Simultaneous COD, Nitrogen, and Phosphate Removal by Aerobic Granular Sludge

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Received 25 August 2004; accepted 18 January 2005

Abstract: Aerobic granular sludge technology offers a possibility to design compact wastewater treatment plants based on simultaneous chemical oxygen demand (COD), nitrogen and phosphate removal in one sequencing batch reactor. In earlier studies, it was shown that aerobic granules, cultivated with an aerobic pulse-feeding pattern, were not stable at low dissolved oxygen concentrations. Selection for slow-growing organisms such as phosphate-accumulating organisms (PAO) was shown to be a measure for improved granule stability, particularly at low oxygen concentrations. Moreover, this allows long feeding periods needed for economically feasible full-scale applications. Simultaneous nutrient removal was possible, because of heterotrophic growth inside the granules (denitrifying PAO). At low oxygen saturation (20%) high removal efficiencies were obtained; 100% COD removal, 94% phosphate (P-) removal and 94% total nitrogen (N-) removal (with 100% ammonium removal). Experimental results strongly suggest that P-removal occurs partly by (biologically induced) precipitation. Monitoring the laboratory scale reactors for a long period showed that N-removal efficiency highly depends on the diameter of the granules. © 2005 Wiley Periodicals, Inc.

Keywords: aerobic granular sludge; efficiency; granule size; nutrient removal; simultaneous nitrification/denitrification (SND)

INTRODUCTION

Present sewage treatment plants require large surface areas. This is mainly due to the need for large settling tanks to maintain the biomass in the system, in combination with the low biomass concentration in the reaction tanks. A conceptual design study pointed out that presently used activated sludge plants could be intensified by a factor 4 with biomass grown in compact aggregates (granules) instead of flocculated sludge (De Bruin et al., 2004). These aerobic granules are biomass aggregates grown under aerobic conditions without a carrier material.

Laboratory studies have indicated a potential to grow stable aerobic granules under a feast/famine regime at high dissolved oxygen concentrations (Beun et al., 1999, 2000; Etterer and Wilderer, 2001; Tay et al., 2002). However, maintaining high oxygen concentrations requires a high energy input and is economically unfeasible. Moreover, the design of a compact installation is based on the possibility of simultaneous nitrification/denitrification (SND) within the granules (Beun et al., 2001; De Bruin et al., 2004), which only can occur at moderate oxygen concentrations.

As is known from activated sludge and biofilm studies, SND requires an aerobic zone in the biofilm or floc for nitrification and an anoxic substrate-rich interior for denitrification (Pochana and Keller, 1999; Third et al., 2003; Satoh et al., 2003). In the case of SND, the oxygen penetration depth is controlled by the oxygen concentration in the bulk liquid, such that the anoxic volume is large enough for nitrate reduction (Fig. 1). Such a low oxygen saturation (40%) in an aerobic granule reactor based on a feast/famine regime led to instability and outgrowth of filamentous structures (Dangcong et al., 1999; Mosquera-Corral et al., submitted). Based on theoretical concepts for biofilm morphology (Van Loosdrecht et al., 1995a; Picioreanu et al., 1998), it was postulated that one of the important parameters for this phenomenon was a high potential growth rate on the applied substrate in combination with a relative low oxygen concentration (Mosquera-Corral et al., submitted). During the feast period in an aerobic SBR, substrate is partly stored (30–70%). The subsequent growth on the storage polymers in the famine phase occurs at a strongly reduced growth rate. As a result, a low DO in the famine period has no negative effect on the granule stability.

Based on theoretical concepts for biofilm morphology (Van Loosdrecht et al., 1995b; Picioreanu et al., 1998) or filamentous sludge (Martins et al., 2004) it was expected that selecting for a population with a low maximal growth rate, would lead to improved granule stability. Enhancement of this effect could be achieved by feeding the substrate under anaerobic conditions, allowing only storage of substrate without growth. It was shown that this indeed leads to stable

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Contract grant sponsor: Dutch Foundation for Water Research (STOWA) and Dutch Technology Foundation (STW)
Contract grant numbers: STOWA, TNW99.262; STW DPC5577

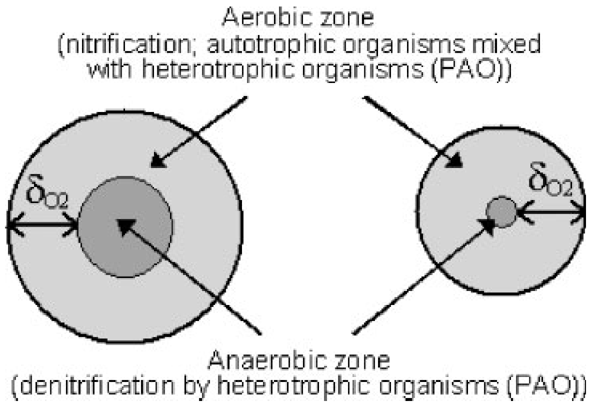

Figure 1. Decreased anaerobic or anoxic zone with a decreased granule diameter at constant DO.

granule formation at low oxygen concentrations (De Kreuk and Van Loosdrecht, 2004). Interestingly, the use of an anaerobic feeding period allows to have combined biological nitrogen and phosphate removal. As long as sufficient phosphate is present, phosphate-accumulating organisms will dominate (Mino et al., 1998). If sufficient biomass is removed, and thus enough stored poly-phosphate (poly-P), the P-removal capacity will be maintained (Morgenroth and Wilderer, 1999). If there is a lack of phosphate, the granules will be dominated by glycogen-accumulating organisms (De Kreuk and Van Loosdrecht, 2004; Zeng et al., 2003b).

This study investigates the important factors for simultaneous nitrogen and phosphate removal in granular sludge sequencing batch reactors (SBRs). Special attention is given to the effect of the required dissolved oxygen concentration for optimal nitrogen (N-) removal.

MATERIALS AND METHODS

Reactor System

Two double-walled 3-L sequencing batch airlift reactors (SBARs) were used, with an internal diameter of 6.25 cm. Both reactors contained a 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 reactors (4 L/min). Dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration ($100\% = 9.1$ mg/L). Oxygen concentration and pH were measured online. To control the oxygen concentration properly, the gas phase was recirculated through the reactor. Dosing extra air or nitrogen gas in the gas recycling flow controlled the oxygen concentration. Experiment 1 (stage A) was performed without oxygen control (DO 100%), experiment 2 (stage B) was performed at 40% oxygen saturation, and experiment 3 (stage C) at 20% oxygen saturation. The pH was maintained at 7.0 ± 0.2 by dosing $1M$ NaOH or $1M$ HCl. Temperature was kept at 20°C. Hydraulic retention time (HRT) was 5.6 h and substrate load, measured in chemical oxygen demand (COD), was 1.6 kg/m^3/day.

The reactor was operated in successive cycles of 3 hours: 60 min feeding 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.

The composition of the influent media were (A) NaAc 63 mM, $MgSO_4 \cdot 7H_2O$ 3.6 mM, KCl 4,7 mM; and (B) NH_4Cl 35.4 mM, K_2HPO_4 4.2 mM, KH_2PO_4 2.1 mM, and 10 mL/L Trace element solution. From both media 150 mL per cycle was dosed together with 1300 mL tap water. During the start-up period (day 1 to 52) nitrification was inhibited by dosing allylthiourea (ATU) at a concentration of 100 mg/L. Simultaneously, a sequencing batch bubble column (SBBC) was operated, with the same operation parameters and dimensions as the SBAR.

Measurements

Morphology of the granules (particle diameter, aspect ratio and shape factor), density, dry weight and ash content of the granules; total organic carbon (TOC), biomass concentration, NH_4^+-N, NO_2^--N and NO_3^--N concentration in the bulk were measured as described in Beun et al. (2002). Poly-b-hydroxybutyrate (PHB) content of the granules was measured according to Smolders et al. (1994). The solid retention time (SRT) of the aerobic granules is not controlled by sludge wasting, but follows from the suspended solids in the effluent, divided by the amount of biomass in the reactor. N_2O in the off-gas was measured, by analyzing the off-gas using gas-chromatography.

PO_4^{3-}-P concentration in the bulk liquid was determined spectrophotometrically by use of standard test kits (Dr. Lange type LCK; manufacturer: Hach Lange, Düsseldorf, Germany). Phosphate in the granules was measured according to the method described by Uhlmann et al. (1990). 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. Calculations (penetration depth into the granule, ammonium oxidation rate, nitrogen removal efficiency, and ratio between acetate rich biomass and aerobic biomass) were performed as described in Mosquera-Corral et al. (submitted).

Fluorescent in situ hybridization (FISH) was performed on thin layers of the granules to determine the location of the phosphate-accumulating organisms (PAO) (mixture of probes PAO462, PAO651, and PAO 846), nitrifying organisms (mixture of probes NSO1225 and NSO 190), and most eubacteria (Mixture of EUB338, EUB338-II, and EUB338-III) within the granule structure (Crocetti et al., 2000; Daims et al., 1999; Mobarry et al., 1996).

RESULTS

The reactor was started up without oxygen control, which means that the dissolved oxygen concentration during aeration was close to saturation. The feeding period was anaerobic and the influent flow could be considered as plug-flow through the settled bed of granules. The development of the granules is described in De Kreuk and Van Loosdrecht (2004). A short summary of the development of the particle characteristics is given below. In this article we focus on the conversion processes at different oxygen concentrations rather than on the granule characteristics.

Granule Formation at Different Oxygen Concentrations

During start-up and the first steady-state period (233 days), the SBAR was operated without oxygen control. Three weeks after inoculation with activated sludge, the aerobic granules had already a size of 1.1 mm. During the experiment, this granule size fluctuated in time between 1.1 mm and 1.6 mm, the average shape factor (capriciousness of the surface) fluctuated between 0.6 and 0.7, and the aspect ratio (roundness of the particle) between 0.66 and 0.76 ($0 = \text{line}$ and $1 = \text{circle}$). The density of the granules increased in time from 56 to 97 g TSS/L biomass. The dry weight in the reactor was 8.5 g VSS/L, ash content 41%. The average suspended solids concentration in the effluent was 0.049 gVSS/L. The sludge volume index (SVI_8) stabilized at 24 mL/g, which is very low compared to the values of activated sludge (100–150 mL/g). To determine the sludge volume index of granular sludge, 8 min of settling time was chosen instead of 30 min, as is often used in case of activated sludge. Schwarzenbeck et al. (2004) showed that granular sludge has a similar SVI after 60 min and after 5 min of settling. This different settling behavior of granules and activated sludge, could be used to distinguish more clearly granular growth from flocculated growth.

After 233 days, the oxygen saturation level was decreased to 40%. This induced breaking of granules during the first 2 weeks, but the granules recovered to the same characteristics as they had at a saturated oxygen level. The average sludge age further increased from 40 days to 67 days and the dry weight in the reactor increased from 8.5 to 12 g VSS/$L_{reactor}$ (granule density—89 and 87 g TSS/L biomass). The ash content of the granules decreased to 34%, and the average suspended solid concentration in the effluent remained similar (0.048 gVSS/L). Also reduction of the oxygen saturation level to 20% did not have large influences on the granule characteristics nor the effluent suspended solids concentration (0.050 gVSS/L), and even had a small positive effect on the sludge volume index ($SVI_8 = 14$ mL/g) and the dry weight in the reactor (16.5 g VSS/$L_{reactor}$, ash content 30%, granule density 78 g TSS/L biomass). Again, the granules stayed stable and there was no growth of filamentous organisms or structures at the surface of the granules. Fluorescent in situ hybridization (FISH) on a cut granule grown with an anaerobic feed clearly showed a layered structure, with a mixture of heterotrophic PAO and autotrophic nitrifyers in the outer layers of the granule and PAO inside the granule. The average granule size fluctuated between 0.4 and 1.8 mm during the long-term (300 days) operation at 20% oxygen saturation.

Long-Term Effects of the Oxygen Concentration on Reactor Operation

After 52 days of reactor operation at saturated oxygen level (stage A), granules were matured and full acetate uptake occurred during the anaerobic period. After 65 days, 95% phosphate removal was measured. The influent phosphate concentration was 20 mg P/L. During the period from 65 to 233 days, the average phosphate release into the bulk liquid during the anaerobic feeding period was 86 mg P/L, while the average effluent concentration was as low as 0.4 mg P/L. The ratio of phosphate released and acetate taken up was 0.44 P mol/C mol, comparable to the ratio of 0.5 at pH 7 for a highly enriched PAO culture (Mino et al., 1998; Smolders et al., 1994). During the first 52 days after start-up, the ammonium-oxidizing organisms were inhibited with allylthiourea (ATU) to prevent nitrification. This prevents the presence of nitrate during the anaerobic feeding period, which would hinder development of PAO. Thirty-nine days after the dosage of ATU was stopped full ammonium oxidation was reached (Fig. 2). However, it took around 100 days before full nitrite oxidation was reached; neither ammonium nor nitrite could be measured in the effluent after day 154. Day 154 to 233 were used to operate the reactor under stable conditions in steady state. Due to the operation at saturated oxygen levels, incomplete denitrification takes place (34% total N-removal, while 27% N-removal is caused by biomass growth).

To increase denitrification by decreasing the oxygen penetration depth and thus the aerobic volume of the granules, the oxygen concentration was lowered to 40% of the saturation level (stage B) (Beun et al., 2001; Mosquera-Corral et al., submitted). This did not have any influence on the phosphate removal efficiency, which remained 97% on average (effluent concentrations <0.8 mg P/L), removing 78 g P m^3/day. Acetate remained fully removed from the influent during the anaerobic period, while the average ratio of phosphate release and acetate consumption did not change (0.45 P mol/C mol).

During the first period of operation at a dissolved oxygen saturation of 40%, denitrification efficiency slowly increased and after 64 days, measured effluent concentrations of nitrate were below 5 mg NO_3^--N/L. Also all ammonium and nitrite were oxidized, which caused an average nitrogen removal of 98% during 35 days (Fig. 2). After this period, the morphology of the aerobic granules started to change from spherical particles to more irregular shaped particles, showing cracks towards the middle of the granule. Among others, this reflected in the N-removal capacity, which decreased to values between 50% and 70%, giving nitrate effluent concentrations between 11 and 19 mgNO_3^--N/L.

Because of insufficient N-removal at an oxygen saturation level of 40%, oxygen was further decreased to 20% saturation level (stage C). The phosphate removal efficiency remained high (94% on average) during the first 90 days after lowering

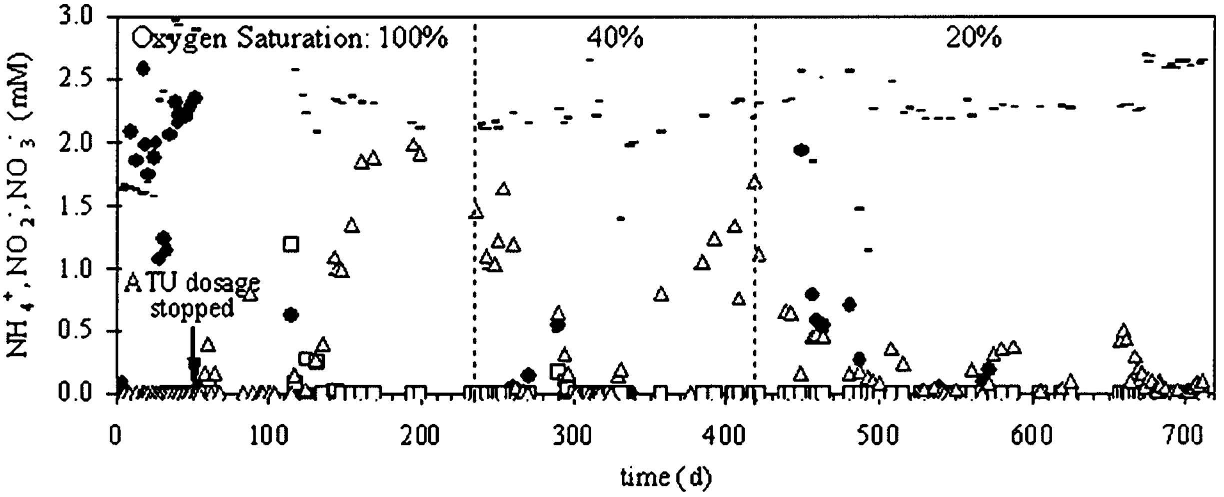

Figure 2. Evolution of nitrogen compounds concentrations: NH_4^+-N (–)(m*M*) in the influent and NH_4^+-N (●), NO_3^--N (△) and NO_2^--N (□) (m*M*) in the effluent of the SBAR.

the oxygen concentration. Hereafter, the effluent phosphate concentrations started to increase, most likely due to the low growth yield of the biomass (SRT was 71 days). The biomass production approximated 80 mg per cycle, while the amount of phosphate that had to be removed was 30 mg per cycle. The maximum poly-phosphate content of biomass in an acetate-fed SBR system reported in literature is 0.38 gP g VSS (Wentzel et al., 1989). To remove phosphate completely, the sludge age should be controlled at a lower value.

After decreasing the oxygen saturation from 40% to 20%, the effluent nitrogen concentrations (NH_4^+-N, NO_2^--N and NO_3^--N) did not show immediate response (Fig. 2). After 30 days of operation at 20% oxygen saturation, a decreased nitrification efficiency was observed, indicated by a sudden increase of the ammonium concentration in the effluent. Seventy-eight days after changing the oxygen concentration (approximately one sludge age), the nitrification capacity recovered and no effluent ammonium was detected. The denitrification capacity was large enough to remove the extra produced nitrate and the total nitrogen removal from the system increased to an average of 94% over 300 days (average effluent concentration 1.8 mg NO_3^--N/L). Typical patterns of phosphate, acetate, PHB and nitrogen concentrations during a cycle are given in Figure 3.

Zeng et al. (2003a) reported the formation of nitrous oxide during simultaneous nitrification, denitrification, and phosphate removal in a lab-scale SBR. Therefore, off-gas has been analyzed several times, but N_2O was not detected.

Short-Term Effects of Increased and Decreased Oxygen Saturation Levels

The direct effect of oxygen concentration on the conversion processes was studied in a separate set of experiments. Decreasing the oxygen concentration will result in a smaller aerobic zone in the granules, which will influence the nitrification–denitrification process. The oxygen saturation was changed during one cycle (100%, 40%, and 10%). The experiments were performed during the steady-state operation of stage B (oxygen saturation level = 40%). The gas flow was kept constant in all cases to obtain the desired oxygen concentration without changing the mixing conditions, and thereby the external mass transfer rates (Nicolella et al., 1999). The biomass concentration and granule composition were comparable in all experiments.

The phosphate uptake rate was influenced by the oxygen concentration (Table II). The phosphate uptake rate under anoxic conditions is lower than under aerobic conditions (Murnleitner et al., 1997). When oxygen penetration depth decreased at lower oxygen concentrations, the anoxic volume increased and the aerobic volume decreased as expected leading to lower overall phosphate uptake rates. In Figure 4 the phosphate removal at 100% oxygen saturation with

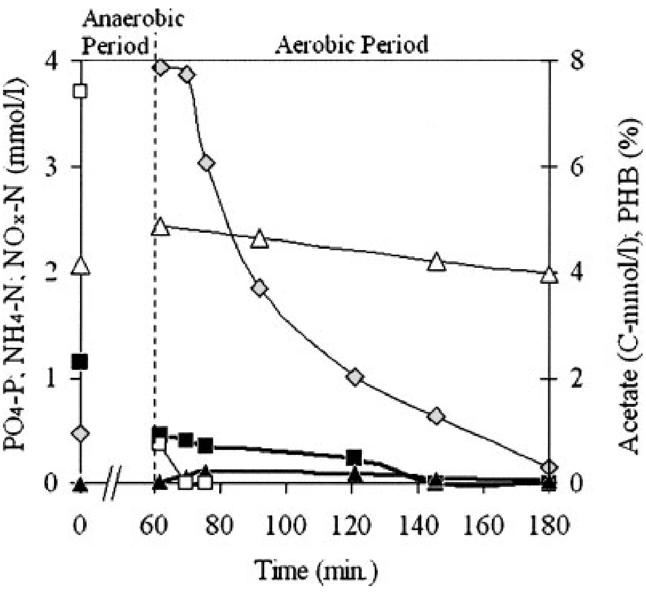

Figure 3. Typical patterns of phosphate (◇), acetate (□), PHB (△), ammonium (■) and nitrate plus nitrite (NO_x, ▲) concentrations during a cycle at 20% oxygen saturation.

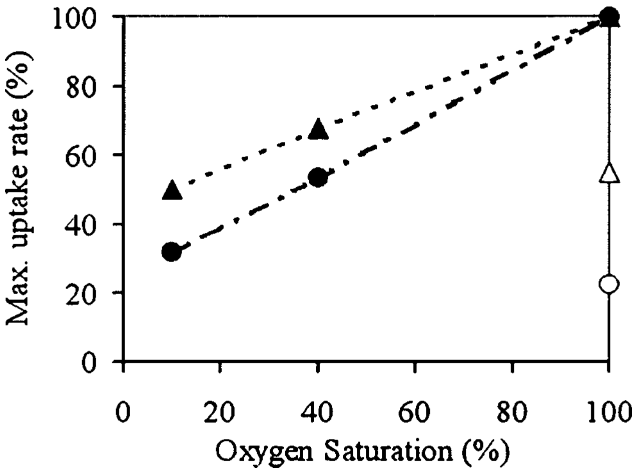

Figure 4. Uptake rate of phosphate [with nitrate present (▲) and without nitrate (△)] and uptake rate of ammonium [with nitrification (●) and without nitrification (○)] at different oxygen concentrations during one cycle; indexed to the value at 100% oxygen saturation.

nitrification/denitrification activity is indexed at 100%. The other rates are presented as a fraction of this 100%. In the absence of nitrate (nitrification inhibition with ATU), the phosphate uptake rate is decreased by 45%. Extrapolating the P-uptake rate to anoxic conditions (0% O_2 in Fig. 3) also indicates that roughly 45% of the P-uptake occurs anoxically.

The trend of the total nitrogen removal during cycle measurements at different oxygen concentrations (Table I), follows the results of the modeling performed by Beun et al. (2001) and previous results of similar experiments in a fully aerobic reactor without bio P-removal (Mosquera-Corral et al., submitted). The highest total nitrogen removal in this experiment is obtained at an oxygen saturation of 40%, which was also the long-term saturation level at which the granules were cultivated. The ammonium uptake rate is also plotted in Figure 4, in which the ammonium uptake rate at 100% oxygen saturation is indexed as 100%. The ammonium uptake rate due to growth is 22% of the total uptake rate, measured at 100% oxygen saturation with ammonium oxidation inhibition (ATU dosage). This value corresponds with extrapolated N-removal if the oxygen is absent (Fig. 3).

DISCUSSION

Introducing an anaerobic feeding period in the cycle of the aerobic granular sludge reactor was advantageous according to granule stability, biological phosphate removal, and simultaneous nitrification/denitrification (SND). Contrary to full aerobic pulse-fed operation (Mosquera-Corral et al., submitted), stable granule formation at low oxygen levels was possible. A 60-min anaerobic feed, followed by an aerobic period with oxygen saturation of 20% resulted in maximum simultaneous COD (100%, acetate), phosphate (94%), and nitrogen removal (100% ammonium removal by nitrification and 94% total N-removal). The biomass concentration that can be maintained in this type of SBR reactor with an exchange ratio of 50% was around five times higher than in an activated sludge system with flocculated biomass. Because of these high biomass concentrations in combination with the extraordinary settling capacity of granular sludge (no external settler needed and high height/diameter ratio possible), aerobic granular sludge systems can be built very compact (De Bruin et al., 2004). These results showed the potential of this process for wastewater treatment systems.

Phosphate Removal With Granular Sludge

Enrichment of phosphate-accumulating organisms in aerobic granular sludge by introducing alternating anaerobic feeding and aeration periods, resulted in stable granules at low dissolved oxygen concentrations. Furthermore, high phosphate removal efficiency (94%) was achieved by these PAO-enriched granules. Besides improved granule formation and P-removal, the problems of pulse feeding at full-scale installations (i.e., oversized pump capacity and large buffer tanks) also are solved. Feeding during 33% of the cycle would allow for a system of three reactors to be operated with a continuous feed.

Table I. Characteristics of granular sludge grown in an airlift reactor: Effect of dissolved oxygen concentration and feeding patterns.

	Anaerobic feed			Aerobic feed[a]	
	DO 100%	DO 40%	DO 20%	DO 100%	DO 40%
Average diameter (mm)[b,c]	1.3	1.1	1.3	1.6	5.0
Particle density (g TSS/1 biomass)[b,c]	89	87	78	53	13
Dry weight in reactor (g VSS/1 reactor)[b,c]	8.5	12	16.5	5.1	0.9
SVI (ml/g)[b,c]	24	20	14	50	200
Average SRT (days)[c]	40	67	71	8	<5
Mg NH_4^+-N/L (effluent)[c]	<0.01	0.03	0.08	0.13	1
Mg NO_3^--N/L (effluent)[c]	27	15	1.7	22	21
Mg NO_2^--N/L (effluent)[c]	0.1	<0.01	<0.01	3.9	1.5
N-removal efficiency	34%	56%	94%	16%	45%

[a] Mosquera et al. (submitted).
[b] De Kreuk and Van Loosdrecht (2004).
[c] This study.

Table II. Effect of dissolved oxygen concentrations on nitrogen removal during a short change in a SBAR with long anaerobic feeding time (steady state at 40% oxygen saturation).

DO	10%	40%	100%
VSS/L in reactor	12.9	12.6	13.9
% NO_x-N effluent	0.2%[a]	17.1%[b]	28.5%[b]
% NH_4^+-N effluent	24.5%	0%	0%
N-removal	75.3%	82.9%	71.5%
P-uptake rate (mmol/gVSS/h)	0.36	0.51	0.62

[a] In effluent as NO_2^--N.
[b] In effluent as NO_3^--N.

With the high phosphate concentration in the influent (19.6 mg PO_4^{3-}-P/L, COD/P = 20.2) and the low growth yield of the granules (measured as 0.25 g VSS/g COD), the calculated P-content of the granules would be 0.20 g P/g SS. In literature, reported values are lower, especially for biofilm systems, ranging from 0.02–0.14 (Falkentoft, 2000). Therefore, it should be considered that part of the phosphate removal could be caused by precipitation inside the granule. In the past, phosphate precipitation in biofilm and EBPR systems has been described as well (Arvin, 1983; Maurer et al., 1999). This possibility was supported by the increased ash content of the granules, which was only 6% when pulse feeding aerobically and 30%-41% with an enriched PAO culture. Also the color of most granules was white, although some light brown granules still occurred in the system. The ash content of light brown granules was determined as 17.2%, while the white granules had an ash content of 50.4%, which also suggests the presence of precipitates in the white granules.

The concentration of calcium in the influent tap water is high enough for the formation of apatite (≈50 mg Ca^{2+}/L), especially when excessive amounts of solute phosphate are available inside the granule during the anaerobic feeding period. A small increase of the pH inside the granule can already cause precipitation of apatite. At pH 7.2 at 20°C, and a phosphate concentration higher than 70 mg/L, precipitation can occur (Maurer et al., 1999). The phosphate concentration measured in the bulk liquid after the anaerobic period exceeds 100 mg/L and because acetate is dosed as a salt and taken up as acetic acid, the pH inside the granule is expected to increase during the anaerobic period (Smolders et al., 1995). Extraction of potential precipitated phosphate with bicarbonate-dithionite (ferrous-bound phosphate), sodium hydroxide (polyphosphate, organic phosphate and aluminium-bound phosphate) and hyperchloric acid (phosphate bound to calcium and magnesium) (Uhlmann et al., 1990) was carried out on the biomass. The average total precipitated phosphate content of the samples at the end of the anaerobic phase was 2.6% (PO_4^{3-}-P/VSS), consisting of 0.8% ferrous-bound, 0.3% organic or aluminium-bound, and 1.3% calcium- or magnesium-bound phosphate. Clearly, a significant part of phosphate can be removed by biologically induced precipitation. The growth of PAOs in granules or biofilms will enhance this precipitation relative to conditions in activated sludge flocs.

In a sewage treatment plant, precipitation of apatite inside the granules could occur as well. This could be advantageous, since the efficiency of the phosphate removal could increase and the granules become heavier, which improves the settling velocity of the granules. However, phosphate concentrations in the influent in practice are lower than used in this experiment and also temperature will be lower than 20°C, both decreasing the precipitation ability. On the other hand, the pH of most treatment plants is higher than in this experiment. The effect of precipitation in practice and the fraction of phosphate removal caused by precipitation will therefore strongly depend on local conditions.

Influence of Oxygen Saturation on Nitrogen Removal

Oxygen concentration is of major importance for aeration energy requirements and therefore for the economic feasibility of aerobic granular sludge technology (De Bruin et al., 2004). Also nitrogen removal by simultaneous nitrification/denitrification (SND) is dependent on the oxygen concentration in the system (Beun et al., 2001; Mosquera-Corral et al., submitted; Pochana and Keller, 1999; Satoh et al., 2003), by which the ammonium is oxidized in the outer layers while the NO_x is reduced in the inner layers of the biofilm, granule, or floc.

In most biofilm systems, consisting of heterotrophic and autotrophic organisms, heterotrophs dominate in the outermost layers, since they outcompete the nitrifiers for dissolved oxygen and space. This phenomonon was shown experimentally (Okabe et al., 1995) and has been described in biofilm model simulations (Wanner and Reichert, 1996). This distribution is disadvantageous for SND in continuous biofilm systems, since most COD will be consumed in the outer aerobic layers and thus cannot be used as an electron donor during denitrification in the inner part of the biofilm. Furthermore, the system is more sensitive to oxygen concentrations, because the nitrifyers are easily outcompeted on growth rate by the heterotropic organisms in the aerobic layer.

The use of biological phosphate removal can simplify the SND process. Figure 5 clearly shows a layered structure within the granules, with a mixture of heterotrophic PAO and autotrophic organisms in the outer layers of the granule and PAO inside the granule. According to the similar growth rate of autotrophic organisms and PAO (Brdjanovic et al., 1998), their existence in the same outer layer, competing for space and oxygen, was expected (Van Loosdrecht et al., 1995b). Contrary to nitrifyers and most heterotrophs, the possibility of PAO to use nitrate as an electron acceptor (DPAO) in combination with their anaerobic COD storage capacity, allows their existence in the anoxic core of the granule. From the conversion rates of the different components during the cycle measurements at 20% oxygen saturation and the stoichiometry of the different processes, the oxygen uptake rate could be calculated, which was 4.9 mg O_2/g VSS/h when phosphate uptake occurs and 3.3 mg O_2/g VSS/h when

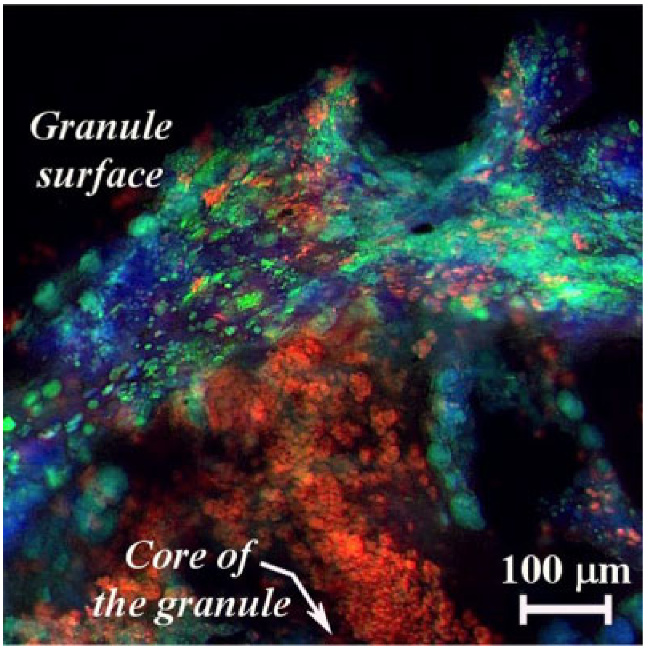

Figure 5. Impression of the layered structure of the granule (20% oxygen saturation in bulk liquid) by applying FISH techniques (— = 100 µm; green = ammonium oxidizing bacteria; blue = eubacteria; red = PAO). [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

phosphate is taken up completely. Together with the diffusion coefficient, biomass density (78 g/L) and oxygen concentration, the oxygen penetration can be calculated. With and without phosphate present the oxygen penetration depth in the biofilm approximates, respectively, 190 µm and 235 µm. This oxygen penetration depth is similar to the size of the layer in which autotrophic organisms occur (Fig. 5).

The layered structure of the aerobic granules underlines the expected and measured improvement of the N-removal efficiency at decreased oxygen concentrations during the short-term experiments (Table II). A lower oxygen concentration leads to an increased anoxic, storage polymer-rich biomass volume. This caused the observed increased denitrification at decreased oxygen concentrations (decreased effluent NO_x). Furthermore, a decreased oxygen concentration will lead to a decreased aerobic outer layer in which the autotrophic organisms are active. Therefore, at 20% oxygen saturation during the short-term experiment, ammonium was measured in the effluent. This effect of oxygen on simultaneous nitrification/denitrification is comparable to the behavior reported in literature for activated sludge flocs (Pochana and Keller, 1999; Satoh et al., 2003), EBPR flocs (Zeng et al., 2003a), biofilm systems (Garrido et al., 1997) or aerobic granules grown under fully aerobic conditions (Beun et al., 2001; Mosquera-Corral et al., submitted).

Steady-state operation at 20% oxygen saturation resulted in the highest nitrogen removal efficiency. With granule sizes larger than 1.3 mm, the anoxic volume containing active DPAO inside the granule is large enough for denitrification, leading to 94% nitrogen removal and stable granules. This result underlines that operation of large-scale reactors at low oxygen concentrations will not be a problem with respect to granule stability.

Influence of Granules Diameter on Nitrogen Removal

In the long-term experiments it was shown that an oxygen saturation of 20% resulted in the highest nitrogen removal. The ratio of the volume of the aerobic layer and the anoxic core is important for the SND efficiency (Fig. 1). A higher oxygen concentration leads to a higher oxygen penetration depth at the same overall oxygen uptake rate and thus to a smaller anoxic volume inside the granules. This phenomenon is also described for activated sludge flocs by Pochana and Keller (1999), showing that physically broken sludge flocs led to lower denitrification rates, due to their decreased anoxic zone. In this respect, the size of the granules will be an important variable in the reactor operation, but so far it is an unknown and unpredictable variable.

During the experiment at an oxygen saturation of 20%, average diameters ranged from 0.4 to 1.8 mm; the smallest average diameters were found in the bubble column (Fig. 6), while all other factors were kept the same. So far, there is no good explanation for this varying granule diameter. Lin et al. (2003) reports different particle sizes at different P/COD ratios. At a P/COD ratio as used in this study (5/100), a subsequent diameter of 1.03 mm was found, but this reactor was only operated for 2 months. In the present research, it was observed that this diameter fluctuates in time during longer operation under the same conditions. Not only process conditions play a role in the granule diameter; the inside of large granules tend to destabilize by endogeneous respiration and granules fall apart in small fractions that can grow out to form large granules again.

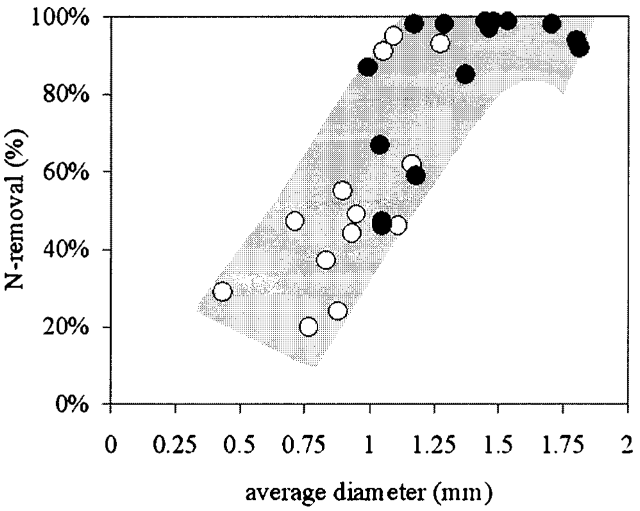

Figure 6. Measured N-removal efficiency at different granule diameters (20% oxygen saturation in the bulk liquid) in the SBAR (●) and in the SBBC (○).

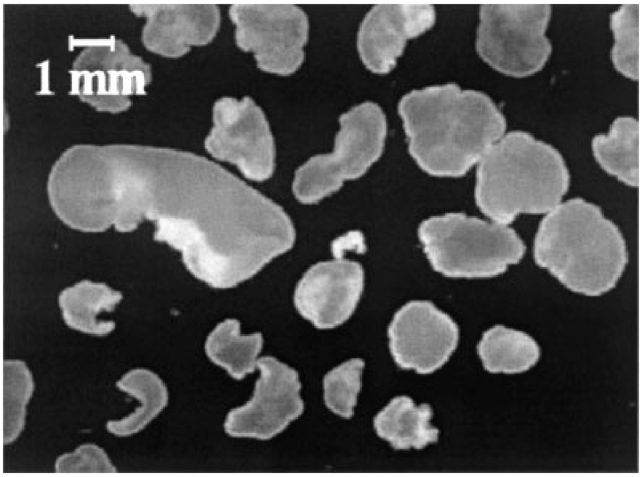

Figure 7. Broken granules, leading to flat or kidney-shaped structures.

The performance of two different aerobic granular sludge reactors at 20% oxygen saturation has been monitored for 2 years (SBAR) and for 1 year (SBBC). The average particle diameter was plotted to the nitrogen removal efficiency (Fig. 6). From this graph a dependency between those factors can be observed; a smaller granule diameter leads to decreased N-removal efficiency. Increased nitrate concentrations in the effluent indicated the decreased denitrification efficiency while ammonium oxidation rates were not affected by the different granule diameters. An optimum N-removal was found with granules larger than 1.3 mm on average for the loading conditions applied in this study. The observed decreased N-removal efficiency at high granule diameters was mainly due to decreased shape factors. At particle diameters larger than 1.75 mm, the particles start to break, leading to big pores in the granule and flattened or kidney-shaped structures (Fig. 7). Although the particle diameter is still large according to the image analysis, the effective anoxic zone will be small.

This observation of dependency between particle diameter and nitrogen removal efficiency should be taken into account when this system is applied in full-scale plants. For economically feasible systems, oxygen concentration in the reactor has to be small. Therefore, an optimum between granule size and oxygen supply has to be found in such way that the high N-removal capacity as found in this study can be achieved. To be able to design a reliable and robust system, the parameters that influence the particle size have to be studied more.

CONCLUSIONS

This study showed that stable granules were formed and high nutrient removal efficiencies were obtained by selecting for slow-growing organisms, such as PAO. Simultaneous COD, N, and P removal was possible at low oxygen saturation (20%), because of heterotrophic growth inside the granules (denitrifying PAO). Results suggest that phosphate is partly removed by (biologically induced) precipitation.

Monitoring the laboratory-scale reactors for a long period showed that N-removal efficiency highly depends on the diameter of the granules. Therefore, an optimum between granule size and oxygen supply has to be found and parameters influencing granule size have to be studied more to design a robust system.

Furthermore, allowing long anaerobic feeding periods, leading to the ability to form stable granules at low oxygen concentrations, is highly advantageous for economic feasible full-scale applications.

The project is in close co-operation with DHV-Water in Leusden, The Netherlands.

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