A comparison of autotrophic ammonia-oxidizing bacteria in full- and laboratory-scale wastewater treatment reactors

A.K. Rowan*, J.R. Snape**, D. Fearnside***, T.P. Curtis*, M.R. Barer**** and I.M. Head*

* Fossil Fuels and Environmental Geochemistry, Civil Engineering, Microbiology and Centre of Molecular Ecology, University of Newcastle, Newcastle-upon-Tyne NE1 7RU, UK
** Brixham Environmental Laboratory, AstraZeneca, Brixham, Devon TQ5 8BA, UK
*** Yorkshire Water Services, Western House, Halifax Road, Bradford, West Yorkshire BD6 2LZ, UK
**** Department of Microbiology and Immunology, University of Leicester, Leicester LE1 9HN, UK

Abstract Lab-scale reactors are commonly used to simulate full-scale plants as they permit the effects of defined experimental perturbations to be evaluated. Ideally, lab- and full-scale reactors should possess similar microbial populations. To determine this we compared the diversity of the β-proteobacterial autotrophic ammonia-oxidizing bacteria (AOB) in a full-scale biological aerated filter (BAF) using PCR with AOB selective primers combined with denaturing gradient gel electrophoresis (DGGE). PCR amplified 16S rRNA gene fragments from the nitrification unit of the lab- and full-scale BAF were subjected to cloning and sequencing to determine the phylogenetic affiliation of the AOB. A high degree of comparability between the lab- and full-scale BAF was observed with respect to AOB populations. However minor differences were apparent. The importance of these minor constituents in the overall performance of the reactor is unknown. Nonetheless the lab-scale reactor in this study did appear to reflect the dominant AOB community within the full-scale equivalent.

Keywords Ammonia-oxidising bacteria; comparative analysis; nitrification; rRNA

Introduction Laboratory-scale simulations of wastewater treatment processes are frequently used to gain insight into the potential and limitations of full-scale treatment systems. Experimental manipulation of full-scale reactors is rarely possible hence lab-scale experiments are generally employed. One assumption inherent in this approach is that the microbial populations present within the laboratory- and full-scale reactors are comparable. In this study the β-proteobacterial autotrophic ammonia-oxidizing bacteria (AOB) present in a full-scale biological aerated filter (BAF) designed for efficient nitrogen removal were compared with those present in a laboratory scale simulation of the BAF reactor.

The BAF reactor comprised three units, for denitrification, carbon removal and nitrification. The AOB present in each of the three units of the reactor were compared within the lab- and full-scale reactor and comparisons between the lab- and full-scale reactors were also made. The polymerase chain reaction (PCR) was used to amplify 16S ribosomal RNA (rRNA) gene fragments using primers selective for the β-proteobacterial AOB (Kowalchuck et al., 1997). The PCR-amplified fragments were analysed by denaturing gradient gel electrophoresis (DGGE) to allow comparative analysis of the dominant AOB populations. In addition, the PCR-amplified 16S rRNA gene fragments obtained from the nitrification unit of both lab- and full-scale reactors were cloned and sequenced to determine the phylogenetic affiliation of the predominant AOB-like sequences recovered from the reactors.
Methods

Reactors

BAF samples were collected from a treatment plant operated by Yorkshire Water, which receives a mixed waste of domestic and industrial origin. The BAF reactor comprised three linked units, for denitrification (anoxic), carbon removal (oxic) and nitrification (oxic). Typical loadings for the plant are: TBOD, 3,400 kg/d; COD 12,200 kg/d; NH$_4$-N 650 kg/d; TSS, 3,400 kg/d and typical values for the final effluent quality for the plant are: TBOD 9.3 mg/l; NH$_4$-N, 1.2 mg/l; TSS, 15.7 mg/l; COD 107.4 mg/l. Duplicate samples of biomass were obtained from the backwash flow from each distinct unit. A lab-scale model that simulated the loadings of the full-scale BAF was also sampled in a similar manner.

DNA extraction and polymerase chain reaction

DNA was extracted from biomass using the method described by Curtis and Craine (1998) except that mechanical disruption was achieved with a Ribolyser instead of a bead beater. PCR amplification of 16S rRNA gene fragments was performed using primers CTO189f (5′-CCGCCCGCCGCGCGGCGGCGGGCAGCGGGGAGRAAGYAGGGGATCG-3′) and CTO654r (5′-CTAGCYTTGTATTTCACCGCG-3′) (Kowalchuck et al., 1997) that are specific for the β-subdivision AOB. For DGGE analysis, nested amplification of the PCR products obtained with the CTO primers was done using primer 2 (5′-ATTACCGCGGTAGCTGG-3′) and primer 3 (5′-CGGCCCGCAGCGGGGCGGCGGCCGGCGGGCAGCGGCG-3′) (Muyzer et al., 1993). PCR was conducted using identical conditions to Kowalchuck et al. (1997) and Muyzer et al. (1993).

Denaturing gradient gel electrophoresis

DGGE was conducted as described by Muyzer et al. (1993) except that a 10% polyacrylamide gel was used with a range of denaturants of 30-60%. DGGE analysis was conducted using the D-Gene system (Biorad, Hemel Hempstead, UK). Gels were run for 4 h at 200 V constant voltage and stained for 30 minutes in SYBR Green I (Sigma, Poole, UK). Stained gels were viewed with an ultraviolet transilluminator (UVP, San Gabriel, California) and photographed with a Polaroid camera (CU-5, GRI, Great Dunmow, Essex).

Cloning and sequencing

PCR products amplified with the CTO primers were cloned using an AdvanTAge™ PCR Cloning Kit (CLONTECH laboratories Inc.) according to the manufacturer’s instructions. A sample of 30 clones from each library was screened using DGGE which proved to be more discriminatory than amplified ribosomal DNA restriction analysis (ARDRA). A representative of each clone type identified was sequenced. The partial rRNA sequences (~460bp) were aligned with published 16S rRNA sequences from β-proteobacterial AOB and a selection of γ-proteobacterial AOB and related non-AOB as outgroup sequences. A phylogenetic distance tree was generated using the Jukes and Cantor correction (Jukes and Cantor, 1969) and the neighbor-joining algorithm (Saitou and Nei, 1987) with the TREECON software package (van de Peer et al., 1994).

Results and discussion

DGGE analysis revealed that several populations of putative AOB were present in both the lab- and full-scale reactors. One DGGE band was dominant in all units of both the full- and lab-scale reactors. However, the full-scale denitrification unit contained several bands that were not present in the lab-scale reactor (Figure 1A Lane 1 and B Lane 1) but the DGGE profiles of putative AOB in the carbon removal units were very similar (Figure 1A Lane 2
and B Lane 2). Comparison of AOB present in the nitrification unit revealed that although the same predominant AOB population was present in both lab- and full-scale reactors a population unique to the lab-scale reactor was detected (Figure 1A Lane 3 and B Lane 3), subsequent analysis of cloned rRNA gene fragments (BAF clone 6l; 30% of lab-scale clones) indicated that this represented an organism most closely related to *Nitrosomonas*.
aestuarii. Analysis of the rRNA gene clone libraries revealed that AOB populations in both the lab- and full-scale reactors were dominated by *Nitrosococcus mobilis*-like organisms (93.4% of clones from the full-scale reactor, \( n = 30 \); 53.4% of clones from the lab-scale reactor, \( n = 30 \)). DGGE analysis of cloned sequences revealed that the dominant clones co-migrated with the intense band observed in all DGGE gels.

**Conclusions**

DGGE profiles and 16S rRNA gene sequence data indicate that the dominant AOB were comparable between the full- and lab-scale reactors and hence differences in AOB diversity are not likely to cause scale up problems in this instance. However it is not clear what influence the presence of other AOB in the lab-scale reactor, albeit probably at low levels, might have on nitrification in the reactor. It is also of note that the dominant AOB was most closely related to *Nitrosococcus mobilis*, a halotolerant AOB. The effluent treated by the reactors examined in this study contains high levels of sodium chloride from a chemical manufacturing process that contributes to the wastestream. Furthermore *Nitrosococcus mobilis* was identified in an earlier study of a nitrification-denitrification plant treating animal waste (Juretschko *et al.*, 1998) and more recently in a rhizoremediation-based wastewater treatment system (Haleem *et al.*, 2000).

**Acknowledgements**

We are grateful to AstraZeneca and Yorkshire Water Services for funding of the project and supplying samples.

**References**


