

### Biofilm thickness modulates community assembly processes and functionality in wastewater treatment

Jane Fowler, PhD

Department of Environmental Engineering, Technical University of Denmark

#### Abstract

Niche and neutral theories provide diverging viewpoints on the importance of selection and neutral processes in community assembly. In practice, both deterministic and stochastic factors play a role in microbial community assembly, though little is known about manipulating their relative importance, or the relationship between assembly processes and community functionality. We investigated the effect of biofilm thickness on community assembly using Z-carriers<sup>®</sup>, biofilm carriers where biofilm thickness is controlled by grid height. Duplicate Z-carriers of five thicknesses (50, 200, 300, 400 and 500 µm), influent and effluent were sampled at intervals from steady state reactors fed with wastewater effluent with added ammonium. Extracted DNA was subject to 16S rRNA gene amplicon sequencing and qPCR for total Bacteria. The biofilm communities were distinct from influent and effluent communities. Biofilm communities were strongly influenced by selection, but the small number of sequence variants (SVs) shared between carriers and influent increased with biofilm thickness. Neutral modelling revealed that a greater percentage of shared SVs were neutrally assembled with increasing thickness, corresponding to a linear relationship between biofilm thickness and migration rate. These observations suggest that biofilm thickness modulates the relative contribution of neutral and deterministic processes on community assembly, with stochastic factors playing a greater role in thicker biofilms. In addition, we quantified nitrification and micropollutant removal kinetics for each thickness. Nitrification and biotransformation rates of 4 micropollutants were inversely correlated with biofilm thickness while the biotransformation rates of a further 14 micropollutants were correlated with biofilm thickness. We propose that the small, active volume in thin biofilms is subject to greater competition, resulting in efficient nitrification, while in thicker biofilms, the greater volume and presence of less active lower layers increases the contribution of neutral processes in community assembly, and results in a wider range of functionalities.

#### Bio

I am a postdoctoral researcher at the Technical University of Denmark in Environmental Engineering. My interests revolve around sustainable biotechnologies for biological drinking water and wastewater treatment using mixed microbial communities. My research ultimately aims to manage microbial communities using principles of ecology, bioenergetics and bacterial physiology to optimize performance, contaminant biodegradation and greenhouse gas mitigation. I obtained my PhD at the University of Calgary in 2014 where I studied syntrophic hydrocarbon biodegradation under methanogenic conditions.



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