Occurrence of nitrifiers and diversity of ammonia-oxidizing bacteria in developing drinking water biofilms

Mari T.T. Lipponen, Pertti J. Martikainen, Ritva E. Vasara, Kristina Servomaa, Outi Zacheus, and Merja H. Kontro

Laboratory of Environmental Microbiology, National Public Health Institute, P.O. Box 95, FIN-70701 Kuopio, Finland
North Savo Regional Environment Centre, Research Department, P.O. Box 1049, FIN-70101 Kuopio, Finland
Department of Environmental Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland
Department of Ecological and Environmental Sciences, University of Helsinki, Niemenkatu 73, FIN-15140 Lahti, Finland

Received 11 June 2003; revised 23 June 2004; accepted 11 August 2004. Available online 13 October 2004.

Abstract

We studied the population dynamics of nitrifying bacteria during the development of biofilms up to 233 or 280 days on polyvinylchloride pipes connected to two full-scale drinking water distribution networks supplying processed and chloraminated surface water. The numbers of nitrifiers in biofilms were enumerated at intervals of 10–64 days by the most probable number (MPN) method at waterworks and at...
several study sites in distribution network areas. The numbers of nitrifiers increased towards the distal sites. The highest detected MPN counts of ammonia-oxidizing bacteria (AOB) for study areas 1 and 7 were 500 MPN cm$^{-2}$ and 1.0×10$^6$ MPN cm$^{-2}$, and those of nitrite-oxidizing bacteria (NOB) 96 MPN cm$^{-2}$ and 2.2×10$^3$ MPN cm$^{-2}$, respectively. The diversity of AOB was determined by PCR amplifying, cloning and sequencing the partial ammonia monooxygenase (amoA) gene of selected biofilm samples presenting different biofilm ages. The PCR primers used, A189 and A682, also amplified a fragment of particulate methane monooxygenase (pmoA) gene of methane-oxidizing bacteria. The majority of biofilm clones (24 out of 30 studied) contained *Nitrosomonas* amoA-like sequences. There were only two pmoA-like sequences of Type I methanotrophs, and four sequences positioned in amoA/pmoA sequence groups of uncultured bacteria. From both study area very similar or even completely identical *Nitrosomonas* amoA-like sequences were obtained despite of high difference in AOB numbers. The results show that the conditions in newly formed biofilms in drinking water distribution systems favor the growth of *Nitrosomonas*-type AOB.

**Keywords:** Drinking water; Biofilm; Ammonia-oxidizing bacteria (AOB); Nitrite-oxidizing bacteria (NOB); Diversity; Ammonia monooxygenase (amoA); Particulate methane monooxygenase (pmoA)

**Article Outline**

1. Introduction
2. Materials and methods
   2.1. Study sites and sample collection
   2.2. Microbiological, physical and chemical analyzes
   2.3. DNA extraction from biofilm samples
   2.4. PCR amplification of amoA and pmoA
   2.5. Cloning and sequencing of amplification products
   2.6. Phylogenetic analysis
   2.7. Nucleotide accession numbers
3. Results and discussion
   3.1. Microbiological, chemical and physical characteristics of drinking water
   3.2. Nitrifying and heterotrophic bacteria in biofilms
   3.3. Phylogenetic analysis of biofilm amoA and pmoA sequences
4. Conclusions

Acknowledgements

References

Corresponding author. North Savo Regional Environment Centre, Research Department, P.O. Box 1049, FIN-70101 Kuopio, Finland. Tel.: +358 40 7412318.