Bacterial community diversity in a full scale biofilter treating wastewater odor
M. J. Allievi, D. D. Silveira, M. E. Cantão and P. B. Filho

ABSTRACT

Constantly, the odors coming from sewage plants are considered a problem by the population. The purpose of this study was to evaluate the microbial community present in a full scale biofilter used for odor treatment. The filter was packed with peat. The main gas treated was hydrogen sulphide (H2S). The removal efficiency reached 99%, with an empty bed residence time of 30 seconds. Molecular analysis can enhance our understanding of the microbial communities in biofilters treating wastewater odor. The analysis made to characterize microbial community was High-throughput 16S rRNA sequencing analysis MiSeq® Illumina. The sampling, carried out in the year 2015, was seasonal (summer and winter) and spatial (depth and position in the biofilter). In this study, a total of 206,174 raw sequence reads for six samples were analyzed using Mothur software (v 1.33.3) based on MiSeq SOP protocol. After Mothur analysis, the results of the bacterial community were explored at the Phylum and Genus levels. In this study, the efficiency removal of hydrogen sulfide reached values greater than 99% during the monitoring, and the main bacterial genera found were Acidotermus, Telmatobacter, Methylovirgula and Bryobacter representing the bacterial community active in the transformation of H2S into a system with long operating time.

Key words | bacterial community, biofilter, odor, sulphide gas, wastewater

INTRODUCTION

Wastewater treatment and industrial processes generate odors that can contribute significantly to atmospheric pollution (Omri et al. 2011). The emission of malodorant gases by wastewater treatment systems is one of the major concerns of the people who live near these areas (Alfonsín et al. 2015). In those systems, the odors come from the organic matter degradation in anaerobic condition, which has sulfide as a byproduct. Hydrogen sulfide is a colorless gas, insoluble in water, highly corrosive, toxic, and malodorant (Pantoja Filho et al. 2010). To Sigurdsson et al. (2015), wherever this gas is present, there is imminent health hazard. Even in low concentrations, inhaling H2S can cause nausea, headache, dizziness and sleepiness.

The control of odorant gases emission has been emphasized in recent years, as most developed and developing countries have faced problems with changes in air quality. Many governments have enacted laws and policies to enforce sewage sludge treatment plants to reduce air contaminating emissions and this is mainly carried out by installation of air pollution control systems (Fulazzaky et al. 2014); for this reason, the search for efficient removal technologies for odors has grown recently (Schlegelmilch et al. 2005).

Physical-chemical techniques applied to odor treatment generate byproducts, which require further treatment, increase operating costs, and have high-energy requirements (Gómez-Cuervo et al. 2017). Thus, the biological processes applied to odor treatment have been diffused in the last decades, becoming attractive treatment alternatives for odors coming from wastewater systems. Biological techniques operate under normal conditions of temperature and pressure, and are capable of treating large gas flows with low concentrations of pollutants (Fulazzaky et al. 2014). In addition to these characteristics, biological processes have advantages such as being eco-friendly, not generating toxic byproducts, and having low operating costs and energy requirements (Alfonsín et al. 2015). Biofiltration happens in a fixed filtration medium, in which a biologically active layer, the ‘biofilm’, grows. In this layer, the adhered microorganisms biologically oxidize the polluting compound,
transforming it into less toxic compounds (Fulazzaky et al. 2014).

In the biofiltration system, the main agents of sulfide gas degradation are the bacteria. They use the H$_2$S as source of energy and electrons, and through oxidizing reactions the sulfide gas is converted into odorless byproducts like S or SO$_4^{2-}$, carbon dioxide, water vapor and organic biomass (Chouari et al. 2015). These reactions are presented below (Tang et al. 2009).

$$H_2S + 0.5 O_2 \rightarrow S^0 + H_2O$$

$$S^0 + H_2O + 1.5 O_2 \rightarrow SO_4^{2-} + 2H^+$$

The malodorant gases’ treatment is intimately linked to the microorganisms that make the compounds’ conversion, that is why the knowledge of the bacterial community structure present in the biofiltration systems, as well as the linking of this community with the physical-chemical and environmental factors, is crucial in comprehending the processes that occur inside these odor treatment systems (Ferrera & Sánchez 2016). Functional stability is a major concern of bioreactor design, especially when the system is operated in full scale transient conditions. Investigating possible links between environmental fluctuation and bacterial community structure is one of the most challenging issues in biological systems (Briones & Raskin 2003). The bacterial community plays a key role in maintaining the functional stability under variations like richness, uniformity, dynamics, functional redundancy, microbial composition and interactions. These variations seem to be of extreme importance to control the reactors and ecosystems functions (Wittebolle et al. 2009). In the last decade, the number of studies about the bacterial fauna in biofilters that process the atmospheric pollution has grown exponentially, since these treatment systems are one of the most commonly implemented technologies for reduction of odors (Cabrol et al. 2012). Knowledge of the bacterial community can be used to improve the bioaccumulation of desirable organisms, increasing the degradation of specific pollutants, but can also help in the modelling, monitoring and operation of real scale systems (Cydzizk-Kwiatkowska & Zielinska 2016). Sophisticated molecular techniques, like 16S rRNA sequencing, has been used to study bacterial diversity and reveals an unknown universe, since most of the identified organisms, often non-cultivable, are responsible for most of the key processes that occur in the system (Wagner & Loy 2002). Bacterial community characterization in odor treatment systems by means of prominent techniques, such as the High-throughput 16S rRNA sequencing used in this work, are rare. Other studies that evaluated the bacterial dynamics using several techniques were Cabrol et al. (2012), Chouari et al. (2015) and Omri et al. (2011).

In this study, the efficiency of H$_2$S removal and the bacterial community were analyzed temporally and spatially in a biofiltration system.

**METHODS**

**Biofiltration system**

A full scale biofiltration system was set up at the wastewater pumping station (WPS) installed in a residential zone in Florianopolis, Santa Catarina, Brazil. The odor emitted by the pumping station was not a controllable parameter and was characterized mainly by the presence of sulfide gas in concentrations that varied in the range of 0.002–5.66 mg·m$^{-3}$.

The biofilter has a rectangular structure, closed with a working volume of 6 m$^3$. It was operated in the upward flow mode and equipped with sampling points at the inlet, the middle and the outlet of the filter bed. It was packed with peat up to a height of 1 m. Never used before, the peat came packed from a garden shop. The empty bed retention time (EBRT) was about 30 seconds and the air stream was about 720 m$^3$·h$^{-1}$. The gases were sucked in through PVC pipes by a centrifuge fan that directed them to the biofilter. Besides that, the biofiltration system was irrigated in the upper area of the peat. This provided the necessary humidity for the microorganisms’ growth while the peat, organic matter, provided the necessary nutrients.

The peat has a porosity of 60%, pH of 4–6, moisture content of 60–80% and bacterial concentration of $7 \times 10^8$ UFC/mL. Peat was supported by a layer of wood chips with a height of 0.15 m. That layer contains orifices to distribute the gas evenly throughout the filtration layer.

**Sampling and analyses**

The analyses carried out in this study include the H$_2$S concentration, at the inlet and outlet of the biofilter system, and molecular analyses to characterize the diversity and richness of the bacterial community. The samples for molecular analyses were collected with the spatial differences within the biofilter: 0.15 m depth (upper) and 0.75 m (deep) of the
filter bed; and seasonal differences: winter (W) and summer (S). The startup of the biofilter system was in July 2013 and the analyses for this study were carried out in the year 2015.

Gas collection and analyses

The gases were analyzed with the Hydrogen Sulfide Analyzer Jerome® (651-X – Arizona Instruments) device. This device is portable and measures the concentration of hydrogen sulfide in ppm in a range of 0.003–50 ppm. Sampling was done in triplicate, and the mean of concentrations was considered for calculation of the system efficiency. The removal efficiency of sulfuric gas was calculated by the efficiency equation.

Analysis of the bacterial community

The diversity and richness of the bacterial community were analysed by High-throughput 16S rRNA sequencing for the different depths and seasons. The samples were collected at two sites in the biofilter at a distance of 2 m from each other and at 0.50 m from the walls of the biofilter. In addition to point sampling, a bacterial pool was carried out for each season.

Winter and summer seasons were studied because the biofilter is located in an area with a subtropical climate, where the seasons are well defined, which can cause a considerable difference in the bacterial community. In addition to seasons, the depth criterion was adopted. Points with different depths were studied because the difference in the bacterial community could be explained by the temperature, which is higher in the upper part of biofilter, since it receives more solar incidence than the lower part; besides that, the bacterial community can have differentiation due to the direction of the gas flow, since the sulfuric gas first comes into contact with the bottom of the biofilter. Thus the analyzed samples are presented in Table 1.

DNA extraction

Genomic DNA was extracted from a pellet of a concentrated effluent sample using the Power Soil™ DNA Isolation Kit (Mbio, laboratories, Inc., Carlsbad. CA), according to the protocol recommended by the manufacturer, with slight modifications. After DNA extraction, the material was stored at –80°C until the moment of use. The quality of the extracted DNA was verified by a 1.5% agarose gel run, and after verification, the DNA was quantified by the Invitrogen QUBIT®.

High-throughput 16S rRNA sequencing analysis

All 16S rRNA reads were analyzed using Mothur, version 1.33.3 (Schloss et al. 2009), following the MiSeq Standard Operating Process (SOP) (http://www.mothur.org/wiki/MiSeq_SOP). Sequences were clustered into operational taxonomic units (OTUs) at 0.03 (97% similarity), and bacterial taxonomic classifications of each OTU were performed in Mothur, using the SILVA Database release 119 as a reference. For the alpha diversity analysis, samples were normalized to the lowest number of sequence reads obtained. Alpha diversity was estimated using Chao1, Good’s coverage, and the Shannon diversity index. A comparative analysis of different samples was determined by the Statistical Analysis of Metagenomic Profiles (STAMP) software, using G-test corrected for sampling size using Yates’ continuity correction. P value correction was calculated using Storey’s FDR approach and then a graphic principal component analysis (PCA) was conducted.

RESULTS AND DISCUSSION

Removal efficiency of hydrogen sulfide

In this study, 213 analyses of the concentrations of H₂S were carried out during 365 days of operation of the biofiltration system. Figure 1 shows the concentrations for the inlet and outlet of the analyzed samples. During the monitoring period, inlet concentrations of H₂S varied in the range of 0.01 mg·m⁻³ to 5.70 mg·m⁻³, and output concentrations varied in the range of 0.00 mg·m⁻³ to 0.21 mg·m⁻³. For the monitoring period inlet gas flow ranged from 660.0 m³·h⁻¹ to 1,233.0 m³·h⁻¹. The biofiltration system removed H₂S with efficiencies up to 100% most of the time.

This biofiltration system had a quick startup (few days) without the use of inoculum; in other biofiltration systems,
as in the work of Duan et al. (2006) and Li et al. (2013), fast startup occurred due to the use of inoculum from sewage treatment systems. The removal efficiency in this study was greater than Duan et al. (2006), 94–99%, and Alfonsín et al. (2013), 94%. The biofilters of these studies were filled with organic material, biological activated carbon (BAC) and peat, respectively.

**Bacterial community – high-throughput 16S rRNA sequencing analysis**

**Bacterial community diversity and estimated richness**

In this study, a total of 125,796 high-quality sequence reads were obtained from bacterial 16S rRNA. Among the samples evaluated, the peat sample from the PS (Pool summer) showed the highest number of bacteria OTUs (435 sequences at 97% similarity) and the sample containing less OTUs number was PDW (Pool deep Winter, 393 sequences) (Table 2).

According to Shannon index, bacterial diversity from highest to lowest was PS > PDS > PW > PUS > PUW > PDW. Predominance of diversity was not observed in a specific sample; however, sample PS presented a higher Shannon index, indicating that summer has a greater bacterial diversity or dominion of certain groups. This result is in agreement with that expected, where the higher temperature interfered positively in the bacterial behavior, since it increased the diversity of the PS sample in relation to the others. These results confirm what Omri et al. (2011) claim, that the bacterial community is greatly affected by system conditions, such as the temperature, oxygenation and moisture content.

As for the peat sample, this has the lowest value for the Chao1, Shannon and OTUs number (173, 3.55 and 167, respectively). Therefore, there is less diversity and bacterial richness in this sample, or some groups were dominant prior to system startup.

The Chao1 estimator is based upon the number of rare classes (i.e. OTUs) found in a sample. This index showed that bacterial community richness ranged from 400 to 444 taxa in the evaluated samples, which indicates a small richness variation in the system. This result shows that there was not much richness variation in samples analysed for the year 2015, although the summer samples, except for PUS, show the highest Chao1 index.

This result can also be observed in the Venn diagram (Figure 2), which shows unique and shared OTUs. Summer and winter samples had similar OTUs, 632 and 628, respectively.

For the peat sample, in general, the number of shared OTUs is low, strengthening the thesis that the peat had a different bacterial community before system startup; this also appears in the PCA chart. The total number of OTUs for all samples was only 44 in winter and 41 in summer, showing the bacterial diversity in temporal variation. For the depths, the Venn diagram shows there was no differentiation between samples, which indicates homogeneity of the system in relation to OTUs.

The distributions of bacterial phylogenetic similarity found in the evaluated samples were classified into distinct groups by

<table>
<thead>
<tr>
<th>Samples</th>
<th>Raw reads</th>
<th>Effective</th>
<th>Norma Reads</th>
<th>OTUs</th>
<th>Norma Chao1</th>
<th>Norma Shannon</th>
<th>Norma Good’s (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>61,928</td>
<td>41,387</td>
<td>1,530</td>
<td>167</td>
<td>173</td>
<td>3.55</td>
<td>99.96</td>
</tr>
<tr>
<td>PDW</td>
<td>23,806</td>
<td>15,304</td>
<td>1,530</td>
<td>393</td>
<td>400</td>
<td>4.86</td>
<td>99.87</td>
</tr>
<tr>
<td>PDS</td>
<td>42,739</td>
<td>25,499</td>
<td>1,530</td>
<td>413</td>
<td>423</td>
<td>4.94</td>
<td>99.86</td>
</tr>
<tr>
<td>PUW</td>
<td>36,239</td>
<td>22,945</td>
<td>1,530</td>
<td>409</td>
<td>422</td>
<td>4.89</td>
<td>99.83</td>
</tr>
<tr>
<td>PUS</td>
<td>40,825</td>
<td>24,056</td>
<td>1,530</td>
<td>400</td>
<td>413</td>
<td>4.89</td>
<td>99.84</td>
</tr>
<tr>
<td>PW</td>
<td>29,314</td>
<td>18,436</td>
<td>1,530</td>
<td>409</td>
<td>421</td>
<td>4.92</td>
<td>99.84</td>
</tr>
<tr>
<td>PS</td>
<td>33,251</td>
<td>19,556</td>
<td>1,530</td>
<td>435</td>
<td>444</td>
<td>5.04</td>
<td>99.85</td>
</tr>
</tbody>
</table>

*aNormalized.*
PCA (Figure 3). It is possible to notice that the PCA result coincides with the results of other analyses performed in this study. The summer samples occupy a different quadrant in relation to the winter samples, in which show variation of the bacterial community. It is also possible to observe the separation between the evaluated samples, in which winter samples are closer to each other. In addition, summer samples are furthest from each other, indicating that in summer there was greater diversity in the bacterial community or there was a predominance of certain bacterial groups in the biofiltration system.

Spatial and temporal bacterial community structure

The phylogenetic diversity of bacterial communities was compared using phylum and genus readings. The relative abundance of the selected Taxons for all samples with spatial and temporal variation is shown in Figures 4 and 5.

Phylum level

A total of 20 phyla represent groups with a higher number of sequences. The phyla found in this study were also reported in a study by Chouari et al. (2015) and Omri et al. (2011). Figure 4 shows the phyla Proteobacteria, Acidobacteria and Actinobacteria were already present before the system startup, and after the startup they expanded their bacterial communities. This indicates the gas was being treated mainly by microorganisms of these phyla. In contrast, the phylum Bacteroidetes was not favored with the startup of system, resulting in a drastic decrease of its bacterial community. Studies indicate this phylum, when present in soils, is highly affected by the pH of the medium. In pH <4, its relative abundance can reach less than 1% (Lauber et al. 2009). The low pH in this system can be explained by conversion of H2S into H2SO4, an acidic compound that reduces the pH of the system.

The Proteobacteria was the most abundant phylum in the system, and it accounted for 51% (for PW and PUW), 48% (for PS), 48% (for PDW and PUS) and 45% (for PDS) of the total reads. Chouari et al. (2015) also report the prevalence of this phylum in biofiltration systems. Proteobacteria comprise a major proportion of the known gram-negative organisms, including phototrophs, heterotrophs and chemolithotrophs. This phylum is commonly found in sewage treatment system sampling.

Phylum Acidobacteria was the second largest bacterial group in samples, with 34% (for PUS), 32% (for PDS), 27% (for PS), 25% (for PDW) and 23% (for PW and
PUW). Summer samples had the highest relative abundances; among them, the PUS sample had the highest value, indicating the higher temperature caused by the higher solar incidence in the upper part of the biofilter influences this bacterial community. The phylum Acidobacteria was also identified in the study of Chouari et al. (2015). According to Foesel et al. (2013), this phylum is present in soils with a relative abundance of 20%, only surpassed by the phylum Proteobacteria.

Only a few representatives of this phylum have been isolated and validated so far; the genus *Bryocabter* forms part of this phylum and is characterized by slow-growing aerobic chemoorganoheterotrophs, which use a narrow spectrum of carbon as energy sources and are able to grow in low-nutrient media, being considered oligotrophic organisms (Foesel et al. 2013; Pascual et al. 2015).

The Bacteroidetes phylum had a small difference in relative abundance between the seasonal samples, ranging from 0.5–1.5%. This small difference indicates that temperature is not a determining factor for this phylum. Bacteroidetes phylum encompasses gram-negative, chemo-heterotrophic filamentous bacteria capable of degrading complex organic matter (Adrados et al. 2014).

The relative abundance of the phylum Actinobacteria remained practically the same in all summer samples (PS, PUS, PDS), between 15% and 16%; the same trend was observed for winter samples (PW, PUW, PDW) where relative abundance varied between 11% and 12%. According to Ye et al. (2011), this phylum is present in wastewater treatment systems and in biofilters filled with organic matter (Chouari et al. 2015).

The others phyla were less than 1% and only 11% of all were unclassified at the phylum level, suggesting that the identities of these bacteria are unknown.

### Genus level

The relative abundance of the evaluated samples is shown in Figure 5, where we observed the largest sequences were uncultured (21% for winter samples and 26% for summer samples) and uncultured bacterium (ranging from 18–21% in samples). Subsequently, the reading belonging to genus *Chitinophaga* had 47% relative abundance in the peat sample. However, this genus was not visualized for other samples evaluated, probably due to injection of hydrogen sulfide.

Members of genus *Chitinophaga* are chitinolytic, known for their chitin degrading potential, fungal cell wall constituent (Weon et al. 2009). The genus appears to be particularly enriched in soil samples where dead fungal mycelia are
being actively decomposed (Larsbrink et al. 2017), being as this degradation is hugely significant in the recycling of carbon in the natural environment. Other genera were also found in peat, but with relative abundance below 1%.

Besides the genus Chitinophaga, the genera that stood out most in the samples were Acidothermus, Methylovirgula, Telmatobacter, Methylovirgula, Bryobacter and Rhizomicrobium. Other genera had relative abundance less than 5% and the unclassified bacteria represented 1.7% of total reads.

Acidothermus genus had close values in summer and winter samples, ranging from 17–22%, indicating that temperature was not a determining environmental factor. With only 0.5% of relative abundance in the peat sample, this genus was considerably benefited by the injection of the H₂S, becoming the fourth genus with greater relative abundance, and thus we can conclude that these microorganisms are fundamental for the removal of H₂S from the system. The genus Acidothermus is considered to be gram-negative, obligate aerobes, which grow at thermophilic temperatures (Mohagheghi et al. 1986).

The third genus in rank was the Telmatobacter. However, in seasonal samples, relative abundance varied between 8% and 9%, indicating the temperature factor did not affect its presence. This genus, although little studied, is widely distributed in soils and is highly related to the nutrient cycle (Li et al. 2017). This genus is gram-negative, facultatively anaerobic chemooorganotrophs, which grow in acidophilic and mesophilic media (Pankratov et al. 2012).

Methylovirgula genus was the fourth genus in the rank. As for the other genres mentioned above, the system startup favored the increase of this bacterial community. A slight increase in abundance was observed in summer samples and at the top of the biofilter, reaching 10%, while in winter samples the maximum value was 5%. These genes are gram-negative, aerobic, acidophilic and mesophilic (Ling et al. 2017).

The Bryobacter genus showed a relative abundance of less than 1% in the peat sample, and values between 5% and 7% for the other samples, with higher values in the summer period, showing the same behavior as the other genera for variables analyzed. Bacteria of this genus are strictly aerobic, gram-negative, acidotolerant and mesophilic and are found in different soil types (Kulichevskaya et al. 2010).
CONCLUSIONS

The biofiltration system showed a removal efficiency of hydrogen sulfide above 99%, showing that, despite a long operating time, H₂S removal was stable and the filter material remained with an adequate lifespan favoring the system. As for the molecular analyses, we can conclude the depth variable was not a determinant factor for structuring and diversity of the bacterial community; that is, the bacterial community was homogeneous along the filter bed. In relation to the seasonal variable, the phylum Acidobacteria and Bacteroidetes, and the genus uncultured were favored in the summer season, and had a significant increase in their relative abundance. We conclude that the variation of the bacterial community was more significant in their relative abundance. We conclude that the variation of the bacterial community was more significant in the seasonal than in the spatial variable. This result may be related to the system having a long operating time making the bacterial community stable and adapted to operating conditions. The relative abundance data showed the presence of sulfuric gas was a determining factor for the change in bacterial community, favoring organisms that are likely to convert H₂S.

The major genera of bacteria found after the system startup were gram-negative, aerobic, facultative anaerobes, acidophilics and mesophilics, such as *Acidothermus*, *Methylovirgula*, *Telmatobacter*, *Methylovirgula*, *Bryobacter* and *Rhizomicrobium*.

This work might add some new insights into the microbial community and further research is needed in order to better elucidate these bacterial groups and their functions within the biofiltration system.

ACKNOWLEDGEMENTS

The authors thank the EMBRAPA for cooperation with the bioinformatics professional and Wastewater Company (CASAN) for giving the space for installing the biofilter. We are also grateful for funding by Coordination of Improvement of Higher Education Personnel of Brazil (CAPES) and Multiágua Environmental Engineering Company.

REFERENCES


Lauber, C. L., Hamady, M., Knight, R. & Fierer, N. 2009 Pyrosequencing-based assessment of soil pH as a predictor of
soil bacterial community structure at the continental scale. *Applied Environmental Microbiology* 75, 5111–5120.

Li, L., Han, Y., Yan, X. & Liu, J. 2013 H₂S removal and bacterial structure along a full-scale biofilter bed packed with polyurethane foam in a landfill site. *Bioresource Technology* 147, 52–58.


