



Microbial diversity and control of the denitrification process during drinking water conditioning using biodegradable polymers

Anja Karolewicz^{1,2}, Thomas Schwartz¹, Martin Kieninger³, Wolf-Rüdiger Müller³, Ursula Obst¹

¹ Institute of Functional Interfaces, Microbiology of Natural and Technical Surfaces Department, Karlsruhe Institute of Technology

² Competence Center for Material Moisture, Karlsruhe Institute of Technology

³ Institute for Sanitary Engineering, Water Quality and Solid Waste Management, Stuttgart

INTRODUCTION

- **Microbial denitrification** activities are used for nitrate degradation in contaminated raw water sources for drinking water conditioning. Adapted bacterial communities are forming biofilms on a biodegradable, non-toxic synthetic material like **Polycaprolactone**, which is used as **carbon source** and **substrate for biofilm growth**, respectively. Therefore, there is no need for extra addition of a carbon source associated with **dosing problems**.
- To monitor **biofilm compositions and dynamics** on the Polycaprolactone particles, rDNA based **populations analyses** were performed at different points of time.
- To **control the process**, different **gene expression analyses** were performed with specifically designed primers targeting key genes involved in the denitrification process.
- Samples were taken from three different raw water denitrification reactors.

METHODS

Samples:

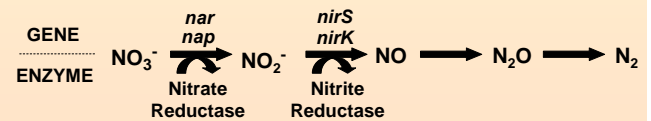
- Up-scaled **Rotobio reactor** (fig. 1) 2 m³ (cooperation with the Water Technology Center Karlsruhe),
- **Rotobio reactor** (fig. 2), and **Dynasand reactor** (fig. 3) 0,05 m³ and 0,8 m³, respectively (cooperation with the University of Stuttgart)

Bacterial population analyses:

16S rDNA PCR, DGGE and sequencing techniques.

Gene expression:

- of 2 different nitrate reductase genes (*nar* and *nap*)
- and of 2 different nitrite reductase genes (*nirS* and *nirK*)
- were quantified in the specific reference bacteria *Pseudomonas aeruginosa* and *Acidovorax caeni*
- and in natural biofilm communities grown on Polycaprolactone in the three denitrification reactors.



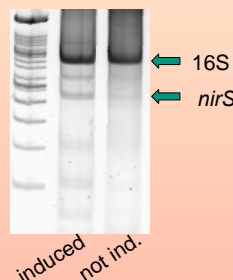
Bacterial population analyses:

Frequently found bacteria are shown in the table below. Most of them are β -Proteobacteria.

	Frequently found bacteria
α -Proteobacteria	<i>Rhodobacter sp.</i>
	<i>Acidovorax ssp</i>
β -Proteobacteria	9 uncultured species
	<i>Aquaspirillum ssp.</i>
	uncultured
γ -Proteobacteria	<i>Xanthomonas sp.</i>
	<i>Pseudomonas sp.</i>
α -Proteobacteria	uncultured
ϵ -Proteobacteria	uncultured
Flavobacteria	<i>Flavobacterium sp.</i>
Bacteroidetes	Cytophagales

Expression analyses:

- No consistent expression pattern for the nitrate reductase within the reference strains.
- The biofilm on the particles exhibited a constitutive like expression of the nitrate reductase gene and a significant up-regulation of nitrite reductase when nitrite accumulated in the system.



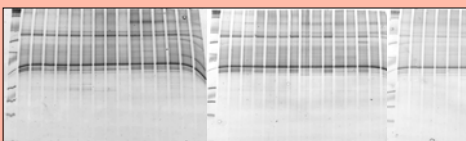
RESULTS

Nitrate reductases		Nitrite reductases	
<i>nar</i>	<i>nap</i>	<i>nirS</i>	<i>nirK</i>
→	↓	↑	↑

Regulation of the nitrate and nitrite reductases during induced conditions in the 2 m³ Rotobio reactor

Continuous analyses:

Biofilm over 17 weeks showed a **stable diversity**.



CONCLUSIONS

- The stable biofilm diversity is a precondition for a stable denitrification and for the safety of the following drinking water conditioning.
- With our methods used for expression analyses of genes involved in the denitrification process we have a toolbox to understand and control this process for optimization and monitoring even in reactors.