WATER RESEARCH 42 (2008) 3552–3560

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Microbial population dynamics during aerobic sludge granulation at different organic loading rates

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ARTICLE INFO

Article history:
Received 12 December 2007
Received in revised form
19 April 2008
Accepted 7 May 2008
Available online 17 May 2008

Keywords:
Aerobic granulation
Biological wastewater treatment
Microbial community
PCR–DGGE
Sequencing batch reactor (SBR)

ABSTRACT

Laboratory experiments were carried out to investigate the evolution of the bacterial community during aerobic sludge granulation. The experiments were conducted in three 2.4 L sequencing batch reactors (SBRs) that were seeded with activated sludge and fed with glucose-based synthetic wastewater. Three different influent organic concentrations were introduced into the three SBRs, R1, R2 and R3, resulting in chemical oxygen demand (COD) loading rates of 1.5 (R1), 3.0 (R2) and 4.5 (R3) kg/m^3 d, respectively. Changes in bacterial diversity throughout the granulation process were monitored and analysed using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) techniques. The experimental results demonstrate that glucose-fed aerobic granules could be formed without significant presence of filamentous bacteria. Granules formed at different loading rates had different morphology, structural properties and bacterial species. A higher loading rate resulted in faster formation of larger and loose granules, while a lower loading rate resulted in slower formation of smaller and more tightly packed granules. The biomass underwent a dynamic transformation in terms of bacterial species richness and dominance during the granulation process. The reactor with the highest substrate loading rate had the lowest species diversity, while the reactor with the lowest substrate loading rate had the highest species diversity. Different dominant species of *β*- and *γ*-*Proteobacteria* and *Flavobacterium* within the granule communities from the three different SBRs were confirmed by analysis of 16S rDNA sequences of the PCR products separated by DGGE. It is apparent that a few common bacterial species play an important role in the formation and growth of aerobic granules and help sustain the granular sludge structure in the bioreactors.

1. Introduction

Aerobic sludge granulation transforms microbial flocs into granules, which may eliminate the biomass-effluent separation problems that occur frequently in biological wastewater treatment. Because of attributes such as its compact structure and fast settling velocity (Morgenroth et al., 1997; Beun et al., 1999; Dangcong et al., 1999; Arrojo et al., 2004), granular sludge allows a high level of biomass enrichment and hence a much higher organic loading rate in bioreactors. Thus, aerobic granulation has a potential role in the development of novel, compact and high-rate biological wastewater

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doi:10.1016/j.watres.2008.05.005

treatment systems (Moy et al., 2002). However, unlike biofilms or anaerobic granules, aerobic granules are a newly acknowledged microbial structure, and understanding is lacking about the mechanisms of aerobic granulation (Liu and Tay, 2004). Change in the microbial community of the sludge is expected to be essential to granule formation. However, bacterial population dynamics during the granulation process under different operating conditions have not been well characterised. The correlation between the rate of aerobic granulation and the change in the microbial community during granule formation also needs to be further investigated.

A number of factors, such as the type of organic substrate, the loading rate, aeration intensity and hydraulic washing rate, influence the sludge granulation process (Liu and Tay, 2002, 2004; Liu et al., 2003). Operational adjustments can be made to create a more favourable environment for the selection and growth of granule-forming species in a bioreactor. The organic loading rate is believed to be essential to sludge granulation, and granule formation has been achieved in a chemical oxygen demand (COD) loading range between 1 and 15 kg/m^3 d (Arrojo et al., 2004; McSwain et al., 2004; Liu and Tay, 2004; de Kreuk et al., 2005). However, given the same substrate fed into the reactors, it is unclear whether the granules formed at different loading rates will have the same microbial communities and structural properties.

DNA-based molecular techniques offer a valuable tool for the characterisation of the bacterial population diversity in biological wastewater treatment systems. Molecular analysis of activated sludge has revealed the effect of the feed strength and operating conditions on the population structure of the biomass (Eichner et al., 1999; Stamper et al., 2003). Although the bacterial diversity of aerobic granules has been studied using DNA-based molecular tools (Zhuang et al., 2005), the dynamics of microbial evolution during the sludge granulation process remain to be characterised. In the present experimental study, aerobic sludge granules were cultivated in sequencing batch reactors (SBRs) under different organic loading conditions. The morphology and structural characteristics of the granules were examined. Changes in bacterial diversity throughout the granulation process were analysed using polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), and subsequent sequencing of selective DNA bands. The aims of the laboratory study were to investigate the effect of the organic loading rate on the formation of aerobic granules to reveal the evolution in the microbial population during the granulation process at different organic loading rates, and to characterise the formation rate and structural features of the granules formed under different loading conditions.

2. Materials and methods

2.1. Experimental set-up and SBR operation

Three identical columns (6 cm in diameter and 80 cm in height) with a working volume of 2.4 L each were used as SBRs for the sludge granulation experiments. The reactors were supplied with an airflow rate of 4.0 L/min during the aeration phase. The three reactors were operated in a sequential mode for a 4-h cycle with 4 min of feeding and 4 min of effluent withdrawal from the middle ports of the columns. The sludge settling time was reduced gradually from 20 to 2 min after 120 SBR cycles in 20 days, and the aeration time was increased accordingly from 212 to 230 min. Activated sludge from a full-scale sewage treatment plant (Stanley Sewage Treatment Works, Hong Kong) was used as the seed sludge for the reactors at an initial sludge concentration of 3000 mg/L in mixed liquor suspended solids (MLSS). The reactors were fed with synthetic wastewater that consisted of glucose and other nutrients according to the chemical composition given by Tay et al. (2002). Three different organic concentrations—500, 1000 and 1500 COD mg/L—were fed into the influents of three SBRs, R1, R2 and R3, resulting in COD loading rates of 1.5 (R1), 3.0 (R2) and 4.5 (R3) kg/m^3 d, respectively. $NaHCO_3$ was dosed into the feeding solution to maintain the reactor pH in the neutral range between 7.0 and 8.0. The reactors were operated at room temperature, and the water temperature was 20–22 °C.

2.2. Analytical methods

The COD concentration, sludge MLSS concentration, sludge volume index at 10 min (SVI_{10}) and effluent suspended solids (ESS) were measured regularly according to the Standard Methods (APHA-AWWA-WEF, 1998). The morphology of the sludge flocs and granules in the reactors was observed under an optical microscope (BX60, Olympus, Tokyo, Japan) equipped with a digital camera (Infinity 3, Lumenera Scientific, Ottawa, Canada). Photographs of aerobic granules were also taken with a digital camera (Kodak V530, Kodak, Rochester, NY, USA) for size measurement. The projected images of the granules were analysed for their shape factor and roughness using a computer-based image analysis system (analySIS 3.1, Olympus Soft Imaging Solutions, Germany). The shape factor of a granule was calculated as the ratio of its minimum diameter to its maximum diameter. The granule roughness was determined from the ratio between the actual boundary of the granule image and the perimeter of a circle that covers the same area of the granule. In addition, the microstructure of mature granules was examined with a scanning electron microscope (SEM) (Cambridge S440, Oxford Instruments, Cambridge, UK) following the sample treatment procedure detailed by Diao et al. (2004).

2.3. DNA extraction, polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE)

The genomic DNA of the biomass in sludge and granule samples was extracted following the protocol described by Zhuang et al. (2005) using a beadbeater (Mini-beadbeater™, Biospec, Bartlesville, OK, USA) and a microcentrifuge (MiniSpin plus®, Eppendorf, Hamburg, Germany). Subsequently, the variable V3 region of the bacterial 16S rDNA gene sequence (corresponding to positions 341–534 of *Escherichia coli* sequence) was amplified by PCR (Muyzer et al., 1993). The forward primer sequence was 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′,