

TOXIC COMPOUNDS REMOVAL PERFORMANCE OF A FULL-SCALE DOMESTIC WASTEWATER TREATMENT PLANT AND THE MICROBIAL COMMUNITY INVOLVED

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ABSTRACT

Wastewater treatment management strategies have become a global challenge because of the fast increase of urban population. The release of sewage, with or without treatment, and rich in toxic compounds as for example nitrogen, phosphorus, and chemical oxygen demand (COD) has been a constant concern, since it can cause the eutrophication of water sources. To obtain the phosphorus removal, the reactors can be designed coupled to an enhanced biological phosphorus removal (EBRPs) that promotes the formation of a microbial community rich in polyphosphate accumulating organisms (PAOs). This study evaluates the efficiency of a full-scale wastewater treatment plant (WWTP) for removing organic loads and toxic compounds from sewage and the bacterial community involved. The waste treatment in the anaerobic followed by aerobic reactors, with physical/chemical treatment by using coagulant and ultrafiltration membranes was carried out. A great diversity of bacterial communities was identified. PAOs identified were responsible by phosphorus removal efficiency in the sludge. However, partial nitrification with simultaneous accumulation of NO₂⁻ in the treated effluent was observed and it could be a result of low nitrite-oxidizing bacteria (NOB) activity in the reactors evaluated.

Keywords: Full-scale domestic WWTP. Polyphosphate accumulating organisms (PAOs). Nitrification. Denitrification. Bacterial community.

1 INTRODUCTION

The environmentally friendly management of wastewater has become a challenge because of limited resources, an ever-increasing population, rapid urbanization, and industrialization. To reduce the effect of these on natural resources and mainly on water quality, different actions have been developed. The improvement of wastewater treatment plants (WWTPs) has been carried out to reduce the effect of the treated sewage released in freshwater and its implications for ecological communities.¹ The release of sewage containing toxic compounds as for example nitrogen, phosphorus, and chemical oxygen demand (COD) has been a constant concern due to the eutrophication of the water.^{2,3} Organic loads based on carbon and nitrogen may be removed by biological WWTPs with a denitrification system. In addition, to obtain the phosphorus salts removal, the reactors also have been designed coupled to an enhanced biological phosphorus removal (EBRPs) that promotes the formation of polyphosphate accumulating organisms (PAOs). The PAOs can store polyphosphate as intracellular granules that can be used as energy source by the cells, removing it efficiently from the wastewater. In many cases, an additional physical-chemical treatment is carried out followed by total phosphorous (TP) and suspended solids (TSS) removal by precipitation.^{4,5} Into anaerobic \ aerobic reactors of the WWTP, a great number of microorganisms drive the conversion of biomass and the treatment of toxic compounds of the wastewater. Different metabolic interactions among bacterial groups can be observed. This study investigated the operation of a full scale EBPR-WWTP treating domestic sewage to allow the release of the treated effluent into body of water with low flow and high sensitivity to impacts. Furthermore, the bacterial community involved in the toxic compounds and COD removal during the domestic wastewater treatment in a full-scale WWTP was evaluated from 16S rRNA gene amplification and sequencing using the next generation sequencing (NGS) technology.

2 MATERIAL & METHODS

2.1 Full-scale wastewater treatment plant (WWTP), sampling and chemical analysis

The present study evaluated the performance of a full-scale WWTP for domestic sewage treatment. The WWTP release the treated wastewater in São Bento River in São Paulo state, Brazil, with a body of water with low flow (average flow = f 44 Ls⁻¹) and high sensitivity to impacts. The WWTP has treated a maximum flow of 2.44 L s⁻¹ of domestic sewage and a maximum organic load of 45.00 kg of BOD₅₋₂₀ per day, 4.09 kg of total Kjeldahl nitrogen (TKN) per day of sanitary effluent, with a sludge production of 15.00 kg per day. The sewage and river samplings were carried out from August 2021 to June 2023, and the parameters evaluated were total nitrogen (TN), total Kjeldahl nitrogen (TKN), ammoniacal nitrogen (dissolved ammonia [NH₃] and ammonium ion [NH₄⁺]), nitrites (NO₂⁻), nitrates (NO₃⁻), biological oxygen demand (BOD₅₋₂₀), chemical oxygen demand (COD), total phosphorus (TP), pH and dissolved oxygen (DO).

2.2 Bacterial community analysis involved in the domestic sewage treatment in the WWTP

The samples were collected in triplicates into the two reactors (anaerobic and aerobic) from full-scale WWTP. The total DNA was extracted using NucleoSpin® Soil KIT (Macherey-Nagel). The partial amplification of 16S rRNA genes from Bacteria was

performed by conventional PCR using the specific primers: Bakt_341F- CCTACGGGNGGCWGCAG and Bakt_805R- GACTACHVGGGTATCTAATCC. The amplicons were used for libraries construction using the TrueSeq DNA PCR Free LT sample preparation kit (Illumina, San Diego, CA, USA) and sequenced by the DSMA company using the Illumina HiSeq (Illumina, CA, USA). The reads generated were trimmed using the "Pipeline Initial Process" using RDP Pyrosequencing Pipeline online tools (www.pyro.cme.msu.edu) considering quality coefficient > 20 (probability of 1% of error). The reads were distributed into Operational Taxonomic Units (OTUs) using MOTHUR and based on Ribosomal Database Project II database (RDP II) for phylogenetic analysis. Chao-1 method was used to richness and Shannon-Winer and Simpson methods to diversity index. The rarefaction curves and the PCA graphs were calculated using the PAST software (Download PAST 4.03 – LO4D.com). The GenBank accession number for the SRA data determined in this study is PRJNA1054602.

3 RESULTS & DISCUSSION

The WWTP operated at mesophilic conditions (35°C) with pH at ~ 6-9 over the time, with an average flow of 1.56 L s⁻¹ (90 m³ d⁻¹). The removal efficiency of BOD and COD in treated effluent ranged 98.3% to 99.4% and 81.9% to 95.3%, respectively. TN removal efficiency ranged from 72.4% to 98.8%, resulting in the ammoniacal nitrogen, NO²⁻ and NO³⁻ ranging from <0.1 to 44.8 mg/L, from 0.067 to 8.4 mg/L, from 1.6 to 11.8 mg/L, respectively. The TP level removal efficiency ranged from 38.5% to 96.2%. Finally, the DO ranged from 2.9 to 6.0 mg/L in the treated effluent. The treated effluent was released into a water body in São Bento River, which has a minimum flow of 44.0 L s. The high efficiency of sewage treatment in the full-scale WWTP was observed and organic loads based on carbon, nitrogen and phosphorus salts were removed; with exception of NO²⁻, accumulated in the effluent.

The treatment performance of WWTPs designed depends on the coordination among different functionally relevant groups of microorganisms, and its diversity is closely related to the system stability.⁶ Diversity and dynamics of bacterial communities identified in the aerobic and anaerobic reactors in the full-scale WWTP was presented and discussed to understand how phosphorus and nitrogen removal is occurring in these systems. The partial sequencing of the 16S rRNA gene from Bacteria using the Illumina MiSeq platform was performed. A high-quality sequencing was obtained with a total of 461,399 sequences which were organized into OTUs. Good's coverage of 75 % and 81 % suggested that the major bacterial diversity was detected in the anaerobic and aerobic reactors, respectively. 39 bacterial phyla were identified being *Pseudomonadota* (62.4 % of the reads in the anaerobic to 51 % in the aerobic reactors) and *Bacteroidota* (15.5% to 20.8%) covering almost 78% of the sequenced reads. The bacterial community abundance increased from the anaerobic to aerobic conditions for almost all representatives, except for those belonged to *Pseudomonadota* (62.41% to 51.04% of the reads), *Gemmatimonadota* (2.27% to 1.75%) and *Nitrospirota* (1.53% to 1.57%) phyla. The highest abundant identified genera were *Dechloromonas* sp., *Comamonas* sp., *Tolomonas* sp., *Zoogloea* sp., uncul. *Micavibrionales* (all of them affiliated to *Pseudomonadota*), *SJA-28*_ge. *Niabella* sp., uncul. *Microscillaceae* (*Bacteroidota*), uncul. *Caldilineaceae*, *ADurb.Bin180*_ge (*Chloroflexota*), *Candidatus Falkowbacteria*_ge (*Candidatus Falkowbacteria*), *Nitrospira* sp. (*Nitrospirota*) and *Gemmatimonas* sp. (*Gemmatimonadota*) (Figure 2). In both reactors PAOs reached almost 20% of the bacterial community identified in the samples, being *Dechloromonas* sp. the most abundant, followed by *Zoogloea* sp., *Candidatus Accumulibacter*, uncul. *Caldilineaceae*, uncul. *Gemmatimonadaceae* and *Gemmatimonas* sp. The abundance of PAOs increased from anaerobic to aerobic conditions. 3.0% of the reads covered genera related to nitrite reduction in both reactors, including nitrite-oxidizing bacteria (NOB) and ammonia-oxidizing bacteria (AOB).

Bacterial genera affiliated to *Pseudomonadota*, *Bacteroidota*, *Bacillota* and *Chloroflexota* were identified in high number in the sludge samples, being responsible by COD and BOD removal efficiency in the effluents.^{7,8} *Bacteroidota* and *Bacillota* include most of the known hydrolyzing bacterial species in WWTPs.⁹ *Comamonas* sp., *Tolomonas* sp. and *Dechloromonas* sp. (belonged to *Pseudomonadota*) responsible by denitrification in different WWTPs were the most abundant genera identified in the sludge samples from anaerobic and aerobic reactors.¹⁰ *Dechloromonas* sp. is a facultative anaerobic bacterium that take part of the oxidation of substrates to the reduction of NO³⁻ to either N₂O or N₂. AOB such as *Nitrosomonas* sp. and *Nitrospira* sp., were identified but, in very low number. Together with denitrification which soluble forms of nitrogen, i.e., NO⁻³ and NO⁻², are sequentially transformed into NO, N₂O and N₂ gases; nitrification removes N₂ from wastewater to control eutrophication in the receiving water body.¹¹ The nitrification take place by three ways: (i) ammonia oxidation to nitrite carried out by AOB; (ii) the complete ammonia oxidizing by comammox bacteria and (iii) ammonia-oxidizing archaea (AOA) (which was not evaluated in this study). After that, nitrite oxidation to nitrate take place by NOB. In the WWTP, NOB affiliated to phylum *Nitrospinae* were identified, being the *Nitrospira* sp., the most abundant in the anaerobic and aerobic reactors. The low number of NOB identified indicates that conditions of the treatment in the WWTP had a more pronounced inhibition effect on NOB than on AOB and denitrifiers such as *Dechloromonas* sp., resulting in the accumulation of NO²⁻ in the treated effluent.¹² This genus and *Dechloromonas* sp. also are PAOs, and with *Candidatus Accumulibacter*, they dominated the TP removal in the reactor samples.

4 CONCLUSION

The full scale WWTP designed for domestic sewage treatment presented a high performance therefore, without impact on the receiving river. *Pseudomonadota*, *Bacteroidota*, *Bacillota* and *Chloroflexota* were responsible by COD and BOD removal efficiency in the effluent. A great diversity of PAOs removed all TP in the sewage. However, a partial nitrification with simultaneous accumulation of NO²⁻ was observed and occurred due to the low number of NOB identified under WWTP operations condition.

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