ELSEVIER

Water Research 39 (2005) 4476–4484

WATER RESEARCH

www.elsevier.com/locate/watres

Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures

M.K. de Kreuk*, M. Pronk, M.C.M. van Loosdrecht

Department of Biotechnology, Delft University of technology, Julianalaan 67, 2628BC Delft, The Netherlands

Received 11 March 2005; received in revised form 22 August 2005; accepted 30 August 2005

Abstract

Temperature changes can influence biological processes considerably. To investigate the effect of temperature changes on the conversion processes and the stability of aerobic granular sludge, an aerobic granular sludge sequencing batch reactor (GSBR) was exposed to short-term and long-term temperature changes. Start-up at 8 °C resulted in irregular granules that aggregated as soon as aeration was stopped, which caused severe biomass washout and instable operation. The presence of COD during the aerobic phase is considered to be the major reason for this granule instability. Start-up at 20 °C and lowering the temperature to 15 °C and 8 °C did not have any effect on granule stability and biomass could be easily retained in the system. The temperature dependency of nitrification was lower for aerobic granules than usually found for activated sludge. Due to decreased activity in the outer layers of granules at lower temperatures, the oxygen penetration depth could increase, which resulted in a larger aerobic biomass volume, compensating the decreased activity of individual organisms. Consequently the denitrifying capacity of the granules decreased at reduced temperatures, resulting in an overall poorer nitrogen removal capacity. The overall conclusion that can be drawn from the experiments at low temperatures is that start-up in practice should take place preferentially during warm summer periods, while decreased temperatures during winter periods should not be a problem for granule stability and COD and phosphate removal in a granular sludge system. Nitrogen removal efficiencies should be optimized by changes in reactor operation or cycle time during this season.

Keywords: Activity; Aerobic granular sludge; Granule size; Nutrient removal; Temperature; Waste water treatment

1. Introduction

Aerobic granular sludge technology is a promising technology for compact wastewater treatment plants (Bathe et al., 2005). So far, many laboratory scale studies have been performed to investigate the important factors of aerobic granular sludge formation and conversion processes that take place in a reactor based on this type of (activated) sludge (Morgenroth et al., 1997; Liu and Tay, 2004; De Kreuk et al., 2005a). Most of the aerobic granular sludge research was carried out at room temperature (20–25 °C) and as a result it is not known how these systems respond to changes in temperature. In the Netherlands, as in other northern

*Corresponding author. Tel.: +31 15 2781551; fax: +31 15 2782355.

E-mail addresses: m.dekreuk@tnw.tudelft.nl (M.K. de Kreuk), m.pronk@tnw.tudelft.nl (M. Pronk), m.c.m.vanloosdrecht@tnw.tudelft.nl (M.C.M. van Loosdrecht).

0043-1354/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2005.08.031

regions, the temperature of sewage usually varies between 5 and 12 °C in winter and 15–25 °C in summer. Therefore, temperature effects on aerobic granular sludge need to be investigated before the technology can be efficiently scaled-up and put into practice.

Previous experimental and modelling research pointed to the importance of growth rate on biofilm or granule morphology (Picioreanu et al., 1998; Villaseñor et al., 2000). When the maximal growth rate of organisms in aerobic granules decreases, it is expected that the biomass density will increase and that the granule surface will become more smooth. Another factor influencing granule stability is the competition between different organisms for space, oxygen and/or substrate. The growth rate and therefore the competitive advantage of various types of organisms can be differently influenced by temperature changes. In enhanced biological phosphate removal (EBPR) systems with low COD loading, the growth of long filamentous *Microthrixs Parvicella* was described when applying low temperatures ($\leqslant 12$ °C), while at higher temperatures (20 °C), the filaments broke into smaller fragments of 30–80 μm (Knoop and Kunst, 1998). Also competition between glycogen accumulating organisms (GAO) and phosphate accumulating organisms (PAO) is considered to depend on temperature. At neutral pH and 20 °C PAO will be dominant, whilst at 30 °C GAO will be dominant (Seviour et al., 2003). Since morphology of aerobic granules is among others influenced by the type of organisms present in the granule and their growth rates, a temperature change could change the granule stability.

It is generally assumed that the rate of conversion processes in biological systems depends on the temperature (as e.g. in ASM2 and ASM3; Henze et al., 2000), which can be described by a simplified derivation of the Arrhenius equation:

$$k(T) = k(20)\theta^{(T-20)}. \quad (1)$$

In which $k(T)$ (h^{-1}) is the conversion rate at temperature T (°C) and θ is a constant that can be determined experimentally.

Nitrification capacity generally decreases strongly with temperature. Nitrification even stops at temperatures below 5 °C (Henze et al., 1997). Frijters et al. (1997) showed that in biofilm systems the effect of temperature will be around 20% less then for activated sludge systems. For biological phosphate removal the effects are not so clear. Baetens et al. (1999) and Kumar et al. (1996) presented a literature overview of different temperature studies with EBPR systems and found that some authors described better efficiencies at moderate temperatures (20–37 °C), while others observed an improvement at low temperatures (5–15 °C) or no influence at all.

In aerobic granules, a decreased temperature can lead to shifts in population and different changes in bioconversion rates. For an adequate scale-up these effects need to be quantified. Therefore we investigated at laboratory scale the influence of temperature (8–20 °C) on granule formation and conversion. Short term as well as long terms effects were compared.

2. Materials and methods

2.1. Reactor system

A double-walled 3 l sequencing batch airlift reactor (SBAR) was used, with an internal diameter of 6.25 cm. The reactor contained an internal riser (90 cm high, 4 cm internal diameter, bottom clearance 1.25 cm). Air was introduced via a fine bubble aerator at the bottom of the reactor (4 l/min). During the first start-up experiment temperature was controlled at 8 °C (stage A). In the second experiment, temperature was controlled at 20 °C (stage BI), 15 °C (stage BII) or 8 °C (stage BIII). During stage BI several short-term temperature change experiments (at 5 and 8 °C) were carried out. The decreased temperature stabilised overnight (5–6 cycles) after which the experiment was performed. The total time of temperature change was never longer than 24 h. Dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration (100% at 20 °C = 9.1 mg/l; at 15 °C = 10 mg/l and at 8 °C = 11.8 mg/l). Oxygen concentration and pH were measured on-line. During stage A oxygen saturated conditions were used during the aeration period. In order to control the oxygen concentration at 20% (during stages BI, BII and BIII), the gas phase was circulated over the reactor. Dosing extra air or nitrogen gas in the gas recycling flow controlled the oxygen concentration. The pH was maintained at 7.0 ± 0.2 by dosing 1 M NaOH or 1 M HCl. Hydraulic retention time (HRT) and substrate load were respectively, 5.6 h and 1.6 kg COD/m^3/day (stage A, BI and BII) and 7.4 h and 1.2 kg COD/m^3/day (stage BIII).

The reactor was operated in successive cycles of 3 h (stage A, BI and BII): 60 min feeding under anaerobic conditions from the bottom of the reactor (plug-flow through the settled bed), 112 min aeration, 3 min settling (to keep only particles settling faster than 12 m/h in the reactor) and 5 min effluent discharge. During stage BIII, the aeration phase was increased to 171 min (4 h cycle) so all conversion processes could occur.

The composition of the influent media were (A) NaAc 63 mM, $MgSO_4.7H_2O$ 3.6 mM; KCl 4, 7 mM and (B) NH_4Cl 42.8 mM, K_2HPO_4 4.2 mM, KH_2PO_4 2.1 mM and 10 ml/l trace element solution (trace element solution taken from Vishniac and Santer, 1957). From both media 150 ml per cycle was dosed together with 1300 ml tap water.

2.2. Measurements

Morphology of the granules (particle diameter, aspect ratio and shape factor), density, dry weight and ash content of the granules; TOC, biomass concentration and acetate in the bulk liquid were measured as described in Beun et al. (2002).

NH_4^+, NO_2^- and NO_3^- and PO_4^{3-} concentrations in the bulk liquid were determined spectrophotometrically by use of standard test kits (Dr. Lange type LCK). The SVI_8 was determined by reading the height of the settled bed in the reactor after 8 min settling (after the settling phase and effluent withdrawal phase) and calculated from the settled bed volume and the dry weight in the reactor. SVI measurements after short settling are more discriminative for granular sludge (Schwarzenbeck et al., 2004). Calculations (conversion rates and removal efficiencies) were performed as described in Mosquera-Corral et al. (2005). The conversion rates were averaged from two cycle measurements (short term and long term temperature changes) and the removal efficiencies were determined as an average from a stable period in each experimental stage.

3. Results

Start-up of aerobic granular sludge reactors at room temperature was extensively studied before (De Kreuk and Van Loosdrecht, 2004). Initially we compared these previous start-ups with a start up of the system at 8 °C. This experiment was followed by monitoring a system in which temperature was decreased in two steps, from 20 to 15 to 8 °C. Temperature changes were studied during one cycle (short-term) experiments in which no population change or adaptation could take place, as well as during long-term experiments.

3.1. Start-up of granular sludge reactor at 8 °C

The SBAR was started-up at 8 °C without oxygen control (almost saturated oxygen concentration) and a settling time of 10 min (particles with a settling velocity larger than 3 m/h are retained in the reactor), which was gradually decreased to 3 min during the first 4 weeks of operation. The reactor was inoculated with 1 l of activated sludge from a WWTP and 20 ml crushed granules (approximately 1.5 g TSS) from a laboratory scale aerobic granular sludge reactor with EBPR activity (Fig. 1a).

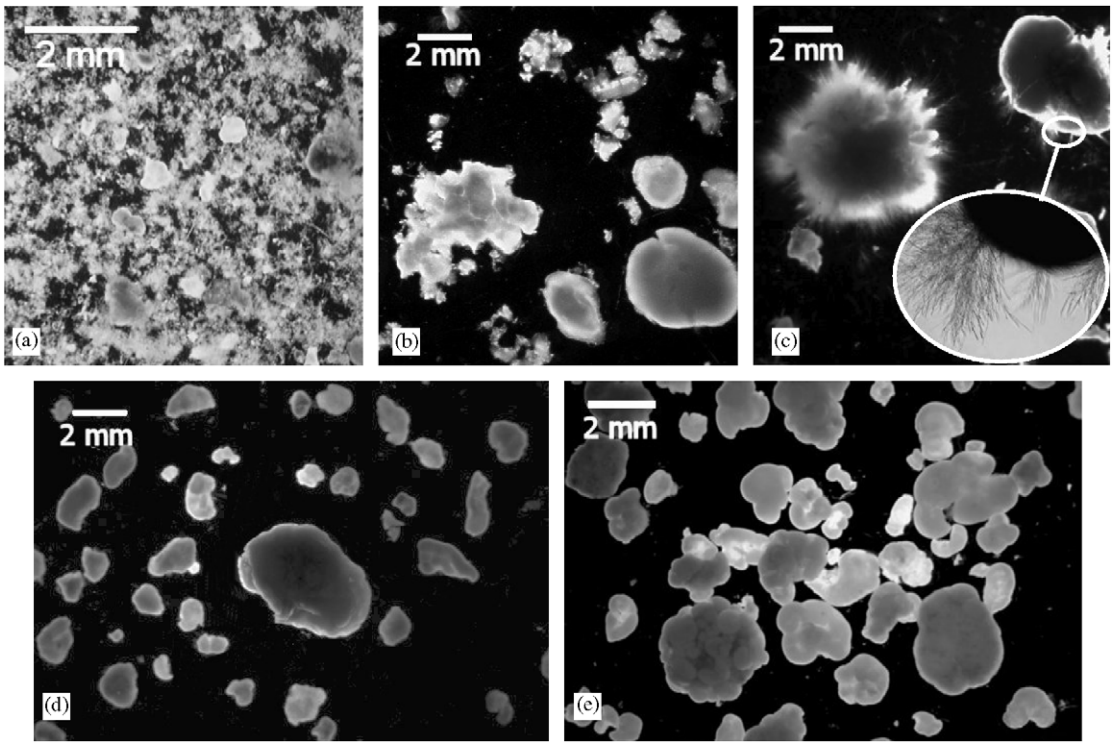

Fig. 1. Start-up material and granules grown at different stages of this study: Inoculation material for start-up at 8 °C (a); irregular granules formed 26 days after start-up at 8 °C (b); granules with fungi/fungi pellet (c); granules formed at 20 °C (d) and granules formed when temperature lowered to 8 °C (e). The size bar is 2 mm.

Three days after start-up, most biomass is washed-out of the reactor and the first irregular granules became visible. Similar to earlier experiences, the reactor had to be cleaned regularly to avoid excessive wall-growth. After 24 days, all biomass was present as large granules. The time needed for total granule formation approximated twice the time that is normally needed at 20 °C. The appearance of the granules also differed from the ones obtained at 20 °C; they flocculated to large structures as soon as aeration stopped. From day 21 to 28, the dry weight in the reactor increased from 0.83 to 1.06 g/l at the same time the SVI_8 increased from 33 to 153 ml/g. The granules changed to fluffy, irregular structures with a low density (Fig. 1b) and after 35 days, most biomass washed-out of the reactor.

The start-up of the reactor at low temperature was initiated again but now approximately 10 ml (approximately 1 g TSS) of intact granules from another laboratory aerobic granular sludge reactor with EBPR activity was used as inoculum. After 27 days, these granules changed again into fluffy, hollow structures that flocculated during settling. At day 33 of the second start-up, an extra amount of 75 ml (approximately 4 g TSS) aerobic granules from another laboratory reactor with EBPR activity was added, which increased the dry weight in the reactor to 3.3 g/l. After 15 days, fungi started to grow on the surface of these granules (Fig. 1c). The first experiments of Beun et al. (1999) also started with fungal pellets, which were overgrown by heterotrophic organisms that changed the pellets into smooth and dense granules. The fungi did not disappear during the experiment and seemed to influence the conversion processes negatively. The dry weight stabilised around 8 g/l and the SVI_8 fluctuated between 114 and 18 ml/g right after granule dosage and stabilised at 34 ml/g at the end of the experiment. Clearly the presence of enough granular sludge at the start of the reactor had a stabilising effect on the granule formation, likely this is related to the sludge/substrate-loading rate.

The conversion processes in the reactor started-up at 8 °C were minimal. During the anaerobic period, hardly any phosphate was released (P concentration is 3 mg/l after the anaerobic period) and most acetate is consumed during the aerobic period. Directly after dosing intact aerobic granules from another laboratory set-up, a high phosphate release took place during the anaerobic period and the ratio of released phosphate and consumed acetate was 0.28, which is comparable to EBPR sludge at 20 °C. However, after 3 days, this ratio decreased to 0.04 and at the end of the experiment it was only 0.02. Acetate at the end of the anaerobic phase was as on average 240 mg COD/l and was aerobically consumed (average effluent concentration 24 mg COD/l). Also nitrification was absent, resulting in high ammonia concentrations in the effluent (43 mg NH_4–N/l). Because of the low activity of the sludge, the experiment was stopped 110 days after the second start-up.

3.2. Long term effects of temperature changes

The SBAR was started-up again, but now at 20 °C and an oxygen concentration of 20% of the saturation concentration. The reactor was started with 27 ml (approximately 2 g TSS) of stable aerobic granules from another laboratory set-up with EBPR activity combined with 200 ml activated sludge to ensure biodiversity at the inoculation. In line with previous experiments, after 55 days the settled granular sludge bed reached a steady-state volume of 1.2 l and a SVI_8 of 15 ml/g. From 20 °C (153 days stable operation), the reactor temperature was decreased to 15 °C (48 days stable operation) and to 8 °C (130 days stable operation).

The morphology of the granules remained similar at all three temperatures (Table 1, Figs. 1d and e). At all temperatures, the average diameter of the granules was 1.2 mm, while shape factor and aspect ratio were both around 0.73. No filamentous structures were observed.

Table 1
Steady-state characteristics of granular sludge grown in an airlift reactor at different temperatures

	20 °C (153 days)	15 °C (48 days)	8 °C (130 days)	Start-up at 8 °C (after addition of granules)
Average diameter (mm)	1.2	1.2	1.2	Not measurable because of flocculation
Aspect ratio	0.72	0.71	0.72	
Shape factor	0.74	0.73	0.74	
Dry weight in reactor (g VSS/l reactor)	18	20	18	7
SVI8 (ml/g)	15	12	14	34
N-removal efficiency (%)	75	65	44	0
P-removal efficiency (%)	97	95	97	11
PO_4^{3-} released per acetate consumed	0.364	0.338	0.339	0.02

The density of the granules at 8 °C was slightly lower than at 20 °C (53 gVSS/l biomass at 8 °C versus 78 gVSS/l biomass at 20 °C). This could be caused by the lower temperature or lower load applied at the lowest temperature.

Steady-state results of the conversion processes are summarized in Table 1 and typical conversions during a steady-state cycle at 20 and 8 °C are shown in Figs. 2(A)–(F). During the stable operation at 20 °C, the nitrogen removal fluctuated around 75%. The

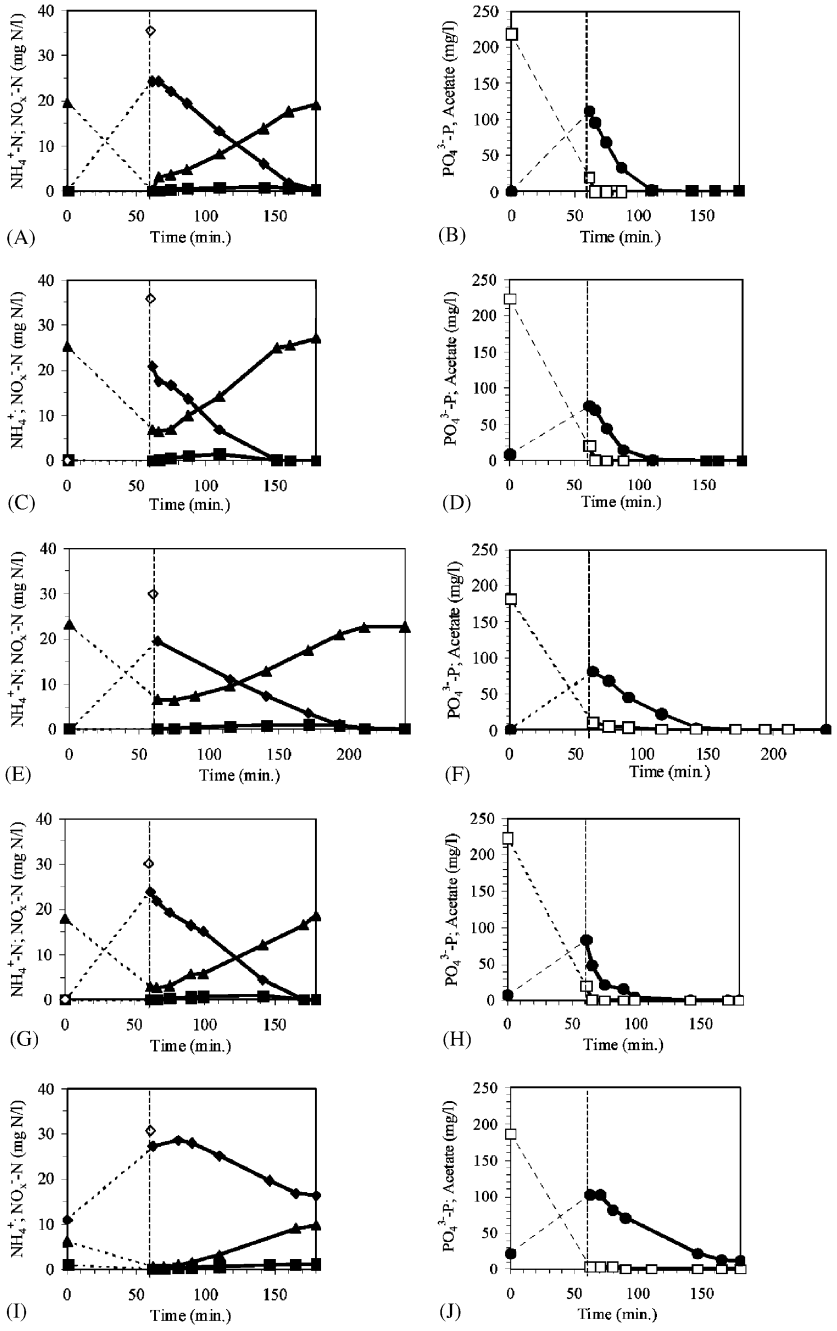

Fig. 2. Conversion in single cycles of the SBAR during the steady-state period at 20 °C (A, B), 15 °C (C, D) and 8 °C (E, F) and during the short-term temperature changes at 15 °C (G, H) and 8 °C (I, J). Symbols: NH_4^+–N (♦), NO_3^-–N (▲) and NO_2^-–N (■) in mg N/l (A, C, E, G, I); PO_4^{3-}–P (●) in mg-P/l; acetate (□) in mg/l. The NH_4^+–N concentration in the reactor after feeding at the start of the aeration period (◇) is calculated by the concentration in the influent and the dilution in the SBAR.

average phosphate removal was 97% and all acetate was consumed during the anaerobic period. Results of the phosphate removal are comparable with previously reported research, the nitrogen removal is lower than the average of 94% measured over 300 days found in previous research, most likely due to the increased nitrogen load in this study (COD/N ratio 6.5 in this study, 7.9 in this previous research) (De Kreuk et al., 2005b).

After 208 days, the temperature was lowered to 15 °C. The suspended solids concentration and SVI_8 remained comparable to the last period at 20 °C, namely 33 gTSS/l (ash content 40%) and 12 ml/g. There were no changes in the observed phosphate and COD removal (total acetate consumption during anaerobic period and P-removal efficiency 95%). However, nitrogen removal decreased to 65%.

The reactor was changed to 8 °C after 48 days of operation at 15 °C. Because of the expected decreased conversion rates at 8 °C, the cycle length was increased to 4 h at this temperature. The anaerobic feeding period remained 1 h, while the aerobic period was increased with 1 h to 171 min in order to preserve the nitrogen removing capacity. This consequently led to a decreased loading rate. During the first 28 days of operation at 8 °C, the granules and effluent composition did not change. However, during the last 102 days of operation, the effluent ammonia concentration remained low (full nitrification), while the nitrate concentration increased. This led to an overall nitrogen removal efficiency of 44%. Dry weight and ash content stayed comparable to those at higher temperatures (30 g TSS/l and ash content 39%) and the average phosphorus-removal efficiency remained 97%. Since most acetate was stored as internal storage-polymers by the PAO during the anaerobic period, substrate was not available for other heterotrophic organisms during the aeration phase. Therefore, fast growing or filamentous organisms were not able to grow and the ratio between phosphate released and acetate consumed remained the same at a low temperature. After 130 days this experiment was stopped.

3.3. Short-term effects of temperature changes

The direct effect of temperature changes on conversions was studied by subjecting the 20 °C steady-state granular sludge reactor for less than 24 h to a decreased temperature. During these experiments it could be assumed that the microbial composition of the granules will be unchanged. The results of representative cycle measurements of these experiments are shown in Figs. 2(G)–(J). The ammonium concentration that is measured at the start of the aeration period was lower than expected based on the influent concentration and the dilution in the reactor. Ammonia can be partly adsorbed to the EPS in the granule during the feeding time (Nielsen, 1996; Temmink et al., 2000), resulting in observed concentrations lower than expected. The mixing time within the reactor can play a role as well; ammonia could be already partly consumed before the reactor is completely mixed.

In line with expectations, the conversion processes at 8 °C were slower than at the higher temperatures. At 20 and 15 °C, all ammonia was converted into nitrite and nitrate and part of the nitrate was denitrified again, respectively leading to 64% and 53% nitrogen removal from the influent. At 8 °C large amounts of ammonia were found in the effluent, together with nitrate and nitrite. This resulted in only 35% nitrogen removal from the influent. Also phosphate was not totally consumed in the aerobic period at 8 °C (phosphate removal efficiency was 37% instead of 100% at 15 and 20 °C).

4. Discussion

The rates of most biological processes depend on temperature. This dependency can be described with a simplified Arrhenius equation (Eq. (1)). In the activated sludge model 2 (ASM2), four groups of temperature dependency are distinguished; (i) $\theta = 1.00$: no dependency, processes such as chemical precipitation; (ii) $\theta = 1.04$: low dependency, e.g. hydrolysis; (iii) $\theta = 1.07$: medium dependency, e.g. heterotrophic conversions and fermentation; (iv) $\theta = 1.12$: high dependency, e.g. nitrification (Henze et al., 2000). Changed conversion rates at low temperatures do not only change nutrient removal efficiencies in aerobic granular sludge, but also granule formation.

4.1. Start-up of the reactor at low temperatures—consequences for large-scale applications

Starting up a laboratory scale reactor at 8 °C resulted in outgrowth of filamentous organisms and irregular structures, leading to washout of the biomass. The reactor was unstable and the experiment had to be stopped because biomass could not be retained in the reactor. Outgrowth of filamentous organisms and irregular structures in biofilms or aerobic granular sludge can be a consequence of concurrent availability of oxygen and readily biodegradable substrate at low concentrations leading to significant gradients of substrate concentration inside the granules (Van Loosdrecht et al., 1997; De Kreuk and Van Loosdrecht, 2004; McSwain et al., 2004). The acetate uptake rate of PAO is strongly dependent on temperature ($\theta = 1.095$, Brdjanovic et al., 1998) and therefore is low at 8 °C. With relatively low concentration of biomass in the reactor during the start-up of the reactor, acetate consumption during the total cycle is limited and most substrate will be available concurrently with electron acceptors

(oxygen and nitrate) during the total aerobic period (effluent acetate concentrations were 24 mgCOD/l). This means that there is no feast-famine regime under these circumstances, which is crucial for stable aerobic granule formation (Beun et al., 1999; De Kreuk and Van Loosdrecht, 2004; McSwain et al., 2004). Because of the substrate availability during the aerobic period and the low temperature, circumstances are advantageous for filamentous growth. In activated sludge systems increased chance of sludge bulking during winter and spring has often been observed (Eikelboom et al., 1998; Kruit et al., 2002). Similar conditions during this experiment led to outgrowth of filaments and formation of irregular structures, bad settling characteristics and biomass washout as well. The results of the start-up period at low temperatures indicate that great care has to be taken by the start-up of full-scale or pilot plants. Preferably, the start of a new system should take place in summer, when temperature is high and processes are fast, resulting in consumption of readily biodegradable COD during the anaerobic phase, in absence of electron acceptors. In this case granules are easily formed, as was shown with the experiments at 20 °C. When this is not possible due to planning, a reactor has to be started with a sufficient amount of granules or very active EBPR sludge from other plants, in order to prevent external COD availability during the aerobic period.

4.2. Temperature dependency of the aerobic granules without adaptation

Decreasing the temperature of a stable operating reactor from 20 to 15 or 8 °C during a short period (24 h), showed a significant decrease in conversion rates (Fig. 3). Temperature coefficients were derived from these experiments (Fig. 3, Table 2), showing a medium temperature dependency for nitrification and for phosphate uptake. The temperature dependency of nitrification in aerobic granules is lower than in a system with suspended biomass. This has also been reported for biofilm systems (Frijters et al., 1997). The aerobic granular sludge consists of a layered structure; an

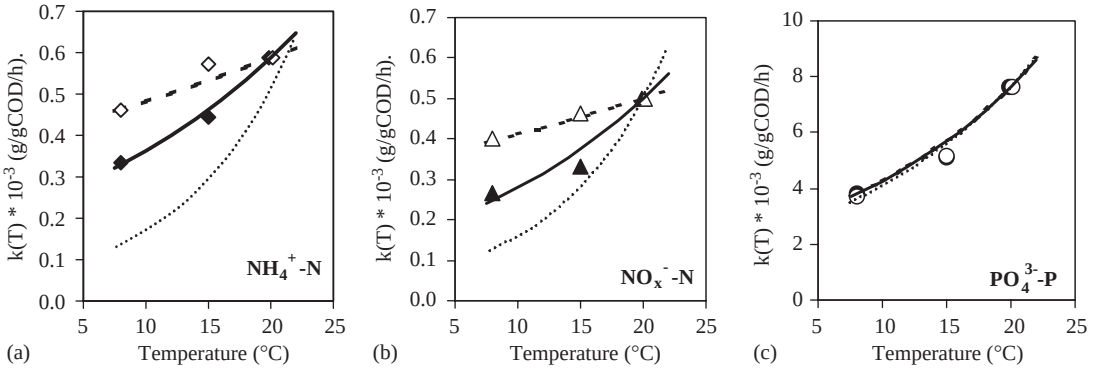

Fig. 3. The maximum measured conversion rates $k(T)$ in g/g biomass COD/h, at different temperatures for NH_4^+–N consumption, NO_x^-–N production and PO_4^{3-}–P consumption for a system adapted to 20 °C with short term changes to low temperatures (◆, ▲, ●) and for systems adapted to the three different temperatures (◇, △, ○). The lines are fitted according to Eq. (1) with the temperature coefficients (θ) for the short-term temperature decrease (solid lines), the adapted system (dashed lines) and the reported values on activated sludge (Table 2, dotted lines).

Table 2
Calculated temperature coefficients (θ; Eq. (1)) for the conversion rates of ammonia and phosphate and the production rate of nitrate and nitrite in a system adapted to the low temperatures and a system adapted to 20 °C

Process	Adapted granules to various temperatures	Short-term tests at changed temperatures	Literature (activated sludge system)
NH_4^+ consumption	1.02	1.05	1.12[a]
NO_x^- production	1.02	1.06	1.12[a]
PO_4^{3-} consumption	1.06	1.06	1.031[b,c]; 1.065[b,d]

[a]Data from ASM2d (Henze et al., 2000).
[b]Data from Brdjanovic et al., 1998.
[c]Long-term experiment.
[d]Short-term experiment.

aerobic outer layer, containing a mixture of heterotrophic and autotrophic organisms, and an anoxic or anaerobic core, in which denitrifying and anaerobic organisms are present. A lower temperature leads to a lower activity in the aerobic layers and thus to a higher oxygen penetration depth. In this situation, the autotrophic organisms existing in the deeper layers of the granule have oxygen at their disposal for nitrification. This extra aerobic volume in the granules compensates for the decreased specific conversion rates. This leads to the overall decreased effect of temperature change for the ammonium oxidation rate. The increased aerobic volume in the granules of course leads to a lower anoxic volume. This results in the observed lower denitrification.

Comparing the temperature dependency for phosphate consumption to data of flocculated systems (Brdjanovic et al., 1998), showed comparable dependencies for both systems. PAO's have the ability to use oxygen and nitrate for phosphate uptake and therefore a change in anoxic and aerobic volume of the granule, does not have an effect on the phosphate uptake rate. Therefore, there is no observed difference between flocculated and granular biomass in the short-term experiments.

4.3. Adaptation of the aerobic granules to low temperatures

The temperature of a steady-state operated granular sludge reactor at 20 °C was reduced to 15 and to 8 °C until steady-state operation. In this way the effects of a long-term temperature change, like during a winter period, were studied.

The temperature dependency of the nitrification rate (ammonium consumption and nitrate production) during this long-term experiment was lower than during the short-term experiment and lower than values for systems containing suspended biomass (Table 2). This is most likely due to the change in population structure inside the granules. The decreased temperature dependency after adaptation for nitrification can be explained by the penetration depth of oxygen into the granule in combination with the enrichment of nitrifying organisms in the aerobic zone. As in the short-term experiment, decreased activity of the organisms at low temperatures will lead to an increased oxygen penetration depth and therefore to an increased aerobic volume. The larger autotrophic population will compensate the decreased specific activity caused by lower temperatures. This effect is larger in the long-term experiment than in the short-term temperature change; since enrichment of autotrophic organisms cannot occur during the duration of such short-term test.

The increased aerobic layer at lower temperatures affects the total nitrogen removal efficiency negatively. The volume of anoxic core, in which denitrification takes place, decreases, resulting in higher nitrate concentrations in the effluent. In practice, the nitrogen removal efficiency can be improved by decreasing the dissolved oxygen concentration for an optimal ratio between aerobic and anoxic biomass (De Kreuk et al., 2005b), in combination with an increase of the aerobic cycle length for total nitrification. Another possibility is incorporating an extra denitrification cycle step during the SBR cycle.

The temperature dependency of the phosphate uptake rate during the long-term experiment did not differ from the temperature dependency during the short-term experiments (Table 2). Since PAO can grow under aerobic and anoxic conditions, PAO are expected to be present throughout the granule and their performance will be insignificantly influenced by a change in oxygen penetration depth. Consequently, the temperature dependency of the phosphate uptake rate during short-term and long-term experiments was similar. However, Brdjanovic et al. (1998) reports adaptation of the PAO during long-term experiments, resulting in a decreased θ-coefficient for the *P*-uptake. However, also in these long-term experiments, strong temperature effects on the other metabolic processes were reported. These experiments were carried out with a system with highly enriched PAO and suspended biomass under fully aerobic conditions. The difference might suggest lower adaptation ability of denitrifying PAO towards their phosphate uptake.

During the long term temperature experiments the granules remained stable over a long period. This indicates that granulation is feasible at low temperature. The problem to achieve good granulation during start-up experiments is therefore indeed likely associated with the low initial biomass content and the occurrence of easy degradable substrate in the aerated phase. During the decreasing temperature experiments acetate was always fully removed in the anaerobic phase.

5. Conclusions

Temperature changes can affect the performance of an aerobic granular sludge reactor to a large extent. The start-up of a reactor at low temperatures led to the presence of organic COD in the aerobic phase and therefore to instable granules that aggregated during settling and biomass washout. Once a reactor is started-up at higher temperatures it was possible to operate a stable aerobic granular sludge system at lower temperatures. Due to an increased oxygen penetration depth at low temperatures, nitrification rates are influenced only limitedly. The increased penetration depth of oxygen leads to decreased nitrogen removing capacity of aerobic granules at low temperature. At large scale, nitrogen

removal should be controlled by adjusting the oxygen concentration in the reactor if the temperature changes. Aerobic granular sludge reactors should preferentially be started-up in warm seasons (spring or summer) or with a substantial amount of aerobic granules from other systems.

Acknowledgements

This research was funded by the Dutch Foundation for Water Research (STOWA, TNW99.262) and STW (DPC5577) within the framework of the "aerobic granule reactors" project. The project is in close co-operation with DHV-Water in Leusden, The Netherlands.

References

Baetens, D., Vanrolleghem, P.A., van Loosdrecht, M.C.M., Hosten, L.H., 1999. Temperature effects in bio-P removal. Water Sci. Technol. 39 (1), 215–225.

Bathe, S., De Kreuk, M.K., Mc Swain, B.S., Schwarzenbeck, N., 2005. Aerobic granular sludge. In: Water and Environmental Management Series (Wems). IWA Publishing, London 170p.

Beun, J.J., Hendriks, A., van Loosdrecht, M.C.M., Morgenroth, M., Wilderer, P.A., Heijnen, J.J., 1999. Aerobic granulation in a sequencing batch reactor. Water Res. 33 (10), 2283–2290.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J., 2002. Aerobic granulation in a sequencing batch airlift reactor. Water Res. 36 (3), 702–712.

Brdjanovic, D., Logemann, S., Van Loosdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J., Heijnen, J.J., 1998. Influence of temperature on biological phosphorus removal: process and molecular ecological studies. Water Res. 32 (4), 1035–1048.

De Kreuk, M.K., Van Loosdrecht, M.C.M., 2004. Selection of slow growing organisms as a means for improving aerobic granular sludge stability. Water Sci. Technol. 49 (11–12), 9–19.

De Kreuk, M.K., De Bruin, L.M.M., Van Loosdrecht, M.C.M., 2005a. Aerobic granular sludge: from idea to pilot plant. In: Bathe, S., De Kreuk, M.K., Mc Swain, B.S., Schwarzenbeck, N. (Eds.), Aerobic Granular Sludge. IWA, London, UK, pp. 111–123.

De Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M., 2005b. Simultaneous cod, nitrogen and phosphate removal by aerobic granular sludge. Biotechnol. Bioeng. 90 (6), 761–769.

Eikelboom, D.H., Andreadakis, A., Andreasen, K., 1998. Survey of filamentous populations in nutrient removal plants in four European countries. Water Sci. Technol. 37 (4–5), 281–289.

Frijters, C.T.M.J., Eikelboom, D.H., Mulder, A., Mulder, R., 1997. Treatment of municipal wastewater in a circox® airlift reactor with integrated denitrification. Water Sci. Technol. 36 (1), 173–181.

Henze, M., Harremoës, P., Arvin, E., 1997. Wastewater treatment: biological and chemical processes. Springer, Berlin Heidelberg, p. 383.

Henze, M., Gujer, W., Mino, T., van Loosdrecht, M., 2000. Activated sludge models; asm1, asm2, asm2d and asm3. Scientific and technical report series. Scientific and Technical Report No.9. IWA Publishing, London. p. 130.

Knoop, S., Kunst, S., 1998. Influence of temperature and sludge loading on activated sludge settling, especially on *Microthrix parvicella*. Water Sci. Technol. 37 (4–5), 27–35.

Kruit, J., Hulsbeek, J., Visser, A., 2002. Bulking sludge solved?!. Water Sci. Technol. 46 (1/2), 457–464.

Kumar, P., Mehrotra, I., Viraraghavan, T., 1996. Biological phosphorus removal: effect of low temperature. Journal of Cold Regions Engineering 10 (2), 63–76.

Liu, Y., Tay, J.-H., 2004. State of the art of biogranulation technology for wastewater treatment. Biotechnol. Adv. 22 (7), 533–563.

McSwain, B.S., Irvine, R.L., Wilderer, P.A., 2004. The effect of intermittent feeding on aerobic granule structure. Water Sci. Technol. 49 (11), 19–25.

Morgenroth, E., Sherden, T., van Loosdrecht, M.C.M., Heijnen, J.J., Wilderer, P.A., 1997. Aerobic granular sludge in a sequencing batch reactor. Water Res. 31 (12), 3191–3194.

Mosquera-Corral, A., de Kreuk, M.K., Heijnen, J.J., van Loosdrecht, M.C.M., 2005. Effects of oxygen concentration on n-removal in an aerobic granular sludge reactor. Water Res. 39 (12), 2676–2686.

Nielsen, P.H., 1996. Adsorption of ammonium to activated sludge. Water Res. 30 (3), 762–764.

Picioreanu, C., Van Loosdrecht, M.C.M., Heijnen, J.J., 1998. Mathematical modeling of biofilm structure with a hybrid differential–discrete cellular automaton approach. Biotechnol. Bioeng. 58 (1), 101–116.

Schwarzenbeck, N., Erley, R., Wilderer, P.A., 2004. Aerobic granular sludge in an sbr-system treating wastewater rich in particulate matter. Water Sci. Technol. 49 (11–12), 41–46.

Seviour, R.J., Mino, T., Onuki, M., 2003. The microbiology of biological phosphorus removal in activated sludge systems. FEMS Microbiol. Rev. 27 (1), 99–127.

Temmink, H., Klapwijk, A., de Korte, K.F., 2000. Feasibility of the biofix-process for treatment of municipal wastewater. Water Sci. Technol. 43 (1), 241–249.

Van Loosdrecht, M.C.M., Picioreanu, C., Heijnen, J.J., 1997. A more unifying hypothesis for biofilm structures. FEMS Microbiol. Ecol. 24 (2), 181–183.

Villaseñor, J.C., van Loosdrecht, M.C.M., Picioreanu, C., Heijnen, J.J., 2000. Influence of different substrates on the formation of biofilms in a biofilm airlift suspension reactor. Water Sci. Technol. 41 (4–5), 323–330.

Vishniac, W., Santer, M., 1957. The thiobacilli. Bacteriol. Rev. 21, 195–213.