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Biofilms in Nitrogen Removal

Bacterial Population Dynamics and Spatial Distribution

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Akademisk avhandling för filosofie doktorsexamen i mikrobiologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras den 3 februari 2012, kl. 10.00 i föreläsningssal Carl Kylberg (K2320), Institutionen för Kemi och Molekylärbiologi, Medicinaregatan 9C, Göteborg.

ISBN: 978-91-628-8420-8

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ABSTRACT

Efficient nitrogen removal at wastewater treatment plants (WWTPs) is necessary to avoid eutrophication of recipient waters. The most commonly used approach consists of aerobic nitrification and subsequent anaerobic denitrification resulting in the release of dinitrogen gas into the atmosphere. Nitrification is a two-step process performed by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) often grown in biofilms at WWTPs. An alternative approach is anaerobic ammonium oxidation (anammox) where anammox bacteria convert ammonium and nitrite directly into dinitrogen gas. These groups of bacteria grow very slowly and are sensitive to perturbations, which may result in decreased efficiency or even breakdown of the process. Therefore, the ecology and activity of these bacteria and the structure of the biofilms in which they grow require detailed investigation to improve the understanding of nitrification and to facilitate the design of efficient nitrogen-removal strategies.

To facilitate such studies of relevance for wastewater treatment, a nitrifying pilot-plant was built where environmental conditions and especially ammonium concentrations could be controlled.

In an experiment on model nitrifying trickling filters (NTFs), it was shown that biofilms subjected to intermittent feeding regimes of alternating high and low ammonium concentration in the water, could maintain a higher nitrification potential than biofilms constantly fed with low ammonium water. Such ammonium feed strategies can be used to optimize wastewater treatment performance.

Different AOB populations within the *N. oligotropha* lineage were shown to respond differently to changes in environmental conditions, indicating microdiversity within this lineage which may be of importance for wastewater treatment. This diversity was further investigated through the development of new image analysis methods for analyzing bacterial spatial distribution in biofilms. The diversity within the *N. oligotropha* lineage was also reflected in the positioning of two such populations in the biofilm, where the vertical distribution patterns and relative positions compared to the NOB *Nitrospira* were different.

In combination with a cryosectioning approach for retrieval of intact biofilm from small biofilm carrier compartments, the new image analysis methods showed a three-dimensonal stratification of AOB-anammox biofilms. This may be of importance for mathematical modeling of such biofilms and the design of new biofilm carriers.

Keywords: AOB, NOB, biofilms, image analysis, FISH, *Nitrosomonas,* population dynamics