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The influence of reactor staging on microbial structure and functions in pre-denitrifying MBBRs

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Introduction

A number of engineering solutions are currently being explored to improve the performance of biological wastewater treatment in terms of conventional and xenobiotic trace pollutant removal. Moving bed biofilm reactors (MBBRs) have recently emerged as a promising option to enhance the elimination of pharmaceuticals under aerobic conditions, as compared to suspended growth systems (Falås et al., 2012). Staging of biological reactors—activated sludge (Plósz, 2007) and biofilm reactors (Plósz and Vogelsang, 2012)—was also hypothesized to optimize pollutant removal processes based on reaction kinetics principles. In staged biofilm systems with enhanced physical retention of biomass, microbial communities in each sub-reactor are likely shaped via exposure to tiered organic substrate availability (in terms of both accessible concentration and degradability). However, it is unclear how this would influence functions performed by microbial communities. In this study, we investigated microbial functions (heterotrophic denitrification, pharmaceutical removal) and community structure in a three-stage pre-denitrifying MBBR, using a single-stage system as reference. Through enhanced biomass retention in biofilm carriers and long-term operation under similar feeding conditions, prolonged biofilm exposure to specific COD availability conditions could be achieved in each MBBR. The objective of our investigation was thus to assess the impact of tiered substrate availability on: (i) nitrogen oxide ($NO_3 + NO_2$) reduction (primary functions) and removal of pharmaceutical residues (secondary functions); and (ii) microbial structure in pre-denitrifying MBBRs.

Material and Methods

Two MBBR systems in single-stage (U) and three-stage (S1+S2+S3) configuration were operated in parallel for ~500 days under continuous-flow conditions, receiving the same influent wastewater (pre-clarified sewage with nitrate dosing at influent concentration 103 mgN L^{-1}, no spiking of pharmaceuticals). The systems were designed to operate at the same working volume (6 L) and hydraulic residence time (8.9 h). A set of batch experiments (48 h) using biofilm grown in S1, S2, S3 and U was performed at day 471 to assess heterotrophic denitrification and pharmaceutical removal in each MBBR. Samples were analyzed using spectrophotometry and HPLC-MS/MS (Escolà Casas et al., 2015) to quantify concentrations of bulk pollutants (COD, NO_3, NO_2) and pharmaceuticals, respectively. The specific denitrification potential of MBBR biofilm (DNP, mgN gTSS^{-1}) was calculated (Eq. 1):

$$DNP = (NO_{X,0} - NO_{X,final}) / X_{TSS} \qquad (1)$$

where $NO_{X,0}$ and NO_X,final denote initial and final NO_X concentration (NO_3-N + NO_2-N) and X_{TSS} the attached biomass concentration (gTSS L^{-1}). Biotransformation rate constants (k_{Bio}, L

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$gTSS^{-1}$ d^{-1}) of pharmaceuticals were estimated from experimental results by assuming pseudo-first-order transformation kinetics. During the 500-d continuous flow operation, biofilm samples were collected from MBBR carriers to assess the temporal evolution of the microbial structure in terms of functional gene abundance and biodiversity. Quantitative PCR (qPCR) was used to determine abundance of denitrifying genes *nirS and nirK* (encoding for two structurally different nitrite reductase) and *nosZ* (encoding for nitrous oxide reductase).

Results and Conclusions

Based on results obtained in the batch experiments, employing carriers from S1 to S3 (Fig. 1a), a progressive decrease was observed for denitrification potential (DNP) and biotransformation rate constants (k_{bio}) of a number of pharmaceuticals (atenolol—ATN, erythromycin—ERY, sulfamethoxazole—SMX, trimethoprim—TMP). Biofilm exposure to tiered substrate availability (declining from S1 to S3) during continuous-flow operation thus caused significant differences in microbial functionalities, i.e. reduction of nitrogen oxides and biotransformation of pharmaceuticals. The analysis of the microbial community using qPCR revealed differences in the abundance of denitrifying genes (*nirS*, *nirK*, *nosZ*) in the three staged MBBRs, suggesting higher abundance of *nir* genotypes in S2 (Fig. 1b). Further microbial characterization through 16sRNA sequencing (Illumina) is currently under investigation to determine whether differences in microbial functions are associated with differences in the microbial diversity in the four MBBRs.
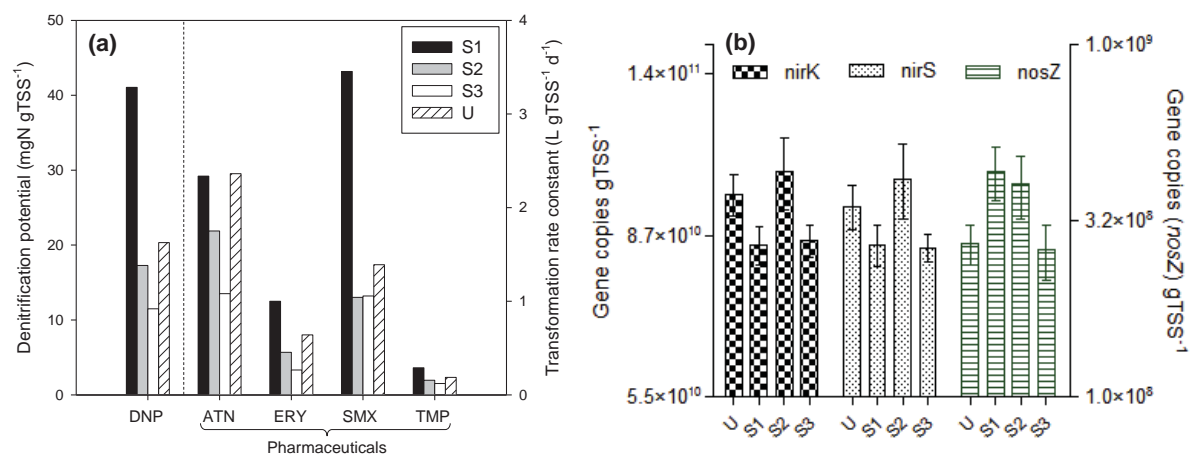

Figure 1 Denitrification potential (DNP) and biotransformation rate constants k_{Bio} for pharmaceuticals (atenolol—ATN, erythromycin—ERY, sulfamethoxazole—SMX, trimethoprim—TMP) estimated from batch experiments (a); gene copies abundance of *nirK*, *nirS* and *nosZ* in the four pre-denitrifying MBBRs (b).

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