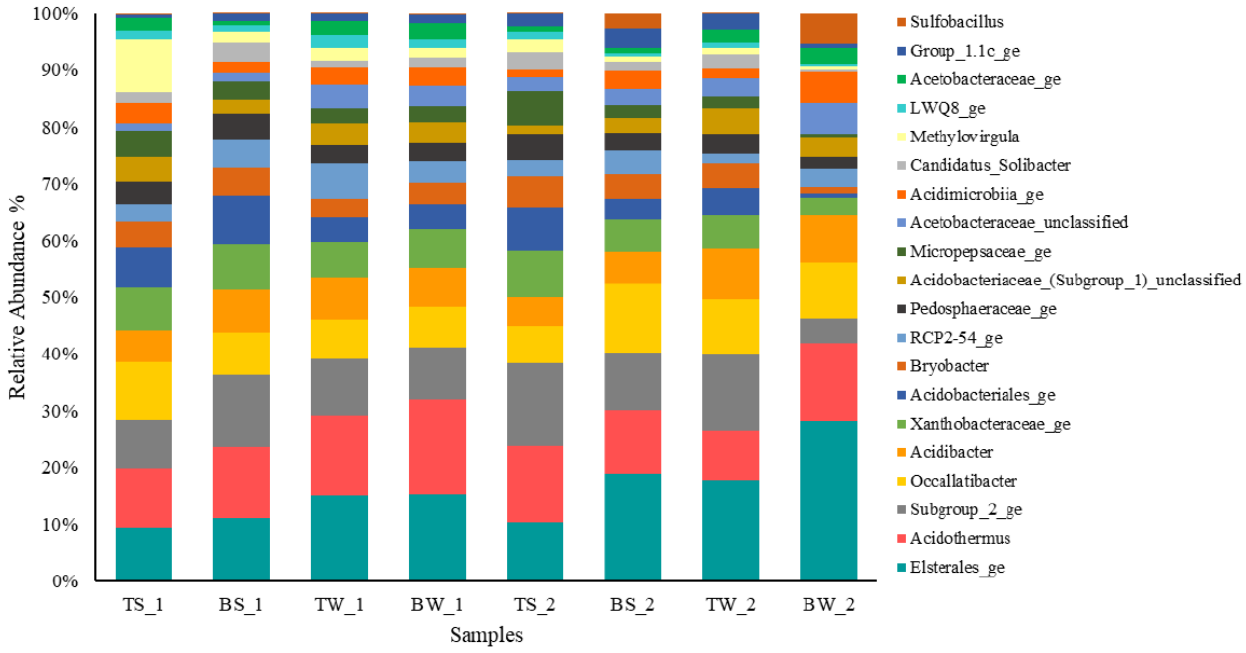

Figure 6 – Relative abundance of the bacterial community at genus level derived from the dataset of the biofilter.

3.4 Predictive functional profiling of microbial communities

To deepen the knowledge of the metabolic behavior of the bacterial community in the system evaluated in this study, the PICRUSt results based on the KEGG database highlighted the functional dynamics of the genes associated with the assimilatory and dissimilatory sulfate pathways (Figure 7).

The assimilatory pathway, which used H_2S, had more genes identified (11 in total) than the dissimilatory pathway (seven in total). Among them, five had greater proportions of the genes, which were cysNC (sulfate adenylyltransferase), cysH (adenylyl-sulfate reductase), cysJ (assimilatory sulfite reductase – NADPH), cysC (adenylyl-sulfate kinase), and cysI (assimilatory sulfite reductase – NADPH), listed in descending order. As shown in Figure 7, the assimilation genes identified did not show relative abundance (spatial and temporal) trends throughout the experiment.

The dissimilatory pathway only had three genes identified (Figure 7): cysD (sulfate adenylyltransferase), cysN (sulfate adenylyltransferase), and sat (sulfate adenylyltransferase). Sat showed a higher proportion in the bottom layer, while the other genes showed no trend. Thus, the genera producing hydrogen sulfide were not identified by taxonomic classification. Figure 8 shows the main functional genes involved in the sulfur metabolism found in this study.

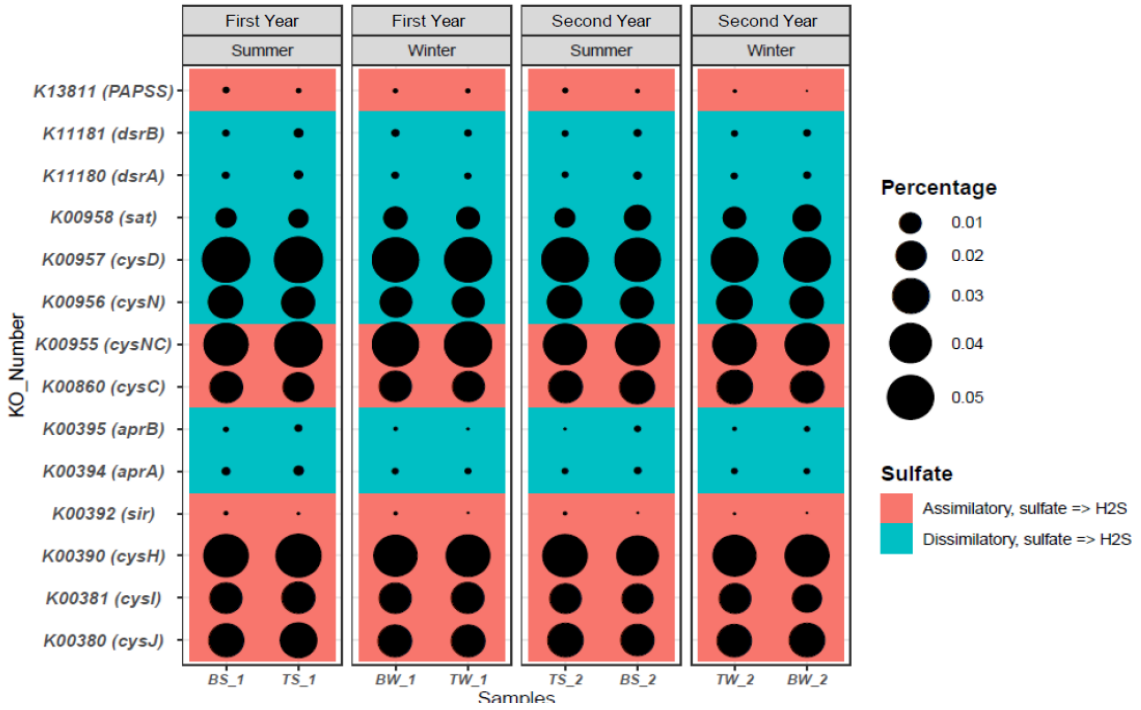

Figure 7 – Bubble plot of the relative abundance of the predicted functional genes related to the microbial sulfur cycle found in the biofilter system datasets, as predicted by PICRUSt.

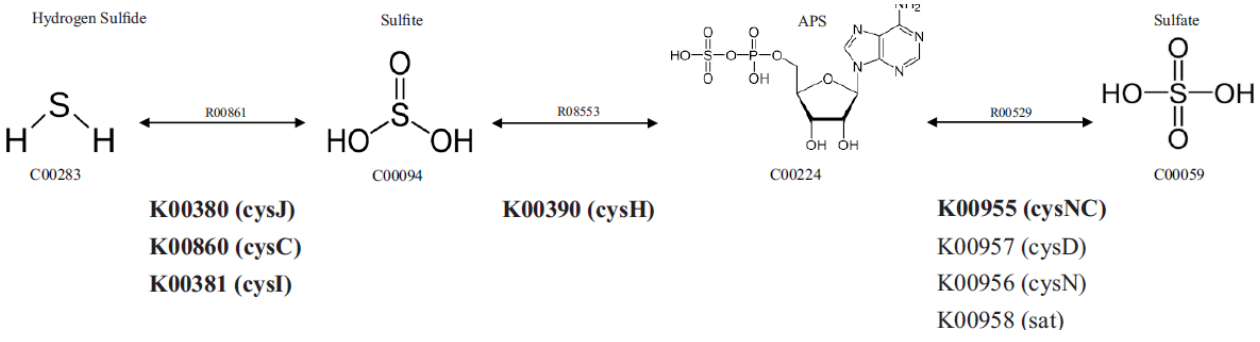

Figure 8 – Dissimilatory and assimilatory pathways in the sulfur metabolism.

Among the five main assimilation genes identified, three are involved in the transformation reaction of H_2S into sulfite, as shown in Figure 8. Even at a low pH, which favors the formation of sulfuric acid, only one gene (cysNC) involved in the sulfite to sulfate reaction was found. Interestingly, the dissimilatory genes identified are responsible for the sulfate to sulfite reaction via adenosine 5'- phosphosulphate (APS). These findings may justify the better RE in the lower part of the system, where a higher concentration of H_2S was available for microbial groups.

As mentioned earlier, H_2S concentrations at the system inlet during the summer reached higher

values than during the winter for most samples, as expected, since the anaerobic bacterial activity in sewage pipes is stimulated by warmer temperatures. Even so, the system remained stable and efficient, with over 99% of H_2S removal (Figure 3). Thus, we can assume that the functional potential found in the bottom layer and along the verticality of the filter, driven by most of the assimilation genes, contributed to the stability of the system.

4 CONCLUSIONS

Studies focused on bacterial behavior and structure in biofiltration systems are still scarce and mostly carried out in a smaller scale and over a short period of time. This study allowed us to better understand bacterial dynamics and metabolism and to advance the idea that microbial analysis can support annual seasonal changes. Therefore, it increases efficiency performance and assists in situations of system dysfunction, with a focus on expanding the applicability of biofiltration systems for the most varied needs. Knowledge of microorganisms can help in the modeling, system recovery, quick startup, and operation of full-scale bioreactors. We also demonstrated that the biofilter used was robust even when a low sulfide loading rate was applied.

5 AUTHOR CONTRIBUTIONS

Conceptualization: Allievi MJ, Belli Filho P; **Methodology:** Allievi MJ, Silveira DD; **Formal analysis:** Allievi MJ, Cruz LMO, Silveira DD; **Bioinformatic analysis:** Cantão ME, Silveira DD; **Picrust analysis:** Delforno TP; **Biological analysis:** Silveira DD; **Investigation:** Allievi MJ, Cruz LMO, Silveira DD; **Visualization:** Allievi MJ, Cruz LMO, Silveira DD; **Writing – original draft:** Allievi MJ, Cruz LMO, Silveira DD; **Writing – review & editing:** Allievi MJ, Cruz LMO, Silveira DD; **Project administration:** Belli Filho P; **Funding acquisition:** Belli Filho P.

6 ACKNOWLEDGMENTS AND FUNDING SOURCE

The authors would like to thank CAPES/Brazil (Finance code 001) and Multiágua Environmental Engineering Company for their financial support. They also thank EMBRAPA for cooperating with the bioinformatics professional and the sanitation company CASAN for providing the space to install the biofilter.

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