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Biodiversity positively associates with biofilm thickness in Moving Bed Biofilm Reactors (MBBRs) – implications on micropollutant removal and nitrification

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Introduction

Nowadays, biological processes in wastewater treatment plants (WWTPs) are mainly designed to remove primary pollutants (e.g., organic carbon and nutrients). However, the optimization of biological wastewater treatment technologies has become necessary due to the upcoming legislation (European Water Framework Directive) targeting a wide range of micropollutants. Biofilm systems such as Moving Bed Biofilm Reactor (MBBR) has been previously observed to enhance the removal of a number of micropollutants (e.g. diclofenac and X-ray contrast media) compared to conventional activated sludge (CAS). However, the underlying mechanisms of the enhanced performance in MBBR are still under investigation. In MBBR, biofilm is growing on specifically designed plastic carriers and are usually operated without a direct control of biofilm thickness due to lack of technology able to develop well-controlled biofilms. However, the increase of biofilm thickness can have a significant effect on the microbial diversity and activity, resulting from diffusion limitation and thus substrate penetration in the biofilm. More specifically, it is hypothesized that, with increasing biofilm thickness, a more heterogeneous and biodiverse biofilm could be developed with "microbial specialist" able to carry out micropollutant biotransformation reactions more effectively. To this end, research has previously shown the importance of biodiversity in ecological systems (Cardinale 2011), observing higher functionality and stability of microbial communities with higher richness compared to microbial communities with lower richness. Biodiversity, which includes species richness (the number of species) and evenness (the relative abundance of the species) has previously observed to maximize removal of a number of micropollutants in activated sludge (Helbling et al. 2012). In our study, we hypothesized that increasing biofilm thickness would lead to increased microbial diversity (both richness and evenness) and that increased biodiversity would associate positively with micropollutant removal in MBBR. The main objective of this study was (i) to investigate the impact of biofilm thicknesses on the ecosystem functionalities in terms of removal of selected micropollutants (pharmaceutical residues) and autotrophic nitrification; and (ii) to assess the impact of biofilm thickness on microbial community structure using laboratory-scale nitrifying MBBRs operated in continuous and batch mode. AnoxKaldnes^{TM} Z-carriers were employed (first time as such) to investigate the impact of biofilm thickness on microbial structure and functionality, thereby allowing a more precise control of biofilm thickness.

Material and Methods

Continuous operation. Two laboratory-scale MBBRs were operated in parallel, where the first reactor (R1, 3 L) contained a mixture of Z-carriers (Z500, Z400, Z300, Z200) with 500, 400, 300, 200 μm thickness (200 carriers of each) and the second reactor (R2, 1.5 L) contained a modified version of Z-carriers (Z50) with 50 μm thickness (260 carriers). The enrichment of nitrifying biofilm was performed under similar conditions in both reactors by

feeding the reactors with effluent wastewater from a local municipal treatment plant (Källby, Lund, Sweden) spiked with additional 50 mg/L of ammonium. *Batch experiments.* Batch experiments were performed in a period of 24 hours with spiking of 22 pharmaceutical chemical compounds (initial conc. ~1 µg L^{-1}). Pseudo first-order biotransformation rate constants (k_{bio}, $LgTSS^{-1}d^{-1}$) were estimated for the different pharmaceuticals using experimental data obtained with three different biofilm thicknesses, i.e. 50 µm (Z50), 200 µm (Z200), 500 µm (Z500). *Microbial and statistical analysis.* Quantitative PCR (qPCR) was used to determine ammonia monooxygenase gene (*amoA*) abundance. 16S rRNA gene sequencing by Illumina MiSeq was used to investigate microbial structure. Shannon diversity and evenness indices were calculated according to Hill (1973*).* Pearson correlation analyses and one way analysis of variance ANOVA (significance level at $p<0.05$) were carried out using the software Prism 5.0. Positive correlations are defined at $r\geq0.9$, whereas no correlation and negative correlation were defined at $r = -0.9$ and $r < 0.1$, respectively.

Results and Conclusions

Our observations (Fig.1) suggested that Shannon taxonomic diversity and evenness index increased with biofilm thickness and were significantly different in Z50 ($P<0.01$) and Z200 ($P<0.05$) compared to thicker biofilms (Fig. 1a). These results suggest that with increasing biofilm thickness it is possible to obtain a more biodiverse but and a more evenly distributed microbial community – with significant alterations observed up to 200 µm as biofilm thickness. Furthermore, biotransformation rate constants k_{bio} for more than 60% of the targeted micropollutants were found positively correlated with biofilm thickness and biodiversity (Pearson´s r >0.9) (Fig. 1b). On the other hand, among the most recalcitrant compounds, diclofenac and three sulfonamides exhibited more efficient removal using the thinnest biofilm. As the thinnest biofilm also presented the highest nitrification rate and abundance of *amoA* during batch experiment (data not shown), this indicated hydroxylation by the mono-oxygenase enzyme reaction as the main removal route for diclofenac and the sulfonamides. Taken together, our results suggested that biofilm technologies with thicker biofilms (~500 µm) and thus exhibiting higher biodiversity may be an effective solution to maximize biotransformation of a number of micropollutants in wastewater treatment plants.

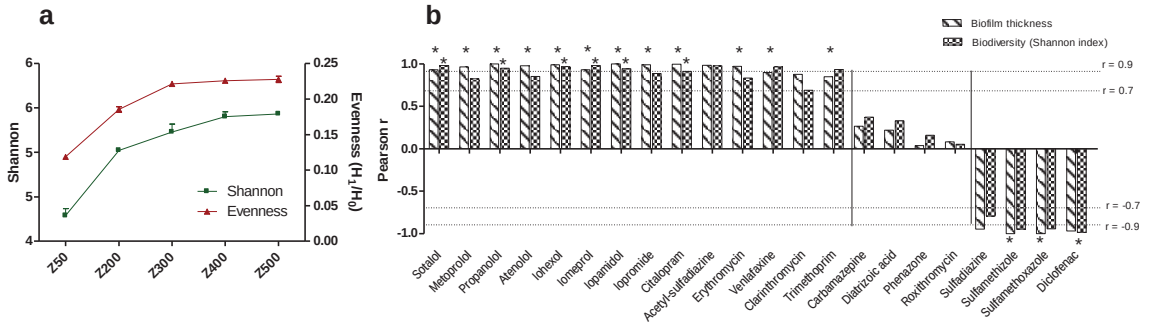

Figure 6. Shannon diversity and Evenness indices at different biofilm thickness (a) and correlation (with Person) of biotransformation rates k_{bio} of 22 micropollutants with biofilm thickness and Shannon diversity index.

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