Characterisation of microorganisms responsible for EBPR in a sequencing batch reactor by using the 16S rDNA-DGGE method

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Abstract

Analysis of the bacterial community in the biological phosphorus removal system is propitious to study the phosphorus-removal mechanism. The activated sludge was acclimated through a repeated anaerobic-aerobic process with glucose as carbon source for 2 months and a stable EBPR was established in an SBR. Total phosphate of the wastewater decreased by 12.43 $mg \cdot \ell^{-1}$ after 4 h aerobic treatment while total P uptake in the raw sludge was 0.57 $mg \cdot \ell^{-1}$ under the same conditions, and the phosphate content of the sludge increased from 1.83% to 6.79%. The protozoa and dominant bacteria of the two sludges were observed by optical and electron microscope. The genomic DNA of samples was extracted as the template and the 16S rDNA genes (V3 region) were amplified; denaturing gradient gel electrophoresis (DGGE) separated these amplified DNA fragments with the denaturant from 35% to 70%. The DGGE profiles showed that the raw sludge, acclimated sludge and dominant bacteria in the acclimated sludge had different band patterns. The results indicated that micro-organisms were selected by the repeated anaerobic-aerobic process and some non-phosphorus accumulating organisms were eliminated. The cultured strains obtained from acclimated sludges were purified and their DNA was amplified using F27 and R1522 to 1.5 kb; the gene sequences were located on the GenBank and they were identified as *Acidovorax* sp.BSB421 and *Sphingomonas* sp.SA-3.

Keywords: EBPR, micro-organism, DGGE, gene sequence

Introduction

Acinetobacter spp. was first identified as the bacterium responsible for enhanced biological phosphorus removal (EBPR) by Fuhs and Chen (1975). Subsequently many researchers reported its predominance in EBPR processes based on culture-dependent identification methods (Buchan, 1983; Lötter, 1985; Wentzel et al., 1988). *Pseudomonas* spp. (Lötter, 1984; Li et al.,2003), *Moraxella* spp. (Lötter, 1984), *Aeromonas* spp. (Lötter, 1984; Li et al.,2003), *Klebsiella* spp. (Gersberg et al., 1985), *Pseudomonas cesicularis* (Suresh et al., 1985), γ-*Proteobacteria* (Schuler et al.,2002; Ahn et al., 2002) and some other bacteria (Ahn et al., 2002) were isolated and identified as having phosphorus-removal ability. Over recent years, very intensive research has demonstrated that no pure cultures of *Acinetobacter* spp. have shown the typical characteristics of EBPR sludges with high P-removal capability (Jenkins et al., 1991; Van Loosdrecht et al., 1997) though there are some different reports (Lin et al.,2003; Okunuki et al.,2004). In fact, the poly phosphate-accumulating organisms (PAOs) in activated sludge are not a sole species and are considered as a group of micro-organisms capable of accumulating phosphate. In the traditional pure-culture methods, only those bacteria that are cultivable on the artificial medium used under the defined conditions can be isolated and identified (Mino et al., 1998). It is likely that only a minor portion of bacteria in activated sludges can grow under such conditions and can thus be detected (Wagner et al., 1993, Kämpfer et al., 1996). Since single pure-culture is not sufficient to determine the predominant bacteria in the EBPR process, micro-organisms responsible for EBPR need further investigation.

To determine PAOs that are responsible for aerobic phosphorus removal in the EBPR process, we adapted the polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) method that has been widely recognised as a powerful tool in the study of microbial ecology. DGGE is based on the electrophoresis of PCR-amplified 16S ribosomal DNA-V3 fragments in polyacrylamide gels containing a linearly increasing denaturant gradient (Muyzer et al., 1993). Different DGGE bands are separated depending on the melting behaviour of the PCR products with the same length but with different sequences, which correspond to the species. Therefore, PCR-DGGE assay allows us to analyse the microbial composition and diversity of a given system without the need to isolate individual species (Muyzer et al., 1995). It is particularly effective in analysing the microbial community structure of micro-organisms responsible for EBPR.

However, no single method is sufficient to analyse complex communities present in the biological wastewater treatment processes as quantitative and detailed information of the total community is necessary to characterise the process performance. The purpose of this study is to characterise PAOs in a sequencing batch reactor (SBR) to analyse the change in microbial community structure and to identify PAOs that perform EBPR by using PCR-DGGE.

Experimental materials and methods

Materials

Wastewater and sludge were taken from Lie-De Municipal Wastewater Treatment Plant (aerobic basin), Guangzhou, China.

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