



STUDY OF THE ACTIVE BACTERIAL COMMUNITY IN TWO MEMBRANE BIOREACTORS



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INTRODUCTION

Membrane bioreactor (MBR) systems allow for the separation of mixed liquor and treated water thanks to membranes. Bacterial communities in MBRs can be quite different from conventional ones due to the long retention time and, therefore, the low metabolic cellular activity. Moreover, MBRs have better treated water quality, are able to operate with high biomass concentrations and their effluent is of great quality and can be recirculated. An extensive research on the bacterial communities in these systems is essential to achieve a good treatment performance.

In this work, bacterial communities from two MBR systems treating leachates were evaluated using the 16S rRNA metagenomics approach, with and without a viability dye (Propidium Monoazide, PMA).

MATERIAL AND METHODS

Two MBR systems (ECO1 and ECO2) were sampled at two different sampling times without and with the addition of PMA to detect active bacteria. PMA was added at a final concentration of 50 μ M, incubated under darkness for 10 min and exposed to blue LED light for 15 minutes. DNA was isolated using FastPrep[®]-24 cell disrupter and prepared for Illumina MiSeq sequencing. PRO341F and PRO805R primers (1) were used to target V3-V4 regions of 16S rDNA for Bacteria and Archaea. Sequenced data was analysed using Qiime 1.8.0 pipeline (2), where they were normalized and assigned taxonomy using Greengenes database clustered at 97% identity. Amplicons obtained from the 8 samples generated 63637 sequences

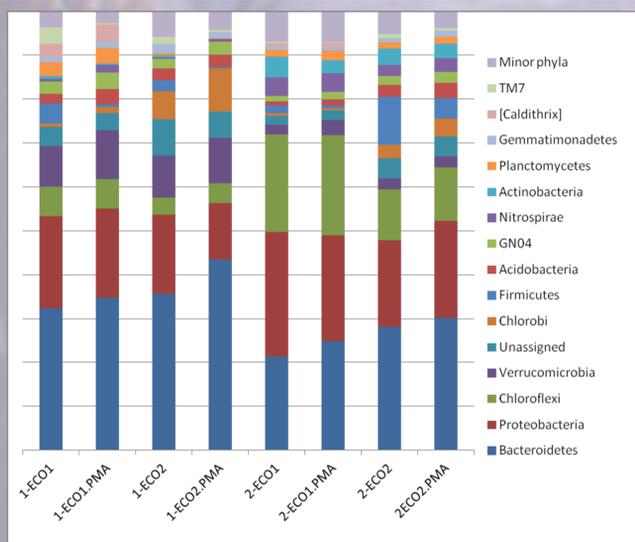
RESULTS

Amplicons obtained from the 8 samples generated a total of 63637 sequences. α -diversity revealed highly diverse microbial communities (41 bacterial and 1 archaeal phyla detected). Generally, the most abundant phyla were *Bacteroidetes*, *Proteobacteria* and *Chloroflexi*, which represented 63.40% of the total population (A).

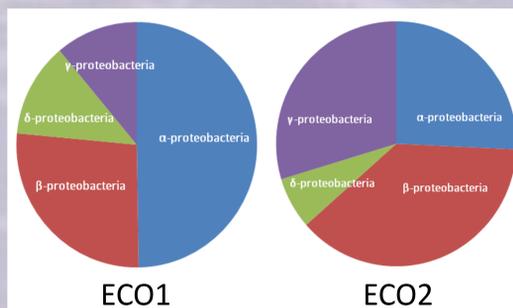
Proteobacteria phyla was 20.8% of the total population (α -proteobacteria: 8.5%, β -proteobacteria: 5.9%, δ -proteobacteria: 2.0% and γ -proteobacteria: 3.8%). The most abundant classes of proteobacteria in the MBR systems (ECO 1 and ECO2) were α and β proteobacteria, showing different distributions (B).

As expected, PMA treatment resulted in a reduction of the total observed species in the rarefaction curves, revealing the potentially active microorganisms (C). *Firmicutes* was the phylum most affected by PMA, thus revealing that many of these bacteria were present but not active in these systems (D).

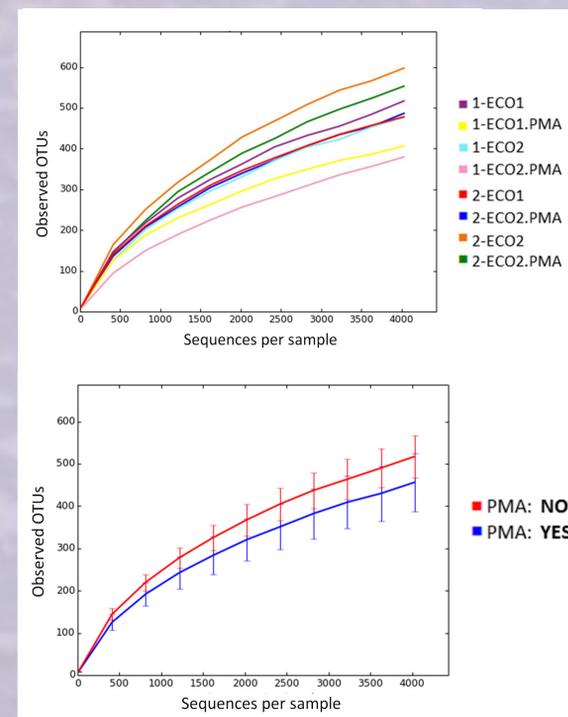
(A) General taxonomic composition at phyla level:



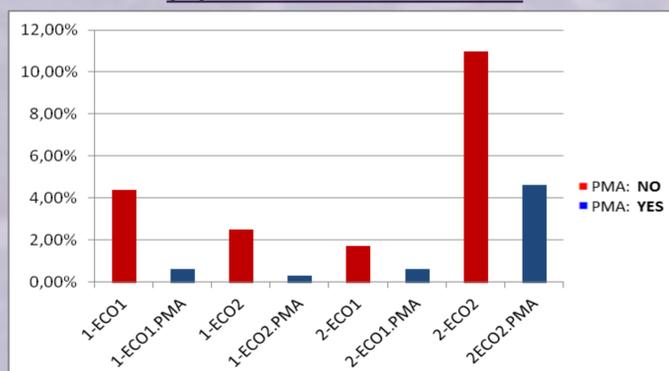
(B) Proteobacteria classes distribution:



(C): Rarefaction curves



(D) Firmicutes abundance :



References

- (1) Takahashi, S., Tomita, J., Nishika, K., Hisada, T., Nishijima, M. 2014. Development of a Prokaryotic Universal Primer for Simultaneous Analysis of Bacteria and Archaea Using Next-Generation Sequencing PLoS One 9: doi: 10.1371/journal.pone.0105592
- (2) Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., et al. 2010 QIIME allows analysis of high-throughput community sequencing data. Nat Methods 5: 335-336