Characterization of sulfate-reducing granular sludge in the SANI® process

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Abstract

The Sulfate reduction, Autotrophic denitrification and Nitrification Integrated (SANI) process is a sulfur cycle-based biological carbon and nitrogen removal process that has been developed in Hong Kong to close the loop between the hybrid water supply and saline sewage treatment. The major bioreactor of this new technology is a Sulfate-Reducing Up-flow Sludge Bed (SRUSB) that convers organics and provides the electron donor for subsequent autotrophic denitrification. In order to minimize footprint and maximize operation resilience granulation of sulfate reducing bacteria (SRB) in the SRUSB was recently conducted. This study investigates the biological and physicochemical characteristics of the granular sulfate-reducing sludge. A lab-scale granular sludge SRUSB reactor was operated with synthetic saline sewage for 368 days. At 1 h nominal hydraulic retention time (HRT) and 6.4 kg COD/m^3-d organic loading rate, the SRUSB reactor achieved 90% COD and 75% sulfate removal efficiencies. Granular sludge was observed within 30 days, and became stable after 4 months of operation with diameters of 400−500 mm, SVI_5 of 30 ml/g, and high cohesion resisting breakage with a shear force G > 3400 s^{-1}. Pyrosequencing analysis of the 16S rRNA gene in the sulfate-reducing granules on day 90 indicated that the microbial community consisted of diverse SRB genera, namely *Desulfobulbus* (18.1%), *Desulfobacter* (13.6%), *Desulfomicrobium* (5.6%), *Desulfosarcina* (0.73%) and *Desulfovibrio* (0.6%). These genera accounted for 38.6% of total operational taxonomic units at genus level and no methanogens were detected. The microbial population and physicochemical properties of the granules explained clearly the excellent performance of the granular SRUSB reactor.

Introduction

The use of seawater for toilet flushing has been practiced in Hong Kong since 1950s (Tang et al., 2007), currently serving 80% of its 7 million inhabitants. This application has helped to relieve its serious water stress problem by saving 750,000 m^3/day, or 22% of freshwater demand, primarily imported from Dong-jiang River of Guangdong Province of China (Leung et al., 2012). This offers an economic and sustainable alternative water resource for water supply and sanitation in water-scarce coastal cities (Chen et al., 2012). Saline sewage resulting from seawater toilet flushing possesses a typical sulfate to chemical oxygen demand (COD) ratio of 0.42 mg S/mg COD, providing an opportunity for sulfur-cycle based treatment as developed in the Sulfate reduction, Autotrophic denitrification and Nitrification Integrated (SANI®) process (Wang et al., 2009) In comparison to conventional biological wastewater treatment processes with sludge incineration, the SANI® process can save approximately 35% of the energy consumption and reduce 36% of the greenhouse gas emissions (Lu et al., 2011). As a result, the combined seawater toilet flushing and SANI® process provides a sustainable new opportunity for urban water management in coastal cities.

Within the SANI® system, a sulfate-reducing up-flow sludge bed (SRUSB) bioreactor is a crucial component. It is responsible for the removal of organics and supplies the electron donors necessary for the subsequent denitrification. The SRUSB performance relies on the content of the sulfate-reducing bacteria. A high concentration of SRB is always desirable because it can mitigate the impact of fluctuations in temperature and/or pH as well as improve the treatment capacity. Granulation of the SRB allows integration of active biological removal and solid-liquid separation. In addition, it provides a better collective defense against environmental impacts on the sludge, allowing the system to operate with higher volumetric loading rates (McHugh et al., 2003).

Although sulfate reduction has many characteristics resembling anaerobic fermentation processes, little literature could be found about the immobilization and granulation of sulfate-reducing bacteria (SRB) in sulfidogenic systems (O'Reilly and Colleran, 2006), nor the time required for SRB to form a biofilm (Visser, 1995). Therefore, granulation of SRB is timely sought. We have recently explored the acclimation and granulation process of a SRUSB reactor (Hao et al., 2013). This paper focuses on the physiochemical properties of sulfate-reducing granular sludge and its microbial community. The results from this work assist in providing a clear understanding of the SRB granules.

Results and discussion

Reactor performance

The 2.85 L lab-scale SRUSB reactor was continuously operated for more than 300 days. The operation of the reactor was divided into four stages in terms of HRT and organic loading rate with the HRT reduced from 6 h initially (1.04 kg-COD/m^3.d) to 1 h by day 111 (6.4 kg-COD/m^3.d). Throughout the study period organic and sulfate removal efficiencies remained around 90 and 70% respectively (see Fig. 1a, b).

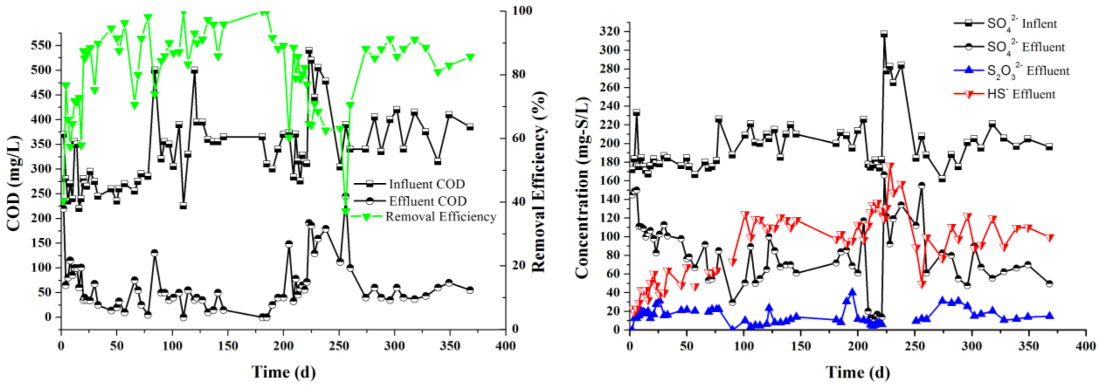

Figure 1. Performance of the SRUSB reactor. (a) influent and effluent COD concentrations and COD removal efficiency; and (b) influent and effluent sulfate concentrations, effluent sulfide and thiosulfate concentrations

A sulfur balance of 80%–116% over the SRUSB operation was obtained based on the sulfate (SO_4^{2-}-S) from the influent, the produced sulfur (sulfide, $S_2O_3^{2-}$-S) and residual sulfate in the effluent.

Characterization of the SRB granules

Settling properties

The change in granular sludge size during the granulation process was recorded, as shown in Figure 2. Granules gradually increased in size from an initial mean diameter of 44±18 μm to a peak mean diameter of 916±256 μm after 4 months. The granular size then decreased to 420±142 μm and maintained at this level. Such phenomenon was probably caused by the self-optimization of the granular size due to substrate diffusion limitations, especially when the diameter of the granules was larger than 400 μm (Lin et al., 2005).

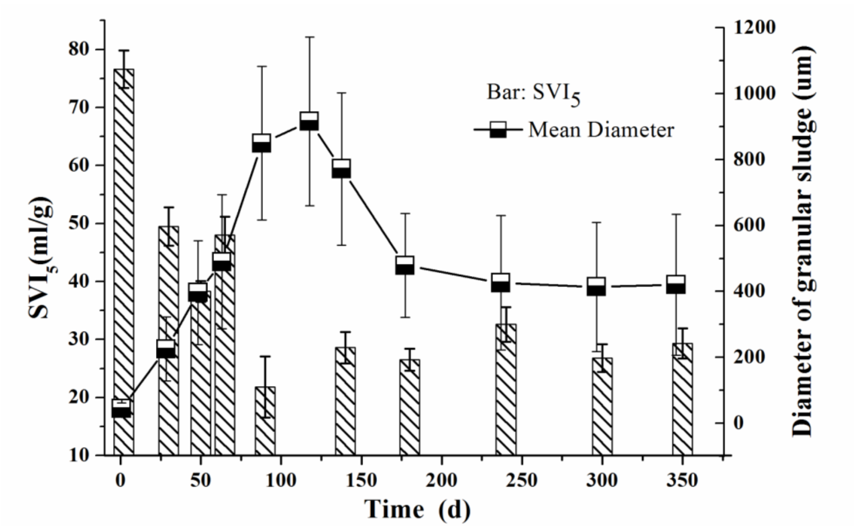

Figure 2. Particle size distribution and SVI_5 of the sludge during granulation. Boxes and **w**hisker plots are the mean and standard deviation values of the particle size distribution while the columns are SVI_5 values.

Granular sludge was well developed with excellent settle ability in terms of SVI_5. The initial SVI_5 of the SRUSB sludge was 78±3 ml/g and decreased to 38±3 ml/g in 50 days (Fig. 2). After 90 days, the SVI_5 and SVI_{30} (data not shown) were close, with a ratio between 1.1 and 1.3, indicating that the sludge bed was predominantly granules (Schwarzenbeck et al., 2005).

Physical strength

The cohesion of an aggregate depends on how a granule can resist shear and elongation forces as well as control surface detachment (erosion) and breakage. This impacts both retention of biomass in the reactor and granule properties such as diameter, shape and density. The SRUSB granules showed a high level of cohesion with no change from the initial particle size distribution (mean diameter 424 µm) when shear force was applied at G values of 250 s^{-1} and 3400 s^{-1} for 45 min each (Fig. 3 phase I and II), indicating that there was no aggregate break-up under these hydrodynamic conditions. A further increase in the impeller rotational speed (phase III: G = 13230 s^{-1}) resulted in an obvious reduction of the particle size to a mean value of 180±97 µm and a new population of small particles less than 90 µm. However, given typical G-values in an aerated activated sludge system range from 80 to 182 s^{-1} (Yoon, 2011) the results indicate not only that the granules are strong enough to resist the applied shear force of operation but that intensive mixing to increase mass transfer could be used to enhance performance.

Following a reduction of the velocity gradient (phase IV: G = 250 s^{-1}) the diameter remained unchanged. This confirms the granular nature of the sludge as flocculent sludge re-aggregates under reduced shear (Wan et al., 2011).

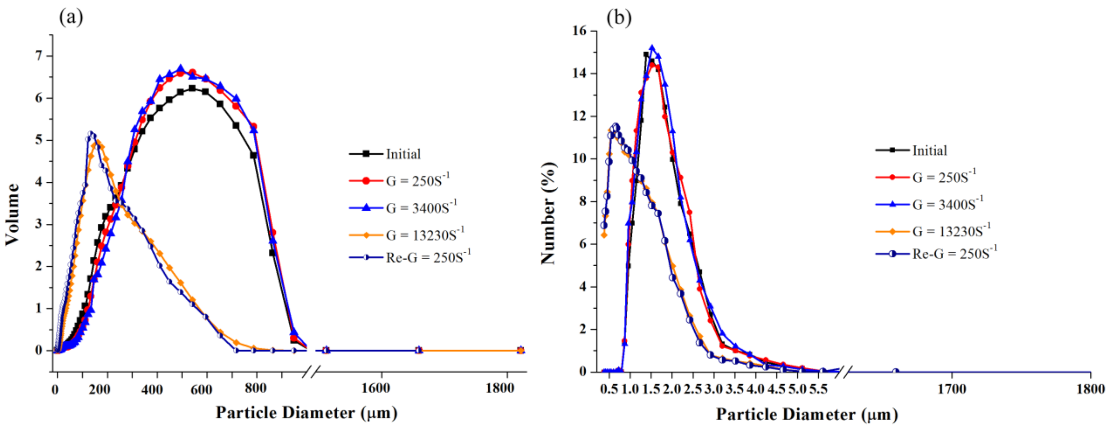

Figure 3 Behaviour of SRB granules when subjected to the cohesion test: (a) PSD by volume and (b) PSD by number.

Phylogenetic analysis and quantification of SRB community

Altogether, 20 bacterial phyla were recovered from the two samples (Fig. 4b). For the seeding sludge, around 90% of the bacterial reads were affiliated with phyla Proteobacteria, Actinobacteria, and Bacteroidetes. Among them, Proteobacteria accounted for 74% of the bacteria community in the seeding sludge. On the other hand, the majority of 16s rRNA gene sequences in the granular sludge at day 90 belonged to phyla Proteobacteria, Firmicutes and Bacteroidetes, which made up to 84% of the total reads. While Proteobacteria was still the major phylum in the granules, the relative abundance in the total bacterial reads had decreased from 74 to 46%. On a finer scale, 37 classes within the 20 phyla were identified for the seeding and granular sludges (Fig. 4c). The bacterial community in the inoculum sample was dominated by Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria and Actinobacteria. In contrast, the bacterial communities in the 90th day granular sludge sample were dominated by Deltaproteobacteria and Bacilli.

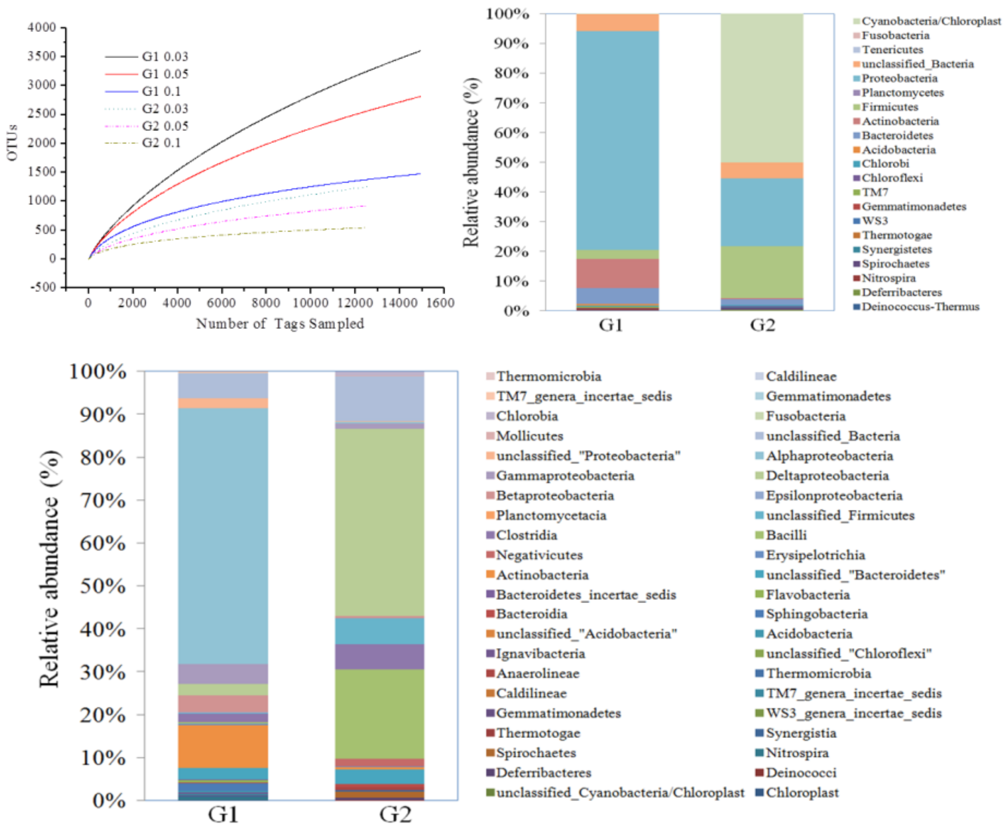

Figure 4. Results of the phylogenetic analysis. (a) Rarefaction curves, G1 – seeding sludge, G2 – granular sludge; (b) Taxonomic classification of bacterial 16S rRNA gene reads retrieved from G1 and G2 at phylum level using RDP classifier with a confidence threshold of 80%; and (c) Taxonomic classification of bacterial 16S rRNA gene reads retrieved from G1 and G2 at class level using RDP classifier with a confidence threshold of 80%.

Most of the sulfate reducers were reported to belong to Deltaproteobacteria (Muyzer et al., 2008). In this study the relative abundance of sequences belonging to Deltaproteobacteria increased considerably with the development of the granules to become the largest class in the granule community (accounting for 43.6 % of total sequences, Fig. 4c), indicating a successful development of SRB within the SRUSB. Six of the ten common reported SRB genera (Warren et al., 2005) within Deltaproteobacteria were identified in the SRB granules, indicating a high diversity of the SRB community. *Desulfobulbus* and *Desulfobacter* were found to be the most dominant SRB genera which accounted for 18.1 and 13.6% of the total bacterial community respectively.

Under anaerobic conditions, organic matter is degraded to CH_4, CO_2, and H_2S via a syntrophic/competitive interaction among fermentative bacteria, acetogens and methanogens, or SRB. In the presence of sulfate, SRB usually out-compete methanogens, while methanogens only dominate in a low-sulfate environment. In our study *Methylocystis* was the only known methanogen identified in the seeding sludge accounting for 2% of total bacteria community. As acclimation proceeded under the high sulfate-to-COD ratio of 0.6 methanogens disappeared completely.

Conclusion

Sulfate-reducing granules were developed in around 30 days in a lab-scale SRUSB. After 4 months of operation, the SRB granules were matured with regular shape, diameters of 400–500 μm, a low SVI_5 of 30 ml/g and the high cohesion resisting breakage with a shear force $G > 3400\ s^{-1}$. Accordingly the SRUSB reactor reached steady state, achieving 90% COD and 75% sulfate removal efficiencies under 1 h HRT and 6.4 kg COD/m^3-d organic loading rate.

454 pyrosequencing revealed that the diversity of the community became simpler with granulation while the relative abundance of SRB related taxa increased and become dominate. A diverse genera of SRBs were identified which may assist in

achieving the high organic removal efficiency of the granules. This study therefore demonstrates that SRUSB granulation is an effective way to enhance SRB population and achieve a stable sludge with high treatment capacity.

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