

## Effects of Fe<sup>2+</sup> on sludge granulation in upflow anaerobic sludge blanket reactors

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**Abstract** Experiments were performed in parallel using six identical UASB (upflow anaerobic sludge blanket) reactors to examine the effects of iron (Fe<sup>2+</sup>) on sludge granulation during start-up. Introduction of Fe<sup>2+</sup> at concentrations of 300 and 450 mg/L enhanced the granulation process in UASB reactors, while dosage of Fe<sup>2+</sup> (150 mg/L) had little effect on the sludge granulation. The Fe<sup>2+</sup> concentration in granules was nearly proportional to the influent Fe<sup>2+</sup> concentration. The specific activity of granules decreased with increasing Fe<sup>2+</sup> concentration in the feed. For high Fe<sup>2+</sup> dosages, a large amount of minerals was deposited within the granules, and FeS and the compounds formed by iron and exopolysaccharide polymers were the main precipitates. This, along with the significant decrease in the water content in granules and the possible toxicity of high-level Fe<sup>2+</sup> accumulated inside granules, reduced bacterial specific activity.

**Keywords** Granulation; iron (Fe<sup>2+</sup>); start-up; upflow anaerobic sludge blanket (UASB) reactor

### Introduction

The upflow anaerobic sludge blanket (UASB) reactor has been used increasingly in recent years to treat a variety of industrial wastes and municipal wastes (Lettinga *et al.*, 1993). The UASB process involves the anaerobic degradation of organic wastes using biomass, which gradually aggregates to produce granules (Schmidt and Ahring, 1996). Microbial granulation involves different trophic bacterial groups, and physico-chemical and microbiological interactions (Hulshoff Pol, 1989). Many factors contribute, in one form or another, to the granulation process (Fang *et al.*, 1995; Schmidt and Ahring, 1996). Granulation may be initiated by bacterial adsorption and adhesion to inert matter, inorganic precipitates and/or to each other through physico-chemical interactions and syntrophic relationships. These substances serve as initial precursors (carriers or nuclei) for new bacterial growth (Uemura and Harada, 1995). These loosely adhered bacterial aggregates are strengthened by extracellular polymers excreted by bacteria and form firmly attached initial granules (Alphenaar *et al.*, 1993). It has been shown that divalent metal ions, such as Ca<sup>2+</sup>, enhance the granulation (Mahoney *et al.*, 1987; Shen *et al.*, 1993). Divalent ions were reported to play an important role in microbial aggregation (Kosaric *et al.*, 1990). It was found that extracellular polymers prefer to bind multi-valent metals due to the formation of stable complexes (Rudd *et al.*, 1984). However, the studies on divalent metal ions have been focused on the effect of Ca<sup>2+</sup> (Van Langerak *et al.*, 1998); information about the effect of Fe<sup>2+</sup> is still sparse. This study was therefore conducted to examine the roles of Fe<sup>2+</sup> in the sludge granulation process in UASB reactors.

### Methods

#### Reactor, wastewater and seed sludge

Experiments were performed in parallel using six identical UASB reactors. The empty bed volume of each reactor was 7.3 litres with an internal diameter of 100 mm and a liquid height of 930 mm. The reactors were housed in a temperature control room maintained at

35±1°C. The reactor (R1) without additional FeCl<sub>2</sub> · 4H<sub>2</sub>O served as control, while the other five reactors with Fe<sup>2+</sup> added 150, 300, 450, 600 and 800 mg/l were designated as R2, R3, R4, R5 and R6, respectively.

Synthetic wastewater was used as feed to the six reactors. Its strength was kept constant at chemical oxygen demand (COD) of 4000 mg/L throughout the study. The organic COD in wastewater was provided by peptone, glucose and meat extract, supplemented with several nutrients and trace elements, including nitrogen, phosphorus, sulfur, calcium, iron and magnesium. The ratios of COD:N:P were approximately 200:4:1. The buffer capacity was provided by the addition of sodium bicarbonate (2000 mg/L). The seeding inoculum was the sludge taken from the anaerobic digester of a local sewage treatment plant. The raw sludge was screened through a 0.2-mm sieve to remove the big debris and fibers before seeding. The sieved sludge had 23.6 g/L of suspended solids (SS), 17.0 g/L of volatile suspended solids (VSS), and 43 mL/g of sludge volume index (SVI). The specific methanogenic activity (SMA) was 0.26 g-CH<sub>4</sub>-COD/g-VSS · d. Each reactor was seeded with 3.5 L of inoculum with biomass content of 8.5 g-VSS/L.

The initial organic loading rate (OLR) to the reactors was set at 2.0 g-COD/L · d. The OLR was then increased in steps to 2.7, 4.0, 5.3 and 8.0 g-COD/L · d. The five OLRs were kept for 23, 22, 31, 32 and 38 days, respectively.

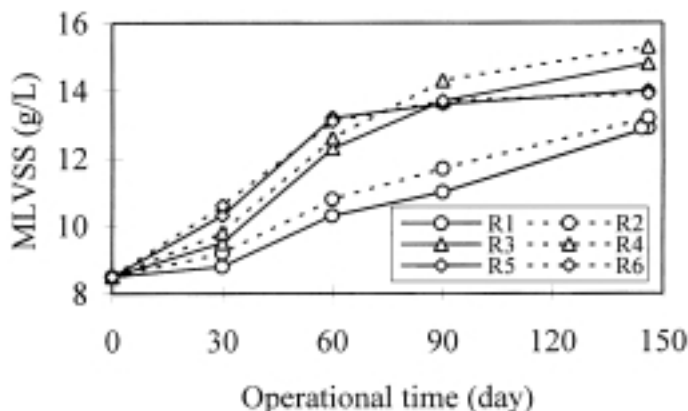
#### Methods of analysis

Analysis of COD, pH, phosphorus, dry weight and density of the biomass and VSS were performed according to the *Standard Methods* (1992). Biomass was periodically taken from the sampling points arranged along the height of the reactors to determine the mixed liquid VSS concentrations. Gas production was recorded by wet gas meters (Shinagawa W-NK-0.5) and gas composition was analyzed by gas chromatography (HP 5890A-GC) with a 2-m long and 3-mm-ID packed column (Haye-Sep Q, 80/100 mesh) and a thermal conductivity detector at 200°C. Helium was used as the carrier gas with a flowrate of 30ml/min. Metals in the liquid samples and biomass were determined by atomic absorption spectroscopy (Perkin-Elmer Model 2280). A known volume of filtrate was dried and the remainder was re-dissolved in hot concentrated HCl solution and filtered into 50 ml volumetric flasks. Samples were analyzed by atomic absorption spectroscopy using hollow cathode lamps and acetylene/air flame. The SMA of sludge samples taken from the bottom sampling points was measured in a 250-ml Kimax flask at 35±1°C under anaerobic conditions, using the method described previously (Fang *et al.*, 1994). For estimating the size distribution, the sludge samples taken from the bottom sampling points were classified into six fractions using laboratory sieves with various openings (0.2, 0.6, 1.0, 2.0, 4.0 mm). The sludge particles were first placed in the sieve with the biggest opening. The particles were gently submerged in water and shaken to let the smaller particles pass through. The procedures were repeated until the five sieves were used.

## Results and discussion

#### Biomass accumulation

The accumulation of biomass for the different influent Fe<sup>2+</sup> concentrations is illustrated in Figure 1. At the initial stages, R5 and R6 had the greatest MLVSS-increasing rates, while R1 and R2 had the lowest MLVSS-increasing rates. During the period between day 30 and day 60, R3 and R4 performed a similar MLVSS-increasing rate to R5 or R6. However, after day 60, the biomass increasing rates in R5 and R6 slowed down, while the MLVSS concentrations in R3 and R4 kept increasing rapidly. By day 90, the MLVSS concentrations in R3 and R4 exceeded those in R5 and R6. Throughout the experiment, the MLVSS concentration in R1 was the lowest, whereas the MLVSS concentration in R2 was slightly higher than



**Figure 1** The MLVSS concentrations in the six reactors

that in R1. The presence of  $\text{Fe}^{2+}$  at 300 and 450 mg/L improved biomass retention of the reactors, while a lower  $\text{Fe}^{2+}$  dosage, e.g. 150 mg/L, did not significantly enhance the biomass retention.

Initially, the biomass was loose and expanded easily. With the proceeding of granulation, the biomass was progressively stratified with the granules settled in the lower part of the reaction zone. When granules were formed increasingly in the reaction zone, a dense sludge bed and a thin sludge blanket were formed with a clear interface between them.

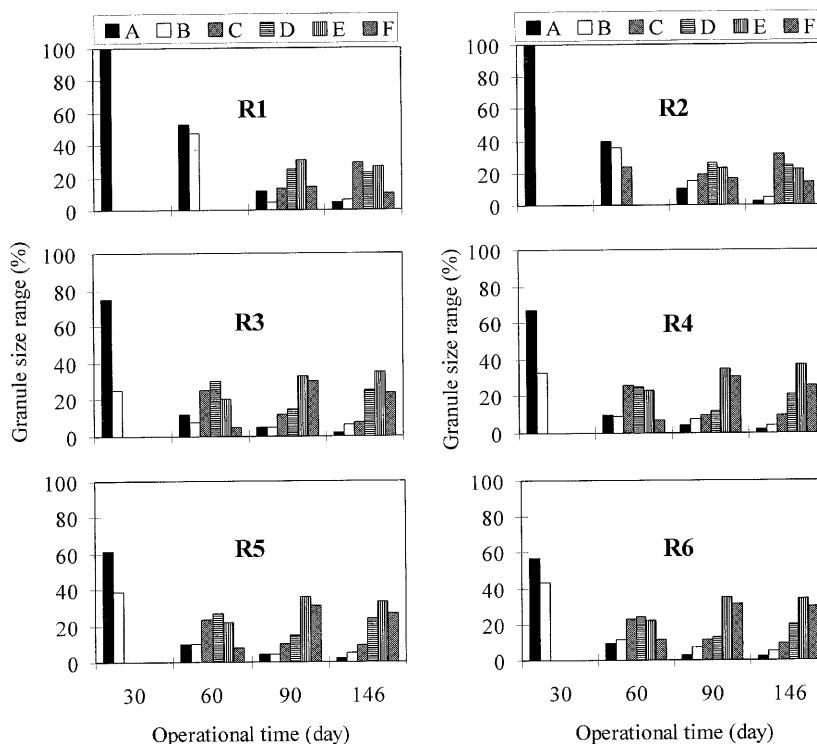
#### Distribution of granules

After 30 days of operation, small granules with diameters of 0.2 to 0.6 mm became visible at the bottom of R3, R4, R5 and R6. These initial granules grew rapidly, and after 30 more days of operation large granules with diameters over 2.0 mm were formed. After about 30 days of rapid growth, the granular growth rate became slower, indicating that a mature and stable granulation had developed. The reactor R6 was the first one in which granules became noticeable (on day 24), whereas R1 was the last one in which granules became noticeable (on day 53).

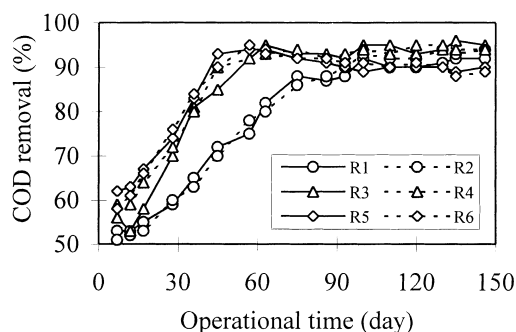
The size distributions of the granules by weight are shown in Figure 2. Sixty days into start-up, approximately 47% of the sludge samples from R1 were in the range of 0.2 to 0.6 mm. On day 146, approximately 50% of the granules from R1 were in the range of 1.0–4.0 mm and only 10% of the sample measured above 4.0 mm. The granule size distribution for R2 was similar to that for R1 at any given stage.

In R3, the granule distribution profile on day 30 was askew with 25% of the sample lying in the 0.2–0.6 mm range. By day 60, about 45% of the granules were measured below 1.0 mm, while the diameters of 25% of the sample measured above 2.0 mm. When the experiment was terminated on day 146, about 24% of granules were larger than 4.0 mm. In R4, the granule distribution profiles were generally similar to those in R3. The average sizes of the sludge samples from R5 or R6 were slightly greater than those from R3 and R4 as shown in Figure 2.

Larger granule sizes in R3 through R6 may be explained by the presence of a better quality or quantity of a binding agent assisted by  $\text{Fe}^{2+}$  as a binding factor. This result implies that the presence of  $\text{Fe}^{2+}$  at concentrations higher than 300 mg/L had promoted granule formation by allowing aggregates to form earlier and achieve a larger size. In terms of granules size distribution, the higher the influent  $\text{Fe}^{2+}$  concentration, the greater the average size of the granules was (Figure 2).



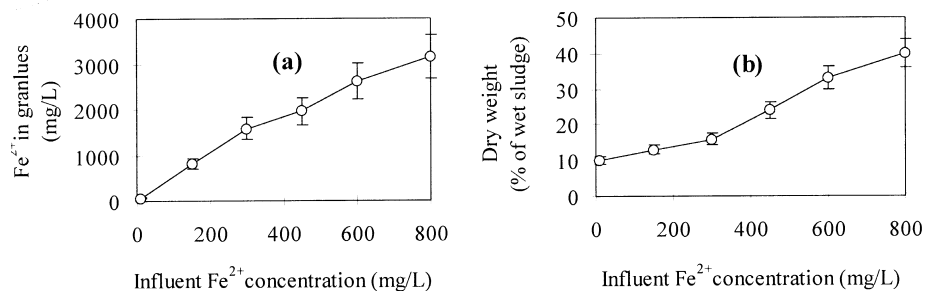
**Figure 2** Size distributions (by weight) of granules of each reactor A:  $d < 0.2$ ; B:  $0.2 < d < 0.6$ ; C:  $0.6 < d < 1.0$ ; D:  $1.0 < d < 2.0$ ; E:  $2.0 < d < 4.0$ ; F:  $d > 4.0$  (unit in mm)



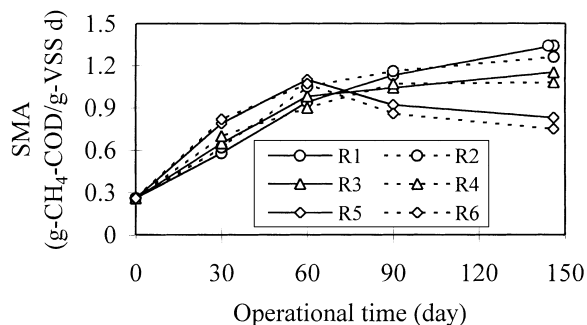
**Figure 3** The COD removal efficiencies of the six reactors

#### COD removal

The COD removal efficiencies of the reactors throughout the experiment are illustrated in Figure 3. During the first two weeks, the COD removal efficiencies of the six reactors were low. With the progress of the experiment, the COD removal efficiencies of all the reactors generally kept increasing. By day 75, the COD removal efficiencies of R1 and R2 were markedly lower than those of the other four reactors. Around day 90, all the reactors achieved almost the same level of COD removal efficiencies. However, when the experiment was terminated on day 146, the COD removal efficiencies of R5 and R6 were slightly lower than those of R1. This may suggest that the COD removal efficiencies had no direct correlation with the formation of granules.



**Figure 4** (a) Iron concentration in granules after 146 days; (b) Dry weight of granules



**Figure 5** SMA on acetate as measured for the sludge from the six reactors

#### Granule composition

Figure 4(a) illustrates that the  $\text{Fe}^{2+}$  concentration in the granules, taken from the bottom sampling points on day 146, was nearly proportional to the  $\text{Fe}^{2+}$  concentration in the substrate. Iron accumulation within granules mostly resulted from the interaction of  $\text{Fe}^{2+}$  with sulfite and exopolysaccharide polymers because  $\text{Fe}^{2+}$  concentration in bacterial cells is very low. The formation of  $\text{FeS}$  and the binding of  $\text{Fe}^{2+}$  to the exopolymers in granules are nonspecific processes and are driven by the  $\text{Fe}^{2+}$  gradient from the bulk liquid phase into the granules. Figure 4(a) shows that the  $\text{Fe}^{2+}$  concentrations in the granules were around five times greater than that in the feeds.

As shown in Figure 4(b), the granules contained 10% to 40% dry mass. When  $\text{Fe}^{2+}$  concentration in the substrate was raised, the water content of the granules decreased and the total dry mass increased. In the dry materials, the proportion of minerals increased significantly while that of organic compounds reduced as indicated by the decrease in VSS/SS (not shown). This indicates that the presence of  $\text{Fe}^{2+}$  increased the dry mass of the granules mainly by increasing the concentration of minerals in the granules. The increased mineral content was very likely the result of more  $\text{FeS}$  and the compounds formed by iron and exopolysaccharide polymers trapped in the granules.

#### SMA measurement and microscopic observation

Figure 5 illustrates the SMA values of the granular sludges, taken on days 30, 60, 90 and 146, using acetate as substrate. The methanogenic activity of granules increased steadily with increasing OLR for all the reactors by day 60. After day 60, the SMA values of R5 and R6 gradually dropped, while the SMA values of R1, R2, R3 and R4 still increased. At the end of the experiment, the SMA value of R1 was 1.34 g- $\text{CH}_4$ -COD/g-VSS · d, higher than the corresponding SMA value of R2 through R6. This result indicates that iron precipitates

had a negative effect on the activity of the biomass. Higher influent  $\text{Fe}^{2+}$  concentration led to higher ash content in granules and severer mass transfer limitations. These lower SMA values of R5 and R6 might be attributed to the presence of larger granules and higher ash content in granules, in which mass transfer was significantly reduced. Mass transfer inside the granules is considered to be solely controlled by diffusion (Pavlostathis and Giraldo-Gomez, 1991). The resistance to substrate diffusion inside granules increases proportionally with physical granular size and ash content, making the substrate less available to the granule core and eventually resulting in substrate deficiency or depletion inside granules (Alphenaar *et al.*, 1993). The order of  $\text{Fe}^{2+}$  dosages was:  $\text{R6} > \text{R5} > \text{R4} > \text{R3} > \text{R2} > \text{R1}$ , while the order of SMA value of the granules in the end of the experiment was:  $\text{R1} > \text{R2} > \text{R3} > \text{R4} > \text{R5} > \text{R6}$  (Figure 5). This suggests that the granules at higher  $\text{Fe}^{2+}$  concentrations had a lower metabolic activity.

Scanning electron microscopic observation revealed that the sludges from all the six reactors had a similar composition and morphology. The granules were predominantly composed of *Methanosarcina*-like species. This implies that the addition of  $\text{Fe}^{2+}$  from 150 to 800 mg/L did not result in a difference in predominant microorganisms, despite the fact that the addition at higher concentrations accelerated the granulation process and led to the formation of larger size granules through physico-chemical functions.

#### Comparison

The present work shows that the addition of  $\text{Fe}^{2+}$  at 300 and 450 mg/L enhanced the sludge granulation. Precipitates of FeS were involved in initial adsorption of cells (Rudd *et al.*, 1984). The positive effects of  $\text{Fe}^{2+}$  in sludge granulation might be explained by the assumption that  $\text{Fe}^{2+}$  condenses the diffusive double-layers, resulting in a relatively strong effect of van der Waals forces. Iron appeared to bridge between negatively charged groups on cell surfaces, which is important in adhesion phenomena. Shen *et al.* (1993) found that  $\text{Fe}^{2+}$  binds to extracellular polymers, and that most  $\text{Fe}^{2+}$  in extracted extracellular polymers might be bound to extracellular polymers. Hence, iron may play an important role in linking extracellular polymers. In addition, iron was found as a component of the essential enzymes that drive numerous anaerobic reactions. The conversion of COD and bacterial growth were limited at iron deficient concentrations as reported by Oleszkiewicz and Sharma (1989).

On the other hand, the presence of high-concentration  $\text{Fe}^{2+}$  in the granules could damage the environment required for maintenance of the granular structure or the bacterial activity. The toxicity of iron accumulated in the granules might also have a negative effect on the biomass activity. Kugelman and McCarty (1965) found that the optimum ion concentration for methanation of acetic acid was 200 mg/L and that the upper limit was from 2000 to 5000 mg/L. In the present study the  $\text{Fe}^{2+}$  concentrations in the granules ranged from 820 to 3160 mg/L [Figure 4(a)]. It is postulated that a high immobilized  $\text{Fe}^{2+}$  concentration in the granules might impose a severe restraint on the specific activity of the granules.

#### Conclusions

In this work, the effects of iron on the sludge granulation process during start-up were investigated in six UASB reactors operated in parallel. Introduction of  $\text{Fe}^{2+}$  at concentrations at 300 and 450 mg/L enhanced the granulation process in UASB reactors, while a low dosage of  $\text{Fe}^{2+}$  at 150 mg/L had little effect on the sludge granulation. The  $\text{Fe}^{2+}$  might accelerate the granulation process through bridging between negatively charged groups on cell surfaces and linking extracellular polymers. The  $\text{Fe}^{2+}$  concentration in granules was proportional to the influent  $\text{Fe}^{2+}$  concentration, and FeS and the compounds formed by iron and exopolysaccharide polymers were the main precipitates in granules. The specific activity of granules decreased with increasing  $\text{Fe}^{2+}$  concentration in the feed. This might be due to the

presence of a large amount of minerals deposited within the granules, the significant decrease in the water content in granules, and the possible toxicity of high-concentration  $\text{Fe}^{2+}$  accumulated inside granules.

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