

Anaerobic ammonium oxidation in the old trickling filters at Daspoort Wastewater Treatment Works

J Wilsenach¹, L Burke², V Radebe², M Mashego², W Stone³, M Mouton³ and A Botha^{3*}

¹Virtual Consulting Engineers, PO Box 323, Groenkloof, Pretoria, 0027, South Africa

²Council for Scientific and Industrial Research, Meiring Naudé Road, Brummeria, South Africa

³Department of Microbiology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

ABSTRACT

The century-old trickling filters at the Daspoort Wastewater Treatment Works in Pretoria (Gauteng, South Africa) are known for their remarkable removal of nitrogen from municipal wastewater. Our study was conducted to identify the microbiological processes responsible for this phenomenon and to establish whether anammox bacteria may be involved. An aerobic and anaerobic bench top reactor, both inoculated with biofilm-covered stones from one of the filters, were spiked with ammonia-nitrogen (NH_4^{+-}N) and nitrite-nitrogen ($\text{NO}_2^{-}\text{-N}$). These reactors were subsequently monitored by conducting stoichiometric analyses of chemical oxygen demand (COD), NH_4^{+-}N , $\text{NO}_2^{-}\text{-N}$, and nitrate-nitrogen ($\text{NO}_3^{-}\text{-N}$). In the aerobic reactor, the COD concentration decreased over the 56 h batch reaction period and nitrification was revealed by a decrease in NH_4^{+-}N and $\text{NO}_2^{-}\text{-N}$ concentrations. However, the $\text{NO}_3^{-}\text{-N}$ concentration showed no notable decrease. In contrast, in the anaerobic reactor the concentrations of COD, NH_4^{+-}N , $\text{NO}_2^{-}\text{-N}$, $\text{NO}_3^{-}\text{-N}$, as well as total nitrogen decreased during the batch reaction period. The decrease of both the NH_4^{+-}N and $\text{NO}_2^{-}\text{-N}$ concentrations, the latter to zero under anaerobic conditions, suggested that, in addition to heterotrophic denitrification, anaerobic ammonium oxidation (anammox) may also occur in the trickling filter biofilm. This was highlighted by the observation that ammonium removal in the anaerobic reactor stopped as soon as the nitrite concentration became zero. The ratio of nitrite:ammonium removal was 1.33 on average, which conforms to anammox behaviour. Gene sequence analysis was used to test for the possible presence of anammox bacteria in the trickling filter biofilm. Genomic DNA was extracted from trickling filter humus sludge and the polymerase chain reaction (PCR) was used to amplify taxonomically informative 16S rRNA gene sequences, using primers specific for selected anammox species. Subsequent sequence analysis, including using the online Basic Local Alignment Search Tool (BLAST), and constructing a phylogenetic tree using a heuristic search strategy for Maximum Parsimony analysis, confirmed the presence of an anammox bacterium closely related to *Candidatus* 'Brocadia anammoxidans' and *Candidatus* 'Brocadia fulgida' on the biofilm-covered stones of the Daspoort trickling filters.