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

TianWei Hao, Li Wei, Hui Lu, HoKwong Chui, Hamish R. Mackey, Mark C.M. van Loosdrecht, GuangHao Chen

ABSTRACT
Hong Kong practices seawater toilet flushing covering 80% of the population. A sulfur cycle-based biological nitrogen removal process, the Sulfate reduction, Autotrophic denitrification and Nitrification Integrated (SANI®) process, had been developed to close the loop between the hybrid water supply and saline sewage treatment. To enhance this novel process, granulation of a Sulfate-Reducing Up-flow Sludge Bed (SRUSB) reactor has recently been conducted for organic removal and provision of electron donors (sulfide) for subsequent autotrophic denitrification, with a view to minimizing footprint and maximizing operation resilience. This further study was focused on the biological and physicochemical characteristics of the granular sulfate-reducing sludge. A lab-scale SRUSB reactor seeded with anaerobic digester sludge was operated with synthetic saline sewage for 368 days. At 1 h nominal hydraulic retention time (HRT) and 6.4 kg COD/m³-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 μm, SVI₅ of 30 ml/g, and extracellular polymeric substances of 23 mg carbohydrate/g VSS. Fluorescence in situ hybridization (FISH) analysis revealed that the granules were enriched with abundant sulfate-reducing bacteria (SRB) as compared with the seeding sludge. Pyrosequencing analysis of the 16S rRNA gene in the sulfate-reducing granules on day 90 indicated that the microbial community consisted of a diverse SRB genera, namely Desulfobulbus (18.1%), Desulfovibacter (13.6%), Desulfomicrobium (5.6%), Desulfoarcina (0.73%) and Desulfouacibio (0.6%), accounting for 38.6% of total operational taxonomic units at genera level, with no methanogens detected. The microbial population and physicochemical properties of the granules well explained the excellent performance of the granular SRUSB reactor.

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1. Introduction

Seawater toilet flushing has been practiced in Hong Kong since 1958 saving 750,000 m³ freshwater every day (Leung et al., 2012; Van Loosdrecht 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–sulfur to chemical oxygen demand (COD) ratio of 0.42 mg S/mg COD, providing an opportunity to develop the novel Sulfate reduction, Autotrophic denitrification and Nitri- fication Integrated (SANI) process (Lau et al., 2006; Wang et al., 2009; Lu et al., 2009, 2011a,b). As demonstrated in a 225-day pilot plant operation, this new process reduces 90% sludge production, 35% energy consumption and 36% greenhouse gas emission compared with conventional biological nitrogen removal (CBNR) processes (Lu et al., 2011a,b).

In this novel process, the sulfate-reducing up-flow sludge bed (SRUSB) acts as the key bioreactor for more than 80% of organic removal, and providing sufficient dissolved sulfide for subsequent autotrophic denitrification (Lu et al., 2011a,b). Since the doubling time of sulfate-reducing bacteria is at least two days (Keith-Roach and Livens, 2002), the sludge yield of SRUSB is only 0.02 kg volatile suspended solids (VSS) per kg COD reduced (Wang et al., 2011). Therefore, maintaining sufficient sulfate-reducing sludge in the reactor is a challenging and important task to reduce reactor footprint. Granulation of sulfate-reducing sludge in the SRUSB is therefore an ideal solution as it can increase sludge concentration significantly (Hulshoff Pol et al., 2004).

Since granulation was first reported in up-flow anaerobic sludge bed reactors (Lettinga et al., 1980), extensive studies were conducted mainly focusing on granulation of methanogenesis sludge (Visser et al., 1993a). 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). This was because most of the studies were focused on the control and prevention of sulfate reduction in anaerobic granulation processes (Liu and Fang, 1998; Lens et al., 1998; Visser et al., 1993b; O’Flaherty and Colleran, 2000). Thus, granulation of SRB needs to be explored. We have recently explored the acclimation and granulation process of a SRUSB reactor (Hao et al., 2012). This paper mainly reports the behavior of sulfate-reducing sludge, its physicochemical properties and microbial community during the granulation of SRUSB. The results obtained from this work would facilitate development of a compact SRUSB for application of the SANI process in densely-populated coastal cities.

2. Materials and methods

2.1. Experimental set-up and conditions

A lab-scale SRUSB reactor was fabricated from a plexiglass column having an internal diameter of 88 mm and a height of 500 mm, with its top covered by a plastic plate and sealed with silicone rubber. The effective liquid volume of this reactor was 2.85 L with headspace of 0.15 L and it was protected from light. 1.21 L of sludge at 18 g VSS/L, taken from a local anaerobic sludge digester, was seeded into the SRUSB reactor resulting in an initial reactor VSS of 7.7 g/L. The synthetic saline sewage was prepared based on our previous research (Wang et al., 2009). Seawater with an average sulfate concentration of 2.7 g/L was mixed with a mixture of fed stock solution and tap water in a volumetric ratio of 1:4.4, resulting in an average influent concentrations of 330 mg COD/L, 540 mg SO₄²⁻ mg/L (or 180 mg S/L) and 30 mg NH₄-N/L, and pH of 7.3, respectively.

At beginning, the SRUSB was continuously fed with the prepared saline sewage under a relatively low organic loading rate of 1.04 kg COD/m³ d and a HRT of 6 h (Fig. 1) for 23 days. During this period, an up-flow velocity of 0.39 m/h was maintained through a re-circulation rate of 4Q (Q = influent flow). After this initial start-up period, the HRT was shortened to 3 h for 37 days with the re-circulation flow rate increased to 5Q. The HRT was further decreased to 2 h and 1 h from days 60 and 111, respectively, while the re-circulation flow rate remained unchanged. The organic loading rate was increased correspondingly with the reduction of HRT during the start-up period.

2.2. Chemical and physical analysis

In this study all the samples (liquid and sludge) were collected and measured immediately. Prior to the microbial analysis and solids sampling, all the sludge in the reactor was well mixed with a stirring rod. Total and soluble organic COD and sulfate COD in the effluent of the SRUSB were measured according to the Standard Methods (APHA, 2005) and Poinapen et al. (2009), respectively. Sludge volume index in 5 min (SVI₅) was measured with a stirring rod. Total and soluble organic COD and sulfide COD in the effluent of the SRUSB were measured according to the Standard Methods (APHA, 2005) while pH and temperature were measured with multimeter electrode (WTW multi3420).

![Fig. 1 – Hydraulic retention time and organic loading rates.](image-url)
The morphology of granules was examined by using a microscope (Olympus CX41) equipped with a digital camera (Olympus C550D Zoom) and a stereo microscope (OLYMPUS SZH10) respectively. The microstructure and bacterial morphologies of granules were observed with a scanning electron microscope (SEM) (JSM 6300F, JEOL) after fixation of sludge sample overnight at 4 °C by immersion in a 2% paraformaldehyde, 2% glutaraldehyde and 1× phosphate-buffered saline (PBS) mixed solution and subsequent lyophilization. Size distribution of the granules was determined from a laser diffraction particle size analyzer (LS13 320). Extracellular polymeric substances (EPS) were extracted from the granules by applying cation exchange resin (DOWEX5, MARATHON®, MSC, Na form) according to Liu and Fang, (2002). The carbohydrate content in EPS was further determined from the anthrone method (Gaudy, 1962) using glucose as the standard.

2.3. Microbial community analysis

2.3.1. Fluorescence in situ hybridization

The composition of the microbial community was first analyzed by using fluorescence in situ hybridization (FISH) (Manz et al., 1992). The seeding sludge samples (G1) and granule samples (G2), collected on day 0 and day 90 respectively, were fixed overnight in 4% (wt/vol) paraformaldehyde under 4 °C, 10–20 μL of each sample was then spotted onto each well of a slide. Prior to the hybridization, samples were dehydrated by sequential immersion in 50, 80 and 100% ethanol for 3 min and air-dried. The 16S rRNA-targeted oligonucleotide probes (TechDragon, Hong Kong) applied on the FISH images were obtained from a confocal laser scanning microscope (CLSM) (LSM Duo7, Carl Zeiss, Germany) under argon laser (488 nm), DPSS-laser (541 nm) and HeNe-laser (633 nm).

2.3.2. DNA extraction, PCR amplification and pyrosequencing

Bacterial genomic DNA was extracted by using the PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA) terms of HRT and organic loading rate (see Fig. 1): 1) Stage 1 (day 1–23), the HRT, organic loading rate and sulfate loading rate were maintained at 6 h, 1.04 kg COD/m³-d and 0.73 kg SO₄²−S/m³-d during which the COD removal efficiency reached 85% on day 20; 2) Stage 2 (day 24–60), the HRT was reduced to 3 h, with organic loading rate increased to 2.08 kg COD/m³-d correspondingly. Although the organic removal and sulfate reduction efficiencies had once decreased to 75.2 and 39.5% respectively, the efficiencies recovered within 3 days; 3) Stage 3 (day 61–110), the HRT was reduced to 2 h, with organic loading increased to 3.2 kg COD/m³-d; and 4) Stage 4 (day 111–368), the HRT was reduced to 1 h during which the organic and sulfate removal efficiencies remained at around 90 and 70% (see Fig. 2a and b).

Fig. 2b shows that sulfate was reduced to sulfide and a small fraction of thiosulfate in the SRUSB. The effluent sulfide concentration gradually increased from 20 to 80 mg-S/L with SRB acclimation from Stages 1 to 3. Subsequently, the effluent sulfide concentration was maintained at around 115 mg-S/L, except during substrate adjustment on day 250. On the other hand, the effluent thiosulfate level remained relatively stable.
at around 20 mg-S/L throughout the entire experiment (Fig. 2b). A sulfur balance of 80–116% over the SRUSB operation was obtained based on the sulfate (SO$_4^{2-}$-S) from the influent and the produced sulfur (sulfide, S$_2$O$_3^{2-}$-S) and residual sulfate in the effluent. Overall, the effluent COD, sulfate, sulfide and thiosulfate concentrations became stable at the end of Stage 3. In Stage 4, the average COD reduction was 270 mg/L while the sulfide and thiosulfate production were 115 mg-S/L and 20 mg-S/L respectively. This indicated that 93% of the stoichiometric electron balances between COD and sulfur were achieved in this stage, or biologically 93% COD being consumed by SRB (Wang et al., 2009; Siu, 1999). The remaining COD could possibly be converted to CO$_2$ directly through heterolactic fermentation as described by Anestis (2006), instead of methanogenesis. This hypothesis is supported by the results of pyrosequencing microbial analysis showing that no methanogens were detected while a small amount of fermentation CO$_2$ producing Lactococcus (1.8% of the total sequences) was found.

### 3.2. SRB granular sludge formation

The seeding sludge flocs had an average diameter of 44 μm (see Fig. 3a). The sludge flocs in the SRUSB (see Fig. 3b) were examined after 7 days of inoculation. Although the sludge bed became dense in the first month of operation, fine sludge flocs were still washed out, resulting in an average total suspended solids (TSS) of 141 ± 8 mg/L in the SRUSB effluent. Granules appeared in around 30 days, as shown in Fig. 3c, much earlier than the reported 2–4 months for granulation of methanogenic bacteria (Liu and Tay, 2004).

The majority of the SRB granules in the reactor was of regular shape with compact structure and a porous but smooth surface (Fig. 3d and e) after 3 months of operation. The morphology of SRB granules was consistent with those reported for slowly growing aerobic granules (De Kreuk and Van Loosdrecht, 2004). During the early granulation stage, a small amount of large, fast-developing granules with a diameter of 2–3 mm was found at the top layer of the sludge bed. These granules disappeared after day 60. Visual and microscopic observations (Fig. 3f and g) showed that these large granules had a hollow internal structure and dense outer layer dominated by filamentous bacteria.

The change in granular size during the granulation process was recorded, as shown in Fig. 4. Granules gradually increased in size from an initial mean diameter of 44 μm and standard deviation (S.D.) of 18, to a mean diameter of 225.2 μm and S.D. of 97 on day 30. The mean diameter peaked at 916 μm with S.D. of 256 after 4 months, during which the maximum size of granules was found to be 1.5–2 mm, though they are still small compared with that of typical methanogenic granules (>2 mm) (Fukuzaki et al., 1990; Yu et al., 1999). The granular size then decreased to a mean diameter of 420 μm due to gradual disintegration of larger particles. This 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; Li et al., 2008), resulting in bacterial lysis in the centre (Alphenaar, 1994), forming large pores, trapping gas and washing out.

Granular sludge had excellent settleability, as measured by SVI$_5$. The initial SVI$_5$ of the SRUSB sludge was 78 ± 3 ml/g, then decreased to 38 ± 3 ml/g in 50 days (Fig. 4). 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 by granules (Schwarzenbeck et al., 2005). The SVI$_5$ maintained at about 30 ml/g, corresponding to a VSS/MLSS ratio of 0.72 ± 0.04. This SVI$_5$ was lower than typical granular sludge of 40–60 ml/g (Henze et al., 2008), meaning that they were more compact, hence developing a more compact sludge bed.

Extracellular Polymeric Substances (EPS) are important materials to maintain the matrix structure and stability of anaerobic granules (Liu et al., 2004). It comprises polysaccharides, proteins and glycoproteins, etc. (Wingender et al., 1999). Failure of microbial aggregation had been reported to be related to the metabolic blocking of EPS secretion (Yang et al., 2004; Hwang et al., 2006). Moreover, salt stress can seriously hamper the performance of an
anaerobic system (Rinzema, 1988), as high salt level may inhibit activities of various enzymes (Rene et al., 2008). As the SANI process was developed to handle saline sewage, EPS monitoring in terms of carbohydrate per g of SRB granule (in VSS) over the 200-day granulation period was therefore measured, as shown in Fig. 5. EPS increased gradually from 12.5 to 25.6 mg/g VSS in 95 days. After full granulation was achieved, i.e. the ratio of SVI₅ to SVI₃₀ close to unity, the EPS became stable at 22 mg/g VSS, which was similar to that of hydrogen-producing granular sludge (Fang et al., 2001), but lower than that of methanogenic granules (23–42 mg/g VSS) (Batstone and Keller, 2001). Nevertheless, it was much greater than that of high sodium-laden wastewater treating anaerobic granules (0.03 mg/g VSS) (Ismail et al., 2010). Therefore, the EPS secretion of SRB granules did not appear to be suppressed by the 2.5 g Na/L saline sewage fed into the SRUSB.

Fig. 3 – Microscope images of SRUSB sludge. (a) Seeding sludge, bar = 500 μm; (b) Small flocs on day 7, bar = 200 μm; (c) Granules on day 30, bar = 500 μm; (d) Granules on day 90, bar = 500 μm; (e) Granule on day 90; (f) Fast-developing granule on day 66; and (g) Surface of fast-developing granules. Images a–d are taken under inverted microscope while images e–g are taken under SEM.

Fig. 4 – Particle size distribution and SVI₅ of the sludge during granulation. Boxes and whisker plots are the mean and standard deviation values of the particle size distribution while the columns are SVI₅ values.
3.3. Biological characterization of SRB granules

3.3.1. SRB community analysis by fluorescence in situ hybridization

The composition of bacterial community in the seeding and granular sludge was analyzed using FISH with combined probes for total bacteria and SRB population (Fig. 6). The fluorescence signals from cells hybridized with SRB385 probe increased in the 90th day granules (Fig. 6b and d), indicating that the SRB bacteria were enriched during the granulation process. The details on the species composition of the bacterial communities along the granulation were further analyzed through pyrosequencing.

3.3.2. Phylogenetic analysis and quantification of SRB community

Approximately 15,000 raw pyrosequencing reads of the 16S rRNA gene were obtained from the inoculums (G1) and the 90th day granular sludge (G2) samples. About 3000 reads were filtered and the remaining 12,000 reads with average size of 398 bp (G1) and 394 bp (G2) were subjected to subsequent analysis.

Rarefaction analysis, based on OTUs at 3%, 5% and 10% dissimilarity, indicated that the recovered sequences well represented the diversity of the microbial communities in the two samples as the rarefaction curves were approaching plateaus with the 91% and 94% coverage under 3% dissimilarity for G1 and G2, respectively (Fig. 7a). In total, 2680 and 1498 OTUs were retrieved from G1 and G2 at 3% dissimilarity level (Supplementary Information, SI. Tables 1 and 2). The estimated species richness of samples was determined by the Chao 1 richness estimator (Chao, 1984). The Chao 1 richness values decreased from 5490 OTUs in the seeding sludge to 2666 OTUs in the 90th day granules (at 3% dissimilarity) (SI. Tables 1 and 2).

Fig. 5 – Changes in extracellular polymeric substances during granulation.

Fig. 6 – Microbial consortium observed under FISH. (a) Seeding sludge – showing SRB detected by Cy3-labeled probe SRB385 (red) and other bacteria detected by fluorescein-labeled EUB mix (green); (b) Seeding sludge – showing only SRB385 probe; (c) SRB granule – showing SRB detected by Cy3-labeled probe SRB385 (red) and other bacteria detected by fluorescein-labeled EUB mix (green); and (d) SRB granule – showing only SRB385 probe. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
 Altogether, 20 bacterial phyla were recovered from the two samples (Fig. 7b). 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. The remaining 26% mainly belonged to Actinobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chlorobi, Nitrospira, TM7 and an unclassified group. On the other hand, the majority of 16s rRNA gene sequences of the 90th day granular sludge 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 drastically from 74 to 46%. Similar to Proteobacteria, the relative abundance of phylum Actinobacteria had also decreased. However, phylum Firmicutes had increased 10 fold, amounting to 35% of the total reads of the granules (Fig. 7b).

On a finer scale, the 16s rRNA sequences belonging to 37 classes within the 20 phyla were identified for the seeding and granular sludge (Fig. 7c). The bacterial community in the seeding sludge sample was dominated by Deltaproteobacteria, Bacilli in Proteobacteria and Firmicutes. The proportion of Alphaproteobacteria, Actinobacteria, Betaproteobacteria and Gammaproteobacteria dropped sharply from 59.7, 9.8, 4.0 and 4.5% to 0.4, 0.6, 0.4 and 1.2%, respectively. The abundance of Deltaproteobacteria, Clostridia and Bacilli increased from 2.7, 1.9 and 0.4% to 43.6, 5.9 and 20.9%, respectively, making them the dominant populations in the granules. In addition to those common classes in both samples, 8 classes disappeared (e.g. Nitrospira, Ignavibacteria, Gemmatimonadetes, etc.) with the development of the granules (Fig. 7c). The results indicate a remarkable shift of bacterial communities from the seeding sludge to granular sludge. Such changes are possibly due to the bacteria aggregation acclimation selection (Zhang et al., 2001). In particular, the increase in the relative abundance of Deltaproteobacteria, a known sulfate reducer (Muyzer and Stams, 2008), in the granular community (account for 43.6% of total sequences, Fig. 7c), indicated the successful development of SRB community in the SRUSB reactor. The presence of six out of ten reported SRB genera (Warren et al., 2005) within Deltaproteobacteria being identified in the SRB granules (see Table 1), also indicated that the granule has a high diversity of the SRB community.

Fig. 7 – 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%.
The high diversity of SRB communities was believed to be the key to high organic removal efficiency of the SRUSB reactor. Fang et al. (1995) reported that the microstructure and composition of the methanogenic granules were highly dependent on the substrates, i.e. the granular microstructure being more complex as the substrates became more complex due to the need for transferring substrate among syntrophic microorganisms (Gujer and Zehnder, 1983). Similarly, a complex microbial consortium was also observed in the SRB granules. In the SRB granules, Desulfobulbus, Desulfofabaer and Desulfolonicrobium were found to be the most dominant SRB genera which accounted for 18.1%, 13.6% and 5.6% of the total bacterial community in granular sludge, respectively. Although the dominant bacteria in these genera can only achieve incomplete oxidation of organic matters (Cord-Ruwisch et al., 1987), the interactions of the diverse genera of SRB bacteria have resulted in high COD removal efficiency of the SRB granular sludge. Indeed, bacteria in the genus Desulfobulbus can consume glucose, propionate and pyruvate, to acetate (Suzuki et al., 2007), the genus Desulfobacter was an efficient acetate-utilizing group (Rabus et al., 2006), whereas Desulforhizobium (5.6%) can effectively oxidize many organic matters (Barton and Tomei, 1995), including the utilization of lactate, pyruvate, glycerol, acetate and ethanol, with sulfate, thiosulfate, or sulfate as electron acceptor (Liu et al., 1999). The large diversity of SRB genera found thus explains the high COD removal efficiency of the granules.

The competition between sulfate reducers for sulfate has remarkably with the development of the granule, as shown by the high diversity of the SRB community in the SRUSB reactor. Laanbroek et al. (1984) reported that Desulfofabaer had higher affinity for sulfate and higher growth rate than other SRB genera such as Desulfobulbus and Desulfofabaer. However, the sequences belonging to genera Desulfobulbus and Desulfofabaer were found to dominate in the granular sludge of SRUSB, while only a small fraction of Desulfofabaer was found (Table 1). The failed competition of Desulfofabaer during granulation was probably due to extreme halophilism of Desulfofabaer and substrate types (Caumette et al., 1991). It was possible that due to the suppression of Desulfofabaer during granulation, other SRB genera were able to develop and gradually led to a diverse SRB community in the granular sludge as we observed (Table 1).

Under anaerobic conditions, organic matter is degraded to CH4, CO2, and H2S via a syntrophic/competitive interaction among fermentative bacteria, acetogens, methanogens, and SRB (Shabir et al., 2008). In the presence of sulfate, SRB usually out-competes against methanogens (Ward and Winfrey, 1985). Methanogens only dominate in a low-sulfate environment (Stams 1994). In our study, methanogens, Methylocystis, accounted for 2% of total bacteria community in the seeding sludge (Table 1). As acclimation proceeded under the high sulfate-to-COD ratio of 0.6 (Wang et al., 2011), methanogens disappeared completely.

In the SRB granules, the genus Trichococcus accounted for 12.5% (Table 1) of the bacterial community, playing a key role in organic reduction in the SRUSB reactor. Trichococcus is able to degrade the complex organic compounds, which are usually difficult for SRB to utilize, into simple organics such as lactate, formate, acetate and ethanol, making them favorable substrates for SRB (Liu et al., 2002; Révézó, 2009). Moreover, Trichococcus has been reported to be active in a wide range of temperature from -5 to 35 °C (Pikuta et al., 2006), which suggested a possible application of the SRB granules for treating wastewaters with high temperature fluctuations. Further studies are needed to validate the ability of the SRB granules developed in this study so as to evaluate its applicability in different wastewater sources.

### Table 1 – Relative abundance of sequences belonging to important functional genera.

<table>
<thead>
<tr>
<th>Sludge sample</th>
<th>G1 – Day 0 (Seeding Sludge) (%)</th>
<th>G2 – Day 90 (Granules) (%)</th>
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<tbody>
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<td>Sulfate-reducing related genera</td>
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<tr>
<td>Desulfobulbus</td>
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<tr>
<td>Desulfobacter</td>
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<td>0.1</td>
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<tr>
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</tr>
<tr>
<td>Trichococcus</td>
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</table>

4. Conclusions

Sulfate-reducing granules were developed in around 30 days and became more compact with time. After 4 months of operation, the SRB granules became stable, regular in shape, with diameters of 400–500 μm and SVI50 of 30 ml/g. They contained EPS of 23 mg carbohydrate/g VSS. Accordingly the SRUSB reactor reached steady state, achieving 90% COD and 75% sulfate removal efficiencies under 1 h HRT and 6.4 kg COD/m³-d organic loading rate.

The fluorescence signals for the SRB population increased remarkably with the development of the granule, as shown by FISH analysis. 454 pyrosequencing further revealed that the diversity of the community became simpler with granulation. The relative abundance of SRB related taxa increased and became dominate. These results indicated that granulation in SRUSB system was efficient in enriching the SRB population. The SRB community in the granule consisted of diverse genera, which was possibly the reason for the high efficiency of the granule in removing organic matter. Co-existence of
non-SRB genera such as Trichococcus in the bacterial community further improved the performance of the SRB granules.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2013.07.052.

REFERENCES


