Bacterial Community Structure of an IFAS-MBRs Wastewater Treatment Plant

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Abstract. In this work, the bacterial community putatively involved in BNR events of a UCT-MBMBR pilot plant was elucidated by both culture-dependent and metagenomics DNA analyses. The presence of bacterial isolates belonging to Bacillus (in the anoxic compartment) and to Acinetobacter, Stenotrophomonas, Rhodococcus, Escherichia and Aeromonas (in the aerobic compartment) is in agreement with the nitrification/denitrification processes observed in the plant. Moreover, the study of bacterial community structure by NGS revealed a microbial diversity suggesting a biochemical complexity which can be further explored and exploited to improve UCT-MBMBR plant performance.

Keywords: Bacterial communities · NGS · Biological nutrient removal · Wastewater treatment plant · Membrane bioreactors · MBBR · Enhanced biological phosphorus removal · IFAS-MBR

1 Introduction

The principal objective of domestic or industrial wastewater treatment is generally to make effluents less hazardous to human or environment health. It is well known that nutrients (particularly, nitrogen and phosphorus compounds) may have adverse environmental impacts (e.g., eutrophication, toxicity towards the aquatic organisms, etc.…) (Wang et al. 2006). Biological nutrient removal (BNR) from domestic wastewater has been extensively investigated and developed in the last years. In systems aimed at the BNR in-series anaerobic, anoxic and aerobic reactors are required (Wanner et al. 1992; Mannina et al. 2016) where nitrogen (N) and phosphorus (P) removal is accomplished by heterotrophic denitrifying bacteria and polyphosphate-accumulating organisms (PAOs), requiring a carbon source (Naessens et al. 2012). In particular, the biological phosphorus removal is usually achieved through the growth of PAOs, able to accumulate P and to store it as intracellular polyphosphate (poly-P) under alternating anaerobic/aerobic conditions (Li et al. 2013). Thus, knowledge of bacterial communities

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that take root in wastewater treatment plant is indispensable for a better understanding of the biological processes that allow the nutrient removal and perspectively for improving plant BNR performance. In this study bacterial communities of an Integrated Fixed Film Activated Sludge (IFAS) University Cape Town (UCT) membrane bioreactor (MBR) was investigated.

2 Methods

2.1 Plant Design

The IFAS-UCT-MBR pilot plant was characterized by three in-series reactors: one anaerobic (volume 62 L), one anoxic (volume 102 L) and one aerobic (volume 211 L) compartment according to the UCT scheme (Fig. 1).

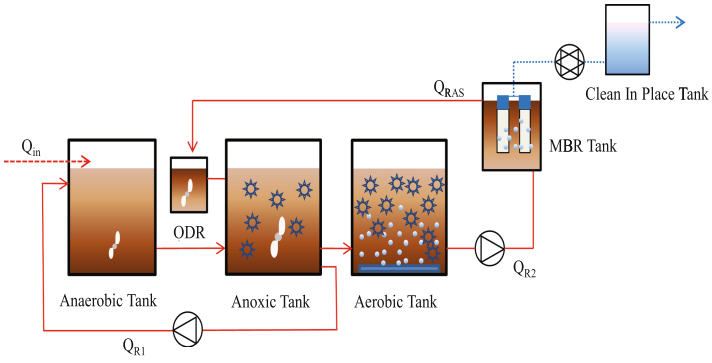

Fig. 1. Schematic lay-out of IFAS-UCT-MBR pilot plant

The IFAS-UCT-MBR pilot plant was operated for 60 days and was fed with a mixture of real wastewater (deriving from the University buildings and characterized by higher ammonia content compared to typical domestic wastewater) and synthetic wastewater. In particular, MBBR processes rely on the use of small plastic carriers elements (courtesy of amitec Co. Ltd., carriers density = 0.95 g cm^3) that are kept in constant motion throughout the entire volume of the reactor, for biofilm growth. The plastic carriers were a 15 and 40% filling fraction, corresponding to a net surface area of 75 and 200 m^2 m^{-3} 120 in the anoxic and aerobic reactor, respectively.

2.2 Identification of Bacterial Isolates

Wastewater aliquots of 100 µL r from the aerobic and anoxic tanks were collected, serially diluted and, then, plated on Luria Bertami (LB), Mannitol Soya flour (MS) and R2YE agar-media (Kieser et al. 2000). The plates were incubated at 30°C until appearance of microbial colonies (2–5 days). The bacterial colonies, selected on the basis of pigmentation and morphology, were repeatedly plated on agar-media to obtain

pure cultures. The bacterial isolates were characterized on the base of their 16S rDNA sequence using the universal bacterial primers 27F and 1492R (Frank et al. 2008) for 16S rDNA amplification by colony PCR as previously described (Gallo et al. 2012; Milanesi et al. 2015). The PCR products were purified by using NucleoSpin Gel and PCR Clean-up (MACHERY-NAGEL, Germany). Sequencing was performed by BMR Genomics srl. Phylogenetic relationships to known species were inferred by neighbour–joining, using the software Mega6 (Tamura et al. 2013).

2.3 Metagenomic Analysis Based on 16S rDNA Sequencing

Metagenomic DNA was extracted from 50 mL aliquots of wastewater from aerobic, anoxic and anaerobic compartment, respectively. In addition, two colonized carriers from aerobic and anoxic compartments were used to obtain Metagenomic DNA. Since CTAB performed better in reducing humic contamination, it was used in the buffer for sodium dodecyl sulfate (SDS)-based DNA extraction. The metagenomic analysis was performed through new generation sequencing (NGS) by Illumina platform (BMR Genomics srl).

3 Results and Discussion

After 2–5 days of incubation, bacterial colonies appeared on the surface of agar-medium plates inoculated with serially diluted wastewater aliquots. The colonies showed different phenotypes: most had a white translucent pigmentation, someone white with a matt pigmentation and one showed orange pigmentation. A total of 14 isolates (8 and 6 from aerobic and anoxic tank, respectively) were selected and obtained as pure cultures. From all the 14 isolates, a 16S rDNA sequence was obtained to carry out phylogenetic analysis. As all the isolates from anoxic tank belonged to *Bacillus* genus, the isolates from aerobic tank belonged to five different bacterial genera: *Acinetobacter* (2 isolates), *Stenotrophomonas* (2 isolates), *Rhodococcus* (2 isolates), *Escherichia* (1 isolates) and *Aeromonas* (1 isolates). Interestingly, they are all implied in nitrification/denitrification processes (Di Bonaventura et al. 2004).

The 16S rDNA-based metagenomic analysis revealed 12 (Operational taxonomic unit) OTU. In general, the bacterial community composition of anaerobic, anoxic and aerobic compartments are quite similar; indeed, the most relevant differences are quantitatively observed for the less represented OTUs. In particular, the most representative OTUs were: Saprospiraceae (27.30%), Rhodocyclaceae (26.61%), Sphingobacteriales (12.96%) in aerobic compartment (Fig. 2a); Rhodocyclaceae (22.69%), Saprospiraceae (19.99%), and Sphingobacteriales (10.54%) in anoxic compartment (Fig. 2b); Rhodocyclaceae (21.60%), Saprospiraceae (19.20%), and Sphingobacteriales (10.29%) in anaerobic compartment (Fig. 2e). Concerning the colonized carriers, the bacterial community composition almost paralleled that one of the corresponding compartment, with Saprospiraceae and Rhodocyclaceae the most abundant OTUs in both cases. However, the less abundant OTUs Rhodanobacter, in aerobic and anoxic conditions, and Thermomonas and Clostridium sensu strictu, in aerobic condition, were observed only in the carries.

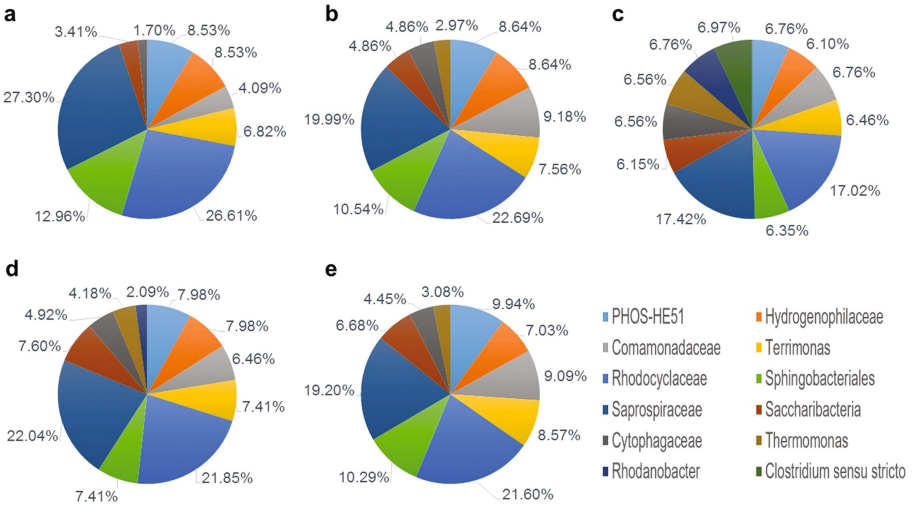

Fig. 2. Bacterial OTU relative abundance in (a) aerobic tank, (b) anoxic tank, (c) aerobic tank carriers, (d) anoxic tank carriers (e) anaerobic tank

4 Conclusions

The bacterial community putatively involved in BNR events of a IFAS-UCT-MBR pilot plant was elucidated by both culture-dependent and metagenomics DNA analyses. The presence of bacterial isolates in the anoxic and aerobic compartments is in agreement with the nitrification/denitrification processes observed in the plant (Mannina et al. unpublished results). Moreover, the study of microbial community structure by NGS revealed a microbial diversity and suggests a biochemical complexity which has to be further explored and exploited to improve IFAS-UCT-MBR plant performance.

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References

Di Bonaventura G, Spedicato G, D'Antonio D, Robuffo I, Piccolomini R (2004) Biofilm formation by *Stenotrophomonas maltophilia:* modulation by Quinolones, Trimethoprim-Sulfamethoxazole, and Ceftazidime. Antimicrob Agents Chemother 48(8):151–160

Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ (2008) Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol 74:2461–2470

Gallo G, Baldi F, Renzone G, Gallo M, Cordaro A, Scaloni A, Puglia AM (2012) Adaptative biochemical pathways and regulatory networks in Klebsiella oxytoca BAS-10 producing a biotechnologically relevant exopolysaccharide during Fe (III)-citrate fermentation. Microb Cell Fact 11:152

Kieser Y, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical Streptomyces genetics. The John Innes Foundation, Norwich

Li C, Wang T, Zheng N, Zhang J, Ngo HH, Guo W, Liang S (2013) Influence of organic shock loads on the production of N2O in denitrifying phosphorus removal process. Bioresour Technol 141:160–166

Mannina G, Capodici M, Cosenza A, Di Trapani D (2016) Carbon and nutrient biological removal in a University of Cape Town membrane bioreactor: analysis of a pilot plant operated under two different C/N ratios. Chem Eng J 296:289–299

Milanesi C, Cresti M, Costantini L, Gallo M, Gallo G, Crognale S, Faleri C, Gradi A, Baldi F (2015) Spoilage of oat bran by sporogenic microorganisms revived from soil buried 4000 years ago in Iranian archaeological site. Int Biodeter Biodegr 104:83–91

Naessens W, Maere T, Nopens I (2012) Critical review of membrane bioreactor models Part 1: biokinetic and filtration models. Bioresour Technol 122:95–106

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Wang XJ, Xia SQ, Chen L, Zhao JF, Renault NJ, Chovelon JM (2006) Nutrients removal from municipal wastewater by chemical precipitation in a moving bed biofilm reactor. Process Biochem 41(4):824–828

Wanner J, Cech JS, Kos M (1992) New process design for biological nutrient removal. Water Sci Technol 25(4–5):445–448