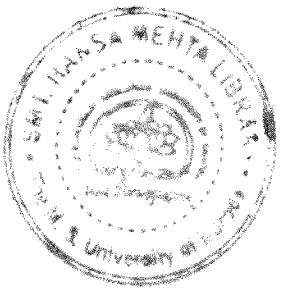

Summary

Assessment of denitrifier community composition in activated sludge and studies with *Paracoccus* sp. W1b biofilm

Chapter 2:

Composition of denitrifying bacteria in activated sludge and the denitrifying activity of selected isolates

- Four different activated sludge samples were collected to investigate the denitrifying bacterial composition. The sludge samples were designated as DaS (Denitrifying reactor sludge from a fertilizer industry), GS (An aeration tank sludge from a fertilizer factory), NL (An aeration tank sludge from CETP) and WL (An aeration tank sludge from domestic sewage). The proximate composition of the sludge samples was also determined.
- Abundance of culturable denitrifiers in the four different activated sludge samples tested, were in the range 2.28 X 10^8 to 2.8 X 10^9, as assessed by the MPN assay.
- Abundance of denitrifying functional genes in the sludge samples showed *nir*S in the range of 10^4-10^5 per ml, *nos*Z with 10^4-10^6 per ml and 16S rRNA gene in the range 10^9-10^{10} copy number per ml of sludge, as analysed by quantitative real-time PCR.
- The ratio of the *nos*Z and *nir*S genes of 0.5 in the NL sludge sample indicated that it contains more number of denitrifiers truncated in the *nos*Z gene.
- The abundance of culturable denitrifiers and the functional genes suggested high number of unculturable denitrifying bacteria to be present in the DaS, denitrifying reactor sludge sample.
- The cultivation of the denitrifying bacteria from the four different activated sludge samples revealed *Pseudomonas* sp. and *Alcaligenes* sp. to be numerically dominant.
- Denitrifying bacterial isolates, possibly truncated in the nitrate reduction step, were also obtained from the activated sludge samples.
- *nos*Z gene library was constructed from the fertilizer factory activated sludge samples (DaS and GS) yielding 114 clones from DaS sludge and 104 clones from the GS sample

- RFLP analysis of the clones with *Alu*I enzyme yielded 10 OTUs in DaS sample and 13 in the GS sample sludge.
- Rarefaction analysis showed that the sludge sample DaS was nearly reaching the asymptote, unlike the GS sample where increasing the clone number would have shown more diversity.
- The Shannon-Weiner and the Simpson's reciprocal diversity were high in GS sludge sample than DaS, refurbishing that the *nos*Z diversity is high in GS sludge sample.
- The translated protein sequences of the *nos*Z gene clones suggested Betaproteobacteria to be numerically dominant in the sludge.
- *Paracoccus* sp., *Comamonas* sp. and *Pseudomonas fluorescens* isolates showed efficient denitrification with negligible amount of nitrite accumulation, while *Diaphorobacter* sp., *Pseudomonas mendocina*, *Pseudomonas stutzeri* and *Brevundimonas diminuta* accumulated nitrite during denitrification.
- The nitrate reduction rate was 1.5 times more than nitrite reduction in *Diaphorobacter* sp. D1, whereas ratio of the rates of nitrate and nitrite reduction in *Paracoccus* sp. W1b was nearly 1.0, as analysed by the resting-state denitrification kinetics.
- Increasing nitrate concentration upto 10 mM in the medium increased the nitrite accumulation in *Diaphorobacter* sp. D1, but not in *Paracoccus* sp.W1b indicating the presence of a sequential denitrification process in the former and a branched electron transfer during denitrification in the latter.
- *Diaphorobacter* sp. D1 was unable to denitrify at high nitrate concentrations from 1M, but *Paracoccus* sp.W1b could denitrify even upto 2 M nitrate.

Chapter 3

Characterization of *Paracoccus* sp. W1b biofilm

- Brightfield and scanning electron microscopy confirmed biofilm formation by *Paracoccus* sp. W1b on polystyrene slides.
- The Plackett-Burman design was shown to be useful for detecting the influence of nutrients on biofilm formation, and the nutrients were also shown to affect the architecture of biofilm.

- In the Plackett-Burman experiment, higher concentrations of succinate, Mg^{++}, Ca^{++} and Mn^{++} enhanced biofilm formation, whereas higher concentration of iron decreased biofilm formation of *Paracoccus* sp. W1b.
- Confocal image quantification of the biofilm formed by *Paracoccus* sp. W1b at high succinate concentrations tested, showed more roughness with high surface to biovolume ratio. The data also suggested a possible production of increased EPS with high succinate concentration.
- Higher Mg^{++} or Ca^{++} concentrations of 10 mM in the medium, induced cohesion of biofilm cells, but contrasting biofilm architectures were detected. Biofilm with subpopulations of pillar-like protruding cells were distributed on a mosaic form of monolayer cells in medium with 10mM magnesium, while 10mM calcium induced a dense confluent biofilm
- Denitrification activity was 5.9 and 6.3 folds increased respectively in the magnesium and calcium induced biofilm of *Paracoccus* sp. W1b.
- Chelator treatment of various biofilm ages indicated that divalent cations are important in the initial stages of biofilm formation of *Paracoccus* sp. W1b.
- EDTA treatment of the magnesium-induced biofilm of *Paracoccus* sp. W1b indicated the presence of subpopulations which was confirmed by the FAME analysis, where the composition of the cellular fatty acids were different in the pillar-like cells from that of the mosaic monolayer.
- The nitrogenous oxides, nitrate, nitrite and nitric oxide at various concentrations did not affect the *Paracoccus* sp. W1b biofilm significantly.

Chapter 4

Influence of carbon sources on the biofilm community grown in a 1L laboratory-scale bioreactor in denitrifying conditions

- Acetate-fed biofilm community showed the highest denitrifying activity with an emergent biofilm structure showing a high thickness and diffusion distance.
- Glucose-fed biofilm community accumulated 213% more ammonium than the influent including accumulation of nitrite was observed, although 99% nitrate was reduced.
- Methanol-fed biofilm accumulated high nitrite during nitrate removal and formed a confluent biofilm without characteristic voids.

- Ethanol-fed biofilm showed relatively higher ratio of denitrifiers and a biofilm of lower thickness and diffusion distance was formed.
- DGGE analysis showed *Pseudomonas* sp. to dominate the acetate and ethanol-fed biofilm, while *Enterobacter* sp. and *Methylobacillus* sp., dominated glucose and methanol biofilms respectively.
- FISH analysis revealed *Pseudomonas* sp. to dominate the biofilm community, possibly due to the colonization of the substratum surface at the early stage of the biofilm development.
- Increasing nitrate concentration in the influent of the reactor increased the abundance of *Paracoccus* sp. relative to *Pseudomonas* sp. However, *Pseudomonas* sp. was found to dominate the substratum surface.