Application of the aerobic granular technology to treat domestic wastewater for biological nutrient removal

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APPLICATION OF THE AEROBIC GRANULAR TECHNOLOGY TO TREAT DOMESTIC WASTEWATER FOR BIOLOGICAL NUTRIENT REMOVAL.

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ABSTRACT

Aerobic granular sludge for treating domestic wastewater was studied to investigate its application for biological nitrogen and phosphorus removal. Simultaneous nitrogen and phosphorus removal was promoted by operating the reactor with a low dissolved oxygen concentration. A fully granular system was achieved after 50 days of operation by progressively decreasing the settling time and increasing the hydraulic residence time (HRT). The reactor operated for 152 days with stable granulation. The HRT was reduced to 9.6 h while achieving 99% and 98% P and N removal respectively. This study demonstrates the key strategies of granule formation and its potential for achieving biological nitrogen and phosphorus removal during the treatment of domestic wastewater.

INTRODUCTION

Treatment of domestic wastewater is essential to maintain surface- and ground-water quality (Zhang et al., 2009), and in industrialised nations the most commonly used approach for that is by activated sludge. Activated sludge can operate for biological nutrient removal (BNR) for efficient removal of chemical oxygen demand (COD), nitrogen (N) and phosphorus (P). These are conventionally operated as floccular biofilm systems which normally have large footprint. This is because floccular sludge requires a relatively long settling time for separation from the treated water, resulting in the need of large sedimentation tanks in the wastewater treatment plants. Recently laboratory-scale systems are operated to form aerobic granules, that are dense aggregates of biomass typically 1-5 mm in diameter, and these reactors can maintain biomass concentrations 10 times that of floccular systems (De Kreuk and Van Loosdrecht, 2004, Liu et al., 2009, Liu and Tay, 2004, Beun et al., 1999). Beneficial treatment properties using aerobic granules include: extraordinary settling capabilities, higher loading capacity, shorter hydraulic residence time and reduced sludge handling. Consequently there are economic advantages to use aerobic granular over conventional floc based systems (de Bruin et al., 2004).

Most research in this field has investigated the performance of aerobic granular systems while treating synthetic wastewater (WW). Recently, some laboratory-scale studies have applied the technology to treat various types of real WW, such as dairy, livestock and industrial WW (Arrojo et al., 2004, De Kreuk and Van Loosdrecht, 2006, Lemaire, 2007, Yilmaz et al., 2008). However, there is very limited study of municipal wastewater treatment by aerobic granules and the few investigations done focus on COD removal and nitrification (Di Iaconi et al., 2008, Ramadori et al., 2006). Additionally, it is reported that low strength wastewater (such as domestic WW) is not favourable for granule formation, which causes long start-up periods (De Kreuk and Van Loosdrecht 2006).

This study investigates the formation and application of aerobic granules to treat domestic wastewater for biological nitrogen and phosphorus removal. Key findings in these experiments were focused on the strategies applied for the successful granule formation from domestic wastewater while maintaining good BNR performance. The results are expected to be beneficial for the future application of aerobic granules for municipal wastewater treatment.

MATERIAL AND METHODS

Sequencing batch reactor operation for aerobic granules performing BNR.

A laboratory scale sequencing batch reactor (SBR) with 2 L working volume was seeded with sludge which taken from Thorneside Wastewater Treatment Plant (WWTP), Brisbane, Queensland. The wastewater used in this study was collected weekly from the WWTP which receives predominantly domestic wastewater. The wastewater was stored at 4°C over the duration of the week, which was effective in preserving the COD and nutrient levels. The level of various parameters of the domestic sewage influent is presented in Table 1. The SBR was operated in 6 hours cycles; the configuration consisted of two feed-anaerobic-aerobic-anoxic phases. In the first sequence, 75% of the wastewater feed was provided, this is where mostly the phosphorus and ammonium removal of the system occurred, while the second feeding sequence is used to enhance denitrification and achieve the BNR performance. Two reactor operation runs were performed. During Run1 the reactor feed was supplemented with acetate to increase the wastewater volatile fatty acid (VFA) levels (Table 1). During Run2 the VFA levels were increased by prefermentation of the wastewater by anaerobic incubation at room temperature for two to three days before it was used as feed for the SBR. Throughout the SBR operations the volume exchange ratio (VER) was varied to achieve different hydraulic residence times (HRT). The VER being the ratio of feed added during a cycle to the total operating volume of the SBR. Also, settling times were reduced consecutively during the SBR operation after monitoring the settling performance to remove some of the slower settling biomass and select the faster settling particles. The settling time was kept to around 2-3 minutes per cycle after the system achieved a fully granular sludge.

Analytical methods

Sludge volumetric Index (SVI), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were periodically analysed using standard methods (APHA, 1995). To monitor the granule structure and characteristics, granule size distribution and density were measured from 30 ml of well mixed SBR sludge using a Malvern Mastersizer 2000 series (Malvern Instruments, Worcestershire, UK).

Concentrations of various components of samples were measured weekly to monitor the reactor performance. The ammonia (NH$_4^+$), nitrate (NO$_3^-$), nitrite (NO$_2^-$) and orthophosphate (PO$_4^{3-}$) concentrations were analysed using a Lachat QuickChem8000 Flow Injection Analyzer (Lachat Instrument, Milwaukee) (APHA, 1995). VFAs were measured by Perkin-Elmer gas chromatography with column DB-FFAP 15m x 0.53 mm x 1.0 µm (length x ID x film) at 140°C, while the injector and FID detector were operated at 220°C and 250°C, respectively. For the analysis, a volume of 1 µl of sample was injected and VFAs detected by closed reflux colorimetric method using standard methods (APHA, 1995).

Microbial community analysis was performed by fluorescence in situ hybridisation (FISH). Sludge samples were fixed and FISH was performed as described by Amann (1995). Oligonucleotide probes used in this experiment were EUBmix for the detection of all bacteria (Daims et al., 1999), PAOmix for *Accumulibacter* spp. also referred to as polyphosphate accumulating organisms (PAO) (Crocetti et al., 2000), the combination of GAOQ989 (Crocetti et al., 2002) and GB_G2 (Kong et al., 2002) for *Competibacter* spp. also referred to here as glycogen accumulation organisms (GAO), and NSO 1225 (Mobarry et al., 1996) for detection of ammonia oxidising bacteria (AOB) from the *Betaproteobacteria*. Probes were labelled with either Cy3 or Cy5. FISH preparations were visualized with a Zeiss LSM 510 Meta confocal laser scanning microscope (CLSM) using Plan-Apochromat 63x oil (NA 1.4) objective. Cy3 and Cy5 were excited with a HeNe laser (543 nm) and red diode laser (637 nm) and collected with 550-625 nm BP and 660 nm LP emission filters respectively. 30 images were taken from each sample for quantification (these were calculated as a percentage of the Bacterial population) using *daime* version 1.2 (Daims and Wagner, 2006).

RESULTS AND DISCUSSION

Formation of aerobic granular sludge

The objective of this experiment was to achieve rapid and stable granule formation, from a floc based sludge, and obtain BNR during treatment of the domestic wastewater. In the first SBR operation, Run1, VFA levels were low in the wastewater, and these were boosted by adding acetate to the feed (Table 1). Run1 occurred over 44 days, during which time the VER and settling times were progressively increased and decreased respectively. However only partial granulation occurred and the BNR performance failed (results not shown). This was likely due to the low biomass concentration. Overgrowth of filamentous bacteria was noticed on the granules, probably due to the occurence of readily degradable COD into the aerobic stage of the SBR cylce. The lesson learnt from Run1 was that manipulation of the VER and settling time had to be performed cautiously.

For Run2 the reactor was reseeded with sludge and operated for BNR as in Run1 except that prefermented wastewater was added to the feed to provide additional VFA (Table 1). This was operated for 152 days during which aerobic granular sludge for treating domestic wastewater was successfully formed in 50 days and reached a quasi-steady state after around 100 days of operation (Figure 1). During the granule formation, the biomass in the reactor decreased rapidly from 4.64 g.L^{-1} (MLVSS) to 0.65 g.L^{-1}(MLVSS) at around day 40 (Figure 2). Once granules had formed the biomass progressively increased to around 3 g.L^{-1} (MLVSS)

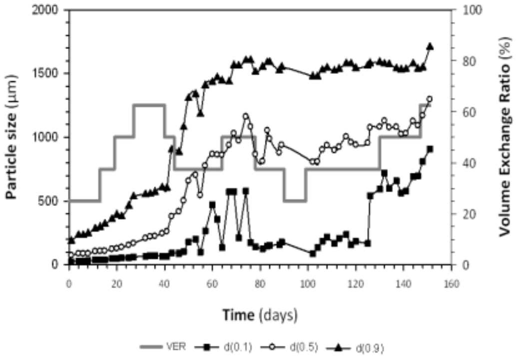


Figure 1. Size distribution profile and volumetric exchange ratio of the SBR treating domestic wastewater during Run2; the d (0.1) d (0.5) and d (0.9) correspond to the percentage of particles (10, 50 and 90 % respectively) that are smaller than the indicated particle size.

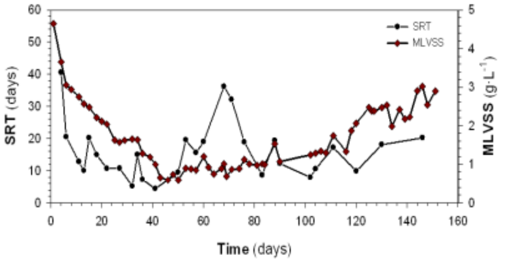


Figure 2. Sludge retention times (SRTs) and mixed liquor volatile suspended solids (MLVSS) profiles during SBR operation Run2.

The strategy used to obtain the granules and maintain BNR was that of progressively decreasing the settling time, washing out the floccular biomass, but maintaining enough biomass in the reactor. The settling time was reduced from the initial 50 minutes to only 2-3 minutes (Figure 3).

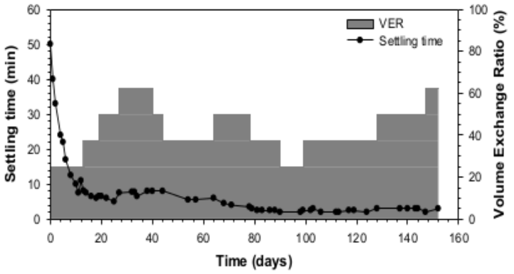


Figure 3. Settling time and volume exchange ratio during SBR operation Run2.

During the granulation process, there are three possible stages in relation to the particle size distributions of the reactors (Figure 1). The first stage is a granulation period (from day 0 to 65), this is followed by a maturation period where a maximum granule sizes appears to be reached (from day 60 to 130). The final period is when flocs are completely lost from the system (from day 130 onwards). The size of the granule was constantly stabile after 60 days.

During the maturation period small particles as floccular sludge were retained in the reactor. Floccular biofilm would compete with granular biofilm for substrate due to diffusion advantages (Wang et al., 2009). At day 130 the VER was increased, and this resulted in removal of the smaller particles (Figure 1). During this stage the reactor MLVSS and the SRT (Figure 2) both increased, indicating further granule formation.

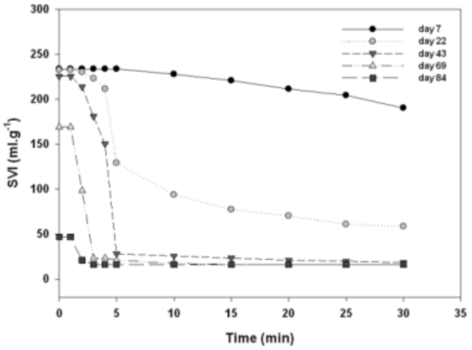
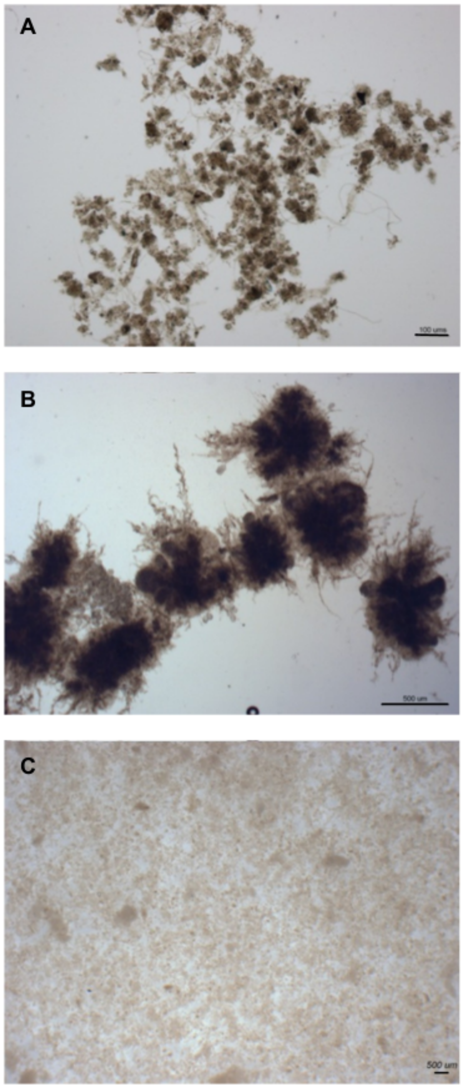


Figure 4. Sludge Volume Index (SVI) of the floccular sludge changes during the granule formation during SBR Run2.

Changes in the sludge volume index (SVI) (Figure 4) and the appearance (Figure 5) of the sludge correlate with the particle size changes during the reactor operation. The initial SVI_{30} of the sludge was 190 $ml.g^{-1}$, which by day 43 it had decreased to around 20 $ml.g^{-1}$ and remained low thereafter.

Changes to the morphology of the sludge during the granulation were evident (Figure 5). The shape of the granules was somewhat spherical with a distinct outline (Figure 5B and 5D). These results further confirm that the formation of aerobic granules was a gradual process from the floccular seed sludge to the compact, granular aggregates. There are suggestions that granule formation from flocs is facilitated by interparticle bridging under the shear conditions of the reactor (Beun et al., 2002). Filaments were observed as part of the granule structure during their development (Figure 5B), and these likely provide structure to the granules (Jang et al., 2003).

After a fully granular system was achieved (from about day 130), the settling time had been reduced to around 2-3 minutes (Figure 3). Also the VER was increased to 50 % and then 63 % (Figure 1). Higher loading is desired for efficient treatment of wastewater. Additionally, it is reported that higher loading is favourable for granule formation (Tay et al., 2004; Liu et al., 2007)). In addition to granule formation, our reactor was operating for BNR and the effect of high organic loading, such as the presence of VFA in the aerobic phase, would be detrimental and had to be considered in the SBR operation.



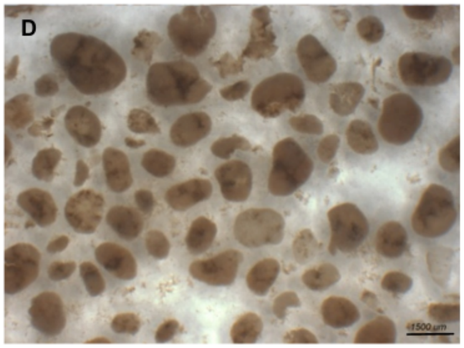


Figure 5. Microscopic images show the evolution of floccular into the granular sludge. Light microscopy images of the sludge at day 0 (A), and day 60 (B). Stereomicroscopic images at day 0 (C) and day 120 (D).

BNR Performance of Aerobic Granular Sludge treating Domestic Wastewater

During the SBR operation of Run1 and Run2 the wastewater contained low VFA levels (Table 1). VFA is a requirement of good BNR (Martin and Wilson, 1994, Abu-ghararah and Randal, 1991, McCue et al., 2003, Kampas et al., 2009), thus the VFA levels were increased during Run1 by addition of acetate, then in Run2 by the prefermentation of the wastewater (Table 1). Although the prefermentation approximately doubled the VFA in the wastewater, a small amount of acetate was added to the feed to facilitate BNR (Table 2). In general the ammonia, and phosphorus concentrations of the wastewater in Run2 was slightly lower than in Run1.

Table1. The concentration of various paramenters of the domestic wastewater used as SBR feed.

Parameters	RUN 1	RUN 2
CODt (mg $O_2 \cdot L^{-1}$)	315 (NA)	317 (97)
CODs (mg $O_2 \cdot L^{-1}$)	183 (21)	177 (64)
VFA_{ww} (mg $C \cdot L^{-1}$)	15.57 (6.39)	31.26 (16.03)
VFA_{IN} (mg $C \cdot L^{-1}$)	111.47 (59.35)	51.59 (15.08)
TKN (mg $N \cdot L^{-1}$)	NA	66.83 (3.85)
NH_4^+-N (mg $N \cdot L^{-1}$)	54.43 (7.76)	44.84 (11.59)
NO_2^--N (mg $N \cdot L^{-1}$)	0.02 (0.02)	0.11 (0.13)
NO_3^--N (mg mg $N \cdot L^{-1}$)	0.13 (0.06)	0.20 (0.25)
N_{TOT} (mg $N \cdot L^{-1}$)	54.58 (7.75)	45.15 (11.58)
TP (mg $P \cdot L^{-1}$)	NA	11.10 (1.15)
PO_4^{3-}-P (mg $P \cdot L^{-1}$)	9.69 (1.23)	8.00 (2.10)

NA: Not applicable
VFA_{WW}: VFA wastewater
VFA_{IN}: VFA Influent wastewater after acetate addition

The profiles of MLVSS and SRT of the reactor during Run2 are presented in Figure 2. The reactor started with a VER of 25%, and this was increased gradually until 62.5% based on the BNR performance (Figure 6). This coincided with biomass washout during the first 50 days, the MLVSS decreasing to as low as 0.6 g.L^{-1}. The SRT also decreased from 30 to 6 days as a consequence of the short settling time and biomass washout. By about day 30 the BNR performance was poor, thus the VER was decreased to enable recovery.

Overall the system showed good BNR performance throughout the operating period once granules had formed and the MLVSS had begun to increase (Figure 6). The system was able to achieve 99% and 98% P and N removal respectively. One period of poor N removal occurred at around day 80, this followed a period of very low strength wastewater.

Microbial community structure in these systems was monitored and quantified by FISH. Changes in populations of PAOs, GAOs and AOB were determined during granule formation period. This SBR was started and seeded with sludge from a wastewater treatment plant operating for enhanced biological phosphorus removal (EBPR). In the seed sludge polyphoshate accumulating organisms (PAOs) were only 10% of the total bacterial population, these then increased progressively to be 75% of the bacteria at day 110 of operation. The glycogen accumulating organsims (GAOs) were less than 5% of the bacteria in the seed sludge. These increased slightly to 25% at day 70, but decreased to only 8% at day 110. The ammonia oxidising bacteria (AOB) in this reactor were very low (less than 5%), this probably due to the aggressive biomass washout during the granule formation, and the slow growth of these organisms. However, good N removal was obtained during most of the operating period. EBPR operation has been found to be favourable for aerobic granular systems (de Kreuk et al., 2005). Suggestions are that slow growing organisms such as PAOs benefit the granule formation and stability. That is supported by the community analysis performed here where PAOs were dominating the granule population.

It appeared that the good BNR performance of this system was achieved through the activity of simultaneous nitrification, denitrification and phosphorus removal (SNDPR). SND involves the processes of ammonia oxidization to nitrite or nitrate, and simultaneous denitrification to dinitrogen. When this is coupled with EBPR then the process is known as SNDPR (Zeng et al., 2003). A representative cycle study performed on day 124 indicates the SNDPR activity (Figure 7). During the aerobic period around 9 mg.L^{-1} of N-NH_4^+ was removed while less than 3 mg.L^{-1} of N-NO_2^- was produced, indicating that simultaneous nitrification and denitrification (SND) occurred. The oxidized nitrogen present at the end of the aerobic period was almost exclusively nitrite (NO_2^-), suggesting that N was removed via the nitrite pathway in this granular SBR (Yilmaz et al., 2008). The high level of PAOs in the system may suggest that these were contributing to the denitrification in this system as has been found in other studies (Lemaire et al., 2006).

CONCLUSIONS

Development of aerobic granules from floccular sludge, while treating low-strength wastewater was achieved. Also good BNR performance was achieved during most of the granulation period. Adjusting the hydraulic loading and the settling times, and monitoring biomass and particle size were important for the success of granulation. The granular sludge was formed in the SBR after about 60 days of operation, and granules were maintained for the complete operation period, until day 152. The stable granular sludge achieved 99% and 98% P and N removal respectively while treating domestic wastewater. The good BNR performance was achieved through the process of simultaneous nitrification, denitirifaction and phosphorus removal (SNDPR). A prefermentation step of the wastewater lowered the necessity of acetate addition for BNR.

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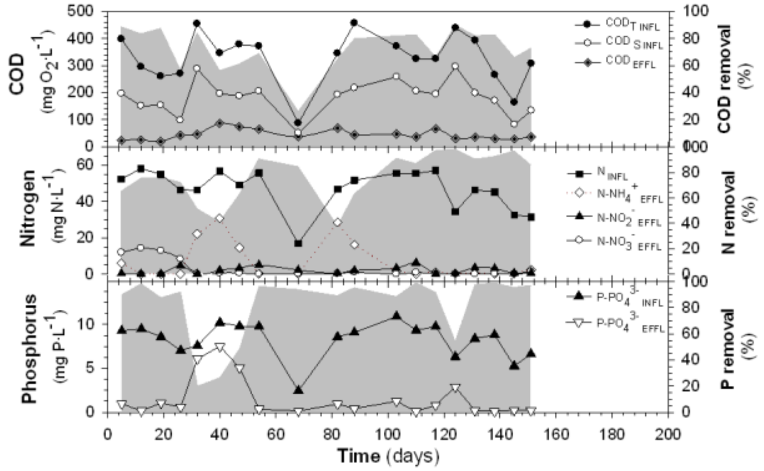

Figure 6. COD, Nitrogen and Phosphorus removal performances on SBR during the 152 days period.

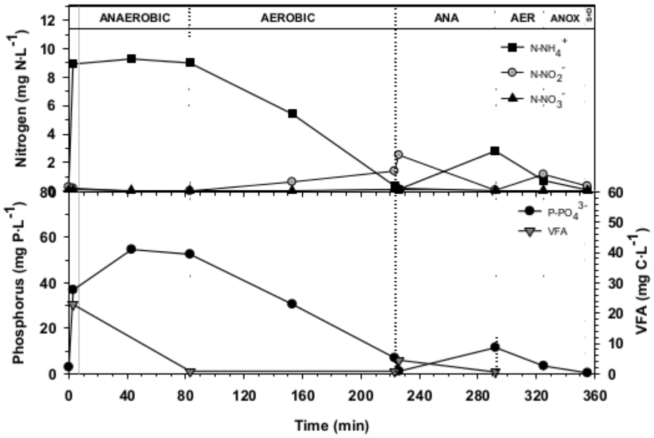

Figure 7. Cycle study performed on the SBR on day 124 demonstrating the occurrence of simultaneous nitrification, denitrification and phosphorus removal (SNDPR). The dotted lines delimit the first anaerobic period (3-80), the first aerobic period (80-220), the second anaerobic period (224-293), the second aerobic period (293-325) and the second anoxic period (325-357).

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