Granulation of anaerobic sludge in the sulfate-reducing up-flow sludge bed (SRUSB) of SANI® process

T. Hao, H. Lu, H. K. Chui, Mark C. M. van Loosdrecht and G. H. Chen

ABSTRACT

This study reports on anaerobic sludge granulation in a laboratory-scale sulfate-reducing up-flow sludge bed (SRUSB) in a novel sulfate reduction, autotrophic denitrification and nitrification integrated (SANI®) process for treatment of saline sewage. Granulation occurred in 30 d and reached full development in 90 d. The sulfate-reducing granules grew up to around 1 mm after 90 d with 21 mL/g SVI_5 (sludge volume index measured after 5 min) and the biomass concentration reached 29 g/L after 4 months' operation. The reactor removed 89% chemical oxygen demand (COD) and reduced 75% sulfate within 1 h of hydraulic retention time, under a COD loading rate of up to 6.4 kg COD/($m^3 \cdot d$).

Key words | anaerobic sludge granulation, saline wastewater, SANI process, SRUSB, sulfate reduction

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INTRODUCTION

A novel sulfate reduction, autotrophic denitrification and nitrification integrated (SANI®) process for treatment of saline sewage resulting from seawater toilet flushing has been recently developed (Lau *et al.* 2006). It has been shown to have minimal biological sludge production through both laboratory and pilot studies (Wang *et al.* 2009; Lu *et al.* 2011, 2012a). The sulfate-reducing up-flow sludge bed (SRUSB) for sulfate reduction plays a crucial role in organic conversion and provision of dissolved sulfide for subsequent autotrophic denitrification using sulfide as an electron donor. However, due to relatively low suspended biomass concentration (~8 g/L mixed liquor suspended solids) in the current SRUSB, there is a need to improve its design and operation towards greater compactness and resilience against shock loadings, such as temperature, pH and hydraulic loads. Granulation of sulfate-reducing sludge in an SRUSB could provide a possible solution. Although use of anaerobic granules for treating sulfate-laden industrial wastewater and heavy metal at a hydraulic retention time (HRT) of 5 h in laboratory-scale up-flow reactors has been reported (La *et al.* 2003), these reactors were not targeted for high organic and hydraulic loadings. Moreover, the organics removal was limited (below 50%) (Goncalves *et al.* 2005). This study focused on the acclimation period and granular sludge development of an SRUSB with a short HRT and very high chemical oxygen demand (COD) loading.

MATERIALS AND METHODS

Experimental setup

A laboratory-scale SRUSB reactor was set up as shown in Figure 1. The reactor had an internal diameter of 88 mm and a height of 500 mm, with both ends covered with plastic plates sealed by silicone rubber. The effective liquid volume was 2.85 L and the headspace was 0.15 L.

Anaerobic digester sludge from a local saline sewage treatment works was used as seeding sludge after sieving through a 180-μm sieve. The SRUSB reactor was operated at 20 °C, with an internal recirculation rate of 4–5 Q (influent flow rate) in order to maintain good mixing conditions in the reactor.

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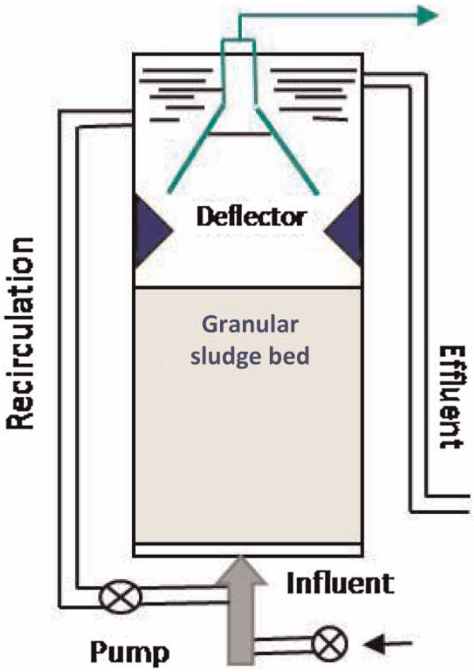

Figure 1 | Schematic of laboratory-scale SRUSB reactor.

Synthetic saline wastewater

An organic stock solution was prepared according to our previous research (Lau *et al.* 2006) with the main components of glucose (19.57 g/L), sodium acetate (26.10 g/L), yeast extract (9.79 g/L), NH_4Cl (18.37 g/L), K_2HPO_4 (1.92 g/L), KH_2PO_4 (0.72 g/L) and trace metals: $FeCl_3{\cdot}6H_2O$ (2,000 mg/L), H_3BO_3 (200 mg/L), $CuSO_4$ (50 mg/L), KI (80 mg/L), $MnSO_4{\cdot}H_2O$ (250 mg/L), $ZnSO_4{\cdot}7H_2O$ (150 mg/L) and $CoCl_2{\cdot}6H_2O$ (200 mg/L), respectively (Lau 2005). To simulate the characteristics of Hong Kong's saline sewage in terms of salinity and sulfate concentration, seawater (with an average sulfate concentration of 2,700 mg/L) was mixed with the stock solution and tap water in a liquid volume ratio of 1:4.4 to achieve the desired average influent COD, sulfate and ammonia nitrogen levels of 330 mg COD/L, 540 mg SO_4^{2-} mg/L (or 180 mg S/L) and 30 mg N/L respectively.

Sample analysis

The experiment was conducted in four stages by varying the COD loading, up-flow velocity and HRT, as shown in Table 1. During the whole experiment, the influent and effluent of the reactor were sampled regularly. The effluent total soluble COD (soluble organic COD + sulfide COD) was measured according to *Standard Methods* (APHA 2005) after sample filtration using 0.45-μm cellulose filter paper (Millipore). Total organic COD was determined after sulfide had been removed by adding excess zinc sulfate ($ZnSO_4$) to samples to precipitate out dissolved sulfide as zinc sulfide (ZnS). Three droplets of 10 M NaOH solution were then added to increase the pH to precipitate residual Zn^{2+} as $Zn(OH)_2$, followed by centrifugation at 3,500 rpm for 5 min and vacuum filtration with a 0.45-μm cellulose membrane filter paper (Millipore). The filtrate was then analyzed for total soluble organics concentration (Poinapen *et al.* 2009). Nitrite, nitrate and thiosulfate were measured by using an ion chromatograph (Shimadzu Corporation, HIC-20A super) equipped with a conductivity detector and an IC-SA2 analytical column. Ammonium nitrogen was determined by using a flow injection analyzer (QuikChem +8000 Series). Dissolved sulfide was measured by the methylene blue method (APHA 2005). Suspended solids, sludge volume index after 5 min (SVI_5) and pH were determined according to *Standard Methods* (APHA 2005). Size distribution and morphology of the sludge were analyzed by using a laser diffraction particle size analyzer (LSI3 320), and a scanning electron microscope (SEM) (JSM 6300F, JEOL) and stereo microscope (Olympus SZH10), respectively. SEM-coupled energy-dispersive X-ray spectroscopy (EDAX) was performed for the elemental analysis with an xFlash detector 4010 (Bruker). Alkalinity was measured by using the five point titration method (Moosbrugger *et al.* 1992; Neytzell-De Wilde *et al.* 1977).

Table 1 | Operating conditions at different stages

Operating parameters	Stage 1	Stage 2	Stage 3	Stage 4
HRT (h)	6	3	2	1
Internal recycling ratio	4	5	5	5
Organic loading rate (kg COD/($m^3 \cdot d$))	1.04	2.08	3.2	6.4
Up-flow velocity (m/h)	0.39	0.78	1.17	2.8
Influent flow rate (L/d)	11.376	22.79	22.79	68.4
Operating period (d)	0–23	24–60	60–110	111–133

RESULTS AND DISCUSSION

Performance of SRUSB

The performance of the SRUSB reactor during the entire experiment is shown in Figures 2 and 3. The SRUSB was operated for more than 130 d with average influent organic and sulfate concentrations of 300–350 mg COD/L and

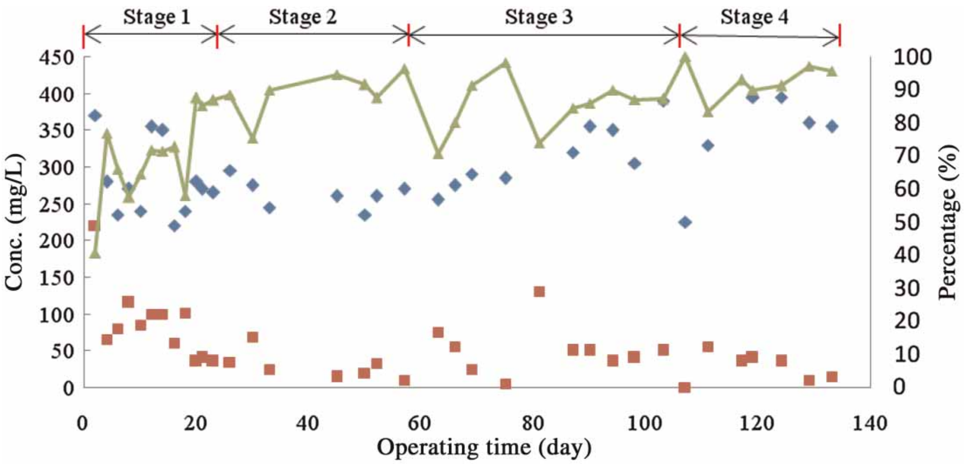

Figure 2 | Concentration of COD in the influent (◆) and effluent (■) and the COD removal efficiency (▲).

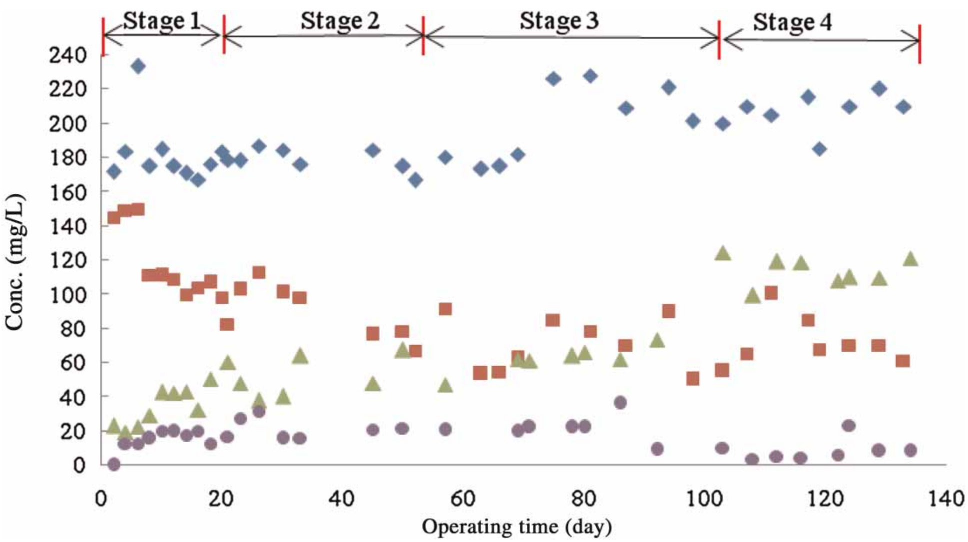

Figure 3 | Concentrations of sulfate in the influent (◆) and effluent (■) and dissolved sulfide (▲) and thiosulfate (●) in the effluent.

180 mg SO_4^{2-} S/L respectively. At Stage 1, the SRUSB was operated at an HRT of 6 h. After an initial acclimation period of 23 d operating at an up-flow velocity at 0.39 m/h, the SRUSB achieved 80% organic COD removal efficiency and sulfate-reducing bacteria (SRB) granules were observed. During this period, 44% of the influent sulfate was reduced to sulfide, of which 75% was present in dissolved form (Figure 3). The average dissolved sulfide and thiosulfate concentrations in effluent were determined to be 45 and 15 mg S/L, respectively.

Between days 24 and 60 (i.e. Stage 2), the HRT was reduced to 3 h, corresponding to an increase in the organic loading rate to 2.08 kg COD/($m^3 \cdot d$) and a higher up-flow velocity of 0.78 m/h. After 9 d of stabilization, the organic COD removal efficiency reached 90%. Moreover, sulfate reduction was enhanced during this period, which was reflected by increased sulfide concentration in the SRUSB effluent.

When the HRT was further reduced to 2 h between days 61 and 110 (Stage 3), the organic loading rate increased to 3.2 kg COD/($m^3 \cdot d$) while the up-flow velocity increased to 2.8 m/h. After 3 d of stabilization, the organic COD removal efficiency was 70%, while after 6 d it reached 85% organic COD removal. The dissolved sulfide and thiosulfate concentration in the effluent increased to 70 and 23 mg S/L, respectively. During days 111–133 (Stage 4), the HRT was further reduced to 1 h. Although both organic loading rate and up-flow velocity were doubled, the reactor performance was satisfactory, with an average of around 89% organic COD removal efficiency.

This stepwise approach in increasing the organic loading rate through shortening of HRT and increasing the up-flow velocity seems to favor the granulation process by applying a selection pressure to methanogenic up-flow anaerobic sludge blanket reactors (Hulshoff Pol *et al.* 1988). However, the physical condition of this system was entirely different to a methanogenic reactor. In a methanogenic reactor, a large amount of biogas is produced resulting in vigorous mixing in the reactor, especially at high organic loading rates, but in the SRUSB reactor, no 'gas' will be formed. Mixing therefore relies entirely on the influent flow and the recirculation of the reactor.

To enable sufficient mixing and formation of a hydraulic shear stress inside the reactor to provide the necessary selection pressure for granulation, the internal recirculation ratio in this experiment was set at 4–5 Q. Despite this high recirculation cycle, a vertical COD and sulfide concentration profile was observed, probably due to the plug flow in the reactor. As sulfate reduction would result in increasing alkalinity, which is an important factor in keeping the H_2S produced completely dissolved (Lu *et al.* 2012a), the combination of a short HRT and a high recirculation cycle would have an added benefit of maintaining a rather uniform pH throughout the reactor. Overall, this start-up approach not only promoted granulation by washing out smaller and less dense sludge flocs, but also provided extra mixing to enable uniform alkalinity distribution through the reactor.

Reactor pH and alkalinity

In saline wastewater treatment, sulfate acts as a terminal electron acceptor during sulfate reduction. Partial monomers, such as amino acids, sugars and long-chain fatty acids, as well as a range of intermediates including acetate, propionate, butyrate, lactate and hydrogen can all be possibly utilized by SRB (Muyzer & Stams 2008), as shown in Figure 4. This type of heterotrophic sulfate reduction reaction can also be expressed by Equation (1) (Lu *et al.* 2012b), based on an overall stoichiometric ratio of COD to sulfate equal to 0.67:

$$100\,g\,COD + 150.2\,g\,SO_4^{2-} + 43.7\,g\,H_2O \rightarrow 53.2\,g\,H_2S + 1.9\,g\,Sludge + 190.9\,g\,HCO_3^- \quad (1)$$

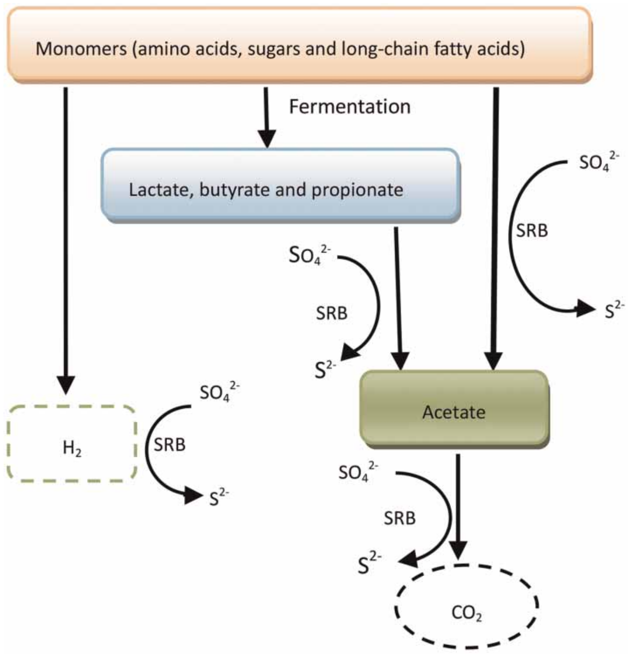

Figure 4 | Microbial degradation of complex organic matter in anoxic environments in the presence of sulfate.

According to Equation (1), for each mole of sulfate reduction, four moles of bicarbonate are generated. During the experiment the influent pH and alkalinity were constantly kept at approximately 7.2 and 200 as $CaCO_3$ mg/L, respectively, as shown in Figure 5.

After completion of the biological reaction, the pH rose to around 8 and alkalinity increased to 600 as $CaCO_3$ mg/L. This pH value is within the optimal pH range of 5–8 for SRB metabolism (Willow & Cohen 2003). The generated bicarbonate raised reactor pH and alkalinity significantly, thus keeping the produced H_2S completely dissolved, which effectively controlled the H_2S odor problem. Overall, the experiment showed that over 89% of dissolved sulfide will leave the reactor when the HRT is reduced to 1 h, thus providing sufficient sulfide for subsequent autotrophic denitrification (Figure 3).

Biomass concentration

With the organic loading rate gradually increased from 1.04 to 6.4 kg COD/($m^3 \cdot d$) and reduction of the HRT over a period of 130 d, the biomass concentration increased from 10 to 29 g/L, as shown in Figure 6. The ratio of mixed liquor volatile suspended solids to mixed liquor suspended solids remained in the range 0.68–0.77. Throughout this

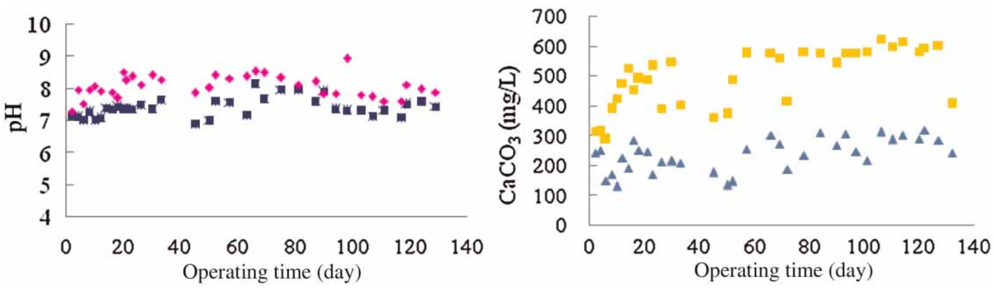

Figure 5 | Influent (◆) and effluent (■) pH and influent (■) and effluent (▲) alkalinity (as $CaCO_3$).

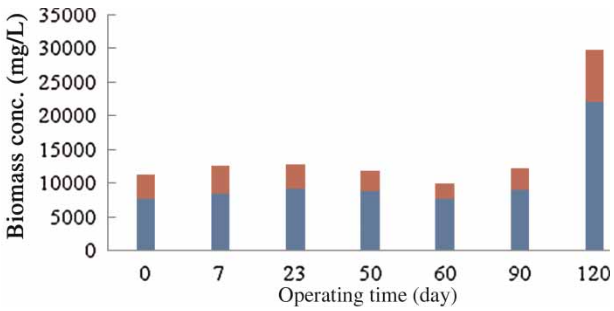

Figure 6 | Biomass concentration of the SRUSB reactor: (■) mixed liquor volatile suspended solids and (■) mixed liquor fixed suspended solids.

entire period, no sludge withdrawal was conducted for the purpose of sludge wastage. Taking account of the limited amount of sludge extracted for sampling, the overall sludge retention time was estimated to be about 38 d.

SVI_5 and diameter of granules

Granular sludge was first observed around day 30, and then achieved almost full granulation on day 60. Figure 7 shows the change in the SVI_5 and average size of the granular sludge during the start-up period. SVI_5 decreased from the initial value of 78 to 21 mL/g in the final stage. This value is quite close to that of the SANI aerobic granules, i.e. 17–30 mL/g (unpublished data). The diameter of the granules increased gradually from 44 to 830 μm on day 90. The high rate of granulation was thought to be due to the appropriate operating conditions facilitating a fast SRB granulation within 30 d, which was much shorter than the reported average of 2–4 months (Liu & Tay 2004).

Morphology of the granules

The morphology of the granular sludge changed progressively, as shown in Figure 8. The shape of the granules is nearly spherical with a clear outline. The average diameter of the granules at the end of the experiment was roughly 1 mm, which was smaller than other reported values of anaerobic granular sludge (Hulshoff Pol *et al.* 2004). Although the granular sludge had a mean diameter of 200 μm and SVI_5 of 49 mL/g at day 30, the structure of the granules was not compact, exhibiting clear crevices on the surface (Figure 8(a)). Following another two months of acclimation, the granules showed increased compactness, regularity and granular surface with large pores but a smooth shell, as shown in SEM images (Figure 8(d)). Channels running into the granules were observed, which was possibly an important feature to facilitate substrate transport within the granules.

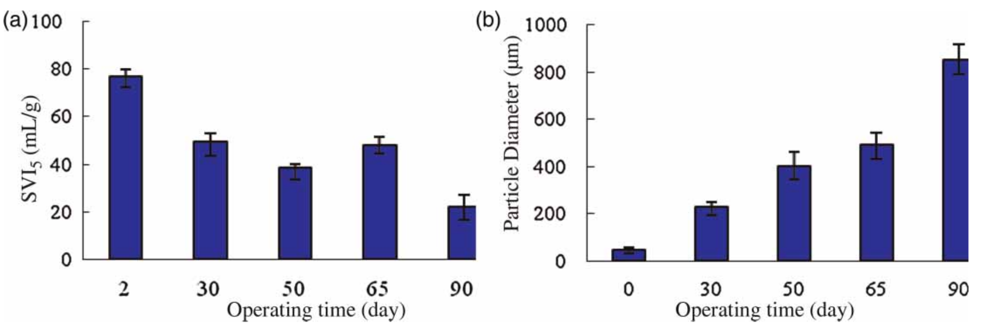

Figure 7 | (a) SVI_5 and (b) mean diameter of the SRB granules.

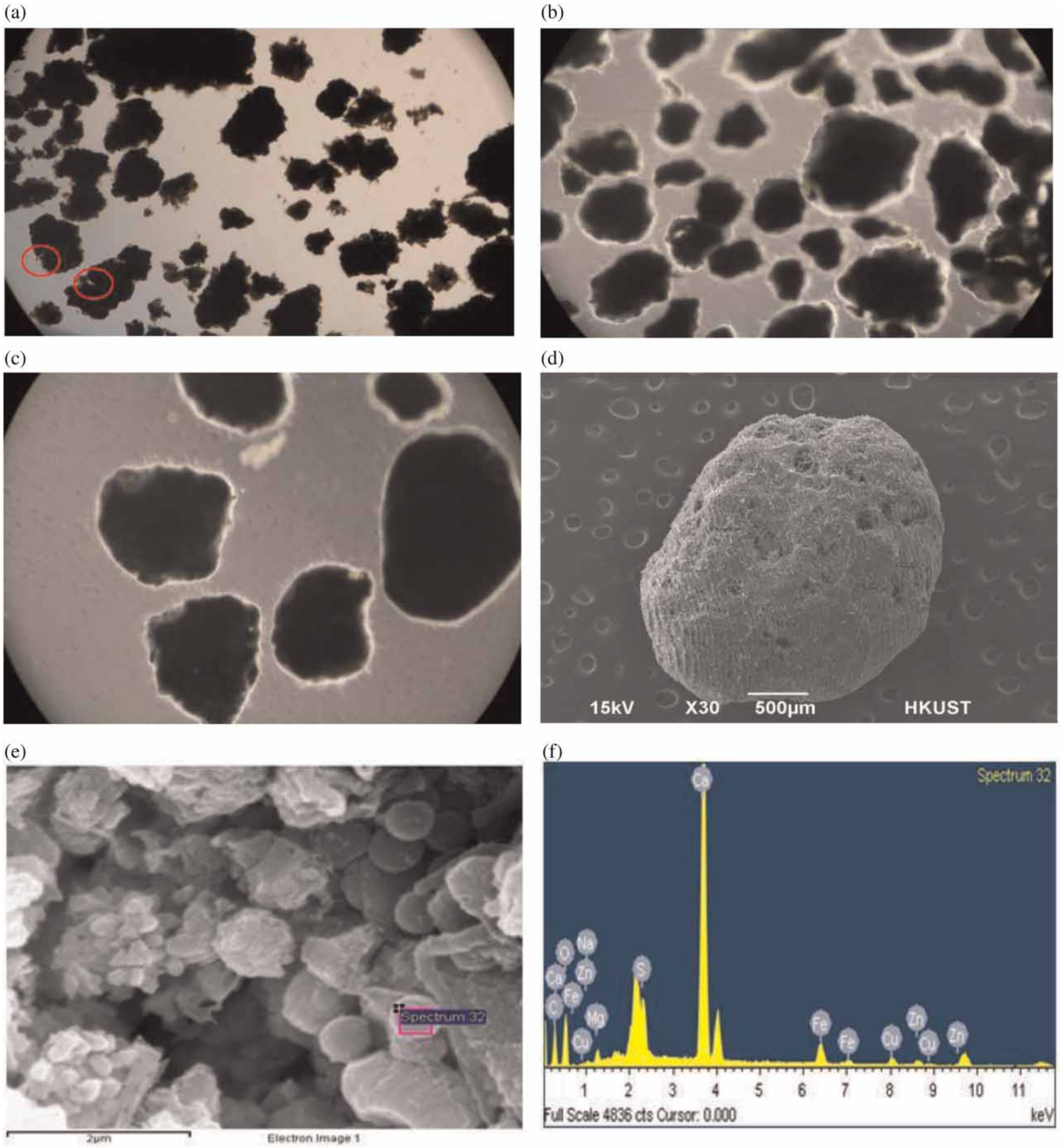

Figure 8 | Micrographs of the granular sludge: stereo micrographs (×10 magnification) taken on (a) day 30, (b) day 60 and (c) day 90; and ((d), (e)) SEM and (f) EDAX images taken on day 90.

SEM images of the interior of the granule (Figure 8(e)) indicated clustered globose objects extensively adhered to the inside surfaces. EDAX results showed that the main component of these clusters is calcium. The accumulation of calcium inside acetate-fed aerobic, anaerobic granules and biofilms has previously been confirmed by Kemner *et al*. (2004). Nevertheless, previous studies indicated that calcium precipitation generally occurred in the central part of the granules whereas the shells of granules are almost calcium free, no matter whether they are aerobic or anaerobic granules (Batstone *et al.* 2002; Wang *et al.* 2007). The distribution of calcium in SRB granules found in the present study is different from that in previous studies as calcium accumulation is rather evenly distributed inside the whole granules. The SEM examination also revealed that there was no calcium precipitation blocking the channels of granules. Hence, it appeared that calcium precipitation has not limited the diffusion within the SRB granules. Although there has been a hypothesis indicating that the precipitation of calcium in the center cores would favor anaerobic granulation (Batstone *et al.* 2002), it is not verified in the granulation procedure of SRB in the present study.

Further studies on the distribution of accumulated calcium need to be conducted in future.

CONCLUSIONS

The sulfate-reducing granules in the SRUSB were first observed around 30 d and became well established within 60 d, enabling sludge concentration of ~30 g/L to be established in roughly three months. The reactor achieved 90% removal of COD and 75% sulfate reduction. In about three months' acclimation, the COD loading rate increased to 6.4 kg COD/($m^3 \cdot d$) with a very short HRT (1 h) without affecting the sludge settleability. No excessive sludge withdrawal was made throughout the entire experimental period. The SEM results showed that the channels in the SRB granules are not clogged, which is different from previous reports.

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