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Can we enhance the biotransformation of pharmaceutical micropollutants by controlling biofilm thickness in MBBR?

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Summary:

The removal of pharmaceuticals was investigated in nitrifying Moving-Bed-Biofilm Reactors (MBBRs), containing carriers with different biofilm thicknesses (50-500 µm), and treating real wastewater. The biofilm with the thinnest thickness (50 µm) was found to have the highest nitrification rate (both in terms of surface- and biomass-normalized) and biotransformation rate coefficient (k_{bio}) for some of the key pharmaceuticals (i.e. sulfonamides). However, the majority of micropollutants (e.g beta-blockers, X-ray contrast media) were found to be positively correlated with biofilm thickness. Molecular analysis used to assess the microbial structure of biofilm revealed different relative abundance of nitrifying guilds in the different carriers, thereby suggesting the impact of biofilm thickness on the microbial community and on the associated microbial functions.

Keywords: removal of trace pharmaceuticals residues (micropollutant), MBBR, nitrification, microbial structure

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 targeting a wide range of micropollutants. MBBR has been found to have considerably higher micropollutants removal potential compared to activated sludge, potentially due to their higher solid retention time and thus the capability to enrich microbial communities responsible for the higher cometabolic biotransformation of micropollutants, e.g., nitrifiers (Falås et al., 2012). MBBR are attached growth systems based on biofilm growing on especially designed plastic floating in water volume (Ødegaard 2006). As the biomass is retained in the system at extremely long observable SRT, MBBR are in particularly advantageous to enrich the microbial community of slow growing bacteria (i.e. nitrifiers). In biofilm systems, functionality strongly depends on resource availability (e.g., oxygen, ammonium, phosphorus) in the bulk liquid. Diffusion processes and metabolic activities of the cells result in concentrations gradients of substrates through the biofilm, resulting in unique ecological niches for microorganisms (Stewart & Franklin, 2008). With increasing biofilm thickness, the substrate gradients and metabolic processes can be more pronounced, potentially leading to a more heterogeneous and biodiverse biofilm with "microbial specialist" able to carry out micropollutant biotransformation reactions. Thus, the main objective of this study was to investigate the impact of biofilm thicknesses on the removal of a number of micropollutants as well as on microbial structure in laboratory scale nitrifying MBBRs. Accordingly, we (i) enriched a nitrifying community in MBBR systems using carriers which allowed the growth of 5 different biofilm

thicknesses (AnoxKaldnes™ Z-carriers), (ii) quantified biotransformation rates of 22 micropollutants and (iii) investigated the microbial community using Real-Time (qPCR).

Material and Methods

Control of the biofilm thickness. AnoxKaldnes™ Z-carriers (Fig. 1) with grids of defined heights were used to control maximum biofilm thickness. Unlike the conventional MBBR carriers, the saddled shaped Z-carriers have a grid covered surface allowing the biofilm to grow on the outside of the carrier rather than in the inside void (Piculell et al. 2015). As the carriers continuously scrape against each other during reactor operation, the grid wells height defines the maximum biofilm thickness.

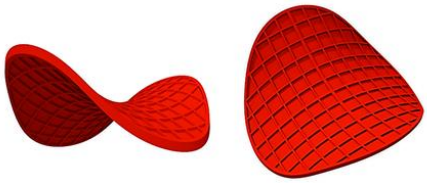

Figure 1. Example of Z-carriers used in the study (Piculell et al. 2015).

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 using the same feed composition and similar operational condition as used during the continuous operation but with spiking of 22 chemical compounds (~1 µg L^{-1}). Pseudo first-order biotransformation rate constants (k_{bio}, $LgTSS^{-1}d^{-1}$) were calculated for different pharmaceuticals and for three biofilm thicknesses, 50 µm (Z50), 200 µm (Z200), 500 µm (Z500).

DNA extraction and Quantitative PCR (qPCR). Duplicates of biomass samples for each Z-carrier were collected stored in sterilized Eppendorf tubes at -20°C. Biomass was detached using a sterile brush (Gynobrush, Dutscher Scientific, United Kingdom) using tap water and consequently centrifuged (10000 rpm for 5 minutes) to remove excess water. The collected biomass was subject to DNA extraction using the MP FastDNA™ SPIN Kit (MP Biomedicals LLC., Solon, USA) following manufacturer's instructions. Quantitative PCR (qPCR) targeting 16S rRNA and functional genes was carried out according to Pellicer-Nàcher et al. 2010 to estimate the abundance of total bacteria (EUB), ammonia oxidizing bacteria (AOB, 16S rRNA), amoA gene, *Archea* (ArcamoA), and nitrite oxidizing bacteria (NOB, *Nitrospira* spp. and *Nitrobacter* spp 16S). Primers are reported in Table S3 in SI.

Statistical analysis. Pearson correlation analyses and one way analysis of variance ANOVA with Bonferroni post-hoc test (significance level at $p<0.05$) were carried out using the software Prism 5.0, thereby determining the significance in terms of differences between the data obtained for the

different Z-carriers. Positive correlations was defined when r was ≥0.9, no correlation when r < 0.1 and negative correlation when r = -0.9.

Results and discussion

After 200 days of continuous operation, a stable nitrification removal rate of 1.7 $gNd^{-1}m^{-2}$ and 2 $gNd^{-1}m^{-2}$ was reached in R1 and R2, respectively.

Batch experiments showed that the carriers with thinnest biofilm (Z50) presented the highest nitrification rate (1.8 gN $gTSS^{-1}$ d^{-1}) compared to other biofilm carriers (< 0.7 1.8 gN $gTSS^{-1}$ d^{-1}). The biotransformation rate constant k_{bio} normalized to the maximum biotransformation rate constant k_{bioMax} for the 22 spiked micropollutants are summarized in Fig. 2. We observed that 13 over 22 of the targeted micropollutants (including beta-blockers and x-ray contrast media) presented higher k_{bio} within the thickest biofilm Z500 (which presented the lowest nitrification rate). Previous studies observed, with exception of atenolol, not direct link to ammonia oxidation and beta-blockers (Sathyamoorthy et al. 2013) or to X-ray contrast media (Casas et al. 2015). Our results suggested that the removal of these groups of micropollutants can be related to a more heterogenic microbial community structure of a thick biofilm unlike nitrification activity. The removal sulfonamides (sulfadiazine, sulfamethoxazole, sulfamethizole) predominantly occurred with Z50. A recent study found enhanced sulfamethoxazole degradation in partial nitrification oxidizing batch reactor (SBR) (Kassotaki et al., 2016), linking its removal to nitrification. Our study supports the hypothesis of a link between nitrification and the three targeted sulfonamides as their removal was enhanced within the thinner biofilm Z50 presenting higher nitrification activity.

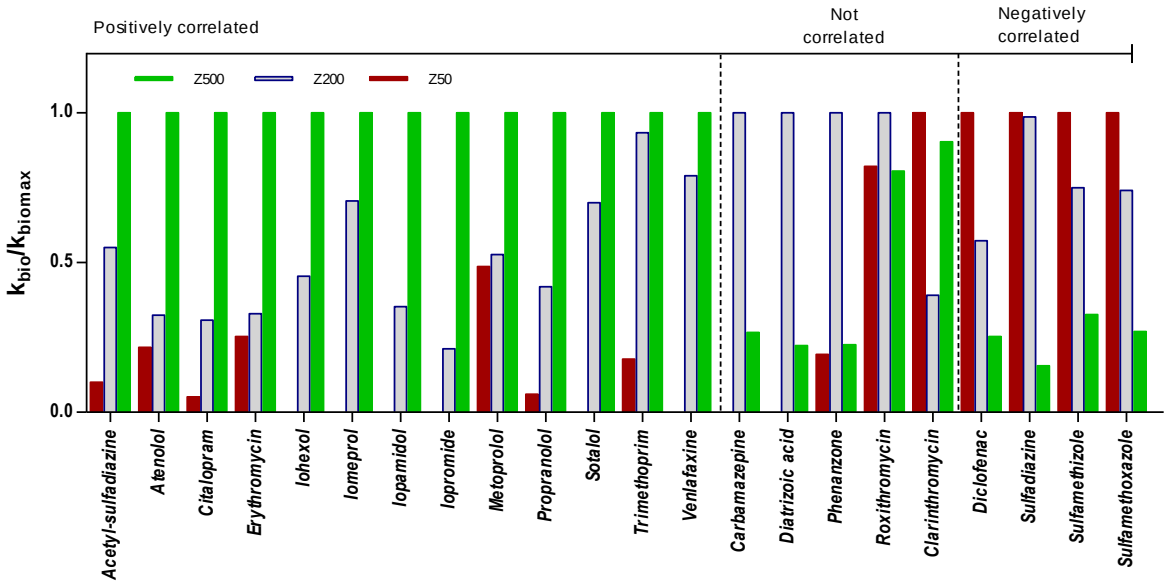

Figure 2. Biotransformation rate constant k_{bio} normalized on the max biotransformation rate constant k_{bioMax} for the 22 targeted micropollutants for Z-carriers carriers with biofilm 500, 200, 50 µm thick.

The microbial community structure, analyzed with qPCR, showed that autotrophic biofilm (AOB plus NOB) accounted for approximately 50% of the biofilm for the Z-carriers, being the smaller for

the Z50 (45%). *Nitrobacter* fraction was lower than 0.05% in all the carriers, while AOA were below the detention limit. Total EUB, AOB, NOB (as *Nitrospira*) and amoA abundances (gene copies $gVSS^{-1}$) were found significantly higher ($p<0.05$) for the Z50 compared with the other Z-carriers (based on the one-way ANOVA tests). The higher microbial abundance (in terms of target genes) in Z50 could be related to the lower diffusion limitation of resources (substrates and oxygen) in the thinner biofilm compared with the other Z-carriers. Strong correlation ($r > 9$) was found between biotransformation rates of diclofenac and sulfonamide and the *amoA* gene abundance, suggesting hydroxylation by the mono-oxygenase enzyme reaction as the main potential removal route.

Conclusion

Overall, our results suggested that biofilm technologies with thicker biofilms (~500 µm) may be an effective solution to maximize biotransformation and removal of several micropollutants. On the other hand, higher nitrification and *amoA* abundance in thinner biofilms could be advantageous for removal of micropollutants which undergo hydroxylation by the mono-oxygenase enzyme reaction. Further microbial characterization investigating the role of the microbial diversity related to the biofilm thickness should be investigated to understand the underlying microbial processes involved in micropollutants biotransformation in biofilm systems.

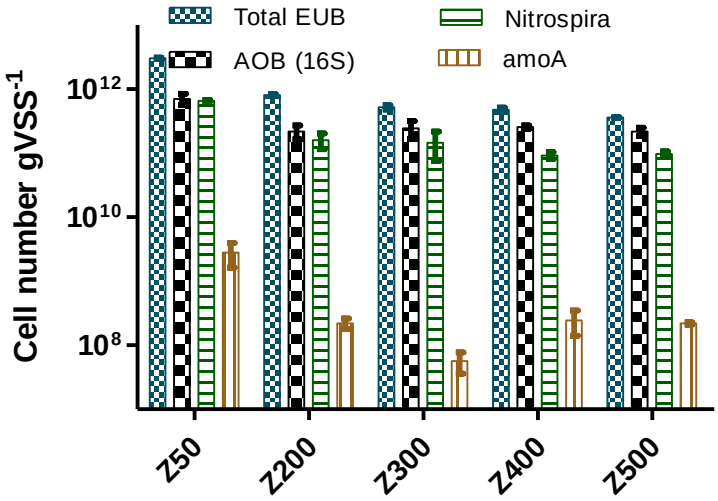

Figure 3. Abundance of targeted guilds and amoA gene in the three Z-carriers.

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