

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 X1, 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 ($\text{NH}_4^+\text{-N}$) and nitrite-nitrogen ($\text{NO}_2^-\text{-N}$). These reactors were subsequently monitored by conducting stoichiometric analyses of chemical oxygen demand (COD), $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, 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 $\text{NH}_4^+\text{-N}$ 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, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, as well as total nitrogen decreased during the batch reaction period. The decrease of both the $\text{NH}_4^+\text{-N}$ 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.

Keywords: Daspoort, trickling filter, anammox, nitrification, denitrification, stoichiometric analyses, polymerase chain reaction

INTRODUCTION

The relationships between hydraulic loading rate, organic loading rate, and efficiency of removal of biochemical oxygen demand (BOD) in trickling filters was first quantified by Schulze (1957) more than 50 years ago. From Schulze's references, it is evident that the formal investigation of these systems had been quite productive since the 1940's. During this period, much of the basic understanding of trickling filters was established; specifically that treatment capacity depends on the mass of active biofilm and the contact time between the liquid and this biofilm. The effect of biofilm thickness on various biochemical processes also seems to be important in understanding trickling filters (Nielsen et al., 1990).

The inner layer of the biofilm in trickling filters is almost always anaerobic, although this zone could also become anoxic, depending on nitrate concentration in bulk liquid, and diffusion into the biofilm. Nielsen et al. (1990), Kuhl and Jorgensen (1992) and Persson et al. (2002) used micro-sensors with high spatial resolution to quantify the concentration of substances at

various depths in biofilms. The same overall picture emerged from their work: oxygen was found only in the upper 0.5 mm of a biofilm, below which anoxic (nitrate reduction) and anaerobic (sulphate reduction) processes occurred, depending on the concentration of diffused nitrate or sulphate. Dalsgaard and Revsbech (1992) studied the effect of micro-zonation on the performance of different processes, especially denitrification, in biofilms. Denitrification was measured as a function of oxygen and nitrate concentrations, organic matter and ammonium. It was found that increased bulk liquid concentrations of nitrate increased the zone within the biofilm in which denitrification took place. In contrast, higher bulk liquid oxygen concentrations increased the aerobic zone thickness within the biofilm, and decreased the overall denitrification rate. This aerobic zone normally extended 0.2–0.3 mm into the biofilm, below which a mostly anoxic/anaerobic zone could be detected. If the oxygen penetration depth was increased artificially, denitrification stopped, but resumed immediately when anoxic conditions re-established. This suggested that the same organisms responsible for aerobic removal of COD were responsible for COD removal with nitrate reduction (denitrification).

Intact biofilm samples from a carbonaceous trickling filter in a bench-scale reactor were investigated by Biesterfeld et al. (2003). The reactors were fed with sterilised wastewater effluent and spiked with nitrate to a final concentration of 16–18 $\text{mg}\cdot\ell^{-1}$

* To whom all correspondence should be addressed.

+27 21 808 5856; e-mail: abo@sun.ac.za

Received 19 March 2013; accepted in revised form 17 December 2013.