Comparison of nitrifying microbial communities of two full-scale membrane bioreactors treating wastewaters from municipal solid waste using 16S rDNA gene amplicon squencing

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Introduction

In the last years, biological treatment plants for the previously separated organic fraction from municipal solid wastes (OFMSW) have gained importance. In these processes a liquid effluent (liquid fraction from the digestate and leachate from composting piles), which has to be treated previously to its discharge, is produced. Landfill leachate is a complex composition containing high levels of ammonia nitrogen. Membrane bioreactor systems (MBRs) are used for this type of complex wastewater treatment, because of their capacity of working at high concentrations levels of suspended solids and high cell retention times. The operation of two full-scale MBRs treating effluents from two OFMSW plants is studied from the point of view of nitrifying bacteria characterization. Comparison is carried out in order to find out the differences in the MBR mixed liquors caused by the different type of process carried out in the OFMSW. Both plants consist of anaerobic digestion (AD) plus composting processes. However, in one plant AD is carried out with a solid concentration higher than 15% (so-called Dry-process) (MBR-HS, MBR1) and, in the other one, the process is carried out with solids at a concentration lower than 10% (Wet-process) (MBR-LS, MBR2) (Table 1).

Table 1. Physico-chemical characteristics of influent wastewaters to MBR1 and MBR2 (n=6).

Parameters	MBR1	MBR2
pH	7,99 ± 0.1	7.99 ± 0.11
Conductivity (mS/cm)	16.26 ± 6.97	40.13 ± 4.65
SS (mg/L)	4401 ± 1812	12940 ± 1301
tCOD (mg/L)	9430 ± 5944	32910 ± 7106
sCOD (mg/L)	3140 ± 1262	20927 ± 7635
TN (mg/L)	1879 ± 990	6267 ± 2666
sTN (mg/L)	790 ± 320	4890 ± 406
NH4-N (mg/L)	767 ± 324	3990 ± 410

Material & Methods

MBR systems and sampling: Both plants consist of anaerobic digestion plus composting processes. Membranes are multichannel tubular and the installed active surface is 127 m2 and 72 m2 in MBR-LS and MBR-HS, respectively. The biological reactors were operated sequentially with external membrane configuration (Figure 1).

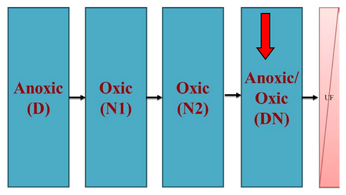

Figure 1. General features of membrane bioreactor systems MBR1 and MBR2. Rectangles refer to the reactors. Red arrow indicates sanpling point for NGS analysis. UF: ultraflitration external membrane.

Biological reactor consists of one anoxic tank, two aerobic tanks and a final tank that can be operated aerobically or anoxically depending of the nitrogen removal efficiencies. Therefore, both plants were designed to eliminate both organic matter and nitrogen. Fifteen samples were taken from the anoxic/oxic reactor (DN) for a year.

DNA extraction and PCR-based Illumina sequencing: Total DNA of 1 ml activated sludge sample was extracted in duplicate. Lysis was performed with the FastPrep® -24 instrument at 6 m/sec for 40 sec (twice) and the DNA was extracted using the FastDNA® SPIN kit for soil (MP Biomedicals) according to the manufacturer's instructions. OneStep™ PCR Inhibitor Removal Kit (Zymo Research) was used in order to remove sample inhibitors. For Illumina amplicon sequencing of the hypervariable V3–V4 region of bacterial 16S rRNA gene, the primers PRO341F and PRO805R were used (Takahashi et al., 2014) .

Bioinformatics analysis: Raw Illumina sequences were analysed using Quantitative Insights Into Microbial Ecology (QIIME™ http://qiime.org/) software package version 1.8.0. Forward and reverse reads were joined. Joined reads were ckecked for chimeras using Usearch61 algorithm against 16S SILVA_123 database (Quast et al., 2013). Remaining sequences were clustered at 97% similarity into Operational Taxonomic Units (OTUs) using the denovoOTU clustering script. The most abundant sequence of each OTU was picked as its representative, which was used for taxonomic assignment against 16S SILVA_123 database at 97% identity (cut-off level of 3%) using default parameters.

Multivariate analysis: Hierarchical cluster analysis was used to evaluate the spatial variability of nitrifying bacterial communities by examining the relative distances among samples in the ordination (abundance square-root transformed data; Bray-Curtis similarity; group-average linking). To assess the contribution of the environmental variables to the variability observed in the nitrifying bacteria community structure, we carried out distance-based linear models (DISTLM), using parsimonious methods (e.g. BIC, AICC). Environmental variables were log-transformed and normalized to eliminate their physical units, prior to multivariate data analyses (euclidean similarity). Distance-based redundancy analysis (dbRDA) was used to visualize the DISTLM. All multivariate analyses were performed with PRIMER v7 (Clarke & Gorley, 2015) with PERMANOVA+ (Anderson et al., 2008).

Results & Discussion

TAOB and NOB OTUs identified in DN reactors of MBR1 and MBR2 plants are indicated in Table 2. Nitrification, the oxidation of ammonia to nitrite and its subsequent oxidation to nitrate, are performed by two functional groups. ammonia oxidizers bacteria (AOB) and nitrite oxidizers bacteria (NOB). The AOB form two monophyletic groups, one within the beta- (*Nitrosomonadaceae*) and one within the gamma-proteobacteria (*Nitrosococcus*). *Nitrosomonadaceae* contains two genera, *Nitrosomonas and Nitrosospira* (Prosser et al. 2014).

Table 1. AOB and NOB OTUs identified in a MBR1 and MBR2

Ammonia oxidizers	MBR1	MBR2
Nitrosomonas	28	20
Nitrosomonas	74	908
Nitrosomonas	92	1115
Nitrosomonas	10	1963
Nitrosomonas	102	-
Nitrosomonas	203	-
Nitrosomonadaceae	103	349
Nitrosomonadaceae	112	409
Nitrosomonadaceae	339	1004
Nitrosomonadaceae	419	1059
Nitrosococcus mobilis	3608	-
Nitrosococcus oceani	6040	18
Nitrosococcus oceani	-	2001
Nitrosococcus oceani	-	3200
Nitrosococcus halophilus	-	1938
Nitrosococcus halophilus	-	2123

Nitrite oxidizers	MBR1	MBR2
Nitrospira	14	13
Nitrospira	225	1423
Nitrospira	-	2964
Nitrolancea	499	205
Nitrolancea	511	308
Nitrolancea	2789	486
Nitrolancea	-	510

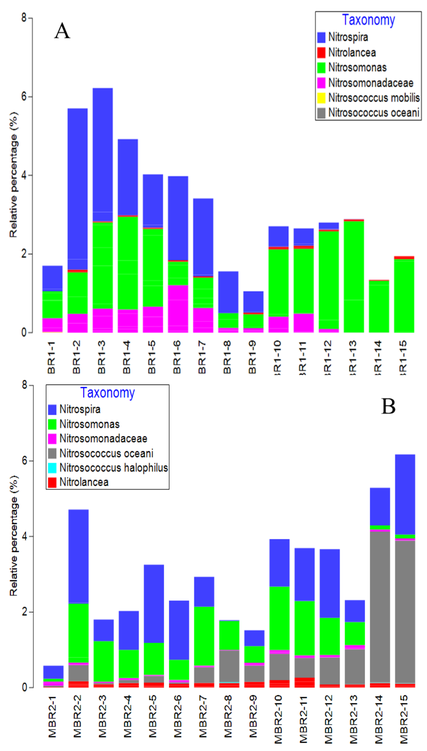

Figure 2. The relative abundance of AOB and NOB in MBR1 (A) and MBR2 (B).

The two MBRs had different nitrifying microbial community compositions. In MBR1 which treats the influent with low solids, the ammonia oxidizing community was predominated by the genus *Nitrosomonas* and the genus *Nitrosococcus* was present in very low abundances (Fig. 2A). In MBR2, which treats influent with high solids, the genus *Nitrosomonas* predominated at the beginning of sampling period with a decline at the end (Fig. 2B). However the relative abundance of *Nitrosococcus oceani* followed an evolution inverse to *Nitrosomonas* (Fig. 2B). In figures 2A and 2B, it can be seen the evolution of NOB during the sampling period. The *Nitrospira* relative abundance follows an evolution inverse to AOB in MBR1, with a higher concentration at the beginning of sampling period and a decline from the M7 sample (Figure 2A). The *Nitrospira* abundance varies throughout the period of sampling in MBR2 DN reactor (figure 3B). The *Nitrospira* OTU14 and OTU225 follows different relative abundance evolution through the period of sampling in MBR1 (Fig. 3).

With few *Nitrospira* OTUs being highly abundant at a particular time, a large diversity of less abundant *Nitrospira* may act as seed bank for compensatory growth after disturbances such as changes in the wastewater compositon in a wastewater treatment plant (WTP) (Gruber-Dorninger et al. 2015). *Nitrolancea*, a chemolitthoautotrophic nitrite-oxidizer, thermotolerant, with high nitrite tolerance (Sorokin et al. 2014) was detected in low relative abundance in activated sludge samples of DN reactors in MBR1 and MBR2 plants (Fig. 2).

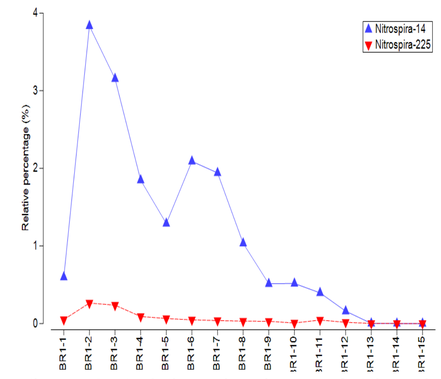

Figure 3. Relative abundance of *Nitrospira* OTUs 14 and 225 in MBR1.

The temperature range of DN reactors of MBR1 and MBR2 plants was 28 to 36 ºC and 32 to 38 ºc, respectively. The MBR2 plant, which uses high solids anaerobic digestion, generates effluents with higher SS and conductivity that the plant with low solids anaerobic digestion (MBR1) (Zuriaga-Agustí et al. 2016). Biomass of MBR2 is subjected to more stress than biomass of MBR1 due to high non-biodegradable suspended solids concentration and salinity (Zuriaga-Agustí et al. 2016).

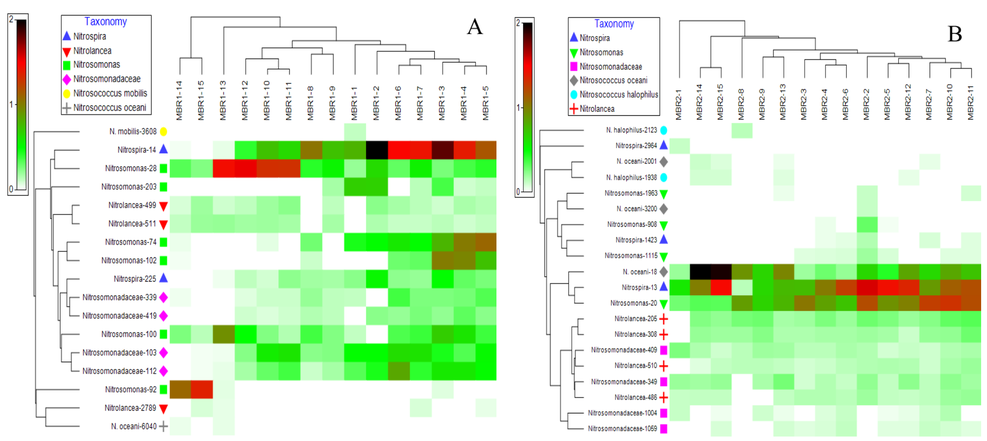

Figure 4. Shade plot illustrating the realative abundance (square root transformation) of nitrifying bacteria (OTUs clustering gives y-axis ordering and samples clustering gives x-axis ordering). A) MBR1 and B) MBR2.

We investigated models of environmental interpretation of nitrifying variables using of distance-based linear models (DISTLM). Figure 4 shows the nitrifying bacteria and samples in different clustering. The samples of MBR1 (Fig 4A) and MBR2 (Fig. 4B) were associated with nitrifying bacteria OTUs relative abundance. Distance-based redundancy (dbRDA) bubble plot illustrating the DISTLM based on the relationship between dissolved COD (sCOD) and the nitrifying bacteria community structure (MBR1) is showed in figure 5A, and the relationship between volatile suspend solids (VSS) and the nitrifying bacteria community structure (MBR2) is represented in Fig 5B. Distance-based redundancy (dbRDA) bubble plot illustrating the DISTLM based on the relationship between nitrite (NO2-N) and nitrifying bacteria community structure (MBR1), and the nitrate (NO3-N) and nitrifying bacteria community structure (MBR2) are showed in Fig 6A and 6B, respectively. The percentage of fitted indicates the variability in the original data explained by the fitted model and the percentage of total variation indicates the variation in the fitted matrix. The length and direction of the vectors represent the strength and direction of the relationship.

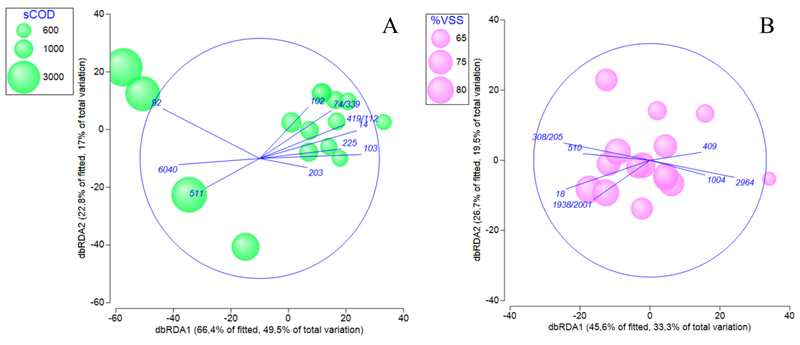

Figure 5. A) dbRDA bubble plot illustrating the DISTLM based on the relationship between dissolved COD (sCOD) and the nitrifying bacteria community structure (MBR1), B) and the relationhip between volatile suspend solids (VSS) and the nitrifying bacteria community structure (MBR2).

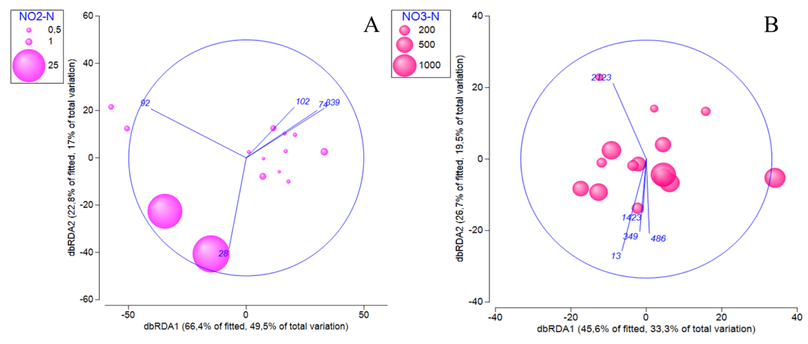

Figure 6. A) dbRDA bubble plot illustrating the DISTLM based on the relationship between nitrite (NO2-N) and nitrifying bacteria community structure (MBR1), B) and the relationship between the nitrate (NO3-N) and nitrifying bacteria community structure (MBR2).

Conclusions

The construction of models allows associating the nitrifying bacteria to environmental ranges, obtaining valuable information to the knowledge of these dynamic populations. This has allowed to carried out an ecological interpretation of the processes that take place in the biological reactors

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