The effect of intermittent feeding on aerobic granule structure

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Abstract Self-immobilized biofilms, or aerobic granules without the addition of carrier material, have only been reported in one suspended growth system, the Sequencing Batch Reactor (SBR) with a very short fill time (dump fill). The SBR utilizes intermittent feeding which creates a period of high load followed by starvation (often referred to as feast-famine). In this experiment, three identical SBRs were operated with different feeding conditions to determine the role of feast-famine on granule formation. All three SBRs were operated with a total volumetric load of 2.4 kg/m^3•d. The 90 minute Fill phase was altered for each reactor, providing an increasing time of Aerated Fill. A dump fill condition was applied for one reactor, while the other two reactors were aerated for different times during Fill, resulting in a smaller COD load at the beginning of each React phase. Aerobic granules formed in all reactors, but the structural properties and content of filamentous organisms were clearly dependent on a high feast condition. Only the reactor with dump fill formed compact, stable granules. It is concluded that intermittent feeding associated with the SBR affects the selection and growth of filamentous organisms and has a critical role in granule structure and composition.

Keywords Aerobic granules; feast-famine; intermittent feeding; sequencing batch reactor (SBR)

Introduction

Granules can be described as a collection of self-immobilized cells into a somewhat spherical form. They are considered to be a special case of biofilm growth (Grotenhuis *et al*., 1991; El-Mamouni *et al*., 1995; Beun *et al*., 2002). It is generally acknowledged that aerobic granules have a wide range of beneficial properties compared to conventional activated sludge flocs. These include a regular, dense, and strong structure, good settling property, high biomass retention, and the ability to withstand a high organic loading rate. Although aerobic granulation in wastewater is a relatively new field, the upflow anaerobic sludge blanket (UASB) bioreactor using anaerobic granules is one of the best-known self-immobilization processes and has been extensively applied to anaerobic wastewater treatment. A brief review of both anaerobic and aerobic granulation shows that a variety of species including methanogens (Lettinga *et al*., 1984), acidogenic bacteria (Vanderhaegen *et al*., 1992), nitrifiers (De Beer *et al*., 1993; Van Benthum *et al*., 1996), denitrifiers (Van der Hoek, 1988), and aerobic heterotrophs (Morgenroth *et al*., 1997) are able to form granules. This observation has led researchers to hypothesize that granulation is not a function of microbiological groups but of reactor operating conditions (Beun *et al*., 1999).

Some aerobic granulation has been reported in continuously-fed Biofilm Airlift Suspension (BAS) reactors which have carrier material added (van Loosdrecht *et al*., 1995; Kwok *et al*., 1998). However, spontaneous aerobic granulation of suspended growth has only been reported in systems applying the SBR with short fill periods. Hitherto, research on the necessary factors for granule formation has focused on the effect of settling time, shear force, and volumetric loading rate on the formation and structure of granules. A short settling time has been utilized to select for fast settling flocs and granules, forcing the

Water Science and Technology **Vol 49 No 11–12 pp 19–25** © IWA Publishing 2004

washout of less dense flocs and suspended organisms (Beun *et al.*, 1999, 2002). Tay *et al.* (2001a) operated four parallel SBRs with increasing aeration rates, showing that granules formed only in reactors with a superficial gas velocity greater than 1.2 cm sec^{-1}. Volumetric loading rate has also been shown to affect the structure of granules, with granules becoming more compact and smooth with increasing loads (Moy *et al.*, 2002). These studies have not addressed the impact of the SBR cycle and intermittent feeding on the formation and structure of granules, although several researchers have observed that a feast/famine regime is important (Tay *et al.*, 2001b; de Kreuk and van Loosdrecht , 2003).

The studies conducted by Chiesa *et al.* (1985) showed that intermittent feeding in the SBR produces feast-famine conditions that select for better settling sludge by selecting against filamentous organisms. Generally, floc-forming bacteria, with relatively high substrate uptake kinetics, have an advantage over filamentous bacteria if food is supplied intermittently, forcing bacteria to acquire and store the food for cell maintenance and growth during periods of starvation (Chudoba *et al.*, 1973). Chiesa and Irvine (1985) showed that the SBR cycle could produce a high feast-famine condition by accumulating the substrate in the reactor before allowing the react phase to begin (termed static fill, which simulates dump fill). In the current experiment, three parallel SBRs were operated with the same settling time, aeration rate, and volumetric loading rate. The nature of intermittent feeding was varied to determine the effect of feast-famine on the formation and structure of granules.

Methods

Three 5 litre column-type SBRs were operated for six months. The reactors were constructed from plexiglass and are shaped as a cylinder (height 100 cm, diameter 9 cm). They were aerated at a rate of 275 L h^{-1} with a 50% volumetric exchange ratio. The reactors were inoculated with 5 litres of activated sludge from a municipal wastewater treatment plant (initial MLSS 2.5 g L^{-1}). The walls of the reactors were cleaned every two weeks, and the biofilm growth was discarded. All reactors were fed from a common feed of glucose and peptone with nutrients (similar to that used by Moy *et al.*, 2002). The three reactors were fed the same total amount of substrate at a rate of 2.4 kg COD m^{-3} day^{-1} over 90 minutes of Fill for 6 cycles per day (90 min Fill, 120 min React, 2 min Settle, 10 min Draw, 18 min Idle). The only variation in operating strategy was the ratio of Static Fill to Aerated Fill for each reactor (see Table 1). Reactor 1 was operated with 90 minutes of Static Fill to simulate a dump fill by maximizing substrate accumulation before initiating React. Reactor 2 was operated with 60 minutes Static Fill followed by 30 minutes Aerated Fill, and Reactor 3 received 30 minutes Static Fill and 60 minutes Aerated Fill. The operating strategies implemented in Reactors 2 and 3 produced a systematic decrease in the substrate load at the beginning of React, or a smaller differential between feast and famine. This is shown in Figure 1 with a typical plot of COD removal during one cycle.

Mixed liquor and volatile suspended solids (MLSS and VSS) content, effluent solids

Table 1 Operating strategy (variation of fill phase to alter feast-famine condition)

Cycle phase	Cycle times (min)		
	Reactor 1	Reactor 2	Reactor 3
Static fill (fill, no aeration, no mixing)	90	60	30
Aerated fill (fill, aeration, mixing)	0	30	60
React	120	120	120
Settle	2	2	2
Draw	15	15	15
Idle	13	13	13
Total cycle time	240	240	240

and volatile solids (ESS and EVSS) content, and the sludge volume index (SVI) were measured once to three times per week, all according to APHA standard engineering methods (*Standard Methods*, 1998). Substrate removal was measured weekly by the chemical oxygen demand (COD) over a cycle and in the effluent using Dr. Lange COD kits (following the colorimetric COD standard method). The oxygen uptake rate (OUR) over a cycle was measured weekly, and the endogenous OUR with ammonium and glucose spikes was measured twice a week (*Standard Methods*, 1998; Manning, 1986). The development of flocs and granules was observed using a stereomicroscope (Leica Wild MPS 46/52), and images were obtained with an attached Kodak digital camera.

Results and discussion

Each SBR yielded predominantly granular sludge within one month of operation. Complete COD removal was achieved within 30 minutes of the beginning of aeration after one week of operation. All reactors had stable and complete COD removal (> 96%). Figure 1 shows the COD accumulation and removal over one cycle on day 30 of operation. The theoretical COD line shows the total COD fed to all reactors over 90 minutes, and the plot for each reactor shows COD removal once aeration began (during Fill for Reactors 2 and 3). Figure 1 also shows how the differential between feast and famine was varied in the three reactors by altering the ratio of static to aerated fill. Reactor 1 accumulated the total COD before aeration began, providing the highest feast condition.

The granules grew and changed considerably in size and morphology over several months, and pseudo-steady state was established after four months based upon the MLSS and SVI measurements. The MLSS and VSS content decreased steadily for Reactors 1 through 3 (9.0, 6.8, and 3.2 g L^{-1} MLSS, respectively). The SVIs for Reactors 1, 2 and 3 increased from 46 mL g^{-1} SS for Reactor 1 to 60 and 114 mL g^{-1} SS, for Reactors 2 and 3. The effluent solids content also increased for Reactors 1 through 3, ranging from 170 mg L^{-1} ESS to 220 and 290 mg L^{-1} ESS for each reactor. Figures 2 and 3 show the average data for MLSS and SVI in all reactors. The error bars indicate the standard deviation for all measurements taken after pseudo-steady state conditions were reached (three months of steady state data).

All three reactors performed well in terms of COD removal, but the performance varied in terms of specific oxygen uptake rate (SOUR) and effluent suspended solids (ESS). When determined, the SOUR was measured at the beginning of React for each reactor, at the end of the React phase, and an endogenous rate was measured after two hours of further aera-

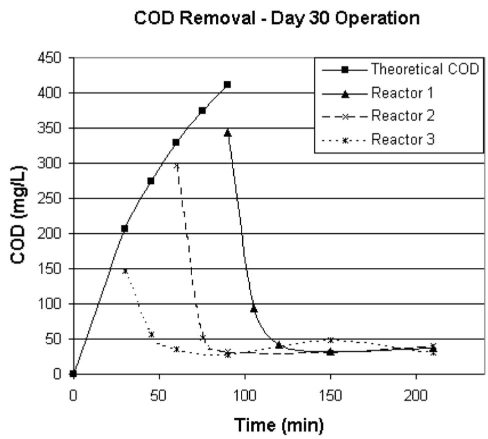

Figure 1 Initial COD at the beginning of React and COD removal during one cycle

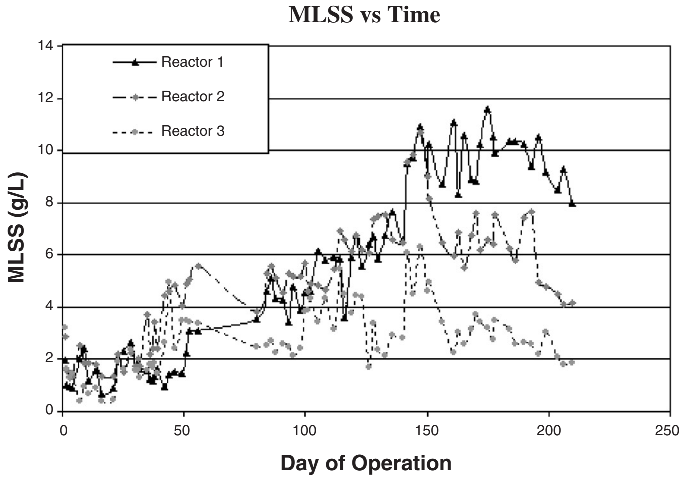

Figure 2 MLSS for Reactors 1, 2, and 3 over time

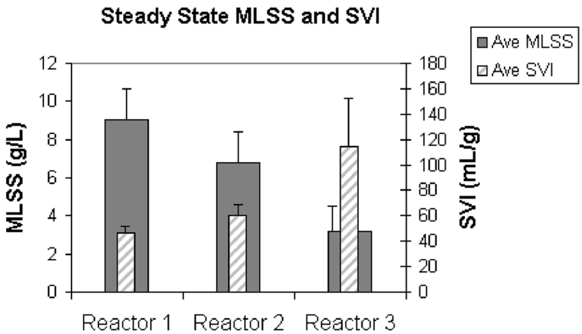

Figure 3 Ave MLSS and SVI at pseudo-steady state

tion. The average steady state values are presented in Table 2. It is clear that all endogenous and end of react SOUR values have no significant difference. However, the maximum SOURs decreased for Reactor 1 through 3, with Reactor 1 having a maximum SOUR of 139 [mg O_2/(mg VSS*h)]. This trend would be expected since the COD is highest in Reactor 1 at the beginning of the React phase. The differences in SOUR may also indicate the different substrate uptake kinetics of the bacteria selected in each reactor. The biomass in Reactor 1 with dump fill had the highest SOUR and substrate uptake rates at the beginning of React.

After 60 days of operation, all reactors contained predominantly granule sludge. The structure of the granules was clearly different in terms of filamentous bacteria. Microscopic observations confirmed that the filamentous content of granules increased with increasing times for Aerated Fill (or, as is shown in Table 2, a smaller feast-famine differential). A typical granule from each reactor after 60 days of operation is shown in Figure 4. Only

Table 2 Ave steady state Specific Oxygen Uptake Rates (SOURS)

		SOUR [mg O_2/(mg VSS*h)]		
Reactor	**Ratio (max/min SOUR)**	**Beginning react**	**End react**	**Endogenous**
1	15	139	11	9
2	11	123	15	11
3	8	112	14	14

Reactor 1, which had a dump fill, contained smooth, dense granules.

After pseudo-steady state was reached, granules in Reactor 1 proved to be very stable, and the MLSS content continually increased to a maximum of 11.5 g L^{-1}, at which point the sludge bed was as tall as the effluent port (50% of the reactor height), and some granules were wasted daily. The granules in Reactor 2 and 3 were not as stable, and they always co-existed with some flocs, which were periodically wasted due to the short settling time of 2 minutes in the reactor. Accordingly, the biomass content of Reactors 2 and 3 was less than Reactor 1. In particular, Reactor 3 had a large number of filamentous organisms, and the average MLSS was 3.2 g L^{-1} compared to 9.0 g L^{-1} in Reactor 1. The structures of the granules after pseudo-steady state was established are shown in Figure 5. These images were taken after 6 months of operation in Petri dishes with a few millilitres of reactor volume. The granules were not separated from co-existing flocs, and the images indicate the relative abundance of granules to flocs.

It is clear from simple microscopy that the granule structure is affected by intermittent or extended feeding. Dense, smooth granules were only formed in Reactor 1, which simulated a dump fill and had the greatest differential between feast and famine. With extended feeding periods during React for Reactors 2 and 3, filamentous organisms were evident in granule structure. These observations correlate well with previous research by Chudoba *et al*. (1982) and Chiesa *et al*. (1985) in suspended growth systems. This early research showed that floc-forming bacteria had a selective advantage over filamentous organisms due to substrate-uptake kinetics and starvation sensitivity. Filamentous organisms were able to compete for substrate in Reactors 2 and 3 since feeding continued during aeration, whereas only the organisms with the highest substrate uptake kinetics competed in Reactor 1 with a stringent intermittent feeding program. The current experiment demonstrates that principles of species selection apply to granule reactors or self-immobilized biofilm systems and have a significant impact on granular structure.

The experiments reported herein also demonstrate that high COD loads or feast conditions, which are established during the pulse feeding strategies easily provided in the SBR, determine granule structure. Previously in research, the settling time, shear force, and volumetric loading rate have been cited as factors controlling granule structure (Beun *et al.*,

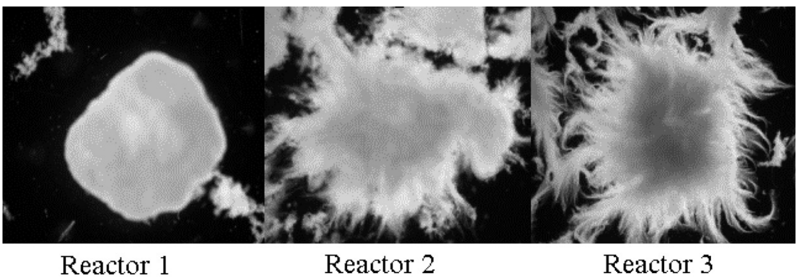

Figure 4 Images of aerobic granules cultivated in each reactor (Reactor 1, 2 and 3 with 0%, 33% and 66% Aerated Fill, respectively). Taken approximately 60 days after start-up

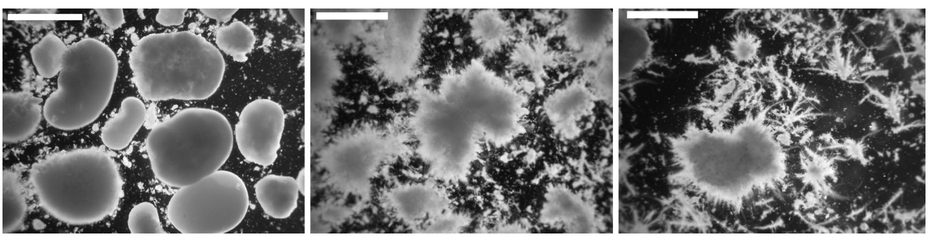

Figure 5 Reactors 1, 2, and 3 at steady state (scale bar = 5 mm)

2002; Tay *et al*., 2001a; Moy *et al*., 2002). Research from Biofilm Airlift Suspension (BAS) reactors has also been used to describe that a balance between shear force and volumetric loading controls the amount of filamentous growth on the surface of granules and the granule density (Tijhuis *et al*., 1996; Kwok *et al*., 1998). However, in this experiment, the settling time, aeration rate, and volumetric loading were constant for all three reactors, and the time for Aerated Fill was the only changing parameter. It can therefore be concluded that the SBR cycle and intermittent feeding is a controlling factor in dense granule formation, due to its role in species selection and growth.

Conclusions

- In three parallel reactors with identical settling time, shear force, and volumetric loading, the structure of granules was completely dependent on intermittent feeding conditions provided by the SBR.
- A high feast-famine ratio, or pulse feeding provided by dump fill in the SBR, was necessary for the formation of compact, dense granules.
- Intermittent feeding affected the selection and growth of floc-forming and filamentous organisms, which affected the structure of aerobic granules.

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