Elucidation of the microbial community structure within a laboratory-scale activated sludge process using molecular techniques

P Padayachee, A Ismail* and F Bux

Centre for Water and Wastewater Technology, Department of Biotechnology, Durban University of Technology, PO Box 1334, Durban 4000, South Africa

Abstract

Microbial community structures were analysed in activated sludge samples from the anoxic and aerobic zones of a laboratory-scale modified Ludzack-Ettinger (MLE) system. Analyses were performed using fluorescent *in situ* hybridisation (FISH) and denaturing gradient gel electrophoresis (DGGE). The r-RNA targeted oligonucleotide probes used for FISH targeted the alpha, beta and gamma subclasses of the gram-negative family *Proteobacteria*, the high G+C content gram-positive bacterium of the actinomycetes branch and the total Eubacteria present. It was found that approximately 75 to 80% of total cells were detected with the DNA specific fluorochrome DAPI, hybridised with a specific eubacterial probe for the anoxic and aerobic zones. Results confirmed the dominance of the alpha and gamma subclasses of the *Proteobacteria* in the anoxic zone whilst the aerobic zone was dominated with the beta subclass of the *Proteobacteria*. The high G+C content bacterium represented the least dominant species present within each of the two zones. The DGGE technique employed in this study analysed the genetic diversity of the microbial community present in each of the anoxic and aerobic zones. The profile for each of the zones revealed a number of consistent bands throughout the duration of the laboratory-scale process. However, the profiles obtained suggested that a diverse microbial community existed within the aerobic and anoxic zones. These results obtained from the application of fluorescent *in situ* hybridisation (FISH) and PCR-DGGE yields a more precise understanding of the microbial community structure and genetic diversity present in domestic wastewater of a laboratory scale treatment process. COD and nitrogen mass balances were conducted to confirm the acceptance of the results obtained for each batch as an indication of the system performance for the MLE model. Nitrogen mass balances indicated an upset in the nitrogen levels for wastewater batches two and seven. The carbon mass balance fell in the range of 92.4% and 105.9% and the nitrogen mass balance fell in the range of 98.4% and 160.0%.

Keywords: fluorescent *in situ* hybridisation (FISH), denaturing gradient gel electrophoresis (DGGE), COD and nitrogen mass balances.

Introduction

Treatment of activated sludge within the modified Ludzack-Ettinger (MLE) system involves the removal of biodegradable organics, unsettleable suspended solids and other constituents. These biodegradable organic compounds are degraded by bacteria in an aerated reactor and the biomass is allowed to settle and concentrate in a clarifier (Muyima et al., 1997). The system is either a continuous or semi-continuous aerobic method for wastewater treatment involving carbon oxidation and nitrification. This process has been developed for the removal of carbon, nitrogen and phosphate and it is well known that prokaryotic micro-organisms catalyses these main biological processes in wastewater treatments (Juretschko et al., 2002). With nutrients and oxygen present the microbial population in the wastewater achieves optimal growth and respiration (Muyima et al., 1997). The dynamics and diversity of the microbial populations in activated sludge have been analysed by culture-dependent methods, however many members of the natural bacterial communities are still un-culturable (Wagner et al., 1994). Hence microscopic identification based on morphological characteristics was researched and developed in a culture-independent manner by direct rRNA sequence retrieval, where nucleic acid probes which are complementary to the rRNA are used as tools to monitor population dynamics amongst bacteria (Amann, 1995). FISH makes use of rRNA targeted probes which are frequently applied in order to quantify the composition of microbial communities present. This procedure is based on the comparative analysis of macromolecules, mostly ribosomal RNA molecules and fluorescent derivatives of such probes. These probes have been applied successfully for *in situ* enumeration of defined groups of micro-organisms present in activated sludge (Manz et al., 1994).

Fluorescently monolabelled, rRNA targeted oligonucleotide probes detect individual cells, allowing whole-cell hybridisation with rRNA targeted probes to be a suitable tool inferring phylogenetic evolution hence the cell morphology of an uncultured microbe and its abundance can be determined *in situ* (Wagner and Amann, 1997). Cell numbers of the bacteria can be obtained by enumeration under an epi-fluorescent microscope. Enumeration procedures involve the use of a semi-automated digital image analysis tools in order to quantify the fluorescently labelled bacteria in samples (Daims et al., 2001).

The molecular technique used for analysing the structure and providing a profile of the microbial population present in wastewaters is the denaturing gradient gel electrophoresis (DGGE) (Muyzer et al. 1993). DGGE that is performed on 16S rRNA genes has been used to produce a genetic fingerprint of mixed microbial communities (Kaewpipat and Grady, 2002). The

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* To whom all correspondence should be addressed.
☎+2731 204-2346; fax:+2731 204-2778;
e-mail: arshadi@dit.ac.za