Biological nitrogen and phosphorus removal by filamentous bacteria in pure culture

TR Ramothokang*, SC Simelane, and F Bux

Centre for Water and Wastewater Technology, Durban Institute of Technology, PO Box 1334, Durban 4000, South Africa

Abstract

The availability of excess nutrients (phosphorus (P) and nitrogen (N)) in wastewater systems causes many water quality problems. These problems include eutrophication whereby algae grow excessively and lead to depletion of oxygen, death of the aquatic life and bad odours. Biological phosphorus removal has gained attention because the condition of wastewater is manipulated in order to facilitate nutrient removal by the microbial communities in the wastewater. It has been reported that filamentous bacteria are capable of removing P at a similar or higher rate to that of heterotrophic bacteria. It has also been reported that conditions that facilitate biological nitrogen removal promote bulking in a biological nutrient removal system. The aim of the project was therefore to evaluate the role of filamentous bacteria in biological nutrient removal (BNR) processes. For denitrification this was achieved by performing the nitrate reduction preliminary screening test followed by batch tests. Neisser staining was used to locate polyphosphate granules in cells. All Neisser positive isolates were evaluated for P accumulation employing batch tests. The findings of this study demonstrated that 29% of the isolates were true denitrifiers, 3% were sequential denitrifiers, 11% were nitrate respirers, 13% were non-denitrifiers and 45% were nitrate respirers at high concentrations (1 g/ℓ and 0.5 g/ℓ) and true denitrifiers at low concentrations (0.2 g/ℓ). The results of the nitrate reduction batch test demonstrated that up to 18.46 mg/ℓ nitrate was reduced to nitrogen gas. 53% of the isolates reduced nitrite, 33% resulted in nitrite accumulation and 9% did not react to nitrite. Of the 38 isolates 16% were positive for the Neisser stain, 34% were positive for the glycogen stain and 79% were positive for the PHB stain. Batch test results showed phosphate accumulation of up to 17.12 mgP/ℓ. It was demonstrated by this study therefore, that filamentous bacteria have the potential to biologically remove nutrients. These research findings will serve as a basis for further investigations.

Keywords: activated sludge, denitrification, glycogen accumulating organisms, filamentous bacteria, phosphorus removal

Introduction

Biological nutrient removal (BNR) has gained attention over chemical nutrient removal because of the high cost of the chemical process and the large sludge volumes produced. (Machnika et al., 2005 and Sarioglu, 2005). BNR has three stages, i.e. the anaerobic compartment, anoxic compartment and aerobic compartment. In the anaerobic compartment phosphate accumulating bacteria (PAOs) are selected for and phosphate is released from the bacteria. Under anoxic conditions denitrification and phosphate uptake take place while nitrification and phosphate uptake take place under aerobic conditions (Van Loosdrecht, 2004).

The process of phosphate uptake involves exposing the PAOs to alternating anaerobic-aerobic conditions. Under anaerobic conditions PAOs take up the carbon source and store it in the form of polyhydroxybutyrate (PHB). This is accompanied by the degradation of internally stored polyphosphate in order to provide energy for carbon source uptake. Phosphate is then released in the form of orthophosphate. Under aerobic conditions PAOs take up orthophosphate to replenish their polyphosphate pools using stored PHB as a carbon and energy source (Barak and Van Rijn, 2000; Mino et al., 1998).

Denitrification is the biological conversion of nitrate to more reduced forms such as dinitrogen gas (N_2), nitrous oxide (N_2O) and nitric oxide (NO). Facultative aerobes that can utilise nitrate instead of oxygen as a final electron acceptor are responsible for denitrification. The breakdown of carbonaceous organics in the denitrification process is similar to that in the aerobic process, the only difference being in the final stages of the electron transfer (Sedlak, 1999). This would indicate the need for strict anoxic conditions in the denitrifying system (Lilley et al., 1997). It has been demonstrated that under acidic conditions denitrification can take place in the presence of oxygen (Martin, 1991). Nitrate will replace oxygen in the endogenous respiration reaction. The rate of denitrification depends on the nature and concentration of the carbonaceous matter undergoing degradation. Most investigations agree that denitrification is a zero-order reaction with respect to nitrate being reduced to very low nitrate concentration levels (Martin, 1991).

A hypothesis for the cause of bulking by the low food-to-microorganism (low F/M) filamentous bacterial group in a BNR system was proposed by Casey et al. (1999). In this hypothesis it is stated that the floc-forming organisms are able to reduce nitrate to di-nitrogen gas through the denitrification intermediates. Casey et al. (1999) state that in a BNR system, competition between floc-forming and filamentous bacteria for mutually growth-limiting substrate is influenced by the inhibition of substrate utilisation of floc-formers under aerobic conditions. If denitrification is incomplete at the onset of the aerobic phase, the intracellular denitrification intermediates inhibit the aerobic cytochrome *o* of floc formers and their substrate utilisation ability is therefore inhibited. At this stage filamentous bacteria utilise the substrate and proliferate in the system, leading to bulking (Casey et al., 1999).

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* To whom all correspondence should be addressed.
☎+2731 204-2345; fax:+2731 204-2778; e-mail: JuniorR@dut.ac.za