

Characterization of eubacterial communities by DGGE and Next Generation Sequencing (NGS) of nitrification bioreactors using young and intermediate leachates as ammonium substrate.

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Landfilling is the most common alternative to dispose waste. Pollution of ground water caused by unmanaged landfills represents significant health and environmental risks. The main cause of this type of pollution is derived from landfill leachate infiltrations. Landfill leachates are the liquids that results from water percolation through the waste deposits and they are mainly characterized by high concentrations of ammonium and organic matter as well as toxic metals and chlorinated salts. Biological treatment of these aqueous effluents is preferred over physico-chemical methods (Show et al., 2019). A commonly accepted indicator to determine and classify the biodegradability of a landfill leachate is the ratio of biological oxygen demand (BOD₅) to chemical oxygen demand (COD). This BOD₅/COD ratio mainly depends on landfill age and can vary from 0.4 in young (< 5 years old) to 0.1 in old leachates (>10 years old). During biological treatment, ammonium is removed from landfill leachates by nitrification which is carried out by ammonium-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). These bacterial communities are highly sensible to environmental factors changes such as salinity, pH, temperature or dissolved oxygen (Yao et al., 2017). Therefore, it is essential to characterize the bacterial communities present during landfill leachate treatment in order to understand the chemical transformations occurred during the process and optimize their operational conditions. Several molecular techniques have been traditionally employed to describe microbial communities in wastewater treatment such as denaturing gradient gel electrophoresis (DGGE) and sequencing of clone libraries (Dong et al., 2010). However, due to the limited information provided by these techniques, new methods such as Next Generation Sequencing (NGS) based on 16r RNA gene region sequencing have been developed. NGS allows to obtain a higher resolution in terms of taxonomy relative abundances of operational taxonomic units (OTUs) compared to DGGE or sequencing of clone libraries. To the best of our knowledge the study of the microbial community composition changes taking place during its acclimatization to two landfill leachates with different ages, have not been carried out so far.

Therefore, the aim of the present work was to elucidate the effect of supplying three different ammonia sources (synthetic wastewater (SWW) using NH₄Cl as ammonium source, young landfill leachate (YLL) with a BOD₅/COD ratio = 0.3 and intermediate landfill leachate (ILL) with a BOD₅/COD ratio = 0.2) on the microbial community composition of a nitrifying consortium. Consequently, three different nitrifying consortiums were generated: (i) Biomass adapted to synthetic wastewater (X_{SWW}); (ii) Biomass adapted to young leachate (X_{YLL}) and (iii) Biomass adapted to intermediate leachate (X_{ILL}).

The acclimatization of these biomasses were carried out in three different bioreactors. Firstly, X_{SWW} was obtained from a continuous stirred tank reactor (CSTR) of 5 L (*Applikon Biotechnology BV, The Netherlands*) equipped with a settler (1.4 L) for biomass recirculation. After 1 year of working operation maintained at room temperature of 24 ± 2°C, pH of 7.8 and DO > 2 mg O₂ L⁻¹, the enriched nitrifying biomass X_{SWW} was inoculated into two different 1 L SBRs (*Infors HT, Switzerland*), in which YLL and ILL were progressively and separately fed to each of them. After three months of acclimatization at the same operational conditions (24 ± 2°C, pH = 7.8), the microbial community of the three different bacterial populations (one from the 5 L CSTR (X_{SWW}), and two from the 1 L SBRs (X_{YLL} and X_{ILL})) were characterized.

The bacterial profiles identification of the three different acclimatized bacterial populations was assessed by analysing the microbial population profiles by 16S rDNA-DGGE and NGS. Firstly, the biological samples were processed for total genomic DNA extraction by using UltraClean™ Soil DNA Isolation Kit (*MoBio Laboratories Inc., USA*). The total extracted DNA was used for amplification of the region V3–V5 of 16S rDNA gen by PCR using the universal primer forward 338 and primer reverse 907 with a clamp of GC region. 1 µg of each product was loaded onto DGGE using the DCode™ system (*Bio-rad, USA*). The similarities among the microbial populations along with the diversity and dominance indexes were calculated from DGGE profiles. In order to further study the microbial communities, X_{SWW} and X_{YLL} biomass were analysed by NGS of V3-V4 region of the

16S rDNA V3–V4 PCR product as input material in STAB Vida (*Portugal*). Resulting reads were compared with a database and organized in features so called OTUs. The number of reads of each OTU is represented as relative abundance with the total of reads being 100%.

Fig. 1a shows the DGGE profiles based on the bacterial V3-V5 region of the 16S rDNA of the three different bacterial communities studied (X_{SWW} , X_{YLL} and X_{ILL}). By banding profile analysis, it is noticeable that the bacterial diversity was sharply reduced when the nitrifying biomass was fed with landfill leachate. While the first lane showed an apparently higher diversity of species, the second and the third lane, which correspond to biomass fed with YLL and ILL, respectively, a lower amount of OTUs is observed. In Fig. 1a, black arrows highlight the bands which are common in the three consortiums, indicating their strength to survive to the different ammonia sources tested. Orange arrows indicate the new species that appeared when leachates were fed, suggesting that its metabolism is related with the organic matter present in both landfill leachates. Therefore, it can be concluded that landfill leachate feeding lead to the loss of several OTUs and appearance of new ones.

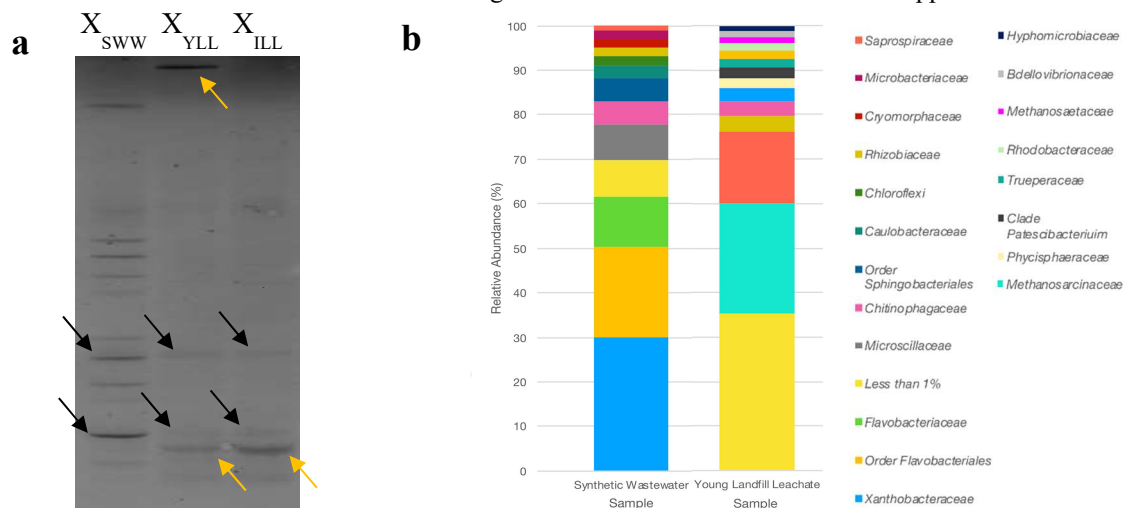


Figure 1. (a) DGGE analysis of 16S rDNA PCR of eubacterial communities. (b) Relative abundances at family level of biomass adapted to synthetic wastewater and young landfill leachate.

To improve the characterization of these microbial consortiums, NGS analysis was then carried out. Due to the similarities of X_{YLL} and X_{ILL} DGGE band profiles, NGS analysis was performed only in X_{SWW} and X_{YLL} samples. NGS results (Fig. 1b) showed that the family Xanthobacteraceae scored the highest relative abundance (30.1%) followed by Flavobacteriales order (20.2%) and Microscillaceae family in sample X_{SWW} all related to nitrogen fixation and metabolism. These data correlate well with those reported by Díaz et al. (2019) in which Proteobacteria and Bacteroidetes phyla achieved 51% and 8% of total relative abundance in raw leachate, respectively. In X_{YLL} , the OTU *Methanosarcina* sp. RPS13 corresponding to the family Methanosarcinaceae recorded with the highest percentage of counts (24.8%) followed by the family Saprospiraceae (14.1%), both related to the breakdown of complex organic compounds and volatile fatty acids conversion into methane. The most abundant families related to the nitrogen metabolism were Rhizobiaceae (2.8%) and Xanthobacteraceae (2.6%) highlighting the great selective pressure applied by leachate feeding. Also, it is important to note that the amount OTUs present in X_{YLL} whose relative abundances are lower than 1% is almost 5-folds higher, revealing the enormous diversity of bacteria present in the microbial consortium but with very low relevance.

In conclusion, according to the results obtained, DGGE-PCR technique allowed to identify significant differences between biomasses fed profiles with SWW and different landfill leachates. Furthermore, NGS analysis revealed that substrate feeding change from SWW to YLL greatly affected the bacterial consortium composition, allowing to gain insights into biological treatment of landfill leachate.

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