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Short communication

Microbial community dynamics in a submerged fixed bed bioreactor during biological treatment of saline urban wastewater

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ABSTRACT

The influence of salt (NaCl) on bacterial and archaeal communities in a submerged fixed bed bioreactor system for the treatment of urban wastewater was determined by DGGE and 454 pyrosequencing of PCR-amplified 16S ribosomal RNA gene fragments. Cluster analysis of DGGE fingerprints showed significant differences in the community structure dependent on the salt concentration in the influent. *Proteobacteria* was found to be the dominant bacterial phylum in all experiments, with *α-Proteobacteria* being the main order at low salinity and *γ-Proteobacteria* the dominant order at high salinity. *Euryarchaeota* was the main archaeal phylum in all experiments, with all microorganisms corresponding to methanogenic archaea. Whereas bacterial α-diversity decreased as salinity increased, archaeal α-diversity increased with higher NaCl concentrations.

1. Introduction

The performance of submerged fixed bed bioreactors in biological wastewater treatment plants depends on the activity of complex microbial communities within the biofilm (Fernández et al., 2008; Yang et al., 2011). Clear links have been reported between community structure and nitrification, denitrification, and phosphorus removal efficiency (Yang et al., 2011; Cheikh et al., 2013). The biofilm composition strongly depends on the type of wastewater as well as reactor design and operating conditions.

In recent decades, the inflow of saline and hypersaline wastewater to treatment plants has increased considerably, represent as much as 5% of the worldwide wastewater treatment streams (Lefebvre et al., 2007). Biodegradation of pollutants in saline wastewaters is often severely impeded due to the toxic effect of the high salt concentration on the biomass. High concentrations of NaCl cause cell plasmolysis due to a dramatic increase in osmotic pressure and result in changes of microbial metabolism, in organisms not previously adapted to saline conditions (Gregory et al., 2010; Cortés-Lorenzo et al., 2012). In this context, salinity has been found to be a major factor affecting the microbial composition across many different habitats (Mohamed and Martiny, 2011).

Knowledge on the composition of microbial communities involved in wastewater treatment processes and the impact of saline wastewater on community structure are regarded as crucially important for the optimization of nutrient removal rates in submerged fixed bed bioreactor systems (SFBBR) and to implement control strategies (Ciesielski et al., 2010). Furthermore, identifying the microorganisms responsible for specific biotransformation processes in complex environments remains one of the major challenges in environmental microbiology and environmental engineering (de los Reyes, 2010; González-Martínez et al., 2014). The aim of this work was to identify the impact of wastewater salinity on the structure of bacterial and archaeal communities in biofilms in SFBBRs. For this purpose, a nested approach using DGGE fingerprinting and 454 pyrosequencing of PCR-amplified 16S rRNA gene fragments was used to characterize the microbial community composition in SFBBRs subjected to urban wastewater at four different NaCl concentrations.

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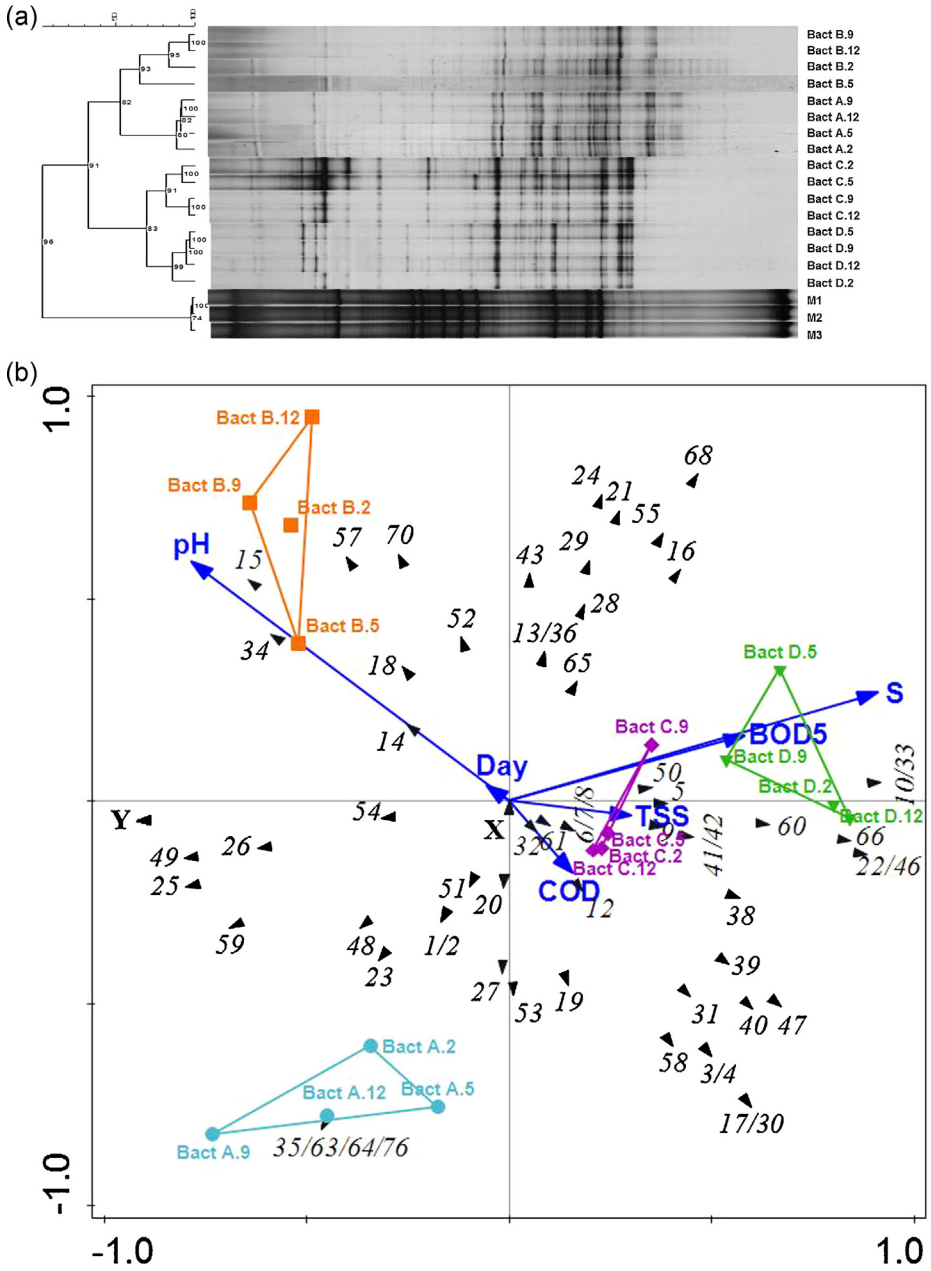

Fig. 1. (a) Evolution of the bacterial community in the submerged fixed bed bioreactor analyzed by DGGE profiling. Pearson coefficient-based analysis of the band patterns generated from all biofilm samples analyzed. Numbers in nodes represent the cophenetic correlation coefficient values. M: ten-species marker (Wako Chemicals GmbH, Germany). (b) Multivariate statistical analysis of bacterial community composition in relation to physicochemical parameters in the influents (COD: chemical oxygen demand; BOD5: biological oxygen demand; pH; TSS: total suspended solids; S: salinity measured as conductivity; sampling day: sampling day of biofilm) during operation of the submerged fixed biofilm reactor (SFBBR) in experiments E0, E3.7, E24.1 and E44.1. First axis is horizontal, second axis is vertical. Redundancy analysis (RDA) ordination diagram (triplot) showing operational parameters (straight arrows); biofilm samples (circles for experiment E0, squares for experiment E3.7, diamonds for experiment E24.1 and down triangles for experiment E44.1) and bacterial bands data (dotted triangles). X: position of bands 11/37/44/45/56/62/67; Y: position of bands 69/71/72/73/74/75/77/78/79/80/81/82.

2. Materials and methods

2.1. Reactor operation and biofilm sampling

The pilot-scale SFBBR used for these experiments and the operational parameters were described previously (Cortés-Lorenzo et al., 2012). The influent urban wastewater was supplemented with salt (NaCl) concentrations of 0, 3.7, 24.1 and 44.1 g/L, according to Cortés-Lorenzo et al. (2012). These conditions (in the following referred to as experiment E0, E3.7, E24.1 and E44.1) were maintained for 45 days each, divided in 3 cycles of 15 days due to clogging of the biofilter. The average values of biological oxygen demand at 5 days (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS) and pH measured in the influents of the four experiments are presented in Supplementary Table S1. Biofilm samples were collected as previously described (Cortés-Lorenzo et al., 2012) at days 2, 5, 9 and 12 of the last of the three cycles of every experiment to assess their biodiversity. Samples from experiment E0 were named A.2, A.5, A.9 and A.12, and accordingly for the other three experiments.