Aerobic Granular Sludge
Scaling up a new technology

Aerobic Granular Sludge
Scaling up a new technology

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de rector Magnificus prof. dr. ir. J.T. Fokkema,
voorzitter van het College voor Promoties
in het openbaar te verdedigen op dinsdag 27 juni 2006 te 17.30 uur

door

Merle Krista DE KREUK

landbouwkundig ingenieur
geboren te Reeuwijk

Dit Proefschrift is goedgekeurd door de promotoren:
Prof. dr. ir. M.C.M. van Loosdrecht
Prof. dr. ir. J.J. Heijnen

Samenstelling promotiecommissie:
Rector Magnificus, Voorzitter
Prof. dr. ir. M.C.M. van Loosdrecht, Technische Universiteit Delft, promotor
Prof. dr. ir. J.J. Heijnen, Technische Universiteit Delft, promotor
Prof. dr. ir. W. Verstraete, Universiteit Gent, België
Prof. Mrs. A. Carucci, University of Cagliari, Italy
Prof. ir. J.H.J.M. van der Graaf, Technische Universiteit Delft
Prof. dr. ir. R.H. Wijffels, Wageningen Universiteit en Research
Ir. B. de Bruin, DHV, adviseur

Het in dit proefschrift beschreven onderzoek is uitgevoerd bij de sectie milieubiotechnologie, vakgroep Biotechnologie, Technische Universiteit Delft, Julianalaan 67, 2628 BC te Delft. Het onderzoek heeft plaats gevonden in nauwe samenwerking met de ingenieurs van DHV, Amersfoort en was gefinancierd door de Stichting Toegepast Onderzoek Waterbeheer (STOWA, TNW99.262) en Technologiestichting STW (DPC5577).

ISBN-10: 90-9020767-8; ISBN-13: 978-90-9020767-4

Voor Martijn, Bart en ons tweede kindje

Summary

Conventional wastewater treatment plants (WWTP's), like activated sludge systems, require uneconomical large footprints, which is mainly caused by the need for large clarifiers and low sludge concentrations in the aeration basins. In the past years continuously operated biofilm reactors were developed, in which the biomass is immobilised on suspended carrier material. The most important aspect of these technologies is the small footprint of the reactors, because of high volumetric loads and high settling velocities of the biofilm-carrier particles. A disadvantage of these continuous systems is the need for complicated integrated three-phase separators (water, air and biomass), which are particularly complex for systems with large hydraulic variations such as municipal wastewater treatment units.

The research described in this thesis aimed at the development of simple, cost-efficient and compact wastewater treatment systems, based on the formation of aerobic granular sludge in sequentially operated batch reactors (SBR) without the use of a carrier material. Sequential operation allows a settling phase as part of the cyclic process and thus avoiding the need for complex and expensive internal settlers in the reactor. Adapting the cycle length and/or the filling height of the reactor can easily compensate for variations in hydraulic loading. Furthermore, integrated nutrient removal can be obtained in the same compartment during different phases of the cycle. In other words, an SBR system based on aerobic granular sludge is a one-reactor system for complete wastewater treatment.

Granular sludge has been applied widely during the last 30 years in the field of anaerobic wastewater treatment. Previous to this research project, laboratory studies already had shown the possibility to produce sludge granules under aerobic conditions. Based on these findings, this research project aimed at the understanding, development and optimization of the aerobic granular sludge technology for COD-reduction and nitrogen and phosphorus removal for wastewater treatment. It focused on understanding the principle of granule formation and the microbial population structure inside aerobic granules, as well as on the scale-up of the technology to full-scale.

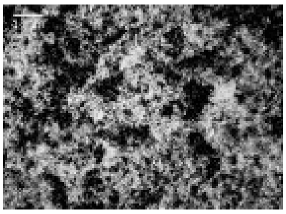
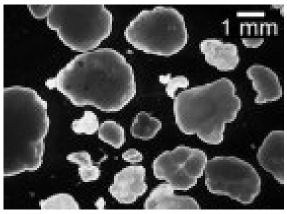
The formation of aerobic granular sludge was demonstrated in preliminary studies at laboratory-scale. In order to be able to enlarge these initial laboratory experiments to a full-grown technology, the bottlenecks for scale-up had to be identified and eliminated and the economic viability had to be evaluated. First ideas of a full-scale system were derived from comparable processes such as large scale SBR's for wastewater treatment, continuously fed biofilm airlift reactors and activated sludge systems. However, due to the unique process conditions for selecting and growing aerobic granules, this process equipment could not be applied one-to-one to aerobic granular sludge systems.
A combined step-by-step and scale-up/scale-down approach was used for this project. Experimental results were translated into a design of a full-scale plant, after which solutions for technical problems and economical bottlenecks were explored experimentally in the laboratory (scale up/scale down). This iterative approach was performed in close cooperation between Delft University and DHV consultants (Amersfoort, The Netherlands). It proved to be very effective, because the full-scale design could be performed in parallel to the laboratory studies and a direct link between theory and practice could be established. In this way, economics, experimental findings and conventional scaling laws were balanced.

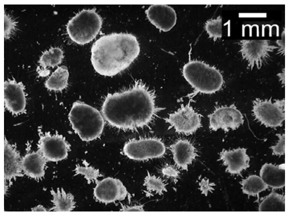
The initial experiments at the start of this research showed stable granule formation when a three-minute pulse-feeding period was applied in combination with a high dissolved oxygen (DO) concentration in an airlift reactor. Earlier performed biofilm modelling simulations of the laboratory set-up suggested high nitrogen removal efficiency at low oxygen concentrations (40% oxygen saturation). The first initial experiments demonstrated however, that at 40% oxygen saturation in combination with pulse feeding, granular sludge became unstable and disintegrated (chapter 2).

For a proper scale-up and full-scale operation with stable granules, it appeared to be essential to allow longer feeding periods than the initially applied pulse feed. To fulfil effluent demands for nitrogen compounds, a low dissolved oxygen concentration is inevitable, which is also favourable for economic reasons. Also, the use of a bubble column is preferred over an airlift reactor, because of its simpler design and significant lower costs at a large scale.

The first bottleneck to be solved at lab-scale was preventing granule instability at low oxygen saturation. From earlier research on biofilm morphology, it was learned

that slow growing organisms increase both density and stability of biofilms. To decrease the growth rate of the organisms in the aerobic granules, easy degradable substrate (e.g. acetate) had to be converted to slowly degradable ones, like microbial storage polymers (e.g. poly-Hydroxy-Alkanoates or PHA). Phosphate- or glycogen-accumulating organisms (respectively PAO or GAO) perform this conversion step most efficiently. Plug-flow feeding of the influent under anaerobic conditions through a settled bed of granules, followed by an aeration period, allowed PAO or GAO to proliferate. It was shown that indeed the selection of such bacteria in aerobic granules led to stable granular sludge, even at low dissolved oxygen concentrations (chapter 3). Moreover, stronger selection pressure for dense and stable granules in this operational mode, made it possible to apply a bubble column instead of an airlift reactor, without leading to instability of granular sludge due to increased stratification in a bubble column.


a)


b)


c)

Figure 1 Granule formation; from activated sludge as inoculum (a) in 11 days to aerobic granules on synthetic influent (b) or in 20 days on pre-treated sewage (c)

Simultaneous nutrient removal was possible, because of heterotrophic growth inside the granules (denitrifying PAO) and autotrophic growth at the aerobic surface. In the experiments with low oxygen concentrations and stable aerobic granular sludge, it was shown that a 60 minutes anaerobic feed period (within a full cycle of 3 hours) and a low oxygen saturation (20%) led to high simultaneous COD, phosphate and nitrogen removal efficiencies (COD 100% (acetate), phosphate 94 % and total nitrogen 94 % with 100% for ammonia) (chapter 4).

Monitoring the laboratory scale reactors for a long period showed that N-removal efficiency highly depends on the diameter of the granules. The biomass concentration was maintained around 5 times higher than in an activated sludge system with flocculated biomass and its sludge volume index after 8 minutes of settling (SVI_8) was only 14 ml/g (compared to 80-150 for well settling activated sludge) (chapter 4). Plug-flow feeding is economically and technically more

attractive than pulse feeding as used in initial experiments. Simultaneous influent feeding and effluent withdrawal would allow operating two or more parallel lines with a continuous influent and effluent flow, which would be far more economic in pre- and or post-processing of the wastewater.
The nutrient removal efficiencies and granule stability, obtained in combination with the technical improvements, strongly improved the feasibility and economics of the process under full-scale conditions.

In northern European countries (as in The Netherlands), wastewater temperatures during winter may drop to 8°C. Since temperature changes may influence biological processes considerably, temperature effects on the conversion processes and the stability of aerobic granular sludge were investigated (chapter 5). A granular sludge sequential 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, leading to severe biomass wash-out and instable operation. However, 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 be retained in the system. The temperature dependency for nitrification was lower for aerobic granules than usually found for activated sludge, because of the stable biofilm matrix and the capacity for simultaneous nitrification and denitrification; autotrophic organisms can enrich in the aerobic part of a granule, compensating for the lower conversion rates at decreased temperatures. The overall conclusion from these experiments was that start-up in practice should take place preferentially during summer periods, while decreased temperatures during winter periods should not pose any problems for granule stability and conversion processes in a granular sludge system.

With the results obtained from laboratory experiments a metabolic biofilm model was made in Aquasim. This complex reaction-diffusion model described the conversions inside the granule by heterotrophic, autotrophic and, and phosphate accumulating bacteria and was used to predict conversions at different process conditions (chapter 6). The model described the experimental data from the GSBR sufficiently. Sensitivities of process parameters on the nutrient removal rates could therefore be reliably evaluated. The influence of oxygen concentration, temperature, granule diameter, sludge loading rate and cycle configuration were analysed. Oxygen penetration depth in combination with the position of the autotrophic biomass played a crucial role in the conversion rates of the different components and thus on overall nutrient removal efficiencies. The ratio between aerobic and anoxic volume in the granule strongly determines the N-removal efficiency as it was shown by model simulations with varying oxygen concentration, temperature and granule size. The optimum granule diameter for maximum N- and P-removal in the standard operating conditions (DO 2 mg L^{-1}, 20°C) was found between 1.2 and 1.4 mm and the optimum COD loading rate was 1.9 kg COD m^{-3} day^{-1}. When all ammonia is oxidised, oxygen diffuses to the core of the granule inhibiting the denitrification process. In order to optimise the process, anoxic phases can be implemented in the SBR-cycle configuration, leading to a more efficient overall N-removal. Phosphate removal efficiency mainly depends on the sludge age; if the SRT exceeds 30 days not enough biomass is removed from the system to keep effluent phosphate concentrations low.

The experimental results obtained at laboratory scale were used for designing two variants of a full-scale sewage treatment plant based on GSBRs in close cooperation with DHV Water, The Netherlands. In order to evaluate the feasibility of this new technology, a desk study was performed in which two types of GSBR's (with pre- or post treatment) at a scale of 119.000 population equivalents were compared with a conventional treatment plant based on activated sludge technology with biological phosphate removal of similar size (chapter 8).
Based on total annual costs both GSBR variants proved to be more attractive than the reference alternative (7-17%). In a sensitivity analysis the GSBR technology appeared to be even more attractive at lower ratios between rain weather to dry weather flow and at higher land prices. Because of the high permissible volumetric load the footprint of the GSBR variants is only 25% compared to the reference.
Since aerobic granular sludge is cultivated in an one-reactor system, contrary to activated sludge plants, no recycling flows of wastewater and sludge are needed. Depending on the design of the aeration in a GSBR, this will lead to around 30% less energy requirement in a system based on aerobic granular sludge compared to activated sludge systems.
A growing number of sewage treatment plants in the Netherlands is now faced with more stringent effluent standards. In general, activated sludge plants will have to be extended with a post treatment step (e.g. sand filtration) or transformed into a Membrane Bio Reactor. In this case a GSBR variant with primary treatment as well as post treatment will be an attractive alternative.

Aerobic granule formation on domestic sewage was studied as the final laboratory experiment before the step to pilot scale studies could be made (chapter 8). Therefore, aerobic granules were produced using pre-settled sewage as influent. After 20 days of operation at high COD-loadings heterogeneous aerobic granular structures were found, with a SVI_8 of 38 $mg \cdot l^{-1}$ and an average diameter of 1.1 mm. Applying a high COD-load (high sludge loads) was found to be the critical factor for the formation of aerobic granules on this type of wastewater. Pilot scale studies were performed at wastewater treatment plant Ede, The Netherlands. Two bubble columns of 0.6 m diameter and 6 m height (effective volume 1.5 m^3) were used. The dry weight concentration was 10 g L^{-1} of which 80% consisted of granular sludge, during steady-state operation. Sludge loading rates up to 0.212 gCOD $gTSS^{-1}$ d^{-1} were applied. The sum of ammonia and nitrate in the effluent were below 10 mg L^{-1} even at average temperatures of 13°C. Average phosphate concentrations in the effluent were 0.9 mg L^{-1} and suspended solids concentrations in the effluent were on average 10 – 20 mg L^{-1}. A simple drum filter could lower this to 4 mg L^{-1} even during additional wash-out of suspended solids during increased influent flow rates.

In this innovative development of a new process for the treatment of municipal and industrial wastewater, the combination of fundamental research at a university and the practical approach of a consultant was shown to be very effective. Knowledge transfer from university to the outside world led to rapid application and its implementation in the design of the required installations. The feed back from practice in its turn helped research to define its course and targets. The cooperation between TU Delft and DHV water and the financial support of Stowa and STW led to the rapid market introduction of an aerobic granular sludge based technology named Nereda™.

Samenvatting

Conventionele rioolwaterzuiveringsinstallaties (RWZI's), zoals actief slib installaties, nemen vaak oneconomisch veel ruimte in. Dit wordt voornamelijk veroorzaakt door de noodzaak om grote nabezinktanks te gebruiken en door de lage actiefslib gehaltes in de beluchtingsbasins. In de afgelopen decennia zijn continu bedreven slib-op-drager reactoren ontwikkeld met gesuspendeerd dragermateriaal. Een belangrijk aspect bij deze technologie is het lage ruimtegebruik door de hoge hydraulische belasting en de hoge bezinksnelheden van het dragermateriaal. Een nadeel van deze reactoren is echter de gecompliceerde, geïntegreerde driefasen scheiders (water, lucht en biomassa), die vooral complex zijn voor systemen met wisselende belasting, zoals bij de behandeling van stedelijk afvalwater.

Het doel van het onderzoek, dat in dit proefschrift wordt beschreven, was de ontwikkeling van een eenvoudige, kostenefficiënte en compacte afvalwater-zuiveringsinstallatie. Deze zou zijn gebaseerd op de vorming van aëroob korrelslib in een SBR (sequentially operated batch reactor), waarbij geen dragermateriaal wordt toegepast. Deze discontinue bedrijfsvoering staat toe, dat iedere cyclus een bezinkfase heeft. Hierdoor is er geen behoefte aan de eerder genoemde complexe en kostbare drie fasen scheiders. Door de cyclusduur en de vulhoogte van de reactor te variëren kan ook eenvoudig een wisselende hydraulische belasting van de reactor worden gecompenseerd. Tevens kan de verwijdering van nutriënten worden bereikt in dezelfde reactor, maar in een andere fase van het proces. In andere woorden, een SBR systeem gebaseerd op het gebruik van aëroob korrelslib is een alles-in-één reactor voor de volledige zuivering van afvalwater.

Gedurende de laatste 30 jaar is korrelslib algemeen toegepast bij de anaërobe afvalwaterbehandeling. Voorafgaand aan dit onderzoek was reeds aangetoond, dat slibkorrels konden worden verkregen onder aërobe condities. Het onderzoek, beschreven in dit proefschrift, mikte op een beter begrip van het principe van de aërobe korrelvorming en de samenstelling van de microbiële populatie binnenin de korrel. Daarbij is de aëroob korrelslib technologie verder ontwikkeld en opgeschaald en zijn CZV-, stikstof- en fosfaatverwijdering geoptimaliseerd.

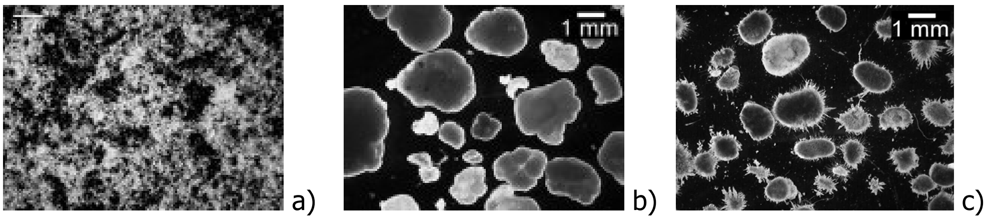
Om eerder uitgevoerde initiële experimenten naar een volwassen technologie te kunnen opschalen, moesten knelpunten worden geïdentificeerd en geëlimineerd en diende ook de economische haalbaarheid van het proces te worden geëvalueerd. De eerste ideeën voor een volle schaal systeem werden ontleend aan vergelijkbare processen zoals SBR's op basis van actief slib, continu bedreven biofilm 'airlift' reactoren en overige actief slib systemen. Door de bijzondere procescondities die nodig zijn voor de vorming van aëroob korrelslib en voor het selecteren van specifieke micro-organismen, konden installaties voor de eerder genoemde processen niet één op één worden vertaald naar apparatuur voor deze toepassing.

Bij dit onderzoek werd een gecombineerd stap-voor-stap benadering met zowel op- als neerschalen van apparatuur gebruikt. Experimentele resultaten werden vertaald naar een ontwerp voor een praktijkschaal installatie, waarna oplossingen voor technische en economische knelpunten werden onderzocht in laboratorium-experimenten. Deze iteratieve benadering werd in nauwe samenwerking tussen de Technische Universiteit Delft en DHV (Amersfoort) uitgevoerd. Dit bleek buitengewoon effectief, omdat op deze manier het praktijkschaal ontwerp parallel met het laboratoriumonderzoek kon worden gemaakt en een directe teugkoppeling tussen theorie en praktijk ontstond. Op deze manier werd een goede balans gevonden tussen economie, experiment en opschaling.

De eerste proeven aan het begin van dit onderzoek lieten een stabiele korrelvorming zien, wanneer een drie-minuten gepulseerde voeding werd toegepast in combinatie met een hoge opgeloste zuurstof concentratie (DO) in een 'airlift' reactor. Eerder uitgevoerde modelsimulaties van aëroob korrelslib in een laboratorium opzet, suggereerden een hoge stikstofverwijdering bij lage zuurstof concentraties (40 % zuurstofverzadiging). Experimenten lieten echter zien, dat bij 40 % zuurstofverzadiging, in combinatie met een gepulseerde voeding, het korrelslib juist instabiel werd en desintegreerde (hoofdstuk 2).

Eerste analyses van een praktijkontwerp brachten een aantal knelpunten aan het licht: i) voor een technisch haalbare opschaling en voor het verkrijgen van stabiel korrelslib, bleek het essentieel om langere voedingsperiodes toe te staan dan de in eerste instantie toegepaste puls voeding; ii) om aan de effluenteisen voor stikstofverbindingen te voldoen, zijn lage zuurstofgehaltes onontkoombaar, hetgeen ook nog economisch aantrekkelijk is; iii) ook zou liever gebruik worden gemaakt van een bellenkolom in plaats van een 'airlift' reactor gezien de eenvoud van het ontwerp en de veel lager kosten bij een praktijkschaal toepassing.

Het eerste knelpunt wat onderzocht werd, was dan ook het voorkómen van korrelinstabiliteit bij lage zuurstofgehaltes. Bij eerder onderzoek naar biofilm morfologie was gebleken, dat de aanwezigheid van langzaam groeiende organismen zowel de dichtheid als de stabiliteit van biofilms deed toenemen. Om de groeisnelheid van de micro-organismen in de aërobe korrels te laten afnemen, diende gemakkelijk afbreekbaar substraat (bijv. acetaat) te worden omgezet in langzaam afbreekbaar materiaal zoals microbiële opslagpolymeren (bijv. poly-hydroxy-alkanoaten of PHA).
Fosfaat- of glycogeen accumulerende organismen (respectievelijk PAO of GAO) voeren deze omzettingen het meest efficiënt uit. Propstroomvoeding van het influent onder anaërobe omstandigheden door een bezonken bed van korrels, gevolgd door een beluchtingsperiode, bevorderden de groei van PAO en GAO. Er werd aangetoond, dat het selecteren van deze typen organismen zelfs bij lage zuurstofgehaltes stabiele korrels opleverden (hoofdstuk 3). De langzame groeisnelheid van de organismen in de korrels maakte het daarbij ook mogelijk om een bellenkolom toe te passen in plaats van een 'airlift' reactor, zonder verlies aan stabiliteit van de korrels door toegenomen stratificatie in een bellenkolom.



Figuur 1 Korrelvorming; van actief slib als inoculum (a) in 11 dagen naar aerobe korrels met synthetisch influent (b) of in 20 days met voorbehandeld rioolwater (c)

Simultane verwijdering van nutriënten was mogelijk door heterotrofe groei van micro-organismen binnenin de korrels (denitrificerende PAO) en autotrofe groei aan het aërobe oppervlak ervan. In de proeven met lage zuurstofconcentraties en stabiel aëroob korrelslib bleek, dat een anaërobe voedingsperiode van 60 minuten (binnen een volledige cyclus van 3 uur) en een lage zuurstofverzadiging (20 %) leidde tot een hoge simultane verwijdering van CZV, fosfaat en stikstof (CZV 100% als acetaat, fosfaat 94 % en totaal stikstof 94 % met 100 % voor ammonium) (hoofdstuk 4).

Wanneer de reactoren op laboratoriumschaal gedurende een langere periode werden gevolgd, bleek dat de stikstofverwijdering sterk afhankelijk was van de diameter van de korrels. De biomassa concentratie werd op een niveau gehouden, dat circa 5 keer hoger lag dan in een normale actiefslib installatie (met slibvlokken) en de SVI na 8 minuten bezinken (SVI_8) was slechts 14 mL g^{-1} vergeleken met 80 tot 150 mL g^{-1} voor goed bezinkend actiefslib (hoofdstuk 4). Propstroomvoeding is economisch en technisch aantrekkelijker dan een pulsvoeding zoals in de eerste experimenten werd gebruikt. Een gelijktijdige voeding van het influent en het aflaten van effluent zou het bedrijven van twee of meerdere parallelle lijnen toestaan, zonder gebruik te maken van een buffertank. Dit is tevens veel economischer voor een eventuele voor- en nabehandeling, omdat het afvalwater gelijkmatig kan worden toe- en afgevoerd. Dit zal uiteindelijk tot kleinere voor- en nabehandelingsinstallaties leiden. De hoge nutriëntenverwijdering en korrelstabiliteit in combinatie met de technische optimalisatie heeft de haalbaarheid en de economie van het proces sterk verbetert voor toepassing op praktijk schaal.

In de noordelijke Europese landen (zoals in Nederland) kan de temperatuur van het afvalwater in de winter afnemen tot 8°C. Aangezien de verandering van temperatuur biologische processen aanzienlijk kunnen beïnvloeden, werden ook temperatuureffecten op de omzettingsprocessen en op de stabiliteit van aëroob korrelslib onderzocht (hoofdstuk 5). Een SBR met aëroob korrelslib werd aan korte en lange temperatuursveranderingen blootgesteld. Opstarten van de reactor bij 8°C resulteerde in onregelmatige korrels, die samenklonterden zodra de beluchting werd gestopt, hetgeen tot een ernstige uitspoeling van biomassa en tot een instabiel proces leidde. Wanneer echter het proces bij 20°C werd opgestart en de temperatuur daarna werd verlaagd naar 15°C en 8°C, had dit geen enkel effect op de korrelstabiliteit en kon de biomassa eenvoudig in het systeem worden gehouden. De temperatuurafhankelijkheid voor nitrificatie was lager in korrelslib dan normaliter wordt aangenomen voor actiefslib. Door de stabiele biofilmmatrix en de capaciteit voor gelijktijdige nitrificatie en denitrificatie kunnen autotrofe micro-organismen zich verder ophopen in het aërobe deel van een korrel, waardoor de lagere omzettingssnelheden bij lagere temperaturen worden gecompenseerd. De totaalconclusie van dit deel van het onderzoek is, dat praktijk installaties bij voorkeur gedurende de zomer moeten worden opgestart, waarbij de afnemende temperaturen in de winterperiode geen problemen hoeven op te leveren voor de korrelstabiliteit en de omzettingsprocessen in een korrelslibinstallatie.

Met de resultaten uit de laboratorium experimenten is een model geschreven in AquaSim. Dit complexe reactie/diffusie model beschreef de omzettingen in het korrelslib door heterotrofe, autotrofe en fosfaat accumulerende bacteriën. Het is gebuikt om omzettingen te voorspellen bij verschillende procescondities (hoofdstuk 6). Het model beschreef de experimentele resultaten voldoende. De gevoeligheden van de procesvariabelen op de omzettingssnelheden van de nutriënten konden derhalve betrouwbaar worden geëvalueerd. Er is een analyse gemaakt van de invloeden van zuurstofconcentratie, temperatuur, korreldiameter, slibbelasting en cyclus opbouw. De indringdiepte van zuurstof, in combinatie met de positie van de autotrofe organismen bleek een cruciale rol te spelen in de omzettingssnelheden en dus in het algehele nutriënten verwijderingsrendement. Modelsimulaties met variërende opgeloste zuurstofconcentraties, temperatuur en korrelgrootte lieten zien dat de verhouding tussen aëroob en anoxisch volume in de korrel sterk het stikstof verwijderingsrendement bepaalt. De optimale korreldiameter voor maximale N- en P-verwijdering bij de standaard procescondities (DO 2 mg L^{-1}, 20°C) lag tussen de 1.2 en 1.4 mm en de optimale slibbelasting was 1.9 kg CZV m^{-3} dag^{-1}. Wanneer alle ammonium is geoxideerd, kan zuurstof naar de kern van de korrel diffunderen waar vervolgens de denitrificatie geremd wordt. Om het proces te optimaliseren, kunnen anoxische fasen in de SBR-cyclus worden opgenomen, wat zal leiden tot een efficiëntere stikstof verwijdering. Het fosfaat verwijderingsrendement hangt voornamelijk samen met de slibleeftijd; als de slibleeftijd niet langer is dan 30 dagen wordt voldoende biomassa uit het systeem verwijderd en zullen fosfaat concentraties in het effluent laag blijven.

De resultaten, verkregen uit de experimenten op laboratorium schaal, zijn gebruikt om twee praktijk schaal varianten te ontwerpen van een huishoudelijke afvalwaterzuivering gebaseerd op de aëroob korrelslib technologie. Dit deelproject is uitgevoerd in samenwerking met DHV. Om de haalbaarheid van deze nieuwe technologie te evalueren is een bureaustudie uitgevoerd, waarin twee typen aëroob korrelslib reactoren (met voor- of nabehandeling) met een capaciteit van 119.000 inwoner equivalenten (i.e) vergeleken werden met een conventionele actief slib installatie met biologische fosfaat verwijdering van vergelijkbare grootte (hoofdstuk 8).

Beide varianten met aëroob korrelslib bleken aantrekkelijker dan de referentie, gebaseerd op de totale jaarlijkse kosten (7-17% lager). In een gevoeligheidsanalyse bleek de korrelslib technologie zelfs nog aantrekkelijker bij kleine verschillen tussen regenaanvoer en droogweer aanvoer en bij hogen

grondprijzen. Door de hoge toegestane volume belasting, bedraagt het benodigd oppervlak van een installatie gebaseerd op de aëroob korrelslib slechts 25% van de referentie.
Omdat bij gebruik van aëroob korrelslib alle processen in één reactor plaatsvinden in plaats van in afzonderlijke tanken, zoals bij actiefslib installaties, zijn geen recycling stromen van afvalwater en slib nodig. Afhankelijk van het ontwerp van de beluchting zal dit, in vergelijking met actief slib systemen, tot een 30% lagere energie behoefte leiden.
Een groeiend aantal huishoudelijke afvalwaterzuiveringen in Nederland krijgt te maken met strengere effluent eisen. Om hieraan te kunnen voldoen, zullen actief slib installaties in het algemeen met een nabehandeling (zoals zandfiltratie) moeten worden uitgebreid of moeten worden omgebouwd in een membraam bioreactor. In dat geval kan een aëroob korrelslib reactor met voor- en nabehandeling een aantrekkelijk alternatief vormen.

Als laatste laboratorium experiment voor de stap naar pilot-schaal werd genomen, is aërobe korrelvorming met huishoudelijk afvalwater bestudeerd (hoofdstuk 8). In dit experiment zijn aërobe korrels gevormd, gebruik makend van voorbezonken rioolwater als influent. Bij een hoge CZV belasting werden na 20 dagen heterogene aërobe structuren gevonden, met een SVI_8 van 38 $mg \cdot L^{-1}$ en een gemiddelde diameter van 1.1 mm. Een hoge slibbelasting werd als cruciale factor gezien voor de korrelvorming op dit type afvalwater; de totale cyclusduur moet zonodig worden verkort tijdens de opstart van de reactor. Pilot-scale onderzoek werd uitgevoerd op de rioolwaterzuiveringsinrichting (RWZI) Ede, Nederland. Hiervoor werd gebruik gemaakt van 2 bellenkolommen, ieder met 0.6 m diameter en 6 m hoogte (effectief volume 1.5 m^3). Tijdens de steady-state fase, was de droge stof concentratie 10 g L^{-1}, waarvan 80% gevormd werd door korrelslib. Slibbelastingen tot 0.212 gCZV $gTSS^{-1}$ d^{-1} werden toegepast. De som van ammonium en nitraat in het effluent was onder de 10 mg L^{-1}, zelfs bij gemiddelde temperaturen van 13°C. De gemiddelde fosfaat concentraties in het effluent waren 0.9 mg L^{-1} en de zwevende stof concentratie in het effluent was gemiddeld 10 tot 20 mg L^{-1}. Een eenvoudige trommelzeef kon dit gehalte tot 4 mg L^{-1} terugbrengen, zelfs tijdens extra zwevend stof verlies bij een verhoogd influent debiet.

Tijdens de innovatieve ontwikkeling van een nieuw proces voor de behandeling van huishoudelijk en industrieel afvalwater, is de combinatie van fundamenteel onderzoek aan de universiteit en de praktische benadering van een ingenieursbureau zeer effectief gebleken. Kennis overdracht vanuit de univeristeit naar de buitenwereld heeft tot een snelle toepassing van deze kennis geleid. Terugkoppeling vanuit de praktijk heeft op zijn beurt het wetenschappelijke onderzoek geholpen door mede de richting en onderzoeksdoelen te bepalen. De samenwerking tussen de TU Delft en DHV met de financiële ondersteuning van Stowa en STW heeft geleid tot een snelle marktintroductie van een technologie gebaseerd op aëroob korrelslib: Nereda^{TM}.

Table of contents

1 General Introduction

History of water treatment

Short History of sanitation1

As societies moved from nomadic cultures to permanent settlements, the concern about solid waste and wastewater disposal became an important issue, as suggested from sewer systems found in Babylonia (Mesopotamian Empire) and in settlements of the Minoan period (3rd millennium B.C.) (Angelakis et al., 2005). The understanding of the Greeks about the relationship between water quality and public health and their sanitation systems were passed on to the Romans. In the city of Rome, underground networks were used to carry most of the wastes to the nearby River Tiber. However, dumping wastes outside the city and sewage into the river was still causing health concerns (Burian et al., 1999).

The fall of the Roman Empire also meant a loss of knowledge about hygienic practice, causing many problems. Waste and sewage was again dumped onto the streets and in the canals at all levels of society. During the Middle Ages, epidemics raged in European cities. But the relationship between the excrements, polluted drinking wells and disease was not recognised yet, although beer became a popular national drink because of its wholesome effect (which was caused by the boiling step in the production process). Although changes in water issues did occur from the 12th to the 15th century, mainly by proclamations of parliaments and religious orders, there were no adequate methods of collection and disposal for the civilians. Using the canals as a sewer and drinking water supply led to many epidemics as the plague and cholera and the unbearable smell in the cities during summer made persons that could afford it to move away in this season. During the Renaissance, concern about waste and health grew. For example in England Henry the VII outlawed slaughterhouses in cities and towns, because of the danger of diseases. During the time of the Republic in The Netherlands, "Hoogheemraden"

[1] Information partly taken from the following internet sites:
http://www.theplumber.com/#history; http://www.waterstaatsgeschiedenis.nl; http://www.scitrav.com/wwater/asp1/

(Waterboards) were allowed to make laws to prohibit certain draining to the surface waters. Furthermore, canals in the cities were flushed with fresh water regularly. Despite these regulations, lack of sanitation became more and more problematic with increasing city populations during the industrial revolution. The world-wide epidemic of cholera in the 19^{th} century led to the awareness of the importance of clean water, when the English physician John Snow discovered the relation between contaminated wells and cholera in 1854. After the Great Plague in London in 1857, the British government decided to use water to transport the pathogen containing waste from the cities to the sea, with the introduction of water closets and sewers ('seawards'). In the decades after the introduction of the London sewer system, in many western cities sewer systems were constructed. Sewer systems became favored over other competing sanitation systems, such as the Liernur vacuum system (separate collection of human excrements via vacuum piping and rainwater) used in several Dutch and German cities, and barrel collection systems e.g. in Sweden, Germany and The Netherlands (Weijers, 2000). In parts of big Dutch cities, barrel collection took place until the 1930's (Figure 1). The increased pollution load to surface water after the introduction of sewers led to a severe decline of water quality in many surface waters. In 1860 treatment of sewage started with flowfields near the city of London. In the same period the septic tank was developed to remove solid debris from the wastewater. This did not solve the problem, since the effluent of the septic tank was still highly untreated. Frankland (1868) developed a trickling sand and soil filter to treat septic tank effluent, mainly to reduce land areas needed for sewage disposal.

Figure 1 "Boldootkar" Barrel collection in Amsterdam in the beginning of the 20^{th} century (Picture from Van Lohuizen, 2004)

Short History of Dutch legislation for wastewater treatment
In the Netherlands it took until 1875 before a commission of medical inspectors proposed new legislation against pollution of soil, air and surface water with faecal matter and other debris. Only in 1897, the Dutch government established a commission to prepare interventions against the pollution of public waters ("commissie ter voorbereiding van maatregelen tegen verontreiniging van de openbare wateren"). However, legislation and regulation remained the responsibility of municipalities and proposed legislation for central regulation of sanitation and sewage treatment were withdrawn in an early stage. It took until 1970 before a legislation against pollution of surface waters (Wet Verontreiniging Oppervlaktewater, WVO) came into practice, in which the responsibility of wastewater treatment and drainage was settled with the waterboards. However, this law was an obvious result of developments in the knowledge development of the importance of sanitation and sewage treatment (Teulings, 1995).
In 1996, a separate legislation for municipal WWTP's was added to the WVO (Lozingsbesluit WVO Stedelijk Afvalwater, LSA), which finally incorporated the European directives (91/271/EEG) in the Dutch laws. This new rules imply that: i) the parameters BOD_5, COD, suspended solids, N-total, and P-total are submissive to the effluent demands as stated in the LSA; ii) if the treatment efficiency is 75% or higher, existing plants or small plants can get less strict effluent demands for N and P removal; iii) However, stricter effluent demands are necessary if the receiving surface water has to approach specific water quality objectives. The specific water quality demands are as decided in a law from 1983, that ascribes certain objectives to surface waters with specific targets (as for salmon containing waters). In 2004 this Dutch law was supplemented with a so called "dangerous components directive", a European directive from 1976 for water quality demands (76/464/EG) and with which the MTR-demands for effluent from the "4de nota waterhuishouding" received a legal status (Backes, 2001).

In the near future, the WVO will be incorporated in the "integrated legislation for water" (intergrale waterwet, IWW), which will recognise only one license for all actions concerning the overall water system in The Netherlands. The legislation for wastewater treatment will change in such a way that effluent demands valid for new plants depend upon the ecological quality of the receiving surface water. This has to be taken into account in the design of such new plants. This is part of an overall directive (Kader Richtlijn Water, KRW; directive 2000/06/EG) that aims at achieving a "good condition" for all surface waters in 2015. This will mean that in the future, WWTP's will face stricter effluent demands to reach this good condition of their receiving surface waters from chemical and ecological viewpoint.

Short history of sewage treatment systems

Before World War II, only several municipal and industrial WWTP's were built, mainly consisting of mechanical separation and settling. These treatment systems could be extended with anaerobic tanks for sludge stabilisation.

The first generation of activated sludge systems was built in the 1920's, after the study to suspended growth treatment and the discovery of activated sludge by Ardern and Lockett in 1914. From the very beginning both basic arrangements of activated sludge process were tested, i.e., the continuous-flow arrangements with separate clarifiers and activated sludge recycle and the fill-and-draw arrangement, nowadays known as a sequencing batch reactor (SBR).

Figure 2 Oxidation ditch in Benschop, The Netherlands, 1955
(Picture from Van Lohuizen, 2004)

From the fill-and-draw systems that were applied from 1914 to 1920, almost all installations were converted to continuously flow systems. Three major reasons were given by Ardern in 1927 (Wilderer et al., 2000):

- The high discharge flow relative to that of the influent;
- The clogging of the coarse bubble diffusers resulting from repeated settlement of the sludge on the diffusers;
- More operator attention due to the need to switch valves and clean diffusers.

It took until the 1940's (Hoover used them in dairy industry) in the USA and until the 1960's (Pasveer ditch) in Europe before the next generation of SBR's were introduced.

The Pasveer Ditch, developed in the Netherlands at the end of the 1950's, was an annular circuit in which a rotating brush takes care of aeration and circulation. The basic idea behind the system was to reduce the treatment of wastewater to a one-reactor system, with addition of raw sewage. The first Pasveer ditches were variable volume options with facilities, operated with intermitted flow, but with constant volume (aeration, sedimentation and effluent displacement with incoming infl0uent). Pasveer developed several versions of the oxidation ditch, among others with intermittent feed, constant feed and with built-in side loops for settling and effluent removal. Pasveers designs incorporated low COD loading rates and static fill phases were long. Therefore, filaments often developed and biomass separation was poor (Irvine and Ketchum, 1989; Wilderer et al., 2000). In 1968, the continuously operated carrousel was developed, based on the Pasveer ditch. Its first application was build in Oosterwolde, The Netherlands (15.000 i.e.) and more than a thousand installations are applied worldwide (Dirkzwager and Kiestra, 1995). Especially in Europe, SBR reactors are only used nowadays in very small WWTP's and industry. However, SBR's do receive worldwide attention and several thousand SBR facilities have been designed, built and put into operation (Wilderer et al., 2000). Most sewage treatment facilities, are still based on continuously operated activated sludge processes.

Characteristics of activated sludge systems

Nowadays activated sludge systems are still the most commonly used systems for biological wastewater treatment. In these systems, a mixed culture of suspended biomass is growing and removing organic carbon and nutrients from the influent. Activated sludge systems consist of two basic treatment steps: an aeration tank in which biochemical processes take place and a settling tank, in which the treated effluent is separated from the biomass (Figure 3). The efficiency of biological wastewater treatment depends upon: i) the selection and growth of microorganisms metabolically capable of converting the polluting agents and growth under given circumstances and ii) upon the efficient separation of those organisms from the treated effluent in the settling tanks.

Biological processes in wastewater treatment

Circumstances in activated sludge systems are designed such that biological processes cause the removal of organic carbon and nutrients (mainly ammonia and phosphate). The microbial community used in these systems is very large and divers, consisting from viruses, bacteria, fungi, algae, protozoa and metazoa. Bacteria are the most common group of organisms in activated sludge systems. By using selection mechanisms, one can regulate the required processes resulting in a clean effluent. The presence of an electron acceptor is one of the greatest regulators in this respect. The biochemical stage of an activated sludge plant can be separated in aerobic tanks (oxidation of organic carbon and ammonia), anoxic tanks (denitrification for complete nitrogen removal) and anaerobic zones (to select for phosphate accumulating organisms (PAO) that are capable of biologically removing phosphate). The choice for these different steps and the configuration of the aeration tanks highly depend on local conditions and effluent requirements.

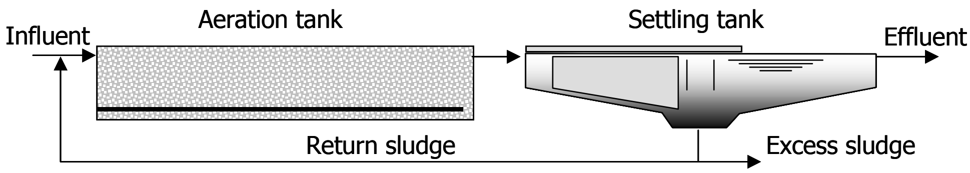


Figure 3 Basic scheme of an activated sludge system

Removal of Organic Carbon

Soluble organic matter in domestic sewage can be roughly divided into carbohydrates, fats and proteins. Most heterotrophic organisms can directly oxidize the organic carbon to CO_2 using oxygen or nitrite/nitrate (denitrification) as an electron acceptor. Part of the organic carbon will be assimilated to new biomass. Particulate and colloidal organic carbon must be hydrolyzed first before the bacteria can use it for their metabolism. Biologically nondegradable carbon will be incorporated in the activated sludge flocs and removed from the system with the excess sludge.

Using a selector (continuously operated system) or a pulse dosing (SBR), the biomass will experience a situation with high concentrations of organic substrates followed by a situation without external organic substrate. Under these circumstances micro-organisms are able to accumulate substrate as internal storage products in their cells. Usually these storage products are glycogen, lipids or polyhydroxyalkanoates (PHA). Organisms can use the stored substrates to

oxidize during famine periods and to regulate their growth rate (Beun, 2001; Martins et al., 2004; Van Aalst-Van Leeuwen et al., 1997).
A special form of storing organic carbon before it is used in the metabolism is performed by PAO and glycogen accumulating organisms (GAO). Their main characteristics will be explained in the sub section about phosphate removal.

Removal of nitrogen compounds: nitrification and denitrification
Nitrification is the oxidation of ammonia into nitrite and nitrite into nitrate. The first conversion is performed by a group of bacteria known as Nitrosomonas, the second step is done by a group of bacteria known as Nitrobacter. Nitrifying bacteria are autotrophic organisms (CO_2 as C-source) and characterized by their low growth rate. The latter is a major concern in activated sludge plants, since this highly determines the solid retention time (SRT or sludge age) in the system.
The second step of the nitrogen removal is the denitrification, in which nitrite or nitrate is used as electron acceptor for the oxidation of organic carbon and is converted to N_2 gas. This process takes place under anoxic conditions. Nitrification and denitrification can occur simultaneously (SND) within one sludge floc, as long as the oxygen penetration depth in the floc is limited and a substrate rich interior is present for denitrification (De Kreuk et al., 2005; Pochana and Keller, 1999; Satoh et al., 2003; Third et al., 2003).

Nitrification
$NH_4^+ + {}^3/_2\ O_2 \rightarrow NO_2^- + H_2O + 2H^+ + 240\ kJ$
$NO_2^- + {}^1/_2\ O_2 \rightarrow NO_3^- + 65\ kJ$

Denitrification
$8\ NO_3^- + 5\ CH_3COO^- + 13\ H^+ \rightarrow 4\ N_2 + 10\ CO_2 + 14\ H_2O + 165.2\ kJ$
$8\ NO_2^- + 3\ CH_3COO^- + 11\ H^+ \rightarrow 4\ N_2 + 6\ CO_2 + 10\ H_2O + 323{}^1/_3\ kJ$

Biological phosphate removal
To achieve enhanced biological phosphorus removal (EBPR), bacteria have to be exposed to changing anaerobic and aerobic conditions, where readily biodegradable substrate must be supplied under anaerobic conditions. During the anaerobic period, easy degradable substrates as acetate are taken up in the cell and stored as PHA's. Acetate will be stored as poly-β-hydroxybutyrate (PHB). The energy of this transport and storage is supplied by the hydrolysis of intracellular stored poly-P to orthophosphate, which is released from the cell into the liquid. The reduction equivalents required for the conversion of acetate to PHB are

supplied by conversion of glycogen, which is stored during the aerobic period from PHB (Blackall et al., 2002; Mino et al., 1998). The anaerobic and aerobic metabolisms are schematically shown in Figure 4. In the aerobic or anoxic phase, without the presence of an external substrate, PHA is used as substrate for cell growth, polyphosphate synthesis and glycogen formation.

GAO's are able to compete for organic substrate with the PAO's during anearobic periods. The only metabolic difference between those organisms is the uptake of phosphate, which doesn't occur during the metabolism of GAO's. However, using acetate as the main substrate favours the development of the PAO's.

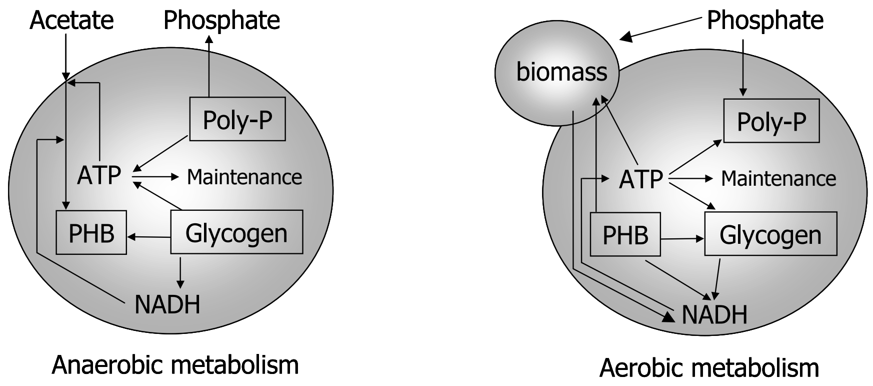


Figure 4 Anaerobic and aerobic metabolism of phosphate accumulating organisms (Smolders et al., 1994a; Smolders et al., 1994b)

Separation of biomass and effluent

In wastewater treatment, biomass and treated effluent need to be separated before effluent can be discharged to the surface water. This is done in a secondary clarifier, settling tank or thickener (Figure 3) that produces clarified final effluent and a thickened flow of sludge that is returned to the activated sludge tank. If the settling tank fails, for example due to sludge bulking, excessive loss of sludge detoriates effluent quality and could lead to uncontrolled low sludge ages and loss of efficiency in the aeration tanks.

Due to the lack of shear stress in a clarifier (no mixing), the suspended biomass usually aggregates to form sludge flocs. These sludge flocs settle relatively slow (velocities <1 m/h), which leads to long needed hydraulic retention times (HRT) in the settling tanks before clear effluent can be released. The sludge volume index (SVI, volume occupied per mass unit of biomass) determines the size of the settling tanks. Well settling sludge from activated sludge systems, usually have a SVI of 90 to 150 $mL \cdot g^{-1}$. According to design guidelines formulated by STOWA (2002) a design based on a loading rate of 0.4-0.5 $m^3\ m^{-2}\ h^{-1}$ would be feasible. This means that the surface area needed for the settling tanks in an average wastewater treatment plant (120,000 population equivalents or p.e.) would be around 8,000 m^2 (rain weather flow taken into account).

Compact wastewater treatment systems

As described above, conventional activated sludge systems require large surface area's. This is caused by the relatively poor settling characteristics of activated sludge, resulting in low permissible dry solids concentrations in aeration tanks (usually around 3 - 4 mg $SS \cdot L^{-1}$) and in a low maximum hydraulic load of secondary sedimentation tanks.
Since wastewater treatment systems are needed in dense populated regions, land is limited. Available space at existing treatment plants is often limited, which can cause problems when treatment plants need to be extended because of new human activities in the surroundings. To avoid large footprints, compact treatment systems are needed. During the last decades different compact treatment systems are developed, as biofilm systems, membrane bioreactors (MBR) and aerobic granular sludge technology (this thesis).

Membrane Bio-Reactors

Membrane bioreactors are based on a combination of activated sludge processes and membrane filtration in one treatment step. An ultrafiltration or microfiltration membrane separates the activated sludge from the effluent. The membrane can be applied within the bioreactor (submerged configuration) or externally through recirculation. Since external settlers, or any other post treatment step, become superfluous by using a membrane for the suspended solid and effluent separation, the required space for an installation is small and sludge concentration in the aeration tanks can be two to three times higher than in conventional systems. Furthermore, the effluent quality is significantly better as all suspended and colloidal material as micro contaminants, bacteria and viruses is removed (Trussell et al., 2005).
Biological processes in a MBR are often comparable or better than in conventional activated sludge systems. Due to the long sludge ages, N-removal is more efficient

because the slow growing autotrophs are kept efficiently in the system. Denitrification can occur by introducing anoxic tanks or intermitted aeration (Drews et al., 2005; Gander et al., 2000).
Despite the excellent effluent quality, a breakthrough for the MBR technology is lacking. This is mainly due to the costs involved with this technology. Due to the high biomass concentration in the system, in combination with the appearance of the activated sludge (more suspended growth) aeration is inefficient. Furthermore, the present generation of membranes shows low permeability due to fouling, operation of membranes is energy demanding and, although prices are decreasing, membranes are still relatively expensive (Brouwer et al., 2005). In order to make MBR technology attractive for a wide range of wastewater treatment applications, these drawbacks should first be eliminated.

Anaerobic wastewater treatment (UASB, EGSB)

To be able to build compact reactors for wastewater treatment systems, sludge with a high settleability is advantageous. With this sludge the biomass can be kept efficiently in the system, making compact reactors with integrated sludge separation feasible. Granular sludge is well known in anaerobic systems (such as UASB (Lettinga et al., 1980) and EGSB (Vanderlast and Lettinga, 1992)), which proved themselves already for over twenty years. The use of granular sludge in anaerobic systems allow for high loading rates (up to 40 kg COD m^{-3} day^{-1}). Furthermore, energy requirements of UASB reactors are very low, since mixing occurs by the upflowing liquid and produced biogas. The latter even generates energy when collected, but also needs good handling and treatment because of its bad smell caused by the produced hydrogen sulphide. The main disadvantage of anaerobic treatment is the needed effluent polishing step. Anaerobic treatment results in relatively high COD concentrations in the effluent, minimal ammonium and phosphate removal and low pathogen removal. Due to the low growth rate of methanogenic organisms, also the start-up takes longer as compared to aerobic processes, when no good inoculum is available. The temperature in the reactor should be relatively high (>20°C) (Seghezzo et al., 1998).
Summarizing, anaerobic treatment is a very good compact method for industrial wastewater with high COD contents, without nitrogen compounds and other nutrient compounds or a very economical step in sewage treatment in tropical and subtropical climates.

Continuously operated biofilm reactors

Aerobic particle based systems started with the development of biofilm reactors. Biofilms are mainly useful to retain slow growing organisms in a reactor and still being able to apply short hydraulic retention times (Mulder et al., 2001; Nicollela et al., 2000). Biofilms grow attached on a static solid surface (static biofilms) or on suspended carriers (particle supported biofilms). Systems with static biofilms (e.g. trickling filters) have small specific biofilm surface areas available for substrate transport and reaction (typically less than 300 m^2 biofilm per m^3 reactor), and thus a limited reactor capacity. When biofilms are grown on small spheres (sand, basalt) specific biofilm surface area can be increased to 3000 m^2 m^{-3} (Nicollela et al., 2000). High settling velocities lead to possible elimination of external clarifiers and therefore to a compact design. Furthermore, high biomass concentrations (30 kg m^{-3}) and large mass transfer areas result in high conversion capacities (for oxygen, 20 kg m^{-3} day^{-1}; compared to 3 kg m^{-3} day^{-1} for activated sludge systems).

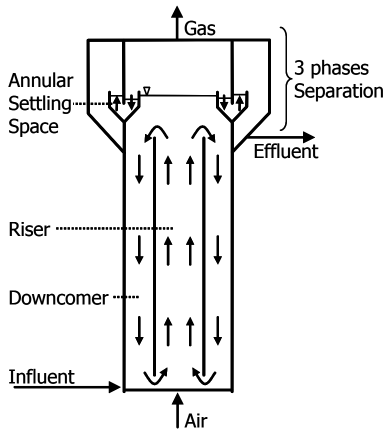


Figure 5 Reactor configuration of a biofilm airlift suspension reactor

In principal, this type of particulate biofilm reactors are continuously operated systems. This causes some disadvantages. First of all, a complex construction is needed at the top of the reactor to separate the particulates and the effluent (Figure 5). Secondly, the reactor is continuously fed, which means that substrate is converted in the outer layers of the biofilms. In these layers, competition for space and oxygen takes place between heterotrophic organisms and autotrophic organisms. Because of the different growth rates, the slow growing autotrophs will be forced to deeper layers of the biofilm. In order to denitrify the formed nitrate, the particles need to be circulated over an integrated anoxic zone with laminar flow (denitrifying CIRCOX reactor), making the reactor design more complex. Doing so, does lead to good COD and N removal efficiencies, comparable to those obtained with an activated sludge installation (Frijters et al., 1997). Phosphate is not removed with this type of reactors.

Sequencing Batch Reactor (SBR)

The Sequentially operated Batch Reactor (Sequencing Batch reactor or SBR) is a simple and compact reactor that is fed discontinuously. It is a time-oriented process that can be designed and operated to simulate virtually all conventional continuous-flow activated sludge systems, from contact stabilization to extended aeration. More simply said, a SBR is a tank, which is operated on a fill-and-draw basis, applying alternating filling, reaction (aerobic, anoxic, anaerobic), settling and withdrawal phases. The length of a cycle is typically a few hours to one day. An example of the cycle operation of a SBR is given in Figure 6. Because of the flexibility associated with working in time rather than in space, the operating policy can be modified to meet new effluent limits, handle changes in wastewater characteristics, and accomodate wide fluctuation in hourly and seasonal flow rates, all without increasing the size of the physical plant (Irvine and Ketchum, 1989). For example, an idle phase can be adapted to control the treatment capacity of a SBR and even long-term idle periods (weeks) do not influence the performance of a SBR negatively (Morgenroth et al., 2000).

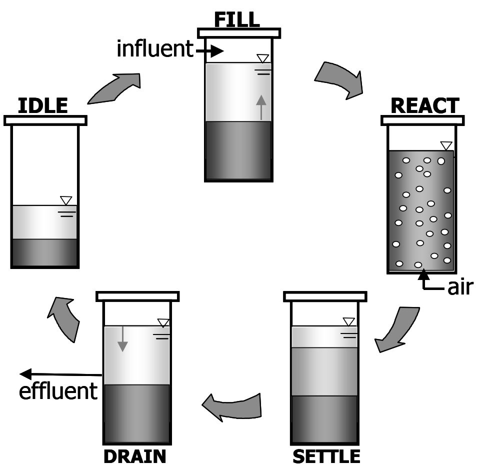


Figure 6 Typical cycle configuration of a SBR

Because all steps are performed in one reactor, external settling tanks are not needed. Although the SBR vessels will be larger than an conventional aeration tank, the total land requirement will be smaller for a treatment plant based on SBR technology. If the supply wastewater exceeds the capacity of the SBR, a buffer tank is needed before the influent can be dosed to the SBR, which decreases the compactness of the system.

Aerobic granular sludge

At the end of the 1990's, research showed the advantageous of a discontinuous fed system (Beun et al., 1999; Heijnen and Van Loosdrecht, 1998; Morgenroth et al., 1997). In these aerobic Sequencing Batch Airlift Reactors (SBAR's), it was proven to be possible to grow stable granular sludge with integrated COD and nitrogen removal. Because of the high settling capacity of the granules, the use of a traditional settler becomes unnecessary and the installation can be designed as a high and slender reactor. A wastewater treatment plant based on granule SBAR technology was expected to be feasible, which led to a successful pilot-plant study (chapter 8) and the technology, named Nereda^{TM}, is only a small step away from the first practical applications.

Aerobic granular sludge

In continuously operated systems, it was possible to grow granular sludge with slowly biodegradable substrates (e.g. methanol or ammonia). In the case of easily biodegradable substrates, these systems can only be used when enough basalt is added to the system (as carrier material and for shear reasons, Kwok et al., 1998). Research concerning the formation of storage polymers (Krishna and van Loosdrecht, 1999; Van Loosdrecht et al., 1997) finally resulted in the idea of growing aerobic granules without carrier material on readily biodegradable substrates in a Sequencing Batch Reactor (Beun et al., 1999; Dangcong et al., 1999; Morgenroth et al., 1997). The conversion of readily biodegradable COD into a substrate yielding a lower maximal growth rate facilitated granule formation. In 1998, an international patent was submitted and granted (Heijnen and Van Loosdrecht, 1998). An extension of this first patent was submitted in 2004, including the description of anaerobic feeding (Van Loosdrecht and De Kreuk, 2004).

This part will form an introduction of aerobic granular sludge, as starting point for this thesis. From the year 2000, aerobic granule formation has been excessively studied worldwide (Figure 7). This development is described in the other chapters of this thesis and only the basic knowledge of aerobic granule formation will be described here, as a starting point for further reading.

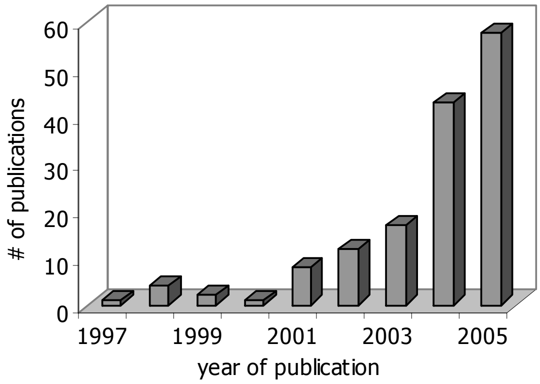


Figure 7 Number of publications about aerobic granular sludge per year

Formation of granular sludge

Granular sludge is well known in anaerobic systems and anaerobic granular sludge reactors (such as UASB, IC and EGSB) proved to perform effectively for several decades. Much research has been carried out on factors concerning granule formation in anaerobic systems. One of the recurring explanations for this granule formation is the occurrence of syntrophic juxtapositioned microcolonies. The hydrogen producing acetogens depend on the hydrogen consuming methanogens or sulphate reducing bacteria. Limiting the diffusion distances for hydrogen and other metabolic intermediate metabolites creates a favorable situation for the acetogens. Methanotrix is supposed to play an important role in the actual granule formation. This genus is present throughout all layers of anaerobic granules and has been observed in all types of anaerobic granules independent from the substrate composition. Therefore, it is often suggested that this organism plays an important role in granule formation (Fang et al., 1994; Guiot et al., 1992).
In aerobic (but also in denitrifying) biofilms or granules syntrophic mechanisms are not at all or to a much lesser extent involved. This implies that other factors are important for granule formation as well (Kosaric and Blaszcyk, 1990).

One of these other process factors is the selection of particles by their settling velocities. In an UASB reactor or other fluid-bed systems, particles are selected by their settling velocity, because only particles with settling velocities higher than the upward velocity of the fluid will stay in the reactor (Alphenaar et al., 1993; Kosaric and Blaszcyk, 1990; Lettinga et al., 1980).
The growth rate of the organisms, however, seems to be one of the main factors responsible for the density of granules or biofilms. Fast growing organisms will produce less dense granules than slow growing organisms (Villaseñor et al., 2000). For example, nitrifiers form a much denser biofilm than heterotrophs under the same circumstances. In anaerobic systems is reported that slow growing methanogens form denser biofilms than fast growing acidifying bacteria. Also, it is reported that an increase of the biomass surface-loading rate (i.e. the growth rate) decreases the biofilm density as well (Van Loosdrecht et al., 1995).
In aerobic biofilm reactors it has been found that shear force is an important factor for the formation of dense aggregates as well (Kwok et al., 1998). The biofilm density as well as the time to develop a fully covered carrier material decreased with a decreasing shear stress (Van Benthum et al., 1996). This shows that shear effects for obtaining dense biofilms have to be balanced with increased detachment of biofilm fragments from the carrier with increased shear stress. The main shear effect in a biofilm reactor results from particle/particle interaction, especially the collisions of bare carrier with the biofilms (Gjaltema et al., 1997; Kwok et al., 1998). Shear stress has also been recognized as an important factor in anaerobic systems. It has been reported that gas production causes enough movement and shear to decrease the average diameter of the anaerobic granules (Arcand et al., 1994).

Based on a wide range of experimental data a general hypothesis for the structure of biofilms was developed (Van Loosdrecht et al., 1995). This hypothesis states that the formation of dense and smooth biofilms occurs when the detachment rates are high compared to the biomass production. This hypothesis was also verified by mathematical modeling of the structure formation in biofilms (Picioreanu et al., 1998). Biofilms and granular sludge can be considered to be the same from a microbiological point of view, although there are obvious differences from a technical standpoint. The hypothesis stated above might be helpful in explaining the conditions required for the formation of good granular sludge.

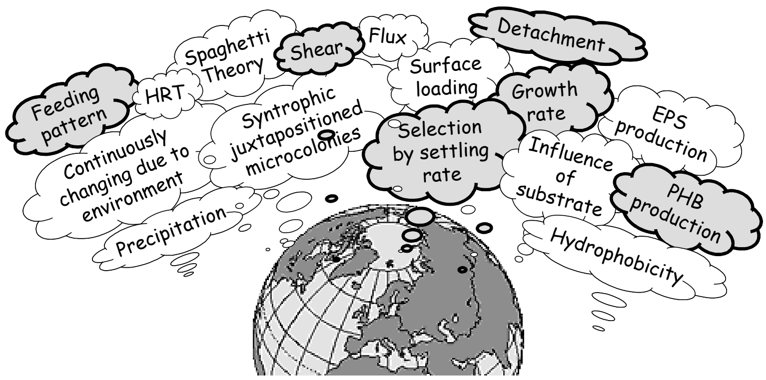


Figure 8 Different ideas about aerobic granule formation exist throughout the world. Research presented in this thesis focused on the gray fields in the picture.

Conversion processes in aerobic granular sludge

When aerobic granular sludge technology is to be applied in sewage treatment, nutrient removal (COD, N and P) will be very important. The mechanisms for nutrient removal with aerobic granular sludge are basically the same as used in activated sludge treatment. The main difference is that it does not occur in different tanks, but simultaneously in different zones inside the granules.

As described in the section above, the formation of stable, dense and smooth aerobic granular sludge is based among others on decreasing the actual growth rate of the organisms involved. A method to achieve this in systems that are fed with readily biodegradable substrate is to convert these substrates into cell-internally stored polymers as PHB. The feast-famine regime (e.g. applied in well working selectors and in discontinuously fed systems) is a good method to accomplish this. During the feast phase, high concentrations of readily biodegradable substrates are available in the liquid and will be stored by the micro-organisms. When the feast phase is anaerobic, selection for phosphate accumulating organisms (PAO, additional phosphate removal from the influent) or glycogen accumulating organisms (GAO) will occur. Aerobic feast phase will lead to the growth of other heterotrophic organisms. Stored substrates can be used during the famine period (no external substrates available) for growth and maintenance. The actual growth rate during the famine period is in general lower than the rate on readily biodegradable external substrates (Beun et al., 2002a; Van Aalst-Van Leeuwen et al., 1997) and therefore formation of storage polymers favors the development of aerobic granular sludge (Beun et al., 2002b and this thesis).

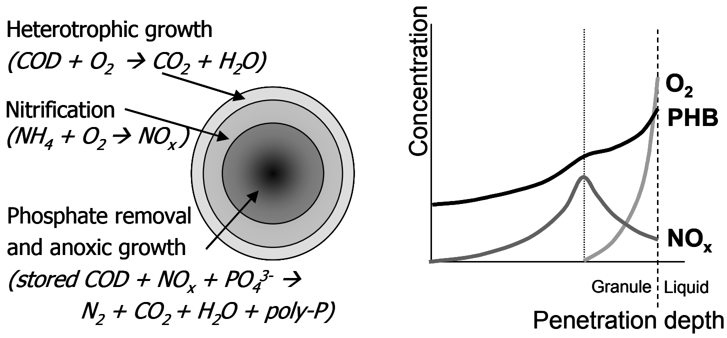


Figure 9 Schematic representation of the layered structure of aerobic granules and of the substrate and electron acceptor concentrations inside the granules during the famine phase.

Simultaneous nitrification/denitrification (SND) is an important mechanism in aerobic granular sludge. Distribution of heterotrophic and autotrophic organisms in granular sludge plays and important role in SND. During the feast period, the concentration of external carbon is high. This substrate will diffuse into the granules completely and will anaerobically (PAO), aerobically or anoxically (other heterotrophs) be stored. During the famine period, cell-internally stored substrate is available throughout the granule (schematically shown in Figure 9). Since autotrophic organisms need oxygen, they will exist in the aerobic layers of the granule. In this layer, ammonium will be converted to nitrate. The nitrate can penetrate to the interior of the granule were the stored substrate can serve as carbon source for denitrification. Optimal nitrogen removal in the system will occur when the aerobic and anoxic volume are well balanced throughout the aeration period (Beun et al., 2001, This thesis).

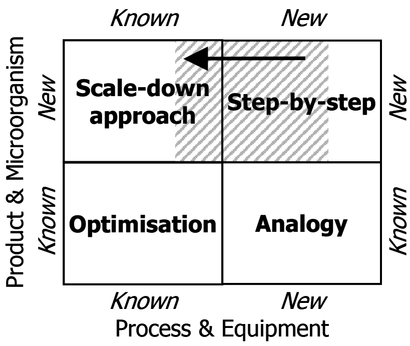
Designing a new wastewater treatment system

At the start of this research, aerobic granular sludge formation was shown as a side effect of PHB formation, in a well-controlled 3-liter laboratory set-up with pulse feeding. Because of the great possibilities for wastewater treatment that this well settling aerobic granules could offer, the aim of this research became translating this first lab results to a robust full-scale concept. Because of the specific circumstances needed for aerobic granular sludge formation, scaling-up an aerobic granular sludge reactor is not only a matter of economical reasoning but moreover a balance between economics and conventional scaling laws. In the scale-up procedure as applied in the scope of the development of aerobic granular sludge technology, practical solutions and economical advantages were coupled to conventional scale-up strategies.

Scaling procedures – an overview

When a new process, micro-organism or product is developed, the process often has to be scaled-up from a laboratory experiment to a full-scale installation. There are different procedures to come to a full-scale design that represents the laboratory circumstances in which the process is developed and optimised.
Before the method of scaling can be determined, the category in which the development is situated has to be clear. Luyben (1992) formulated a possible subdivision of scale-up problems along the dimensions of process & equipment and product & operation (Figure 10). When a new product has to be produced with new equipment, increase can only be achieved by step-by-step enlargement of the production volume (in reality or by modelling). Scale-up of a known product in an already known process can be done by optimisation and a new product with a know process can be done by analogy, since there is already a lot of information on that specific process. The most common situation is the development of a new product with known equipment. In that circumstance, a scale down approach can be used according to the strategy as is given in Figure 10, without the pilot step. The known equipment is scaled-down to a laboratory test-rig in which the process can be simulated and optimised. Finally the optimal process is scaled-up to a full-scale application (Oosterhuis, 1984).

Different methods for a scale-down/scale-up approach have been described in literature with a basic difference in know-how and know-why scaling (Deckwer and Schumpe, 1993). The first group covers the rules of thumb that are often used, like keeping the value of a typical operation or equipment variable constant (for example constant k_la, constant Power/Volume ratio). Know-why methods study the process and take more aspects into account. The fundamental scale method implies solving all micro-balances for momentum, mass and heat-transfer of the system. Since this method is impossible to use for complete microbial processes, a simplified semi-fundamental method is often used, based on flow models in the reactor (Oosterhuis, 1984).



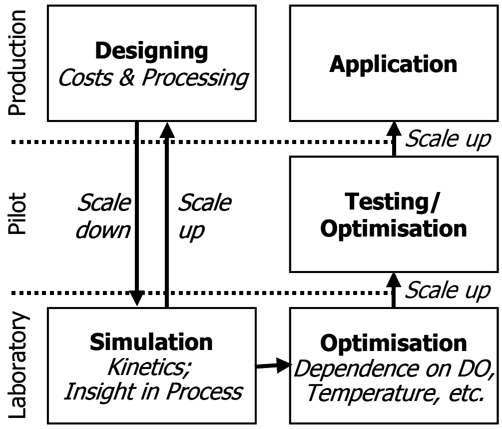


Figure 10 Different approach of scale up approach to scale-up conditions (Luyben, 1992) and the scale down approach for new processes or equipment as used in the development of aerobic granular sludge technology

Besides the use of a know-how method, the most used know-why approach for scale-up is derived from dimensional and regime analysis. This method is based on the analysis of the total system and determining a set of dimensionless numbers from the dimensionless balance equations or from similarity principles.
Similarity in wastewater treatment systems can be found in: geometric; kinematic (similarity in time, velocities); dynamic (forces of the same kind act upon corresponding particles at corresponding times in geometrically similar systems); chemical; biological and/or thermal similarity (a fixed relation between concentration at corresponding points in small and large scale). It can be easily stated that complete similarity in wastewater treatment plants on small and large scale is impossible (Horvath and Schmidtke, 1983). Complete similarity can be mathematical contradictory, technological impossible or economical unfeasible. To make a choice which dimensionless groups can be similar and which not, without affecting the process excessively, the field of regime analysis is entered. This analysis compares characteristic times of subprocesses, to identify the rate-limiting step at production scale. These rate-limiting steps are translated to laboratory scale, thereby ensuring that the rate-limiting step is established. The production on laboratory scale can be optimised or the process can be further developed in the regime of the rate-limiting step, after which the optimised process is scaled up to the full scale application (Groen, 1994).

Development of the aerobic granule technology

A said before, aerobic granule formation was initiated and developed at laboratory scale. The large-scale experience is limited to comparable processes as large scale SBRs for wastewater treatment (Wilderer et al., 2000) and continuously fed biofilm airlift reactors (Mulder et al., 2001; Nicollela et al., 2000). Because of the required process conditions for selecting and growing granules, this process equipment could not be applied one-to-one.

The scale-up problem of the aerobic granular sludge technology can be situated between the two upper quadrants of Figure 10. The first aerobic granules were observed in a biofilm airlift suspension reactor (BASR), formed from biofilm fragments that originated from broken biofilm particles (Tijhuis et al., 1994). Hereafter, research to the new aerobic granule formation process without a carrier was started in a laboratory scale reactor. At the beginning, knowledge about the most favourable reactor type or configuration for granule formation was not available, since the desirable process conditions had to be found. The laboratory scale reactor was not scaled down from a typical large-scale installation, but the dimensions were chosen to be practical. E.g., the laboratory-scale reactor height was one meter to have a good gas-liquid mass transfer and mixing. To be able to work with a limited reactor volume, the diameter was chosen small, which resulted in a height/diameter ratio of 15 (Beun et al., 1999). It is clear that this is not in line with full-scale plants, and that geometrical similarity is not possible.

Since the scaling problem was situated between two variants, a combined step-by-step and scale-up/scale-down approach has been executed as is schematically shown in Figure 10. The scale-up/scale-down is a translation from laboratory experiments into a design of the full-scale plant and visa versa. In other words, the design of a full-scale operational installation occurred at the same time as the laboratory experiments and was done in close cooperation of the engineers of DHV Water (Amersfoort, The Netherlands). The morphology of the granules and the conversion processes at different process conditions were studied at 3-litre scale. The experimental results were translated to a full-scale design and the bottlenecks for the technical design were translated back to experiments.

Scope and outline of this thesis

The main goal of this study was to translate first laboratory scale observations of aerobic granule formation to a robust and economically feasible full-scale process. Therefore, a better understanding of granule morphology, structure and stability was needed. Furthermore the conversion processes in aerobic granular sludge were studied experimentally and by modelling, in order to be able to optimise the nutrient removal efficiency.

Most of the research done and chapters written in this thesis originate from bottlenecks and sensitivities that were studied and/or solved in the scale-up/scale-down process:

- The dissolved oxygen (DO) concentration, and thus the aeration, highly influences the energy requirement and the nitrogen removal efficiency. Beun (2001) showed an optimum in nitrogen removal at 40% dissolved oxygen (DO) by modelling, but experiments (low DO and pulse feed; **Chapter 2**) shows granule instability at this low oxygen concentrations. This was not the case when a long anaerobic feeding period was applied. An anaerobic feeding period selected for phosphate accumulating organisms or glycogen accumulating organisms, resulting in stable, dense and smooth granules. This improvement is described in **Chapter 3**.
- Besides the granule instability at low oxygen concentrations, pulse feeding at large scale is also undesired, because it would imply the need for large buffer tanks. Furthermore a pulse feed is economically and technically unrealistic when 800 m^3 h^{-1} (dry weather flow) needs to be treated in an average WWTP. Increasing the feeding time from a pulse feed, as described by Beun (1999), to an anaerobic feeding period with a duration of 1/n·total cycle time (n = amount of SBR lines applied at full scale) would solve several bottlenecks; a long feeding period is technical simpler, less expensive and less space consuming, since buffer tanks are not needed. This feeding regime also has advantages for the nutrient removal, which are described in **Chapter 4**;
- The temperature during the laboratory scale experiments was well controlled at 20°C. However, this temperature is high for moderate to cold climates as in The Netherlands, were sewage temperature in winter can cool down to 8°C. Therefore, the effects of temperature on reactor start-up, nutrient removal and granule morphology were studied and described in **Chapter 5**;

- Biological processes in the granules are determined by concentration gradients of oxygen and diverse substrates. The concentration profiles are the result of many factors, e.g., diffusion coefficients, conversions rates, granule size, biomass spatial distribution and density. All of these factors strongly influence each other, thus the effect of separate factors cannot be studied experimentally. Moreover, due to the long sludge age in granule systems, lengthy experiments should be carried out before we can speak of a steady state reaching system. Therefore, a good computational model for the granular sludge process provides significant insight in the most important factors that affect the nutrient removal rates and in the distribution of different microbial populations in the granules. Moreover, a model could be used as tool to optimise the design of a full-scale system. **Chapter 6** presents a model for aerobic granule sludge reactors and the influence of oxygen concentration, temperature, granule diameter, sludge loading rate and cycle configuration are analysed in this chapter.
- The actual full-scale design and description of the bottlenecks and cost optimisation is presented in **Chapter 7**
- The final step-by-step approach of the development of this new technology comprised experiments with sewage. This was done first at laboratory scale. Hereafter, the economical and technical feasible full-scale design was translated to a pilot plant reactor, in which tests with sewage were performed at WWTP Ede, The Netherlands. The results from both experiments are described in **Chapter 8**.

The step-by-step approach at laboratory scale in combination with scale-up/scale-down of the technical design, led to insight in process conditions vital for aerobic granule formation and optimisation. This will hopefully lead to full-scale applications in the near future. **Chapter 9** gives the evaluation of the study presented in this thesis and the recommendations of new research directions in this fresh field of aerobic sludge technology.

References

Alphenaar PA, Visser A, Lettinga G. 1993. The effect of liquid upward velocity and hydraulic retention time on granule formation in UASB reactors treating wastewater with a high sulphate content. Bioresource Technology 43:249-258.

Angelakis AN, Koutsoyiannis D, Tchobanoglous G. 2005. Urban wastewater and stormwater technologies in ancient Greece. Water Research 39:210-220.

Arcand Y, Guiot SR, Desrochers M, Chavarie C. 1994. Impact of the reactor hydrodynamics and organic loading on the size and the activity of anaerobic granules. The chemical engineering journal 56:B23 - B35.

Backes C. 2001. Wetgeving op de waterkwaliteit. In: Backes C, Drupsteen TG, Gilhuis PC, Koeman NSJ, editors. Milieurecht. Deventer: Tjeenk Willink.

Beun JJ. 2001. PHB metabolism and N-removal in sequencing batch granular sludge reactors [Ph. D. thesis]. Delft: Technical University.

Beun JJ, Dircks K, Van Loosdrecht MCM, Heijnen JJ. 2002a. Poly-b-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. Water Research 36(5):1167-1180.

Beun JJ, Hendriks A, van Loosdrecht MCM, Morgenroth M, Wilderer PA, Heijnen JJ. 1999. Aerobic granule formation in a sequencing batch reactor. Water Research 33(10):2283-2290.

Beun JJ, Van Loosdrecht MCM, Heijnen JJ. 2001. N-removal in a granular sludge sequencing batch airlift reactor. Biotechnology and Bioengineering 75(1):82-92.

Beun JJ, van Loosdrecht MCM, Heijnen JJ. 2002b. Aerobic granule formation in a sequencing batch airlift reactor. Water Research 36(3):702-712.

Blackall LL, Crocetti G, Saunders AM, Bond PL. 2002. A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 81(1-4):681-691.

Brouwer H, Temmink H, Remy M, Geilvoet S. 2005. MBR technology: future research directions. H2O(4):19-22.

Burian SJ, Nix SJ, Durrans SR, Pitt RE, Fan CY, Field R. 1999. Historical Development of Wet-Weather Flow Management. Journal of Water Resources Planning and Management 125(1):3-13.

Dangcong P, Bernet N, Delgenes J-P, Moletta R. 1999. Aerobic granular sludge--a case report. Water Research 33(3):890-893.

De Kreuk MK, Heijnen JJ, van Loosdrecht MCM. 2005. Simultaneous COD, Nitrogen and Phosphate Removal by Aerobic Granular Sludge. Biotechnology and Bioengineering 90(6):761-769.

Deckwer W-D, Schumpe A. 1993. Improved tools for bubble column reactor design and scale-up. Chemical Engineering Science 48(5):889-911.

Dirkzwager AH, Kiestra A. 1995. Van persleiding naar zuivering. In: van den Berge AP, Groen K, Havekes HJM, Hofstra MA, Teulings JHA, editors. Bestrijding van de watervervuiling - vijfentwintig jaar WVO. Den Haag: Unie van Waterschappen - Ministerie van Verkeer en Waterstaat. p 145159.

Drews A, Evenblij H, Rosenberger S. 2005. Potential and drawbacks of microbiology-membrane interaction in membrane bioreactors. Environmental Progress 24(4):426-433.

Fang HHP, Chui HK, Li YY. 1994. Microbial structure and activity of UASB granules treating different wastewaters. Water science and technology 30(12):87 - 96.

Frijters CTMJ, Eikelboom DH, Mulder A, Mulder R. 1997. Treatment of municipal wastewater in a CIRCOX® airlift reactor with integrated denitrification. Water science and technology 36(1):173-181.

Gander M, Jefferson B, Judd S. 2000. Aerobic MBRs for domestic wastewater treatment: a review with cost considerations. Separation and Purification Technology 18(2):119-130.

Gjaltema A, Vinke JL, Van Loosdrecht MCM, Heijnen JJ. 1997. Abrasion of suspended biofilm pellets in airlift reactors: Importance of shape, structure and particle concentrations. Biotechnology and Bioengineering 53(1):88 - 99.

Groen DJ. 1994. Macromixing in Bioreactors [Phd. Thesis]. Delft: Technical University Delft. 190 p.

Guiot SR, Pauss A, Costerton JW. 1992. A structured model of the anaerobic granule consortium. Water Science and Technology 25(7):1 - 10.

Heijnen JJ, Van Loosdrecht MCM; Technische Universiteit Delft, assignee. 1998 27 August 1998. Method for acquiring grain-shaped growth of a microorganism in a reactor. US, European patent patent WO 98/37027.

Horvath I, Schmidtke NW. 1983. Scale-up and Scale-down Concepts and problems. In: Schmidtke NW, Smith DW, editors. Scale-up of Water and Wastewater Treatment Processes. Edmonton, Alberta: Butterworth Publishers. p 447.

Irvine RL, Ketchum LH, Jr. 1989. Sequencing batch reactors for biological wastewater treatment. Critical Reviews in Environmental Control 18(4):255 - 294.

Kosaric N, Blaszcyk R. 1990. Microbial Aggregates in anaerobic Waste water treatment. Advances in Biochemical Engineering/Biotechnology 42:27 - 62.

Krishna C, van Loosdrecht MCM. 1999. Effect of temperature on storage polymers and settleability of activated sludge. Water research 33(1):2374 - 2382.

Kwok WK, Picioreanu C, Ong SL, van Loosdrecht MCM, Ng WJ, Heijnen JJ. 1998. Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. Biotechnology and bioengineering 58(4):400- 407.

Lettinga G, Van Velsen AFM, Hobma SW, De Zeeuw W, Klapwijk A. 1980. Use of the Upflow Sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. Biotechnology and bioengineering 22:699 - 734.

Luyben KCAM. 1992. Lecture notes: Scale-up. Delft, The Netherlands: Department of Biotechnology, Delft University of Technology.

Martins AMP, Pagilla K, Heijnen JJ, van Loosdrecht MCM. 2004. Filamentous bulking sludge - a critical review. Water Research 38(4):793-817.

Mino T, van Loosdrecht MCM, Heijnen JJ. 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. Water Research 32(11):3193-3207.

Morgenroth E, Obermayer A, Arnold E, Brül A, Wagner M, Wilderer PA. 2000. Effect of long-term idle periods on the performance of sequencing batch reactors. Water Science and Technology 41(1):105–113.

Morgenroth E, Sherden T, van Loosdrecht MCM, Heijnen JJ, Wilderer PA. 1997. Aerobic Granular Sludge in a Sequencing Batch Reactor. Water Research 31(12):3191 - 3194.

Mulder R, Vereijken T, Frijters C, Vellinga SHJ. 2001. Future perspectives in bioreactor development. Water Science and Technology 44(8):27-32.

Nicollela C, van Loosdrecht MCM, Heijnen JJ. 2000. Particle-based biofilm reactor technology. TIBTECH 18(July):312-320.

Oosterhuis NMG. 1984. Scale-up of bioreactors, a scale-down approach [Phd. Thesis]. Delft: Technical University Delft. 152 p.

Picioreanu C, Van Loosdrecht MCM, Heijnen JJ. 1998. Mathematical modeling of biofilm structure with a hybrid differential-discrete cellular automaton approach. Biotechnology and Bioengineering 58(1):101-116.

Pochana K, Keller J. 1999. Study of factors affecting simultaneous nitrification and denitrification (SND). Water Science and Technology 39(6):61-68.

Satoh H, Nakamura Y, Ono H, Okabe S. 2003. Effect of oxygen concentration on nitrification and denitrification in single activated sludge flocs. Biotechnology and Bioengineering 83(5):604-607.

Seghezzo L, Zeeman G, van Lier JB, Hamelers HVM, Lettinga G. 1998. A review: The anaerobic treatment of sewage in UASB and EGSB reactors. Bioresource Technology 65(3):175-190.

Smolders GJF, van der Meij J, Van Loosdrecht MCM, Heijnen JJ. 1994a. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. Biotechnology and Bioengineering 43:461-470.

Smolders GJF, van der Meij J, Van Loosdrecht MCM, Heijnen JJ. 1994b. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. biotechnology and Bioengineering 44:837-848.

STOWA. 2002. Optimalisatie van ronde nabezinktanks - Ontwikkeling van het nabezinktankmodel en evaluatie van de STORA - ontwerprichtlijnen. Utrecht: STOWA.

Teulings JHA. 1995. Het ontstaan van de wet in bestuurlijk perspectief 1873-1970. In: van den Berge AP, Groen K, Havekes HJM, Hofstra MA, Teulings JHA, editors. Bestrijding van de watervervuiling - vijfentwintig jaar WVO. Den Haag: Unie van Waterschappen - Ministerie van Verkeer en Waterstaat. p 3-17.

Third KA, Burnett N, Cord-Ruwisch R. 2003. Simultaneous nitrification and denitrification using stored substrate (PHB) as the electron donor in an SBR. Biotechnology and Bioengineering 83(6):706-720.

Tijhuis L, Van Loosdrecht MCM, Heijnen JJ. 1994. Formation and growth of heterotrophic aerobic biofilms on small suspended particles in airlift reactors. biotechnology and bioengineering 44(5):595-608.

Trussell RS, Adham S, Trussell RR. 2005. Process Limits of Municipal Wastewater Treatment with the Submerged Membrane Bioreactor. Journal of Environmental Engineering 131(3):410-416.

Van Aalst-Van Leeuwen MA, Pot MA, van Loosdrecht MCM, Heijnen JJ. 1997. Kinetic modeling of Poly(b-hydroxybutyrate) Production and consumption by *Paracoccus pantotrophus* under Dynamic Substrate Supply. Biotechnology and Bioengineering 55(5):773 - 782.

Van Benthum WAJ, Garrido-Fernandez JM, Tijhuis L, van Loosdrecht MCM, Heijnen JJ. 1996. Formation and detachment of biofilms and granules in a nitrifying biofilm airlift suspension reactor. Biotechnology Progress 1996(12):764-772.

Van Lohuizen K. 2004. Afvalwaterzuivering in Nederland voor de Pasveersloot van 1954: NVA-De Nederlandse vereniging voor Waterbeheer.

Van Loosdrecht MCM, De Kreuk MK; Technische Universiteit Delft, assignee. 2004 18 Maart 2004. Method for the treatment of waste water with sludge granules. The Netherlands, International patent NL1021466C; WO2004024638 (A1).

Van Loosdrecht MCM, Eikelboom DH, Gjaltema A, Mulder A, Tijhuis L, Heijnen JJ. 1995. Biofilm structures. Water Science and Technology 32(8):35-43.

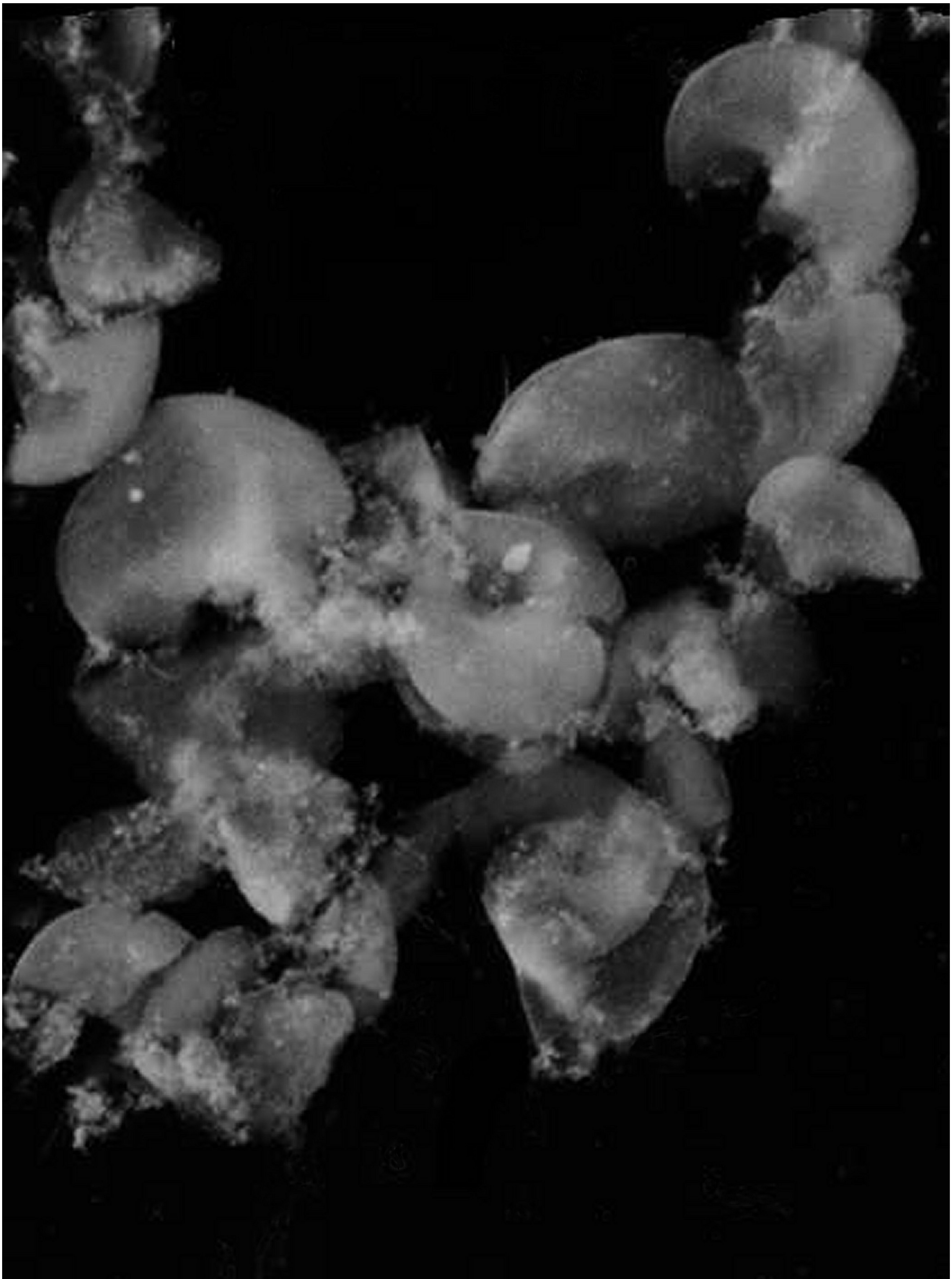
Van Loosdrecht MCM, Pot MA, Heijnen JJ. 1997. Importance of bacterial storage polymers in bioprocesses. Water Science and Technology 35(1):41-47.

Vanderlast ARM, Lettinga G. 1992. Anaerobic Treatment of Domestic Sewage under Moderate Climatic (Dutch) Conditions Using Upflow Reactors at Increased Superficial Velocities. Water Science and Technology 25(7):167-178.

Villaseñor JC, van Loosdrecht MCM, Picioreanu C, Heijnen JJ. 2000. Influence of different substrates on the formation of biofilms in a biofilm airlift suspension reactor. Water Science and Technology 41 (4-5):323-330.

Weijers SR. 2000. Modelling, identification and control of activated sludge plants for nitrogen removal [PhD. Thesis]. Eindhoven: Technische Universiteit Eindhoven. 257 p.

Wilderer PA, Irvine RL, Goronszy M, C. 2000. Sequencing Batch Reactor Technology. Colchester, UK: IWA Publishing. 160 p.



2 Effect of oxygen concentration in combination with pulse feeding on granule stability

Abstract

In order to optimise nitrogen removal in an aerobic granular sludge system, short- and long-term effects of decreased oxygen concentrations on the reactor performance were studied. Operation at decreased oxygen concentration is required to obtain efficient N-removal and low aeration energy requirement. A short-term oxygen reduction (from 100% to 50%, 40%, 20% or 10% of the saturation concentration) did not influence the acetate uptake rate. A lower aerobic acetate uptake at lower oxygen concentrations was obviously compensated by anoxic acetate uptake. Nitrogen removal was favoured by decreased oxygen concentrations, reaching a value of 34% for the lowest oxygen concentration tested. Long-term effects were evaluated at two oxygen saturation levels (100%; and 40%). Nitrogen removal increased from 8% to 45% when the oxygen saturation was reduced to 40%. However, the granules started to disintegrate and biomass washout occurred. It was impossible to obtain stable granular sludge at this decreased oxygen concentration under applied conditions. A solution to obtain stable aerobic granular sludge at low oxygen concentrations is needed in order to make aerobic granular sludge reactors feasible in practice.

This Chapter was published as Mosquera-Corral, A., de Kreuk, M.K., Heijnen, J.J. and van Loosdrecht, M.C.M. (2005). "Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor". *Water Research* 39 (12), 2676-2686

Introduction

Activated sludge systems generally require large surface areas for treatment of wastewater and settling of sludge flocs. In order to reduce space, research is focussed among others on more compact systems. These compact systems in aerobic wastewater treatment are generally based on realising high biomass concentrations, for example via enhancement of the settleability of the biomass. This can be achieved by growing biofilms adhered to a solid surface (airlifts, fluidised beds, etc.) or by cultivating biomass as anaerobic granules (UASB and EGSB reactors). Around ten years ago aerobic granular sludge was grown in an aerobic upflow sludge blanket where pure oxygen was supplied outside the reactor into a liquid recycle flow (Mishima and Nakamura, 1991; Shin et al., 1992). Only recently aerobic granular sludge has been cultured in directly aerated and well-mixed reactors (Morgenroth et al., 1997; Heijnen and van Loosdrecht, 1998; Beun et al., 1999; Dangcong et al., 1999; Etterer and Wilderer, 2001; Tay et al., 2001).

The feeding pattern in biofilm systems influence the appearance of the biomass as well as the COD and nitrogen removal and is even a crucial factor in the growth of aerobic granular sludge (Beun et al. 1999; 2000a). A continuous feed leads to a constant low substrate concentration in the reactor. In these systems, acetate is oxidised by heterotrophic organisms in the outer layer of the biofilm, whilst autotrophic organisms oxidise ammonia in the centre layers in which oxygen is available but acetate is absent (schematically depicted in Figure 1). No zone with substrate and without oxygen exists, so denitrification will not occur. If the rapidly biodegradable substrate concentration (e.g. acetate) in the liquid is high enough to penetrate deeper into the biofilm than oxygen, there is no region without substrate and with oxygen. In this case fast growing heterotrophic organisms will outcompete the ammonia oxidisers. No nitrification will occur.

A sequencing batch airlift reactor (SBAR), in which aerobic granular sludge (Beun et al., 2001), regular biofilm (Arnold et al., 2000) or flocculated activated sludge (Pochana and Keller, 1999) can be grown, is a suitable system for simultaneous COD and nitrogen removal (Simultaneous Nitrification Denitrification, SND). Special circumstances, depending on floc, biofilm or granules size, oxygen concentration and COD/N ratio, can lead to a substrate rich aggregate with an oxygen free zone in the interior (diffusion limitation of oxygen), in which denitrification takes place. Because of the size and structure of granules and biofilms, SND can be maintained and controlled easier in these aggregates than in activated sludge flocs.

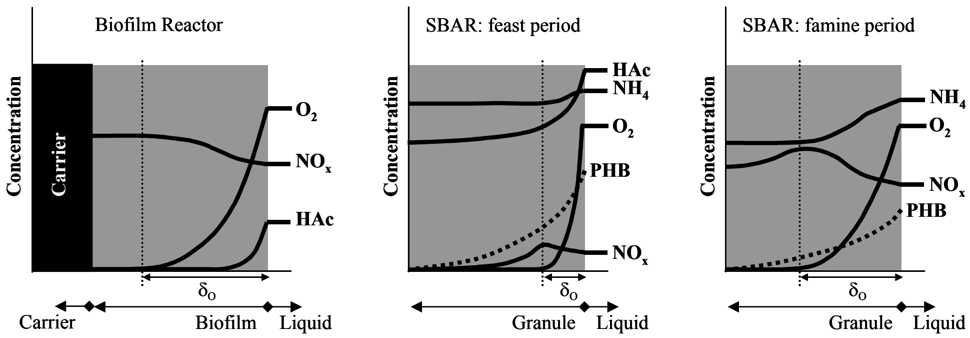


Figure 1. Schematic profile of substrates inside the biomass for a biofilm system and a SBAR (feast and famine periods; external mass transfer neglected)

In the biofilm or granular sludge SBAR system, the substrate (acetate) is supplied in a short period of time. High substrate concentrations in the bulk liquid cause a penetration of substrate deeper into the biofilm than oxygen. During the period in which the external substrate is available (feast period), oxygen is quickly consumed in the outer layers by growth, substrate storage and nitrification. Oxygen has only a limited penetration depth in this period. Acetate storage (as poly-β-hydroxybutyrate or PHB) and growth occur aerobically in the outermost layer or anoxically inside the granule (as indicated in figure 1). During the period without external substrate (famine period), growth takes place on the internally stored PHB at much lower growth rates (Beun et al., 2001). The oxygen penetration depth during the famine period will be higher, because of this decreased respiration rates of the heterotrophic organisms, but will still be limited due to increased nitrification relative to the feast phase (Third et al., 2003). The produced nitrate can be simultaneous denitrified inside the granule using the stored PHB as electron donor. The applied oxygen concentration in combination with the size of the granule or biofilm are closely related to the size of the anoxic zone: the lower the oxygen concentration or the bigger the granule, the larger the anoxic zone and thus, the larger the nitrogen removal capacity. Obviously, the oxygen needed for nitrification limits the decrease in the oxygen concentration.

The distribution of biomass inside the biofilm or granule affects the performance of the processes taking place in the reactors. In aerobic biofilm systems, where COD removal and nitrification take place simultaneously, heterotrophic and autotrophic microorganisms compete inside the biofilms for space and oxygen. Fast growing microorganisms (heterotrophs) will be located in the outer layers while slow growing microorganisms (autotrophs) will be confined to deeper zones of the

biofilm with lower oxygen availability (van Loosdrecht et al., 1995; Okabe et al., 1995). After the feast period the nitrifiers get sufficient oxygen, because of the mentioned increased oxygen penetration depth due to the lower oxygen requirement of heterotrophs growing on PHB (Beun et al., 2001).
Oxygen supply has to be optimised in order to minimise the energy consumption in full-scale aerobic systems. Control of the oxygen concentration is also necessary to optimise nitrogen removal via denitrification. For these reasons it is important to evaluate what the consequences are of operation at low oxygen concentration on granule formation and N-removal in SBAR systems.
Previously, a simulation model has been used to predict the nitrogen removal efficiencies for different oxygen concentrations in a SBAR (Beun et al., 2001). The optimum nitrogen removal efficiency was predicted for an oxygen saturation of 40%, however, this was not experimentally verified. The present work intends to study experimentally the effect of oxygen concentration on N-removal and on the formation and development of the granules inside the SBAR.

Material and methods

Reactor set-up and operation

A sequencing batch airlift reactor (SBAR) with a working volume of 3 litres was used for the experiments, as previously described by Beun *et al.* (2000a). The hydraulic retention time was 5.8 hours, the volumetric exchange ratio per cycle was 55% and the substrate load was 1.6 g COD $(L \cdot d)^{-1}$. The temperature of the reactor was maintained at 20±1°C using a thermostat bath and a water jacket. The pH value was controlled at 7.0±0.1 by addition of acid and base solutions. Air was introduced at the bottom of the reactor using a porous glass stone. The gas flow was maintained at 24 $m^3\ h^{-1}$ or a superficial velocity of 90.8 m h^{-1}, using a mass flow controller (oxygen saturation level 100%) or a membrane pump (oxygen saturation level lower than 100%). Controlling the oxygen saturation inside the reactor at lower levels than 100% was done by a controlled off-gas circulation system (Figure 2). The reactor was inoculated with activated sludge from a municipal wastewater treatment plant. After a period of 65 days sludge from a nitrifying reactor was added to stimulate nitrification. The SBAR was operated in successive cycles of 3 hours each, consisting of four different phases: filling (3 min), aeration (169 min), settling (3 min) and effluent withdrawal (5 min). The short settling time in combination with the height of the reactor allowed only

particles with a settling velocity larger then 12 m h^{-1} to be maintained in the reactor. Due to the operation procedure, the cycle could be divided into two different periods: a feast period, in which acetate was present in the solution and a famine period, in which only cell-internal stored substrate was available (as PHB).
The total operation period consisted of three different stages:
Stage I: start-up and operation for 150 days using similar conditions as described by Beun et al., (2000a). The oxygen saturation level was not controlled and was approximately 75% during the feast period and 100% during the famine period.
Stage II: The oxygen saturation level in the reactor was controlled at 40% during the whole cycle. The other operation conditions were sustained. This stage was performed during 50 days, after which the experiment was interrupted.
Stage III: the SBAR was started up using the same conditions as in Stage II (oxygen saturation level controlled at 40%). The reactor was operated during 30 days, after which the experiment was stopped.
During the stable operation of Stage I, several cycle measurements were performed using different oxygen concentrations in one single cycle (controlled by mixing nitrogen in the gas-phase using a mass flow controller). The used oxygen saturation levels were 100%, 50%, 40%, 20% and 10% saturation. Cycle measurements were also performed during Stage II operation.

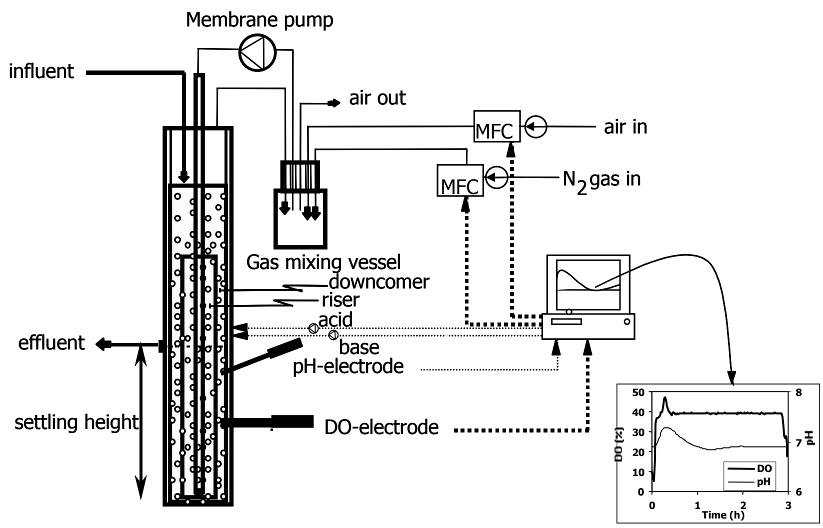


Figure 2. Schematic representation of the Sequencing Batch Airlift Reactor layout

Media

Two different media were used with the following compositions. Medium A: $CH_3COONa{\cdot}3H_2O$ 65.1 mM; $MgSO_4{\cdot}7H_2O$ 3.7 mM; KCl 4.8 mM. Medium B: NH_4Cl 35.2 mM; K_2HPO_4 2.2 mM; KH_2PO_4 4.4 mM; 10 mL L^{-1} trace element solution according to Vishniac and Santer (1957). Each cycle 150 mL of each medium was added to the reactor together with 1300 mL of dilution tap water. The COD-load with this medium was 1.6 g COD $(L{\cdot}d)^{-1}$ and the COD/N ratio was 8.3.

Analytical procedures

Oxygen concentration in the reactor was measured online with a DO-electrode as a percentage of the oxygen concentration at air saturation. The CO_2 content of the off-gas was measured online with an infrared CO_2 analyser. Dry weight concentration (TSS) was determined by filtering a sample of reactor solids or a sample of effluent (Whatman GF/C) and drying the filter with solids during at least 24 h at 105°C. Ash content was measured according to the Dutch standard method NEN6621 (NNI, 1982). The biomass concentration of the effluent after a short settling period (5 minutes) obtained from measuring the total organic carbon (TOC) concentration (DOHRMAN DC-190 analyser) from a filtered effluent sample (0.45 μm filters) and from a sample taken after 5 min of settling, and subtracting these two TOC values. The acetate concentration of filtered samples from the reactor was measured by gas chromatography. Ammonium and nitrite were

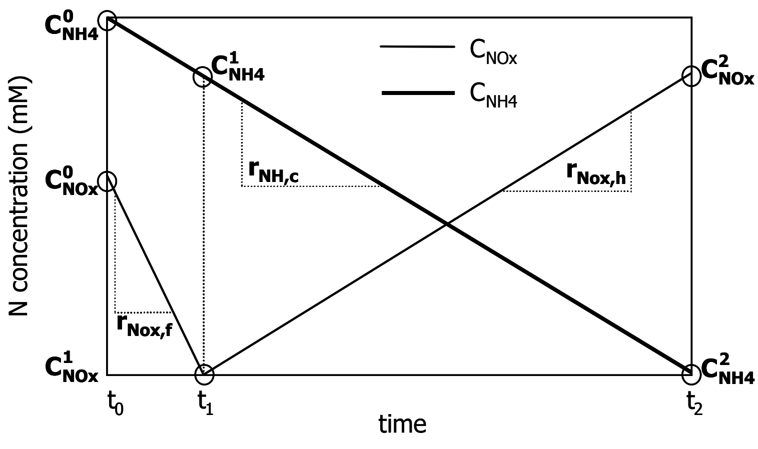


Figure 3. Schematic representation of the nitrogen compounds concentrations during the cycle and definition of the calculated parameters

measured colorimetrically according to the standard methods. Nitrate was measured colorimetrically at 420 nm after reaction (20 min) with a saturated sulfamic acid solution, and 5 % salicylic acid in 98% sulfuric acid and NaOH solution (Strous et al., 1998). The elemental composition (CHNS) of the biomass was determined by flash combustion in a partial oxygen atmosphere at 1020°C using a Carlo Erba EA1108 elemental analyser. The oxygen content of the biomass was obtained by substracting the percentages of C, H, N, S and ash from 100%.

The bedvolume of the settled biomass granules was determined after 5 minutes of settling by reading on a graduated scale placed on the reactor column. This SVI_5 (mL $gTSS^{-1}$) is comparable to the SVI after 60 minutes in case of granular sludge, in contrast to the findings with flocculated sludge (Schwarzenbeck et al., 2003). Due to the compact shape and the high settling velocity of the granules, no substantial change in the volume of the settled bed was observed. The biofilm morphology (particle diameter, aspect ratio and shape factor) was evaluated using an image analyser as described in Kwok et al. (1998). The density of the biomass (the concentration of biomass in the granules) was determined as g TSS $L_{granules}^{-1}$ using the Dextran blue 2000 method (Beun et al., 2002) and measuring absorbance changes at 620 nm.

Calculatory procedures

The solids retention time (SRT), biomass yield (Y), reactor surface specific area of the granules (a) and volume occupied by the biomass (V_x) were calculated according to Beun (Beun et al., 2000a). Penetration depth of the different substrates was calculated according to Tijhuis (Tijhuis et al., 1994), using the equation for spherical particles. A relative ratio between the volumes of biomass penetrated with O_2 and with acetate was calculated from the obtained penetration depths as the volume of the shell of sphere.

N-removal efficiencies and removal and consumption rates were calculated according to the following expressions (for symbols see Figure 3). The removal percentages during the famine period and the total cycle, are calculated according to equations [1] and [2].

$$\%N_f = \frac{C^1_{NOx} + C^1_{NH4} - C^2_{NOx} - C^2_{NH4}}{C^0_{NH4} + C^0_{NOx}} \cdot 100 \qquad [1]$$

$$\%N_c = \frac{C^0_{NOx} + C^0_{NH4} - C^2_{NOx} - C^2_{NH4}}{C^0_{NH4} + C^0_{NOx}} \cdot 100 \qquad [2]$$

The percentages of ammonia and nitrogen oxides, present in the effluent related to the amount of total nitrogen at the beginning of the cycle, were calculated according to equations [3] and [4].

$$\%NH4_e = \frac{C_{NH4,2}}{C_{NH4,0} + C_{NOx,0}} \cdot 100 \qquad [3]$$

$$\%NOx_e = \frac{C_{NOx,2}}{C_{NH4,0} + C_{NOx,0}} \cdot 100 \qquad [4]$$

The consumption rates for ammonia and NO_x^- and the production rate of NO_x^- were calculated using equations [5], [6] and [7] respectively.

$$r_{NOx,f} = \frac{C_{NOx,0} - C_{NOx,1}}{t_1 - t_0} \qquad [5]$$

$$r_{NH4,c} = \frac{C_{NH4,0} - C_{NH4,2}}{t_2 - t_0} \qquad [6]$$

$$r_{NOx,h} = \frac{C_{NOx,1} - C_{NOx,2}}{t_1 - t_2} \qquad [7]$$

Results

Long term effects of the oxygen concentration on reactor operation and granule formation

During 150 days (stage I), an airlift reactor was operated in the same conditions as described by Beun et al. (2000a). The only difference was the lower COD/N ratio in the feed solution (decreased from 14 to 8). This means that the oxygen concentration in the reactor was not controlled, resulting in an oxygen saturation of 75% during the feast period (acetate is present in the liquid) and 100% during the famine period (the organisms use the cell-internally stored substrate (PHB) for growth).

Granules developed similar to the dense and cauliflower shaped granules obtained in previous work by Beun et al. (2000a). Granules of 0.6 mm were first observed 5 days after inoculation, progressively increasing their size to an average steady-state diameter of 1.6 mm at day 30 (Figure 7-a). The average shape factor and aspect ratio were 0.62 and 0.68 respectively, indicating regular and round granules.

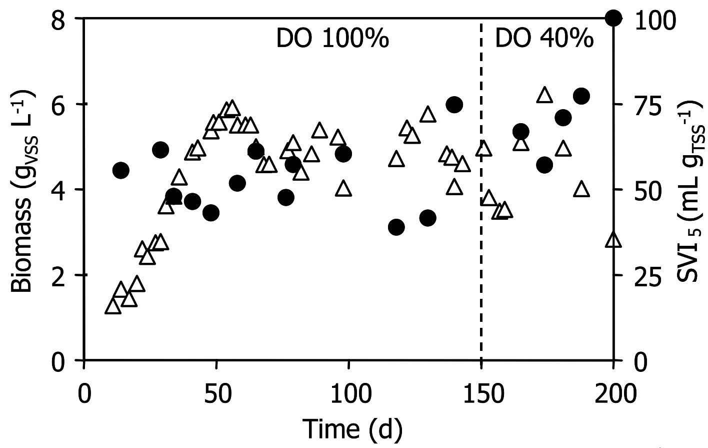


Figure 4. Evolution of the biomass concentration in the reactor (g VSS L^{-1}) (Δ) and of the sludge volume index (SVI_5) (mL g TSS^{-1}) (●)

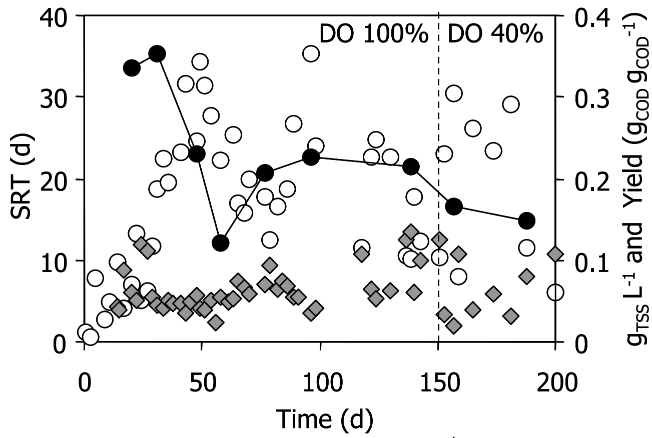


Figure 5. Evolution of the SRT (d) (○), g TSS L^{-1} on the effluent (◆) and biomass yield (g COD g COD^{-1}) (●)

After 50 days, biomass concentrations in the reactor stabilised around 5 gVSS L^{-1} (Figure 4). The sludge retention time (SRT) increased during the first 50 days of operation to 35 days (filling up reactor till effluent withdrawal point). At this time sludge growth was balanced by granule wash-out, stabilising the SRT around 25 days (figure 5). The surface load applied to the granules was 3.5 gCOD $(m^2 \cdot d)^{-1}$ during the major part of the operational period and the maximum density of the granules was 53 gTSS $L_{granule}^{-1}$. High granule density in combination with a regular shape result in a low SVI_5, which fluctuated between 48 and 75 mL $gTSS^{-1}$ (Figure 4). The average biomass yield fluctuated around 0.2 gCOD $gCOD^{-1}$ (Figure 5).

11 Days after start-up, the volumetric acetate uptake rate during the feast period reached a steady state. This rate was deduced from the length of the feast period, which fluctuated between 15 and 20 minutes during the whole operation period.

The fluctuation was mainly due to the fluctuating biomass concentration in the reactor. Complete ammonia removal was reached after 120 days (Figure 6). The average N-removal between 100 days and the end of this stage was 16% of the total amount of nitrogen added. Part of this N-removal was due to assimilation; part was due to denitrification.

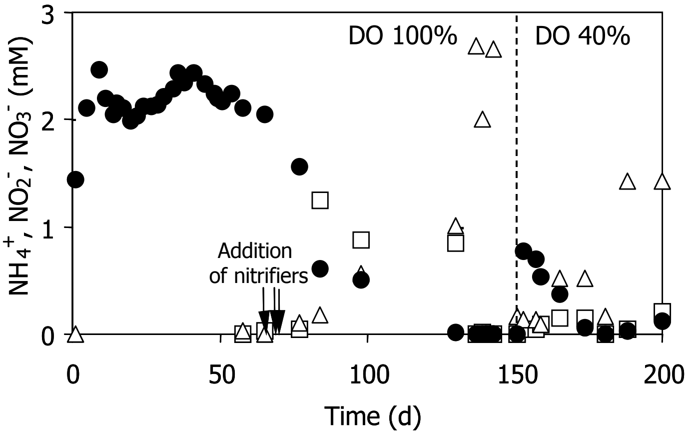


Figure 6. Evolution of nitrogen compounds concentrations NH_4^+-N (●), NO_3^--N (∆) and NO_2^--N (□) (g N L^{-1}) in the effluent of the SBAR

After 150 days of operation, the oxygen saturation was decreased to 40% oxygen saturation (stage II), in order to improve the nitrogen removing capacity of the system corresponding to the modelling results of Beun et al. (2001).

Ten days after the oxygen concentration was decreased, filamentous structures could be observed at the surface of the granules (Figure 7-b). From day 174 the granules started to disintegrate and became hollow structures (Figure 8), resulting in a visually decreased granule size and a decreased shape factor (<0.52). The settling properties of the sludge were highly affected by the disintegration of the granules in combination with filamentous growth. A substantial amount of biomass was washed-out with the effluent. The experiment was stopped at this point.

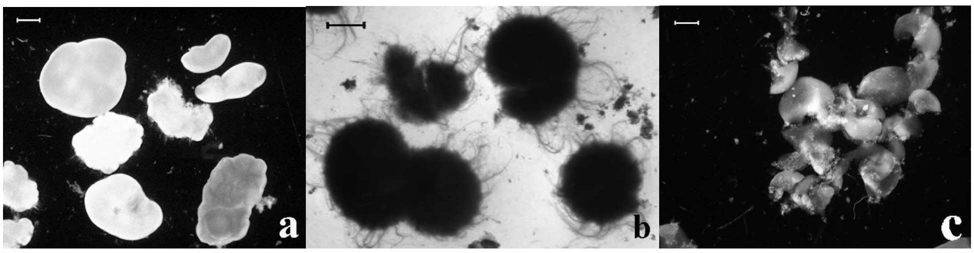


Figure 7. Pictures of the granules during Stage I (DO 100%): days 45 (a) and 181 (b); and Stage II (DO 40%): day 9 (c) (- = 1 mm)

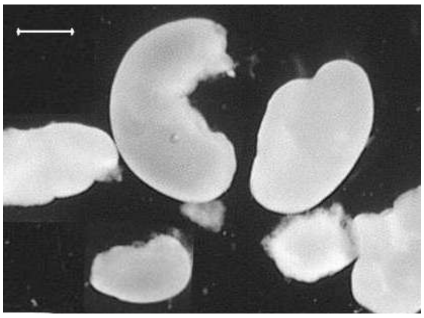


Figure 8. Breaking up of granules and transforming into flat structures (- = 1 mm)

The biomass concentration in the reactor decreased from 5 to 3.5 gVSS L^{-1} (Figure 4), due to wash-out of unstable granules. This was also noticed in an increased solid content in the effluent (0.1 gTSS L^{-1}) and the SRT was reduced to 8 days (Figure 5). The SVI_5 increased to 100 mL $gTSS^{-1}$. This might be a reasonable SVI for flocculent sludge, but under the settling conditions in the aerobic granule reactor, all such sludge will be washed out.
Due to the decreased biomass concentration in the reactor the volumetric acetate uptake rate decreased lightly, observed by an increased length of the feast phase. A lower oxygen concentration led to increased denitrification efficiency and NO_x^- was almost completely depleted during the first days of stage II. Conversely, nitrification efficiency decreased and ammonia was present in the effluent. After 20 days of operation with a decreased oxygen concentration, the nitrification capacity was restored fully and all ammonia was converted, but low concentrations of nitrite and nitrate were present again in the effluent (Table 1). The average nitrogen removal during the total cycle was 63% at oxygen saturation level 40%; clearly a larger part of the nitrite and nitrate formed is denitrified under these conditions.

During the third experiment (stage III), the SBAR was started up at 40% oxygen saturation with further identical conditions as in the other experiments. The first granules were observed 10 days after start-up, but these granules were small, white coloured, unstable and were washed-out frequently (Figure 7-c). During stage III, an important competition between biofilm growth on the reactor surface and granule formation occurred. This is typically a small-scale problem because of the high ratio between reactor wall surface and volume.

The experimental results from this run (Table 1) were obtained while the highest amount of granules was present in the reactor. The maximum biomass concentration measured in the reactor was 0.9 gVSS L^{-1}, when the settled bed levelled the effluent extraction point and biomass production was balanced by wash-out. The average biomass concentration in the effluent was very high (0.144 gTSS L^{-1}). The density of the floc-like biomass was 13 gTSS $L_{granules}^{-1}$. The SVI_5 reached a value of 200 mL $gTSS^{-1}$.
The volumetric acetate uptake rate was very low, indicated by a very long feast period (160 minutes). No nitrification was observed, since the slow growing autotrophic organisms can not be maintained in such an instable system. After 1 month of operation the reactor was stopped due to the impossibility of formation of stable granules under these conditions.

Short term effects of a low oxygen concentration on reactor operation
The effect of a changed oxygen penetration depth on granules grown under high oxygen concentrations was explored by changing the oxygen saturation during one cycle in the reactor operating stable at a high oxygen content (75-100% saturation level). These measurements were performed in the period from day 133 to day 143 of stage I. The biomass concentration in all experiments was comparable. The

Table 1. Results of measured parameters in the reactor during the three operational stages.

Averaged values	DO 100% (I)	DO 40% (II)	DO 40%[2] (III)
Feast phase (min)	15.5	20	160
Bed volume (L)	0.8	1.0	0.6
Density granules (g TSS $L_{granule}^{-1}$)	53	20	13
g VSS L^{-1} (reactor)	5.0	3.5	0.9
g TSS L^{-1} (effluent)	0.065	0.100	0.144
SVI_5 (mL g_{TSS}^{-1})[1]	72	100	200
mg TOC L^{-1} (effluent)[1]	20	20	--
mg NH_4^+-N L^{-1} (effluent)	0.13	(0.7) 1	--
mg NO_3^-N L^{-1} (effluent)	22	(0.13) 21	ND
mg NO_2^-N L^{-1} (effluent)	3.9	(0.04) 1.5	ND

ND Not detected. [1] Measured after 5 min of settling. [2] Values taken from the period when only granules where present, but this value was very variable due to wall growth. Values between brackets were obtained during the first days of Stage II.

Table 2. Resume of the results of tests with low oxygen saturation levels with the reactor operating in steady state at a high oxygen concentration.

Stage	**I**	**I**	**I**	**I**	**I**	**II**[1]
DO (%)	100	50	40	20	10	40
g_{VSS} L^{-1} (initial in reactor)	5.0	4.6	4.8	4.7	4.1	2.69
Feast length (min)	17	15	16	15	15	17
q_{Ac} (mg_{COD} g_{COD}^{-1} h^{-1})[2]	170	180	164	156	154	217
%N_c (N removed cycle)[2]	8.0	21.1	15.2	22.9	34.5	44.8
%N_f (N removed famine)[2]	3.7	2.1	4.7	11.3	21.3	19.5
% $NH4_e$ (NH_4^+ effluent)[2]	0.04	1.9	0.1	12.0	45.0	2.14
% NOx_e (NO_x^- effluent)[2]	92	77	84	65	20	53
% $NO3_e$ (NO_3^- effluent)[2]	88	68	74	47	15	49
% $NO2_e$ (NO_2^- effluent)[2]	4	9	10	18	5	4

[1] Cycle measurement after more than 1 month of operation at oxygen saturation level 40%

[2] Data calculated according to materials and Methods

volumetric acetate uptake rate was observed to be independent of the oxygen concentration and thus comparable in the different experiments, expressed by a similar duration of the feast phase in all cases (Table 2).

Ammonium oxidation and nitrate production rates respectively decreased and increased with a decreasing oxygen level (Figure 9). At oxygen saturations lower than 20%, ammonium concentrations in the effluent increased, which indicates that no complete nitrification was achieved. NO_x^- compounds were denitrified during the feast period using acetate as carbon source. During the famine period of the cycles with high oxygen saturation (40%-100%) denitrification did not take place. At low oxygen concentrations, denitrification occurred during the famine period with previously stored PHB. The nitrogen removal increased with decreasing oxygen concentrations until the maximum value of 34.5% at 10% oxygen saturation (Table 2).

After more than 6 weeks of reactor operation at oxygen saturation of 40% (Stage II) a cycle measurement was performed. The percentage of nitrogen removal obtained in this experiment was 45%, which is higher compared to the nitrogen removal at 100% oxygen saturation (26% average nitrogen removal over the total operation period or 8% measured during the cycle measurement at 100% oxygen saturation). The acetate consumption rate was similar to the operation with a high oxygen concentration (length feast period 17 min). Ammonia was consumed

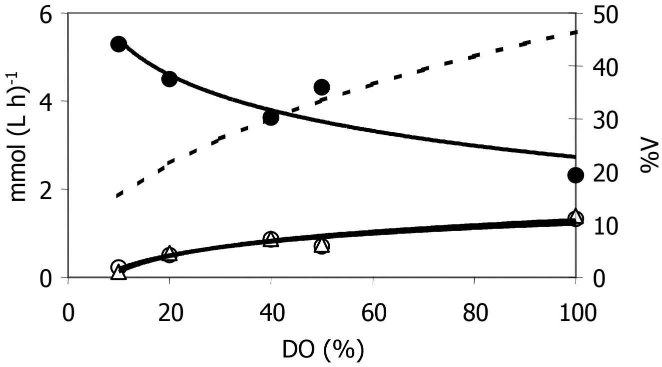


Figure 9. Values of $r_{NOx,f}$ (●), $r_{NH4,c}$ (○) and $r_{NOx,h}$ (∆) (mmol N $(L{\cdot}d)^{-1}$) for every oxygen concentration tested and the ratio of the aerobic biomass volume to the biomass volume in which acetate is present (%V) (▪▪▪). Acetate is present in 62% of the total biomass volume.

during the cycle, while nitrate appeared in the effluent, indicating a non-complete denitrification. In Figure 10 (a, b) the concentrations of the compounds in the liquid phase for the cycles carried out with oxygen saturation of 40% for Stages I and II are shown. The most notorious, but logical difference between both cycles is that the nitrification rate was lower and denitrification was higher in the cycle carried out during Stage II.

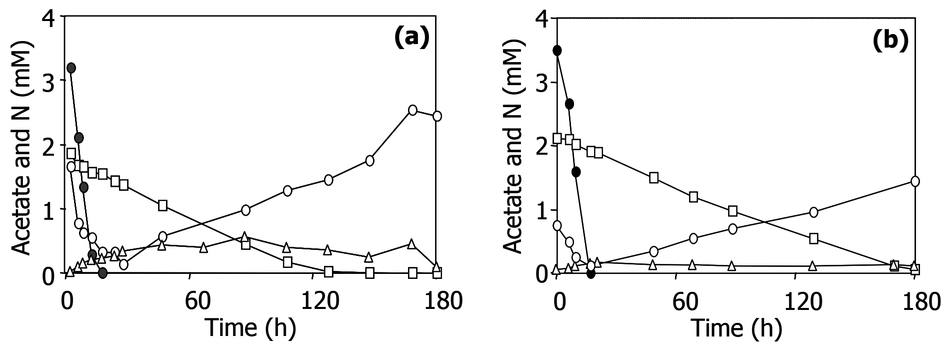


Figure 10. Concentration of acetate (●) and nitrogen NH_4^+ (□), NO_3^- (○) and NO_2^- (∆) during the cycle measurements with oxygen saturation level 40% for Stages I (a) and II (b).

Discussion

Effects of oxygen on acetate uptake

The acetate uptake rate was similar at the different applied oxygen concentrations (Table 2). However, Beun et al. (2000b) showed that the specific anoxic acetate uptake rate is 3 to 4 times lower than the rate under aerobic conditions. At decreased oxygen concentrations, the ratio of the aerobic biomass volume to the acetate-rich volume decreases (Figure 9). In the anoxic biomass with acetate present, acetate will be taken up anoxically at mentioned lower uptake rates than in the aerobic zone. Therefore, a decreased oxygen concentration was expected to lead to a lower acetate uptake rate and thus to a longer feast phase.

The comparable acetate uptake rates during the short-term experiments show that the acetate is indeed taken-up anoxically when oxygen is not available and that this denitrifying activity is also available in the normally aerobic zone of the granules. The acetate penetration depth at the used influent concentration was around 275 µm, which corresponds to an acetate-penetrated or active volume of 62% of the total biomass volume, calculated with the acetate uptake rate of the aerobic process (Beun et al., 2000b) (Figure 9). Lower effective acetate uptake rates in the anoxic zone will lead to a higher penetration depth for acetate and thus to a larger active zone. This indicates the large flexibility of the granules to adapt to a decreased oxygen concentration and their capability to maintain their high substrate uptake rates and efficiency under changing circumstances.

Effect of oxygen saturation on N-removal

In the case of nitrogen removal, as expected, a lower oxygen concentration resulted in a lower nitrification capacity and a higher denitrification capacity (Table 2). Higher N-removal efficiencies were found at lower oxygen concentrations, with the maximum N-removal (34.5%) at the minimum oxygen saturation (10%). The general behaviour is comparable to the modelling results of Beun et al. (2001).

Like in activated sludge flocs, simultaneous nitrification and denitrification (SND) can occur because of the existence of aerobic zones in the outer layers of the granule or floc and anoxic substrate rich layers in the inner zones, in which respectively nitrification and denitrification can occur (Pochana and Keller, 1999; Satoh et al., 2003). The trend of SND efficiency at different oxygen concentrations found in the short-term experiments is also comparable to Third et al. (2003) in

which denitrification takes place in the anoxic zones of sludge flocs using PHB as substrate during the famine phase.
The relation between the oxygen penetration depth and the acetate penetration depth in the aerobic granules is expressed as %V in Figure 9 (%V is the percentage of aerobic biomass volume relative to the acetate or PHB rich biomass volume). The anoxic volume relatively increased at decreased oxygen concentrations, increasing the capacity for denitrification. This leads to higher N-removal efficiencies at decreased oxygen concentrations, which is analogous to the measurements in the short-term experiments. This trend emphasises the importance of the anoxic zone inside the aerobic granules.
The nitrification efficiency decreased at lower oxygen concentrations (Table 2). Competition between ammonium oxidising organisms and heterotrophic organisms in the outer layer for oxygen combined with a decreased oxygen penetration depth, might have contributed to this decrease in nitrification efficiency (Okabe et al., 1995). The volume of aerobic biomass in the outer layer of the granule decreased less than the ammonium oxidation rate. For example, decreasing the oxygen saturation from 100% to 50% caused an aerobic volume decrease of 27%, but an ammonium oxidation rate decrease of 47%. This indicates a layered structure in the aerobic zones, with heterotrophic organisms growing more on the outside than the ammonium oxidisers. This layered structure is mainly caused by differences in growth rate between heterotrophic and autotrophic organisms in combination with competition for oxygen (van Loosdrecht et al., 1995).
The decreased nitrification rate did not lead to decreased total nitrogen removal. In fact, the increased volume of anoxic substrate-rich biomass caused a higher overall nitrate reduction, resulting in decreased effluent nitrate concentrations. Increased overall nitrogen removal arised at decreased oxygen concentrations.
During long-term changes of oxygen concentration, the effect on SND was expected to be different, because of a changing biomass distribution within the granules. This was verified in a long-term experiment at 100% (stage I) and 40% (stage II) oxygen saturation (Table 2). The initial decreased nitrification and increased denitrification capacity was analogous to the short-term experiments. However, after 20 days of operation at low oxygen concentration, granules started to deteriorate and changed into thin and irregular structures. Because of this structure, oxygen could penetrate a larger fraction of the granule, resulting in improved nitrification capacity, but denitrification capacity became comparable to 100% oxygen saturation (Table 1). Hence, the long-term effect of nitrogen

removal at low oxygen concentrations was mainly influenced by the change of the granule structure.

Effects of oxygen saturation on granule formation

Full size aerobic granules were formed within 16 days of operation after inoculation with activated sludge at 70-100% oxygen saturation. Granules had an average diameter of 2 mm and a density of 53 gTSS $L_{granules}^{-1}$, which is comparable to the granules formed in the experiments of Beun et al. (2000a), reporting an average diameter of 2.5 mm and 60 g TSS $L_{granules}^{-1}$. Measured biomass concentrations in this study and in Beun et al. (2000a) were respectively 8 gVSS L^{-1} and 5 gVSS L^{-1}, which is mainly due to the different substrate loading rate of 2.5 g COD $(L \cdot d)^{-1}$ and 1.6 g COD $(L \cdot d)^{-1}$.

Reducing the oxygen saturation to 40% (stage II) caused deterioration, a decreased density and finally breaking of the granules. In combination with outgrowth of filamentous structures, the biomass could not be maintained in the reactor. Also the start up of the reactor with a reduced oxygen saturation (40%, stage III) led to the formation of small unstable granules, with filamentous outgrowth and a low density.

The decreased oxygen penetration depth into the granule grown at 100% oxygen saturation during stage II, might have caused decay or reduced EPS production (Eikelboom and van Buijsen, 1983) in the internal parts of the granule. This weakened the structure of the granules and led to density reduction, breakage and thus to declined settling characteristics and wash-out. Dangcong et al. (1999) reported a study carried out in an SBR at low oxygen concentrations (1 mg O_2 L^{-1}) and observed small "granules" (diameter of 0.3-0.5 mm) that agglomerated into big flocs during settling. The reported settling time was 2.5 hours and the measured SVI was 100 mL g^{-1}, indicating formation of aerobic granules did not really occur. Wilén and Balmér (1999) have observed similar effects studying sludge flocs at low oxygen concentrations.

Picioreanu et al. (1998) postulated smooth biofilms would develop when the ratio of biomass growth rate versus diffusive transport is low (gradients of substrate or oxygen are smooth). When this ratio is high, and thus sharp gradients exist, irregular floc like structures would develop. This is in line with this study, because decreased oxygen concentrations means increased diffusion limitation and therefore to a more sharp concentration gradient in the granule. Martins et al. (2003) recently discussed that this hypothesis for the morphology of biofilm formation could equally well be used for activated sludge morphology. If this proposed ratio determines also the structure of the granules, selection for slow growing organisms (i.e. growth on slower degradable substrates) would lead to stable granular sludge at low oxygen concentrations (de Kreuk and van Loosdrecht, 2004).

Conclusions

The processes of aerobic COD removal, nitrification and denitrification can occur simultaneously in an aerobic granular sludge reactor fed with acetate. The operation of the SBAR with an oxygen saturation of 40% increased the nitrogen removal efficiency (compared to 100% oxygen saturation) due to an increased denitrification. However, the granular sludge stability was negatively influenced. Granules broke up due to a low oxygen concentration. It was also not possible to form stable granules from activated sludge at such a low oxygen concentration. For practical application, operation at a low oxygen concentration is essential for reducing energy demand and obtaining sufficient N-removal. Different operational strategies are needed in order to generate stable granular sludge at low oxygen concentrations.

Acknowledgements: This work was funded by the BIOTOBIO European TMR Program (Contract FMR-CT97-0114), the Dutch Foundation for Water Research (STOWA, TNW99.262) and STW (DPC5577). The authors thank to the analytical staff of the Kluyver Laboratory of Biotechnology of the Technical University of Delft for their analytical assistance.

References

Arnold E., Boehm B. and Wilderer P.A. (2000) Application of activated sludge and biofilm sequencing batch reactor technology to treat reject water from sludge dewatering systems: A comparison. *Wat. Sci. Tech*. **41**(1), 115-122.

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

Beun J.J., van Loosdrecht M.C.M. and Heijnen J.J. (2000a) Aerobic granulation. *Wat. Sci. Tech.* **41** (4-5), 41-48.

Beun J.J., Verhoef E.V., van Loosdrecht M.C.M., Heijnen J.J. (2000b) Stoichiometry and kinetics of poly-β-hydroxybutyrate metabolism under denitrifying conditions in activated sludge cultures. *Biotechnol. Bioeng*. **68**(5), 496-507.

Beun J.J., van Loosdrecht M.C.M. and Heijnen J.J. (2001) N-removal in a granular sludge sequencing batch airlift reactor. *Biotechnol. Bioeng*. **75**(1), 82-92.

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

Dangcong P., Bernet N., Delgenes J.-P. and Moletta R. (1999) Aerobic granular sludge-a case report. *Wat. Res.* **33**(3), 890-893.

De Kreuk M.K. and Van Loosdrecht, M.C.M. (2004) Selection of Slow Growing Organisms as a Means for Improving Aerobic Granular Sludge Stability. *Wat. Sci. Tech.* **49**(11-12), 9-17.

Eikelboom D.H. and van Buijsen H.J.J. (1983) *Micoscopic sludge investigation manual*. TNO Research Institute for Environmental Hygiene. 2nd Ed.

Etterer T. and Wilderer P.A. (2001) Generation and properties of aerobic granular sludge. *Wat. Sci. Tech.* **43**(3), 19-26.

Heijnen J.J. and van Loosdrecht M.C.M. (1998) Werkwijze voor het verkrijen van korrelvormige groei van een micro-organisme in een reactor. NL patent 1005345.

Kwok W.K., Picioreanu C., Ong S.L., van Loosdrecht M.C.M., Ng W.J. and Heijnen J.J. (1998) Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. *Biotechnol. Bioeng.* **58**(4), 400-407.

Martins, A.M.P., Heijnen J.J. and van Loosdrecht M.C.M. (2003) Effect of dissolved oxygen concentration on sludge settleability. *Appl. Microbiol. Biotechnol.* **62**, 586-593.

Mishima K. and Nakamura M. (1991) Self-immobilization of aerobic activated sludge-a pilot study of the aerobic upflow sludge blanket process in municipal sewage treatment. *Wat. Sci. Tech.* **23**, 981-990.

Morgenroth E., Sherden T., van Loosdrecht M.C.M., Heijnen J.J. and Wilderer P.A. (1997) Aerobic granular sludge in sequencing batch reactor. *Wat. Res*. **31**(12), 3491-3194.

NNI. (1982) NEN 6621, Bepaling van de asrest. Nederlands Normalisatie Instituut, Delft.

Okabe S., Hirata K. and Watanabe Y. (1995) Dynamic changes in spatial microbial distribution in mixed-population biofilms: experimental results and model simulation. *Wat. Sci. Tech*. **32**(8), 67-74.

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

Pochana K. and Keller J. (1999) Study of factors affecting simultaneous nitrification and denitrification (SND). *Wat. Sci. Tech.* **39** (6), 61-68.

Satoh H., Nakamura Y., Ono H. and Okabe S. (2003) Effect of oxygen concentration on nitrification and denitrification in single activated sludge flocs. *Biotechnol. Bioeng.* **83**(5), 604-607.

Shin H.S., Lim K.H. and Park H.S. (1992) Effect of shear stress on granulation in oxygen aerobic upflow sludge bed reactors. *Wat. Sci. Tech.* **26**(3-4), 601-605.

Strous M., Heijnen J.J., Kuenen J.G. and Jetten M.S.M. (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Appl. Microbiol. Biotechnol*. **50**, 589-596.

Schwarzenbeck N., Erley R. and Wilderer P.A. (2003) Growth of aerobic granular sludge in an SBR-system treating wastewater rich in particulate matter. IWA Biofilm Conference, Capetown, South Africa, IWA Publishing.

Tay J.H., Liu Q.S. and Lui Y. (2001) Microscopic observation of aerobic granulation in sequential aerobic sludge blanket reactor. *J. Appl. Microbiol.* **91**(1), 168-175.

Third K.A., Burnett N. and Cord-Ruwisch R. (2003) Simultaneous nitrification and denitrification using stored substrate (PHB) as the electron donor in an SBR. *Biotechnol. Bioeng.* **83**(6), 706-720.

Tijhuis L., van Loosdrecht M.C.M. and Heijnen J.J. (1994) Formation and growth of heterotrophic aerobic biofilms on small suspended particles in airlift reactors. *Biotechnol. Bioeng.* **44**(5), 595-608.

Van Loosdrecht M.C.M., Tijhuis L., Wijdieks A.M.S. and Heijnen J.J. (1995) Population distribution in aerobic biofilms on small suspended particles. *Wat. Sci. Tech.*, **31**(1), 163-171.

Vishniac W. and Santer M. (1957) The Thiobacilli. *Bacteriol. Rev*. **21**, 195-213.

Wilén B.-M. and Balmér P. (1999) The effect of dissolved oxygen concentration on the structure, size and size distribution of activated sludge flocs. *Wat. Res*. **33**(2), 391-400.

3 Selecting slow growing organisms as a means for improving aerobic granular sludge stability

Abstract

Recently, several groups have showed the occurrence of aerobic granular sludge. The excellent settling characteristics of aerobic granular sludge allow the design of very compact wastewater treatment plants. In laboratory experiments, high oxygen concentrations were needed to obtain stable granule formation. However, in order to obtain energy efficient aeration and good denitrification low oxygen concentrations would be required. From earlier research on biofilm morphology, it was learned that slow growing organisms influence the density and stability of biofilms positively. To decrease the growth rate of the organisms in the aerobic granules, easy degradable substrate (e.g. acetate) has to be converted to slowly degradable COD like microbial storage polymers (e.g. PHA). Phosphate or glycogen accumulating bacteria perform this conversion step most efficiently. In this paper it is shown that the selection of such bacteria in aerobic granules indeed led to stable granular sludge, even at low oxygen concentrations.

This chapter was published as de Kreuk, M.K. and van Loosdrecht, M.C.M. (2004). "Selection of slow growing organisms as a means for aerobic granular sludge stability". *Water Science and Technology* 49 (11-12), 9-19.

Introduction

In recent years, a significant part of research on wastewater treatment has focussed on development of compact systems. Wastewater treatment plants (WWTP) can be designed compact when biomass is maintained in the system without the use of conventional settling tanks as in flocculated activated sludge systems. Examples are developments in the field of Membrane Bio-Reactors, Biological Aerated Filters and other biofilm systems. For conventional biofilm technology the surface area of the biofilms, needed for mass transfer and therefore the conversion processes, is limited by the reactor surface the biomass can attach to. When biofilms are grown in a granular shape, a maximum surface area per volume of biomass can be obtained. Research showed that a discontinuous influent feed stimulated the growth of dense and smooth aerobic granular sludge (Beun et al. 2000; Etterer and Wilderer, 2001; Tay et al., 2001).
To be able to apply aerobic granule formation technology on a large scale, the most important conditions for the formation of granules have to be understood. So far, the importance of the following conditions at laboratory scale have been reported in literature:

- The conversion of rapidly biodegradable substrates into slow biodegradable stored substrate by applying a feast/famine regime (Villaseñor et al., 2000; Beun et al. 2001);
- Selection of fast settling granules by applying short settling times (Beun et al. 2000);
- Sufficient high shear stress within the reactor during aeration (Beun et al. 2000; Liu and Tay, 2002).

This paper will focus on the first condition and the importance of the growth rate of the micro-organisms for stable granule formation.

Experiments and a full-scale design based on aerobic granular sludge technology showed that simultaneous nitrification/denitrification is an important process to obtain sufficient N-removal. Simultaneous nitrification/denitrification in aerobic granular sludge is based on the oxidation of ammonia in the aerobic outer layer of the granule. Produced nitrate can diffuse to the interior of the granule, where it serves as electron acceptor for maintenance and growth on stored substrate. The lower the oxygen concentration (DO), the lower the oxygen penetration depth and thus the higher the volume of biomass in an anoxic surrounding (Pochana and

Keller, 1999; Beun et al. 2001). Besides the improved nitrogen removal from the influent, a lower oxygen concentration also leads to a better process economy at large-scale application. However, Mosquera-Corral et al. (In Preparation) showed experimentally that a low oxygen concentration (DO 40% of the saturation concentration) in combination with a pulse feed of easy degradable substrate, led to granule instability, filamentous growth and biomass washout.

Control of Biofilm Stability

A suitable parameter to enhance the control of granule or biofilm stability is the actual growth rate of the micro-organisms inside the granule (Van Loosdrecht, 1995). Picioreanu et al. (1998) linked the biofilm stability to diffusion of the substrate combined with the growth rate of the organisms, leading to substrate gradients inside the biofilm. If substrate gradients inside a biofilm or granule are sharp, heterogeneous or floc-like biofilms will develop. If the gradients are gradual, more regular biofilms will be produced. Picioreanu et al. (1998) expressed this in a characteristic number G (growth):

$$G = L_Y^2 \cdot \frac{\mu_m C_{xm}}{D_s C_{s0}} \qquad \text{(eq. 1)}$$

G represents parameters that highly affect biofilm and granule structures, namely: the concentration of soluble nutrients in the bulk, C_{S0}; the diffusion coefficient, D_S; the biomass maximum density in the biofilm, C_{xm}; the maximum specific microbial growth rate, μ_m; and the biofilm thickness or granule radius, L_Y. A lower G will result in smoother biofilms or better granule formation. This means that stable granule formation at a low oxygen concentration should occur when organisms grow with a low rate.

Heterotrophic organisms have decreased growth rates when they grow on the slowly biodegradable storage polymer PHB or glycogen compared to the growth on easy biodegradable substrate as acetate or glucose (Carta et al., 2001). In previous aerobic granular sludge research, this phenomenon was used to obtain stable granule formation. An aerobic pulse-feeding period was applied, in which the substrate could penetrate the whole granule and was partly used for growth with a high growth rate (40%) and partly stored as a storage polymer (PHB; 60%) (Beun et al. 2002). This period was followed by a long aerobic reaction period in which organisms grow on PHB at a lower growth rate. The period in which external substrate is available for growth is called feast phase, the period in which the organisms use their internally stored substrate is called famine phase. Lowering the oxygen concentration led to a longer feast phase (acetate present) and thus a longer period with higher growth rates. Furthermore, it led to a steep oxygen gradient inside the granule. Both cause filamentous outgrowth and granule instability.

To be able to apply low oxygen concentrations, the growth rate of the organisms has to be decreased during the total cycle. This can be done by selecting a different type of organism that converts all easy biodegradable substrates to slowly biodegradable storage polymers, instead of only 60%. Therefore, in this research, a long anaerobic feeding period was applied, followed by an aerobic reaction period. In that way, we expected the selection for slow growing organisms (Brdjanovic et al., 1998) as phosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO), which convert all acetate into glycogen or PHB during the anaerobic period.

Materials and methods

Two double walled 3-litre laboratory scale reactors were used, with an internal diameter of 6.25 cm. The sequencing batch airlift reactor (SBAR) contained a riser (90 cm high, 4 cm internal diameter, clearance 1.25 cm). The sequencing batch bubble column (SBBC) had the same set-up as the airlift, without the riser. Air was introduced via a fine bubble aerator at the bottom of the reactors (4 L min^{-1}). Dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration (100% = 9.1 mg L^{-1}). DO concentration and pH were measured continuously. In order to control the oxygen concentration properly, the gas phase was largely circulated over the reactor. Dosing extra air or nitrogen gas in the gas recycling flow controlled the oxygen concentration. Experiment 1 was performed with no oxygen control (DO 100%), experiment 2 was performed with oxygen saturation 40% or 20%. The pH was maintained at 7.0 $\pm$0.2 by dosing 1 M NaOH or 1 M HCl. Temperature was kept at 20°C. Hydraulic retention time (HRT) was 5.6 hr and substrate load was 1.6 kg COD m^{-3} day^{-1}.

Both reactors were operated in successive cycles of 3 hours: 60 minutes feeding from the bottom of the reactor (plug-flow through the settled bed), 112 minutes aeration, 3 minutes settling (to keep particles settling faster than 12 m/h in the reactor) and 5 minutes effluent discharge.

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 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 of the reactor (day 1 to 52) allylthiourea (ATU) was dosed to a final concentration of 100 mg L^{-1} reactor.
Morphology of the granules (particle diameter, aspect ratio and shape factor), density, dry weight and ash content of the granules; TOC, biomass concentration, NH_4^+, NO_2^- and NO_3^- concentration in the effluent and the PHB content of the granules were measured as described in Beun et al. (2001). The SVI_8 was determined by reading the height of the settled bed after 8 minutes settling and calculated from the settled bed volume and the dry weight in the reactor.
FISH was performed on the crushed granules in order to determine the PAO (mixed probe PAO462, PAO651 and PAO 846) and GAO (mixed probe of GAOQ431 and GAOQ989) in the granules and their approximated ratio of existence (Crocetti et al., 2000; 2002).

Results

The reactor was started up without oxygen control, which means that the oxygen concentration (DO) during aeration was close to saturation. The feeding period was anaerobic. An overview of the particle characteristics during different stages of the experiment is given in table 1. Pictures of the particles grown under different circumstances are presented in figure 1.

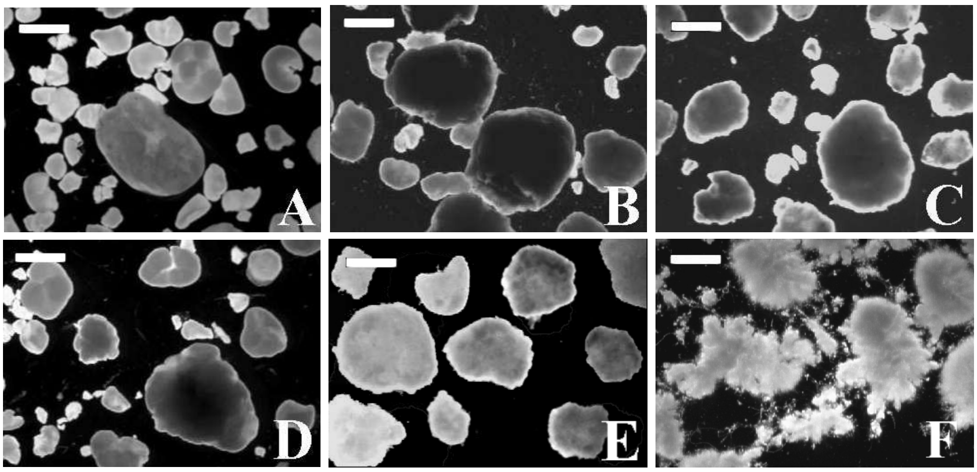


Figure 1 Granules grown under different circumstances, with long anaerobic feeding periods; Reactor types: a,b,c,e,f,: Airlift, d: Bubble column. Feeding: a,b,c,d : Anaerobic, e,f: Aerobic. Oxygen saturation: a) 100%; b) 20%; c) 20% without feeding phosphate; d) 40%. e) 100%; f) 40% (Bar indicates 2 mm)

Start-up period without oxygen control

After inoculation with activated sludge, it generally took 4 days before the first granules appeared while after 9 days all flocs had disappeared. After three weeks, the granules had their average full-grown size of 1.1 mm. The reactor was operated for 233 days without oxygen control, of which the first 52 days were used to stabilise the conversion processes, without nitrification (inhibition by dosage of ATU). At day 52 the dosage of ATU was stopped and from this day to day 115 nitrifying organisms could develop in the granules. Day 115 to 233 were used to operate the reactor under stable conditions in steady state. The size of the granules fluctuated in time between 1.1 and 1.6 mm (average 1.3 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=line and 1=circle). The density of the granules increased from 56 to 97 gTSS L^{-1} biomass. The sludge volume index (SVI_8), estimated from the settled bed volume after the settling and effluent withdrawal period (8 minutes of settling), stabilised at 24 ml g^{-1}. This is very low compared to the values of activated sludge (100-150 ml g^{-1}), indicating the good settleability of this granular sludge. The average solid retention time (SRT), determined by the biomass washout in the effluent was 67 days.

Table 1 Characteristics of granules grown in different reactor types, dissolved oxygen concentration and feeding patterns

Particle characteristics	**Long feeding period**[2]					**Pulse feed**	
	SBAR				SBBC	SBAR[1]	
	DO 100%	DO 40%	DO 20%	DO 20%	DO 40%	DO 100%	DO 40%
Characters in Fig. 1	A	-	B	C	D	E	F
Dominant organism[3]	PAO	PAO	PAO	GAO	PAO	Heterotrophs	
Stability granules	stable	stable	stable	stable	stable	stable	not stable
Local high shear	yes	yes	yes	yes	no	yes	yes
Average diameter (mm)	1.3	1.1	1.3	1.2	1.1	1.6	5.0
Particle density (g VSS L^{-1} biomass)	89	87	78	108	90	53	13
Dry weight in reactor (g VSS L^{-1} reactor)	8.5	12	16.5	15	13	5.1	0.9
SVI_8 (ml g^{-1})	24	20	14	17	19	50	200
Average SRT (days)	40	67	70	71	63	8	<5

1) Mosquera et al. (in preparation); 2) this paper; 3) Determined by FISH analysis

Steady state period with oxygen control

During the second stage of this experiment the oxygen concentration in the reactor was controlled at 40% of the saturation concentration. This change caused a break-up of granules. Contrary to previous results under fully aerobic feast/famine regime, the granules restored within two weeks and the characteristics of the granules were similar to the ones grown at oxygen saturation of 100%. There were no filaments observed at the surface and the settleability maintained very good (SVI_8 = 20 ml g^{-1}).

To be able to reduce aeration costs in large-scale operations and to improve simultaneous nitrification / denitrification the oxygen saturation was further reduced to 20% (stage 3). This also did not have any influence on the formed granules (d = 1.3 mm; shape factor = 0.75; aspect ratio = 0.69). It even did have a slight positive influence on the settleability (SVI_8=14 ml g^{-1}). Again, the granules stayed stable and there was no growth of filamentous organisms at the surface of the granules. Simultaneous nitrification / denitrification improved and almost full nitrogen and phosphate removal was obtained.

Because of the alternating anaerobic feeding (presence of acetate) and aerobic reaction periods and the availability of phosphate in the influent, selection for phosphate accumulating organisms (PAO) took place. All acetate was converted to internal storage polymers (PHB) during the anaerobic feeding period and phosphate was released to the liquid. During the aerobic period, growth took place on the internally stored PHB while phosphate in the liquid was converted into cell-internal poly-phosphates. The ammonia was nitrified in this aerobic period and the produced nitrate was used as electron acceptor in the inner anoxic zones of the particle. In this way, 100% COD removal, 99% P removal and 90% total-N removal (at DO 20%) was obtained.

Selection for Glycogen Accumulating Organisms

When dosage of phosphate was stopped, PAO disappeared and were replaced by glycogen accumulating organisms (GAO), which are able to store acetate anaerobically too. The energy to take up acetate actively is produced by hydrolysing glycogen that is formed during the aerobic period. During the aerobic period PHB is used for formation of glycogen, growth and maintenance. The PAO population disappeared 50 days after P-dosage was stopped. Particle characteristics did not change much when GAO became the dominant organism in

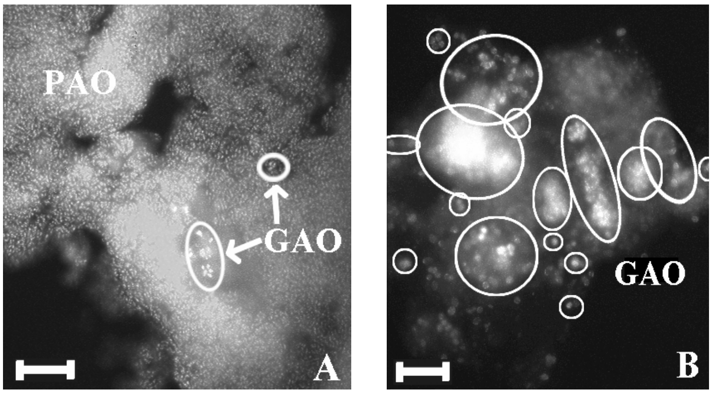


Figure 2 Crushed Granules stained with FISH probes: grown with phosphate, mainly PAO and some GAO present; grown without phosphate, no PAO and mainly GAO present. Bar indicates 20 μm.

the granule (table 1). FISH analysis showed that the biomass with phosphate consisted mainly of PAO, while the biomass without phosphate dosage mainly consisted of GAO (figure 2).

Bubble Column versus Airlift Reactor

The use of a bubble column instead of an airlift reactor would decrease the investment costs of a large-scale installation. In a bubble column (SBBC) combined with a pulse feed under aerobic conditions granular sludge was unstable. (Beun et al. 2000; Liu and Tay, 2002). The stability of these granules in an airlift reactor was likely due to the fact that the airlift reactor has local high shear zones (Gjaltema et al., 1997). If the feed was given under anaerobic conditions in a bubble column stable granular sludge was formed. However, the start-up period was significantly longer. The first particles appeared after 28 days, and the system came into steady state only after 60 days. Lowering the maximal growth rate due to selection of PAO or GAO, thereby converting all acetate in slowly biodegradable storage polymers, seemed to make the high local shear less critical. The same good granular sludge as in an airlift reactor could be obtained (Table 1). However the fact that it took significantly longer to obtain granular sludge is an indication that the higher local shear rates in airlift reactors are preferred. Obviously this is one of the critical aspects in further scale-up of the aerobic granular sludge technology.

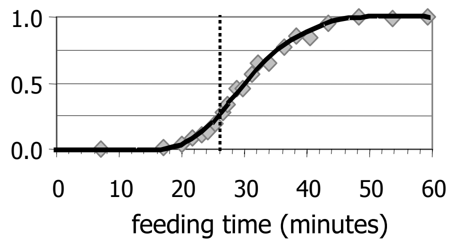


Figure 3 Step-response curve during the feeding phase in a granular sludge reactor. Response measured 10 cm above the settled bed (0 = effluent concentration and 1 = influent concentration)

Plug-flow feeding regime

For a good penetration of acetate through the total granule, a high substrate concentration during the feeding period is advantageous. This can be established by applying a pulse feed, while mixing the reactor, but mechanically mixing of the granules could damage them. Therefore, the reactor was fed in up-flow conditions. The low influent flow (superficial velocity was 0.5 m h^{-1}) results in a plug-flow behaviour of the liquid through the settled bed. Especially granules in the lower part of the settled bed faced a high acetate concentration in this way. The flow pattern was monitored by a Dextran Blue coloured influent flow. The step response curve is shown in figure 3. When there would have been an ideal plug-flow, without preferential channels in the granule bed, the tracer would have been monitored after 27 minutes (dotted line in figure 3) instead of 22 minutes. It can be concluded that as expected little mixing occurs during liquid flow through the sludge bed.

Discussion

Previous research indicated a dependency of the structure of biofilms on shear forces, actual growth rate of micro-organisms and diffusion of substrate into the biofilm (Van Loosdrecht, 1995; Picioreanu et al., 1998). These results were used in this study to obtain stable aerobic granular sludge at low oxygen concentrations.

It has been shown previously that strong concentration gradients, as low oxygen or low substrate concentration (as in continuously fed systems), cannot lead to formation of stable aerobic granular sludge. Previous research underlined that systems with a low maximum growth rate will easier form granular sludge than those with a high maximum growth rate. As example, methanogenic granules are

easier to obtain than acidifying granules. A low maximum growth rate also makes the influence of substrate gradients less evident, since also the outward growth velocities of the surface heterogeneities are lower (Picioreanu et al., 1998).
A smooth surface of the granules is obtained when the outgrowth velocity of the biofilm is properly balanced by detachment forces (shear). In particle based biofilm reactors, these forces are mainly obtained by erosion due to non-colonised carrier particles in the reactor that collide with the colonised particles (Gjaltema et al., 1997). For systems fed with easy degradable substrate as acetate this is a critical design factor (Kwok et al., 1998). In granular sludge reactors, carrier material is absent and therefore erosion is only due to granule-granule collisions, which are less detrimental then collision between biofilm and bare carrier (sand) particles (Gjaltema et al. 1997). This means that extra conditions had to be created that would reduce the growth rate of the organisms in the reactor.

The application of a feast/famine regime in a SBR system lowers the maximum growth rate during the famine phase. The organisms use slowly biodegradable internally stored substrate in this period. This feeding regime allowed formation of stable granular sludge but it was not sufficient to sustain stable granular sludge at low oxygen concentration. One could make the analogy with bulking sludge. Conditions that give rise to bulking sludge are more or less opposite to conditions giving rise to granular sludge.
In order to improve sludge granule formation at low oxygen concentrations easy degradable substrate should first be converted into a substrate leading to low growth rates. In this study it was shown that the use of phosphate or glycogen accumulating organisms indeed provided the conditions for obtaining stable granular sludge. An extra advantage of selection of phosphate accumulating organisms is that besides COD and nitrogen, also phosphate will be removed from the wastewater.
Because of the lower growth rate, the outgrowth of the granule surface is lower and thus, less compensating detachment forces are needed to maintain a smooth surface. This made the high local shear of the airlift reactor redundant and it became possible to use a bubble column. However it should be mentioned that in the scale-up of aerobic sludge technology this aspects needs further attention.

Consequences for the design of aerobic granular sludge systems

The possibility of using a bubble column at a large-scale application instead of an airlift reactor has economical advantages, since the construction of a bubble column is easier and less expensive. Also the constructions for effluent discharge can be easier implemented in a bubble column. In an airlift reactor, the presence of a riser will need extra attention to allow equal discharge from riser and downcomer compartment. If this is not done properly the granular sludge could easily end up in the reactor discharge.

Feeding in an anaerobic period during an extensive period could simplify the operation of a granular sludge SBR. Feeding N reactors in $1/N^{th}$ of the cycle time can be translated in a continuous influent flow to the total installation. As an example, present design is based on 3 reactors with a filling time of 1/3 of the total cycle time (De Bruin et al., This Volume). Plug-flow conditions through the settled granular sludge bed allow a simultaneous feed-discharge period. Approximately 90% of the void volume of the settled bed can be replaced simultaneously with addition of the influent (Arnz et al., 2000). This could make an effluent withdrawal phase superfluous. In such an operation mode the influent and the effluent flow could become continuous, which greatly eases the design of pre- and/or post treatment facilities. Moreover, a greater part of the reactor can be packed by granular sludge, which improves the volumetric conversion rate.

Conclusions

The theoretical framework resulting from previous research, combined with an understanding of the morphology of aerobic granules, let to awareness of the necessity to select for slow bacterial growth within these granules, in order to obtain stable granule formation. In this respect, selection of phosphate accumulating organisms in wastewater with phosphate and/or glycogen accumulating organisms in influent without phosphate resulted in smooth, dense and stable granules at both high and low oxygen concentrations. Besides the advantage of more stable granule formation, the proposed operating procedure with a prolonged anaerobic feeding period has benefits for the process design. Local shear forces become less evident, so a bubble column could be used and the long anaerobic feeding period makes a simplified feeding and withdrawal system possible.

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 was performed in close co-operation with DHV, Amersfoort, The Netherlands.

References

Arnz P., Esterl M.S., Nerger C., Delgado A. and Wilderer P.A. (2000). Simultaneous loading and draining as a means to enhance efficacy of sequencing biofilm batch reactors. *Wat. Res.* **34**(5), 1763 – 1766.

Beun J.J., van Loosdrecht M.C.M. and Heijnen J.J. (2000). Aerobic Granule formation. *Wat. Sci. Tech.* **41**(4-5), 41-48.

Beun J.J., Van Loosdrecht M.C.M. and Heijnen J.J. (2001). N-removal in a granular sludge sequencing batch airlift reactor. *Biotechnol. Bioeng.* **75**(1), 82-92.

Beun J.J., Dircks K., Van Loosdrecht M.C.M. and Heijnen J.J. (2002). Poly-b-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. *Wat. Res.* **36**(5), 1167-1180.

Brdjanovic D., van Loosdrecht M.C.M., Hooijmans C.M., Alaerts G.J. and Heijnen J.J. (1998). Minimal aerobic sludge retention time in biological phosphorus removal systems. *Biotechnol. Bioeng.* **60**(3), 326-332.

Carta F., Beun J.J., Van Loosdrecht M.C.M., Heijnen J.J. (2001) Simultaneous storage and degradation of PHB and glycogen in activated sludge cultures. *Wat. Res.* 35:2693-2701.

Crocetti G.R., Hugenholtz P., Bond P.L., Schuler A., Keller J., Jenkins D. and Blackall L.L. (2000). Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Appl. Environ. Microbiol.* **66**(3), 1175-1182.

Crocetti G.R., Banfielda J.F., Keller J., Bond P.L. and Blackall L.L. (2002). Glycogen-accumulating organisms in laboratory-scale and full-scale wastewater treatment processes. *Microbiol.* **148**, 3353-3364.

De Bruin L.M.M., De Kreuk M.K., van der Roest H.F.R., Van Loosdrecht M.C.M. and Uijterlinde C. (2004). Aerobic granular sludge technology, alternative for activated sludge technology? *Wat. Sci. Tech.* **49**(11-12), 1-7.

Etterer T. and Wilderer P.A. (2001). Generation and properties of aerobic granular sludge. *Wat. Sci. Tech.* **43**(3), 19-26.

Gjaltema A., Vinke J.L., Van Loosdrecht M.C.M. and Heijnen J.J. (1997). Abrasion of suspended biofilm pellets in airlift reactors: Importance of shape, structure and particle concentrations. *Biotechnol. Bioeng.* **53**, 88 - 99.

Kwok W.K., Picioreanu C., Ong S.L., van Loosdrecht M.C.M., Ng W.J. and Heijnen J.J. (1998). Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. *Biotechnol. Bioeng.* **58**(4), 400-407.

Liu Y. and Tay J.H. (2002). The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge - Review. *Wat. Res.* **36**(7), 1653-1665.

Mosquera-Corral A., De Kreuk M.K., Heijnen J.J. and Van Loosdrecht M.C.M. (In Preparation). Effects of dissolved oxygen on N-removal in an aerobic granular sludge reactor.

Picioreanu C., van Loosdrecht M.C.M. and Heijnen J.J. (1998). Mathematical Modeling of Biofilm Structure with a Hybrid Differential-Discrete Cellular Automaton Approach. *Biotechnol. Bioeng.* **58**(1), 101-116.

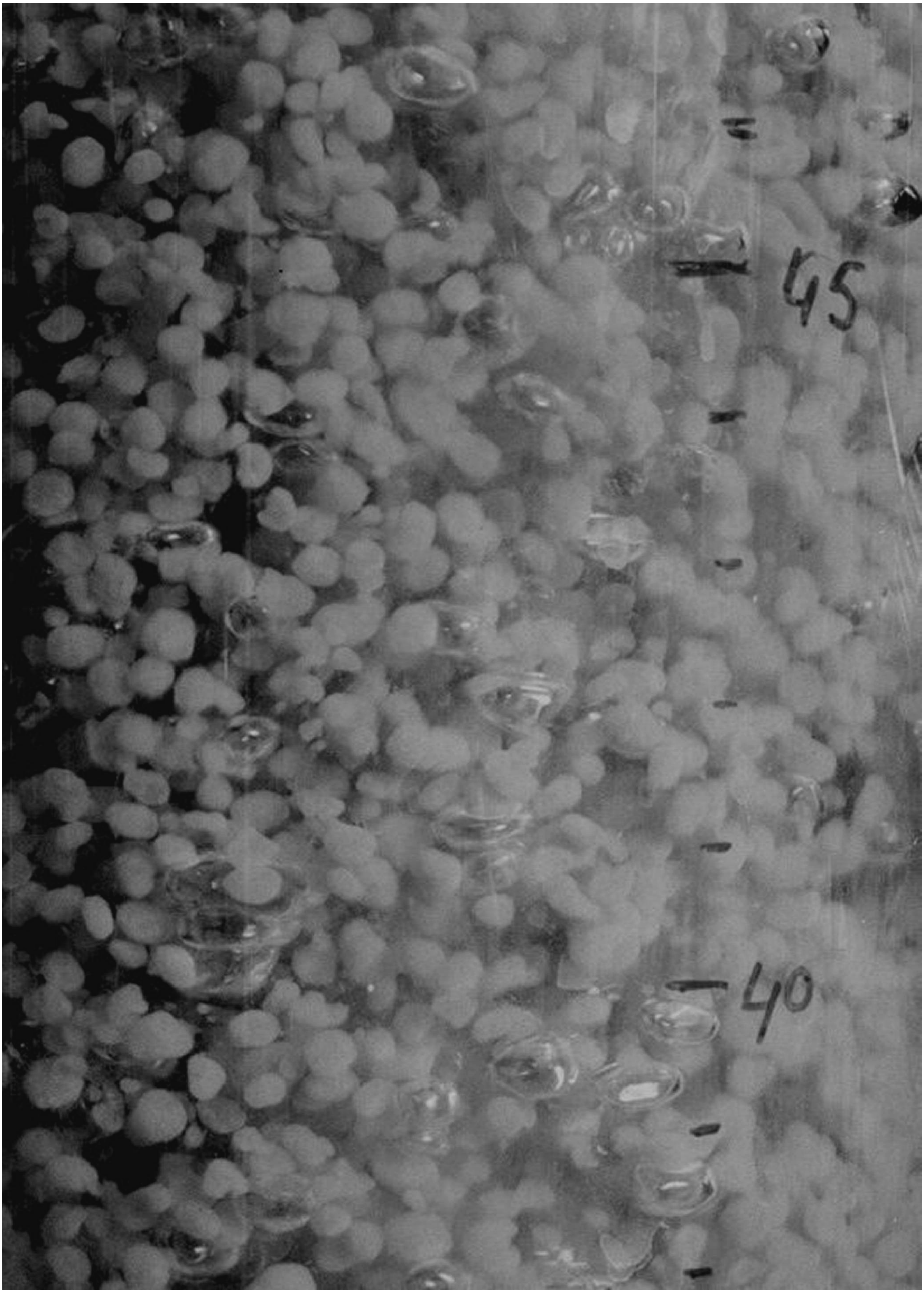
Pochana K. and Keller J. (1999). Study of factors affecting simultaneous nitrification and denitrification (SND). *Wat. Sci. Tech.* **39**(6), 61-68.

Tay J.H., Liu Q.S. and Liu Y. (2001). Microscopic observation of aerobic granule formation in sequential aerobic sludge blanket reactor. *J. Appl. Microbiol.* **91**(1), 168-175.

Van Loosdrecht M.C.M. (1995). Biofilm structures. *Wat. Sci. Tech.* **32**(8), 35-43.

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

Zeng R.J., van Loosdrecht M.C.M, Yuan Z. and Keller J. (2003). Metabolic model for glycogen-accumulating organisms in anaerobic/aerobic activated sludge systems. *Biotechnol. Bioeng.* **81**(1), 92-105.


45
40

4 Simultaneous COD, nitrogen and phosphate removal by aerobic granular sludge

Abstract

Aerobic granular sludge technology offers a possibility to design compact wastewater treatment plants based on simultaneous 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 removal and 94% total nitrogen 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.

This chapter was published as de Kreuk, M.K., Heijnen J.J. and van Loosdrecht, M.C.M. (2005). "Simultaneous COD, Nitrogen and Phosphate Removal by Aerobic Granular Sludge". Biotechnology and Bioengineering *90* (6), 761-769.

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 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 (figure 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., 2005). 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., 2005). 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.

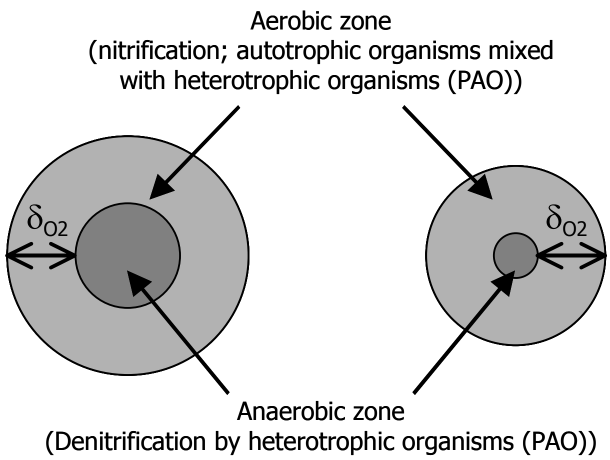


Figure 1. Decreased anaerobic or anoxic zone with a decreased granule diameter at constant DO

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 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-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 SBR's. Special attention is given to the effect of the required dissolved oxygen concentration for optimal N-removal.

Materials and methods

Reactor system

Two double walled 3-litre sequencing batch airlift reactors (SBAR) 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^{-1}). Dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration (100% = 9.1 mg L^{-1}). Oxygen concentration and pH were measured on-line. In order to control the oxygen concentration properly, the gas phase was circulated over 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 $\pm$0.2 by dosing 1 M NaOH or 1 M HCl. Temperature was kept at 20°C. Hydraulic retention time (HRT) was 5.6 hr and substrate load was 1.6 kg COD m^{-3} day^{-1}.

The reactor was operated in successive cycles of 3 hours: 60 minutes feeding from the bottom of the reactor (plug-flow through the settled bed), 112 minutes aeration, 3 minutes settling (to keep only particles settling faster than 12 m h^{-1} in the reactor) and 5 minutes effluent discharge.

The composition of the influent media were (A) NaAc 63 mM, MgSO4.7H2O 3.6 mM; KCl 4,7 mM and (B) NH4Cl 35.4 mM, K2HPO4 4.2 mM, KH2PO4 2.1 mM and 10 mL L^{-1} 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^{-1}. 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; 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). 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. N2O in the off-gas was measured, by analysing 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). Phosphate in the granules was measured according to the method described by Uhlmann et al (1990). The SVI8 was determined by reading the height of the settled bed in the reactor after 8 minutes 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., (2005).
FISH was performed on thin layers of the granules in order to determine the location of the 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 (Mobarry et al. 1996, Daims et al., 1999; Crocetti et al., 2000).

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. This paper focuses 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=line and

1=circle). The density of the granules increased in time from 56 to 97 g TSS L^{-1} biomass. The dry weight in the reactor was 8.5 gVSS L^{-1}, ash content 41%. The average suspended solids concentration in the effluent was 0.049 gVSS L^{-1}. The sludge volume index (SVI8) stabilised at 24 mL g^{-1}, which is very low compared to the values of activated sludge (100-150 mL g^{-1}). To determine the sludge volume index of granular sludge, 8 minutes of settling time was chosen instead of 30 minutes, as is often used in case of activated sludge. Schwarzenbeck (2004) showed that granular sludge has a similar SVI after 60 minutes and after 5 minutes of settling. This different settling behaviour of granules and activated sludge, could be used to distinguish more clearly granular growth from flocculated growth.

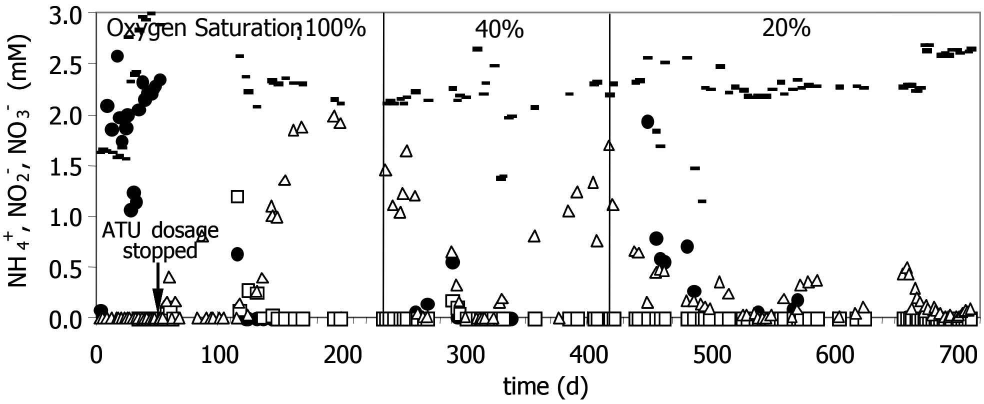


Figure 2. Evolution of nitrogen compounds concentrations: NH_4^+-N (-)(mM) in the influent and NH_4^+-N (●), NO_3^--N (△) and NO_2^--N (□) (mM) in the effluent of the SBAR.

After 233 days, the oxygen saturation level was decreased to 40%. This induced breaking of granules during the first two 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 gVSS $L_{reactor}^{-1}$ (granule density: 89 and 87 gTSS $L_{biomass}^{-1}$). The ash content of the granules decreased to 34%, and the average suspended solid concentration in the effluent remained similar (0.048 gVSS L^{-1}). 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^{-1}), and even had a small positive effect on the sludge volume index (SVI8=14 mL g^{-1}) and the dry weight in the reactor (16.5 gVSS L^{-1}, ash content 30%, granule density

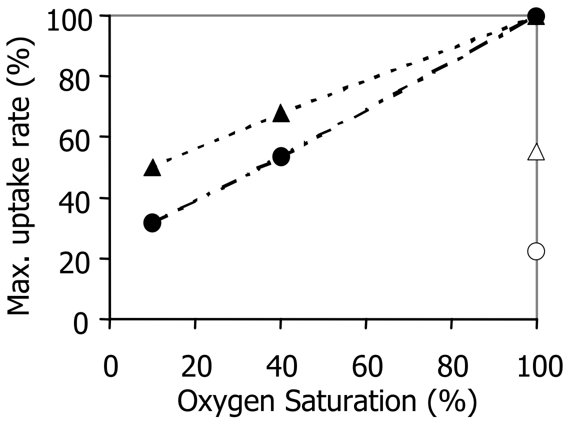


Figure 3. Uptake rate of phosphate (with nitrate present (▲) and without nitrate (△)) and uptake rate of ammonium (with nitrification (●) and without nitrification (○)) at different oxygen concentrations during one cycle; indexed to the value at 100% oxygen saturation.

78 gTSS $L_{biomass}^{-1}$). Again, the granules stayed stable and there was no growth of filamentous organisms or structures at the surface of the granules. Fluorescent In Situ Hybridisation (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 (figure 5). The average granule size fluctuated between 0.4 and 1.8 mm during the long-term (300 days) operation at 20 % oxygen saturation (Figure 6).

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^{-1}. 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^{-1}, while the average effluent concentration was only 0.4 mg-P L^{-1}. The ratio of phosphate released and acetate taken up was 0.44 P-mol C-mol^{-1}, comparable to the ratio of 0.5 at pH 7 for a highly enriched PAO culture (Smolders et al., 1994; Mino et al., 1998). During the first 52 days after start-up, the ammonium oxidising 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. 39 Days after the dosage of ATU was stopped full ammonium oxidation was reached. It took however 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).

In order 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., 2005). This did not have any influence on the phosphate removal efficiency, which remained 97% on average (effluent concentrations < 0.8 mg-P L^{-1}), removing 78 g P m^{-3} day^{-1}. 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^{-1}).

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^{-1}. Also all ammonium and

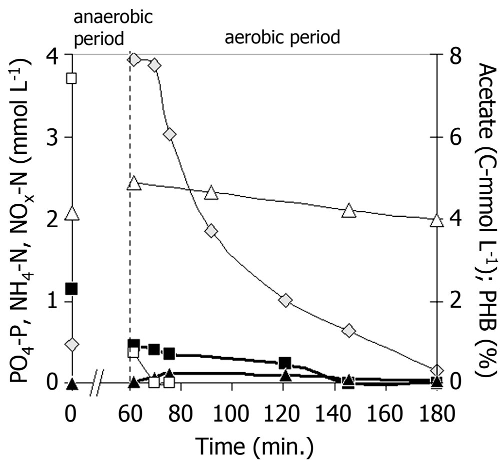


Figure 4 Typical concentration patterns of phosphate (◆), acetate (◻), PHB (△), ammonium (■) and nitrate plus nitrite (NO_x, ▲) during a cycle at 20% oxygen saturation.

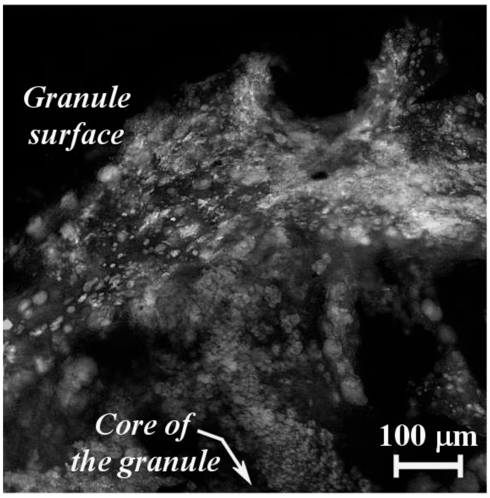


Figure 5 Impression of the layered structure of the granule (20% oxygen saturation in bulk liquid) by applying FISH techniques (--- = 100 µm; green = ammonium oxidising bacteria; blue = eubacteria; red = PAO). See cover of this thesis for colour picture.

nitrite were oxidised, which caused an average nitrogen removal of 98% during 35 days. 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 (figure 7). 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^{-1}.

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 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 $gVSS^{-1}$ (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. After 30 days of operation at 20% oxygen saturation, decreased nitrification

Table 1 Characteristics of granular sludge grown in an airlift reactor: effect of dissolved oxygen concentration and feeding patterns

	Anaerobic feed			**Aerobic feed**[1]	
	DO 100%	DO 40%	DO 20%	DO 100%	DO 40%
Average diameter (mm) [2, 3]	1.3	1.1	1.3	1.6	5.0
Particle density (g TSS L^{-1} biomass) [2, 3]	89	87	78	53	13
Dry weight in reactor (g VSS L^{-1} reactor) [2, 3]	8.5	12	16.5	5.1	0.9
SVI_8 (mL g^{-1}) [2, 3]	24	20	14	50	200
Average SRT (days) [3]	40	67	71	8	<5
mg NH_4^+-N L^{-1} (effluent) [3]	<0.01	0.03	0.08	0.13	1
mg NO_3^--N L^{-1} (effluent) [3]	27	15	1.7	22	21
mg NO_2^--N L^{-1} (effluent) [3]	0.1	<0.01	<0.01	3.9	1.5
N-removal efficiency	34%	56%	94%	16%	45%

1) Mosquera et al. (2005); 2) De Kreuk and Van Loosdrecht (2004); 3) This study

efficiency was observed, indicated by a sudden increase of the ammonium concentration in the effluent. 78 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^{-1}). Typical patterns of phosphate, acetate, PHB and nitrogen concentrations during a cycle are given in figure 4.
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 analysed 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 and 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

Table 2 Effect of dissolved oxygen concentrations on N removal during a short DO change in a SBAR with long anaerobic feeding time (steady state at 40% DO)

Oxygen saturation	10%	40%	100%
VSS L^{-1} in reactor	12.9	12.6	13.9
% NO_x-N effluent	0.2% [1)]	17.1% [2)]	28.5% [2)]
% NH_4^+-N effluent	24.5%	0%	0%
N-removal	75.3%	82.9%	71.5%
P-uptake rate (mmol $gVSS^{-1}$ h^{-1})	0.36	0.51	0.62

[1] in effluent as NO_2^--N, [2] in effluent as NO_3^--N

constant in all cases in order 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 2). The phosphate uptake rate under anoxic conditions is lower than under aerobic conditions (Murnleiter et al., 1997). When oxygen penetration depth decreased at lower oxygen concentrations, the anoxic volume increased and the aerobic volume decreased, leading to lower overall phosphate uptake rates, as expected. In figure 3, phosphate removal at 100% oxygen saturation with nitrification/ denitrification activity is indexed at 100%. The other rates are presented as a fraction of this 100%. In 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), also indicates that roughly 45% of P-uptake occurs anoxically.
The trend of the total nitrogen removal during cycle measurements at different oxygen concentrations (Table 1), follows the results of the modelling 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., 2005). 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 3, 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 the extrapolated N-removal when O_2 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., 2005), stable granule formation at low oxygen levels was possible. A 60 minute 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 reactors with an exchange ratio of 50% was around 5 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, also the problems of pulse feeding at full scale installations, which are among others oversized pump capacity and large buffer tanks, are solved. Feeding during 33% of the cycle would allow that a system of 3 reactors can be operated with a continuous feed.

With high influent phosphate concentrations (19.6 mg PO_4^{3-}-P L^{-1}, COD/P=20.2) and the low growth yield of the granules (measured as 0.25 gVSS $gCOD^{-1}$), the calculated P-content of the granules would be 0.20 g P g SS^{-1}. In literature, reported values are lower, specially for biofilm systems, ranging from 0.02 to 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 colour 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 (app. 50 mg Ca^{2+} L^{-1}), 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^{-1}, precipitation can occur (Maurer et al., 1999). The phosphate concentration measured in the bulk liquid after the anaerobic period exceeds 100 mg L^{-1} and since acetate is dosed as a salt and taken op 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% (gPO_4^{3-}-P $gVSS^{-1}$), 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 PAO's 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, Pochana and Keller, 1999; Satoh et al., 2003; Mosquera-Corral et al., 2005), by which the ammonium is oxidised 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 can not be used as electron donor during denitrification in the inner part of the biofilm. Furthermore, the system is more sensitive to oxygen concentrations, since 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

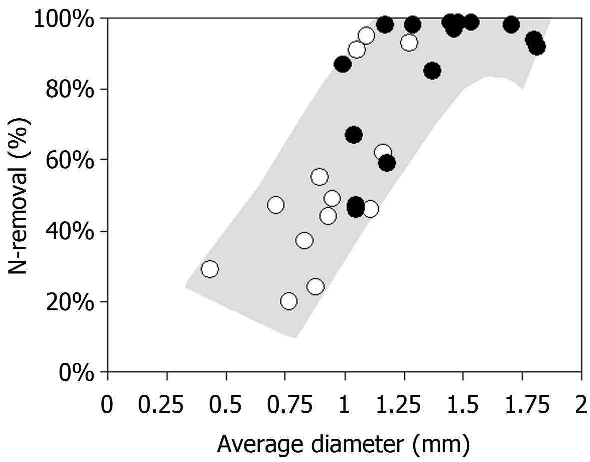


Figure 6 Measured N-removal efficiency at different granule diameters (20% oxygen saturation in the bulk liquid) in the SBAR (●) and in the SBBC (❍)

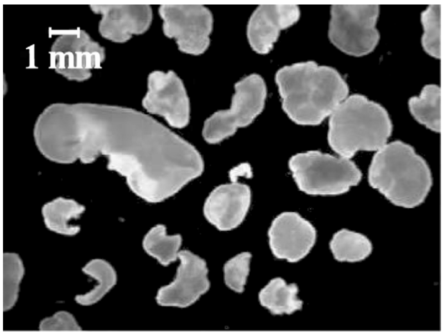


Figure 7. Broken granules, leading to flat or kidney shaped structures

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 $gVSS^{-1}$ h^{-1} when phosphate uptake occurs and 3.3 mg O_2 $gVSS^{-1}$ h^{-1} when phosphate is taken up completely. Together with the diffusion coefficient, biomass density (78 g l^{-1}) 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 (Figure 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 2). 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 behaviour 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., 2005).
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 (Figure 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 (figure 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 ratio's. 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 destabilise by endogeneous respiration and granules fall apart in small fractions that can grow out to form large granules again.
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 (figure 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 (figure 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 suggested 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 in order 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.

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 was performed in close co-operation with DHV in Amersfoort, The Netherlands.

References

Arvin E. 1983. Observations supporting phosphate removal by biologically mediated chemical precipitation - a review. *Wat. Sci. Technol.* **15**(3-4):43-63.

Beun JJ, Hendriks A, van Loosdrecht MCM, Morgenroth M, Wilderer PA, Heijnen JJ. 1999. Aerobic granulation in a sequencing batch reactor. *Wat. Res.* **33**(10):2283-2290.

Beun JJ, van Loosdrecht MCM, Heijnen JJ. 2000. Aerobic Granulation. *Wat. Sci. Technol.* **41**(4-5):41-48.

Beun JJ, Van Loosdrecht MCM, Heijnen JJ. 2001. N-removal in a granular sludge sequencing batch airlift reactor. *Biotechnol. Bioeng.* **75**(1):82-92.

Beun JJ, van Loosdrecht MCM, Heijnen JJ. 2002. Aerobic granulation in a sequencing batch airlift reactor. *Wat. Res.* **36**(3):702-712.

Brdjanovic D, van Loosdrecht MCM, Hooijmans CM, Alaerts GJ, Heijnen JJ. 1998. Minimal aerobic sludge retention time in biological phosphorus removal systems. *Biotechnol. Bioeng.* **60**(3):326-332.

Crocetti GR, Hugenholtz P, Bond PL, Schuler A, Keller J, Jenkins D, Blackall LL. 2000. Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Appl. Env. Microbiol.* **66**(3):1175-1182.

Daims H, Bruhl A, Amann R, Schleifer KH, Wagner M. 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: Development and evaluation of a more comprehensive probe set. *Sys. Appl. Microbiol.* **22**(3):434-444.

Dangcong P, Bernet N, Delgenes J-P, Moletta R. 1999. Aerobic granular sludge - a case report. *Wat. Res.* **33**(3):890-893.

De Bruin LMM, De Kreuk MK, van der Roest HFR, Van Loosdrecht MCM, Uijterlinde C. 2004. Aerobic granular sludge technology, alternative for activated sludge technology? *Wat. Sci. Technol.* **49** (11-12):1-9.

De Kreuk MK, Van Loosdrecht MCM. 2004. Selection of Slow Growing Organisms as a Means for Improving Aerobic Granular Sludge Stability. *Wat. Sci. Technol.* **49**(11-12):9-19.

Etterer T, Wilderer PA. 2001. Generation and properties of aerobic granular sludge. *Wat. Sci. Technol.* **43**(3):19-26.

Falkentoft CM. 2000. Simultaneous removal of nitrate and phosphorus in a biofilm reactor; the aspect of diffusion [Ph. D. Thesis]. Lyngby: Technical University of Denmark. 193 p.

Garrido JM, Van Benthum WAJ, van Loosdrecht MCM, Heijnen JJ. 1997. Influence of dissolved oxygen concentration on nitrite accumulation in a Biofilm airlift suspension reactor. *Biotechnol. Bioeng.* **53**(2):168 - 178.

Lin YM, Liu Y, Tay JH. 2003. Development and characteristics of phosphorus-accumulating microbial granules in sequencing batch reactors. *Appl. Microbiol. Biotechnol.* **62**(4):430-435.

Martins AMP, Pagilla K, Heijnen JJ, van Loosdrecht MCM. 2004. Filamentous bulking sludge - a critical review. *Wat. Res.* **38**(4):793-817.

Maurer M, Abramovich D, Siegrist H, Gujer W. 1999. Kinetics of biologically induced phosphorus precipitation in waste-water treatment. *Wat. Res.* **33**(2):484-493.

Mino T, van Loosdrecht MCM, Heijnen JJ. 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. *Wat. Res.* **32**(11):3193-3207.

Mobarry BK, Wagner M, Urbain V, Rittmann BE, Stahl DA. 1996. Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. *Appl. Env. Microbiol.* **62**(6):2156-2162.

Morgenroth E, Wilderer PA. 1999. Controlled biomass removal - the key parameter to achieve enhanced biological phosphorus removal in biofilm systems. *Wat. Sci. Technol.* **39**(7):33-40.

Mosquera-Corral A, De Kreuk MK, Heijnen JJ, Van Loosdrecht MCM. 2005. Effects of dissolved oxygen on N-removal in an aerobic granular sludge reactor. Wat. Res. 39(12); 2676-2686.

Murnleitner E, Kuba T, van Loosdrecht MCM, Heijnen JJ. 1997. An integrated metabolic model for the Aerobic and denitrifying Biological phosphorus removal. *Biotechnol. Bioeng.* **54**(5):434 - 450.

Nicolella C, van Loosdrecht MCM, Heijnen JJ. 1999. Identification of mass transfer parameters in three-phase biofilm reactors. *Chem. Eng. Sci.* **54**:3143-3152.

Okabe S, Hirata K, Watanabe Y. 1995. Dynamic changes in spatial microbial distribution in mixed-population biofilms: experimental results and model simulation. Wat. Sci. Technol. 32(8):67-74.

Picioreanu C, Van Loosdrecht MCM, Heijnen JJ. 1998. Mathematical modelling of biofilm structure with a hybrid differential-discrete cellular automaton approach. *Biotechnol. Bioeng.* **58**(1):101-116.

Pochana K, Keller J. 1999. Study of factors affecting simultaneous nitrification and denitrification (SND). *Wat. Sci. Technol.* **39**(6):61-68.

Satoh H, Nakamura Y, Ono H, Okabe S. 2003. Effect of oxygen concentration on nitrification and denitrification in single activated sludge flocs. *Biotechnol. Bioeng.* **83**(5):604-607.

Schwarzenbeck N, Erley R, Wilderer PA. 2004. Aerobic granular sludge in an SBR-system treating wastewater rich in particulate matter. *Wat. Sci. Technol.* **49**(11-12):41-46.

Smolders GJF, Bulstra DJ, Jacobs R, Vanloosdrecht MCM, Heijnen JJ. 1995. A Metabolic Model of the Biological Phosphorus Removal Process .2. Validation During Start-up Conditions. *Biotechnol. Bioeng.* **48**(3):234-245.

Smolders GJF, van der Meij J, Van Loosdrecht MCM, Heijnen JJ. 1994. Stoichiometry and pH influence of the anaerobic metabolism. *Biotechnol. Bioeng.* **43**:461-470.

Tay JH, Liu QS, Liu Y. 2002. Aerobic granulation in sequential sludge blanket reactor. *Wat. Sci. Technol.* **46**(4-5):13-18.

Third KA, Burnett N, Cord-Ruwisch R. 2003. Simultaneous nitrification and denitrification using stored substrate (PHB) as the electron donor in an SBR. *Biotechnol. Bioeng*. **83**(6):706-720.

Uhlmann D, Roske I, Hupfer M, Ohms G. 1990. A simple method to distinguish between polyphosphate and other phosphate fractions of activated sludge. *Wat. Res.* **24**(11):1355-1360.

Van Loosdrecht MCM, Eikelboom DH, Gjaltema A, Mulder A, Tijhuis L, Heijnen JJ. 1995a. Biofilm structures. *Wat. Sci. Technol.* **32**(8):35-43.

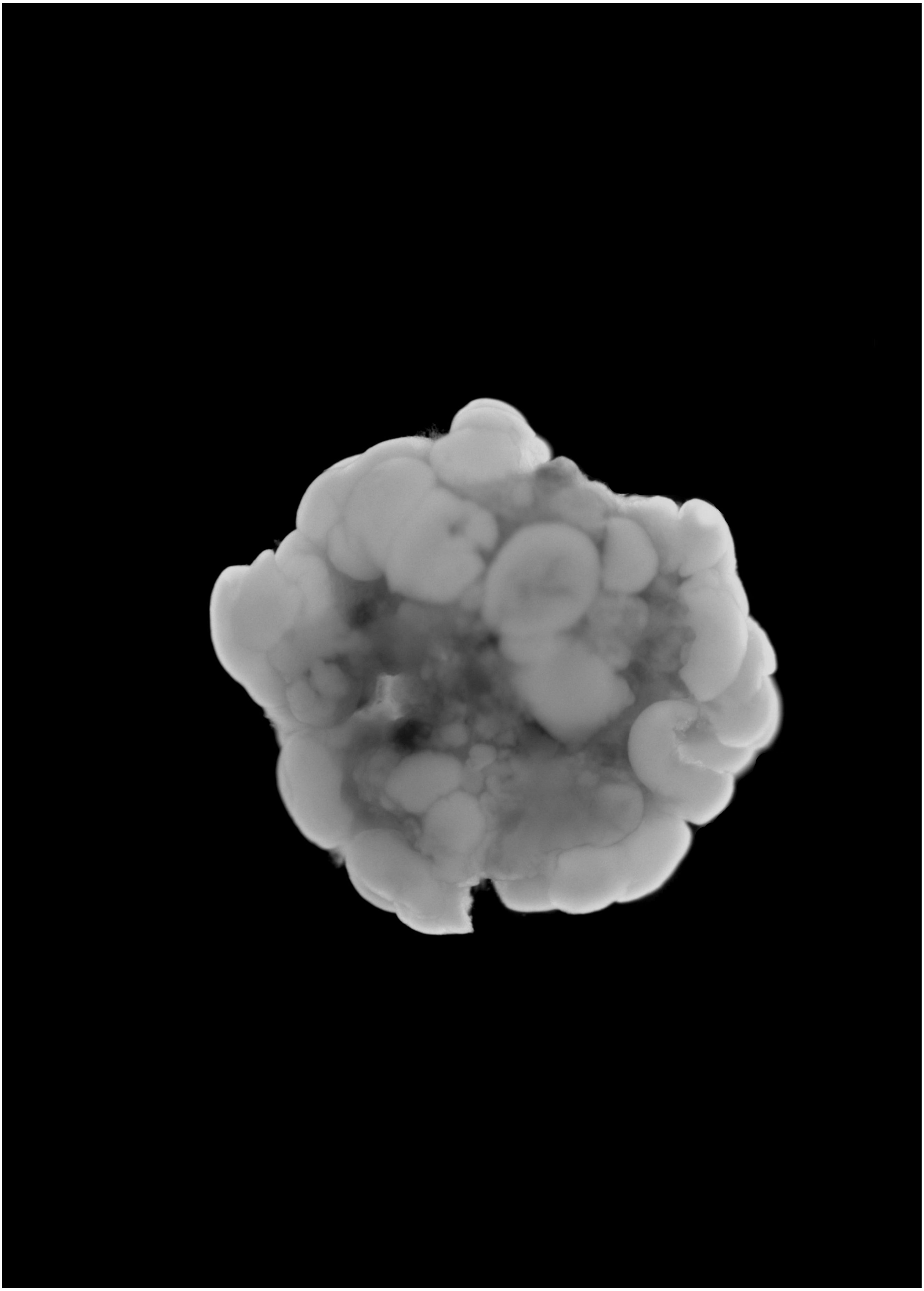
Van Loosdrecht MCM, Tijhuis L, Wijdieks AMS, Heijnen JJ. 1995b. Population distribution in aerobic biofilms on small suspended particles. *Wat. Sci. Technol.* **31**(1):163 - 171.

Wanner O, Reichert P. 1996. Mathematical modelling of mixed-culture biofilms. *Biotechnol. Bioeng*. **49**(2):172-184.

Wentzel MC, Ekama GA, Loewenthal RE, Dold PL, Marais GR. 1989. Enhanced polyphosphate organism cultures in activated sludge systems. Part II: experimental behaviour. *Water SA* **15**(2):71-88.

Zeng RJ, Lemaire R, Yuan Z, Keller J. 2003a. Simultaneous Nitrification, Denitrification, and Phosphorus Removal in a Lab-Scale Sequencing Batch Reactor. *Biotechnol. Bioeng.* **84**(2):170-178.

Zeng RJ, van Loosdrecht MCM, Yuan Z, Keller J. 2003b. Metabolic model for glycogen-accumulating organisms in anaerobic/aerobic activated sludge systems. *Biotechnol. Bioeng.* **81**(1):92-105.



5 Aerobic granule formation and nutrient removal at moderate and low temperatures

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 wash-out 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.

This chapter was published as de Kreuk, M.K., Pronk, M. and van Loosdrecht, M.C.M. (2005). "Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperature". Water Research *39* (18), 4476-4484.

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 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 temperature changes. In the Netherlands, as in other northern regions, the temperature of sewage usually varies between 5-12°C in winter and 15-25°C in summer. Therefore, temperature effects on aerobic granular sludge need to be investigated before the system can be efficiently scaled-up and used in 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, growth of long filamentous *Microthrixs Parvicella* was described when applying low temperatures (≤12°C), while at higher temperatures (20°C), the filaments broke into smaller fragments of 30-80µm (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 activated sludge models; Henze et al., 2000), which can be described by a simplified derivation of the Arrhenius equation:

$$k(T) = k(20) \cdot \theta^{(T-20)} \qquad [1]$$

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.

Materials and methods

Reactor system

A double walled 3-litre 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^{-1}). 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 °C and 8°C) were carried out. The decreased temperature stabilised overnight (5 to 6 cycles) after which the experiment was performed. The total time of temperature change was never longer than 24 hours. Dissolved oxygen (DO) concentration was measured as percentage of the saturation concentration (100% at 20°C = 9.1 mg L^{-1}; at 15°C = 10 mg L^{-1} and at 8°C = 11.8 mg L^{-1}). 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 $\pm$0.2 by dosing 1 M NaOH or 1 M HCl.. Hydraulic retention time (HRT) and substrate load were respectively 5.6 hr and 1.6 kgCOD m^{-3} day^{-1} (stage A, BI and BII) and 7.4 hr and 1.2 kg kgCOD m^{-3} day^{-1} (stage BIII).

The reactor was operated in successive cycles of 3 hours (stage A, BI and BII): 60 minutes feeding under anaerobic conditions from the bottom of the reactor (plug-flow through the settled bed), 112 minutes aeration, 3 minutes settling (to keep only particles settling faster than 12 m h^{-1} in the reactor) and 5 minutes effluent discharge. During stage BIII, the aeration phase was increased to 171 minutes (4 hour 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^{-1} 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.

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^- 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 minutes 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.

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°C to 15°C 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.

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 minutes (particles with a settling velocity larger than 3 m h^{-1} are retained in the reactor), which was gradually decreased to 3 minutes during the first 4 weeks of operation. The reactor was inoculated with 1 litre of activated sludge from a WWTP and 20 ml crushed granules (approximately1.5 gTSS) from a laboratory scale aerobic granular sludge reactor with EBPR activity (figure 1a).

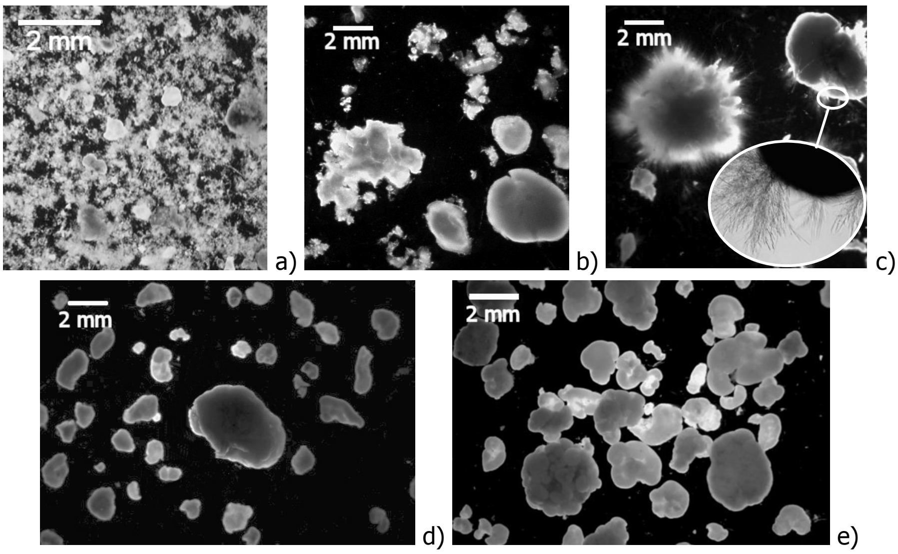


Figure 1 Start-up material and granules grown at different stages of this study: Inoculation material for start-up at 8°C (a); irregular granules formed 26 days after start-up at 8°C (b); granules with fungi/fungi pellet (c); granules formed at 20°C (d) and granules formed when temperature lowered to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

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 (gVSS L^{-1} reactor)	18	20	18	7
SVI_8 (mL g^{-1})	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

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 g L^{-1} to 1.06 g L^{-1} at the same time the SVI_8 increased from 33 mL g^{-1} to 153 mL g^{-1}. The granules changed to fluffy, irregular structures with a low density (figure 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 gTSS) 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 gTSS) aerobic granules from another laboratory reactor with EBPR activity was added, which increased the dry weight in the reactor to 3.3 g L^{-1}. After 15 days, fungi started to grow on the surface of these granules (figure 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^{-1} and the SVI_8 fluctuated between 114 mL g^{-1} and 18 mL g^{-1} right after granule dosage and stabilised at 34 mL g^{-1} 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^{-1} 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 mgCOD L^{-1} and was aerobically consumed (average effluent concentration 24 mgCOD L^{-1}). Also nitrification was absent, resulting in high ammonia concentrations in the effluent (43 mg NH_4-N L^{-1}). Because of the low activity of the sludge, the experiment was stopped 110 days after the second start-up.

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 litres and a SVI_8 of 15 mL g^{-1}. 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, figure 1d and 1e). 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. The density of the granules at 8°C was slightly lower than at 20°C (53 gVSS L^{-1} biomass at 8°C versus 78 gVSS L^{-1} 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°C and 8°C are shown in figure 2 (A – F). During the stable operation at 20°C, the nitrogen removal fluctuated around 75%. The 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 33gTSS L^{-1} (ash content 40%) and 12mL g^{-1}. 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 hours at this temperature. The anaerobic feeding period remained 1 hour, while the aerobic period was increased with 1 hour to 171 minutes 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 gTSS L^{-1} 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.

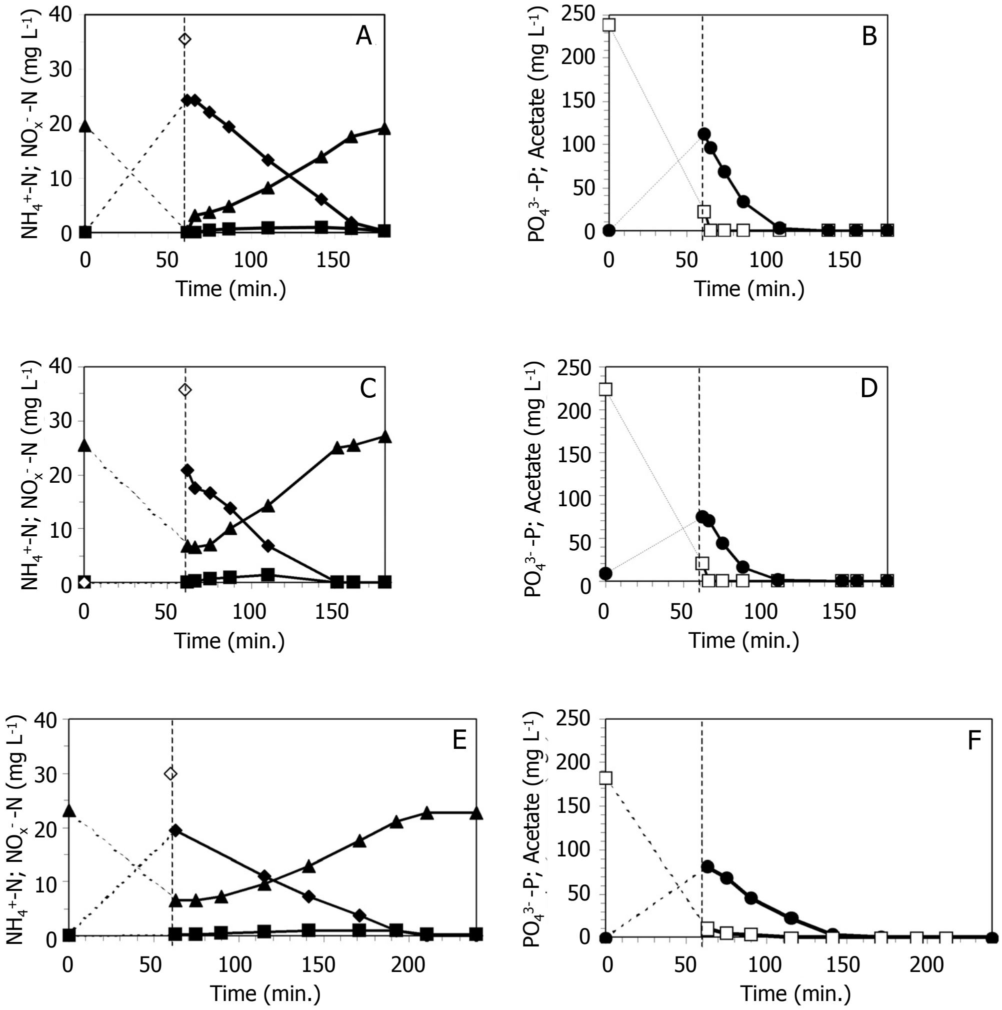


Figure 2 Conversion in single cycles of the SBAR during the steady-state period at 20 °C (A, B), 15°C (C, D) and 8°C (E, F) and (see next page) short-term temperature changes at 15°C (G, H) and 8°C (I, J). Symbols: NH_4^+-N (◆), NO_3^--N (▲) and NO_2^--N (■) in mg N L^{-1} (A, C, E, G, I); PO_4^{3-}-P (●) in mg P L^{-1}; acetate (□) in mg L^{-1}. 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.

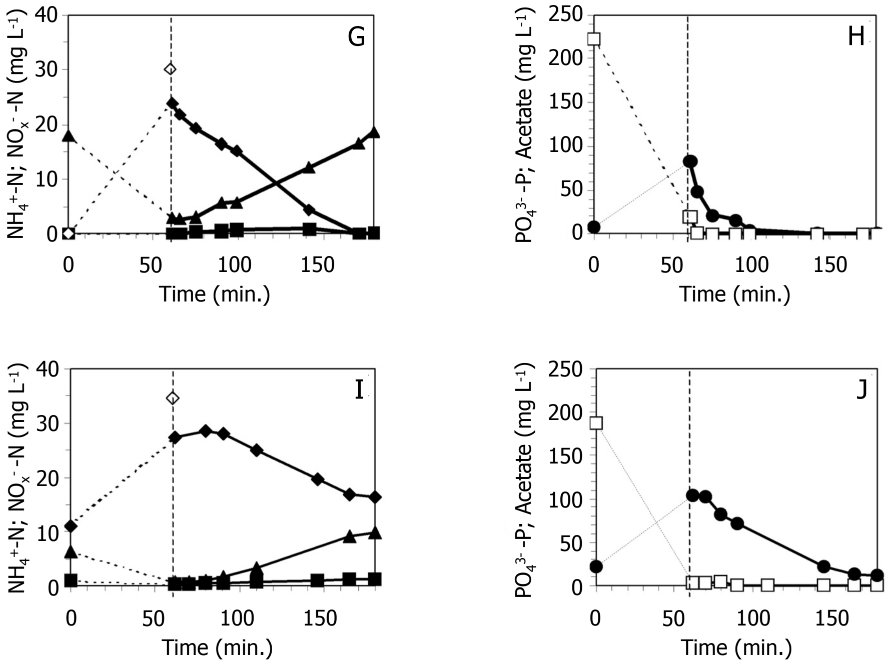


Figure 2 Continued

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 hours 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 figure 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°C 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°C and 20°C).

Discussion

The rates of most biological processes depend on temperature. This dependency can be described with a simplified Arrhenius equation (equation 1). In the activated sludge model 2 (ASM2), four groups of temperature dependency are distinguished; i) θ = 1.00: no dependency, processes such as chemical precipitation; ii) θ = 1.04: low dependency, e.g. hydrolysis; iii) θ = 1.07: medium dependency, e.g. heterotrophic conversions and fermentation; iv) θ = 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.

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 (θ=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^{-1}). 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 wash-out 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.

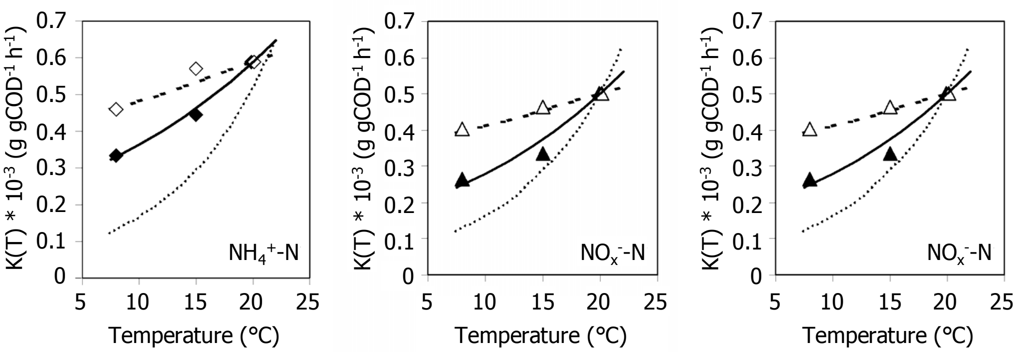


Figure 3 The maximum measured conversion rates k(T) in g $g_{biomass\ COD}^{-1}$ h^{-1}, at different temperatures for NH_4^+-N consumption, NO_x^--N production and PO_4^{3-}-P consumption for a system adapted to 20°C with short term changes to low temperatures (◆, ▲, ●) and for systems adapted to the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

Temperature dependency of the aerobic granules without adaptation

Decreasing the temperature of a stable operating reactor from 20°C to 15°C or 8°C during a short period (24 hours), showed a significant decrease in conversion rates (Figure 3). Temperature coefficients were derived from these experiments (Figure 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 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.

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°C 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

Table 2 Calculated temperature coefficients (θ, equation 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[1]
NO_x^- production	1.02	1.06	1.12[1]
PO_4^{3-} consumption	1.06	1.06	1.031[2,a]; 1.065[2,b]

[1] Data from ASM2d (Henze et al., 2000); [2] Data from Brdjanovic et al., 1998;
[a] long term experiment, [b] short term experiment

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.

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 wash-out. 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 was performed in close co-operation with DHV, Amersfoort, The Netherlands.

References

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

Bathe, S., De Kreuk, M.K., Mc Swain, B.S. and Schwarzenbeck, N. (2005). Aerobic granular sludge. In: *Water and environmental management series (wems)* (170 p. IWA Publishing: London.

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

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

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

De Kreuk, M.K., De Bruin, L.M.M. and 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. and Schwarzenbeck, N. (Eds.), *Aerobic granular sludge* (111-123 pp.). IWA: London, UK.

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

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

Eikelboom, D.H., Andreadakis, A. and Andreasen, K. (1998). Survey of filamentous populations in nutrient removal plants in four european countries. *Wat. Sci. Technol.*, **37** (4-5), 281-289.

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

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

Henze, M., Harremoës, P. and Arvin, E. (1997). Wastewater treatment: Biological and chemical processes. (p. 383). Springer-Verlag: Berlin Heidelberg.

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

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

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

Liu, Y. and 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. and Wilderer, P.A. (2004). The effect of intermittent feeding on aerobic granule structure. *Wat. Sci. Technol.*, **49** (11), 19-25.

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

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

Nielsen, P.H. (1996). Adsorption of ammonium to activated sludge. *Wat. Res.*, **30** (3), 762-764.

Picioreanu, C., Van Loosdrecht, M.C.M. and 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. and Wilderer, P.A. (2004). Aerobic granular sludge in an sbr-system treating wastewater rich in particulate matter. *Wat. Sci. Technol.*, **49** (11-12), 41-46.

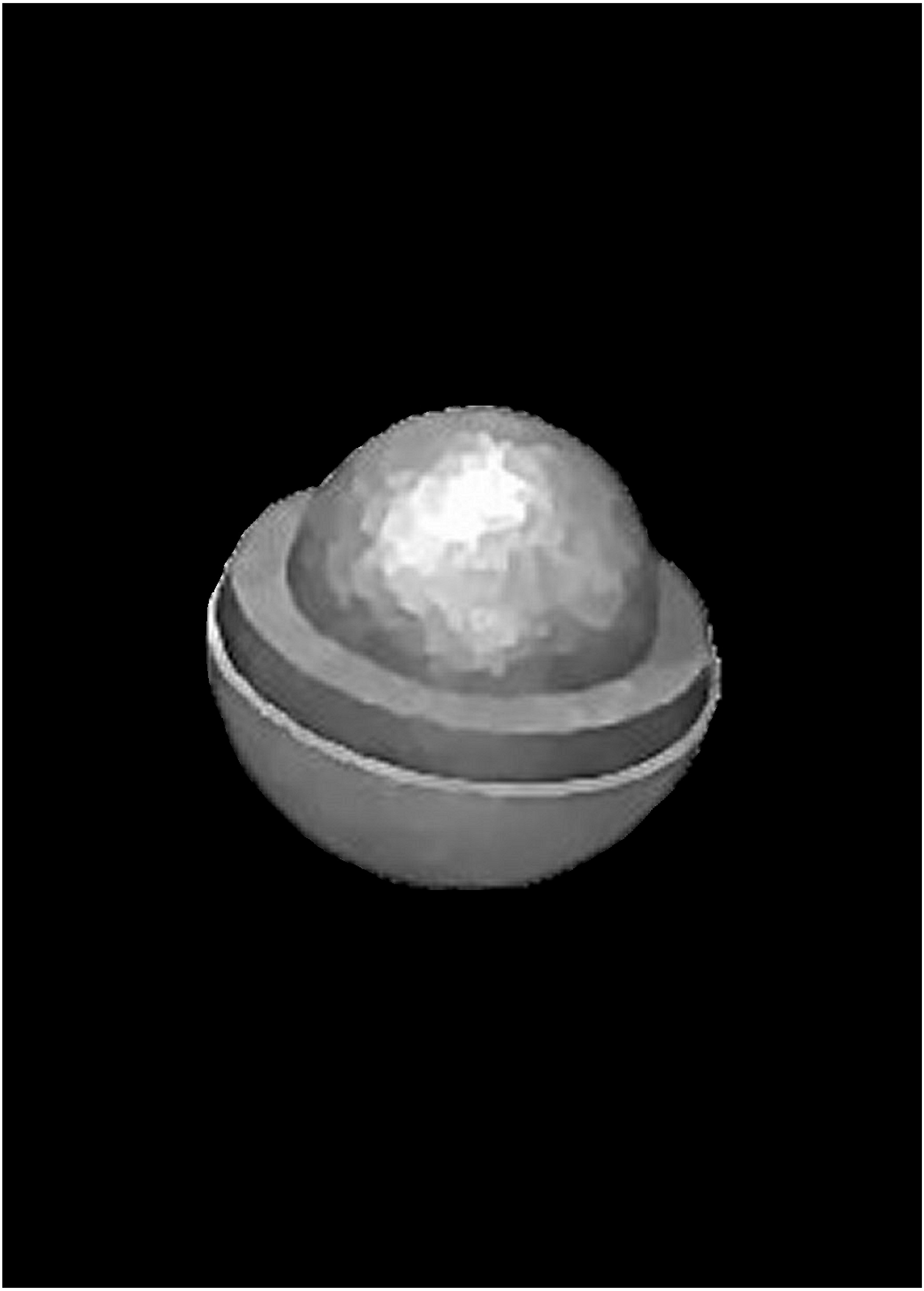
Seviour, R.J., Mino, T. and Onuki, M. (2003). The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiology Reviews*, **27** (1), 99-127.

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

Van Loosdrecht, M.C.M., Picioreanu, C. and 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. and Heijnen, J.J. (2000). Influence of different substrates on the formation of biofilms in a biofilm airlift suspension reactor. *Wat. Sci. Technol.*, **41** (4-5), 323-330.

Vishniac, W. and Santer, M. (1957). *The* thiobacilli. *Bacteriol. Review*, **21**, 195-213.



6 Kinetic model of a granular sludge SBR – Influences on nutrient removal

Abstract

A mathematical model was developed that can be used to describe an aerobic granular sludge reactor, fed with a defined influent, capable of simultaneously removing COD, nitrogen and phosphate in one sequencing batch reactor (SBR). The model described the experimental data from this complex system sufficiently. Sensitivities of process parameters on the nutrient removal rates could therefore be reliably evaluated. The influence of oxygen concentration, temperature, granule diameter, sludge loading rate and cycle configuration were analysed. Oxygen penetration depth in combination with the position of the autotrophic biomass played a crucial role in the conversion rates of the different components and thus on overall nutrient removal efficiencies. The ratio between aerobic and anoxic volume in the granule strongly determines the N-removal efficiency as it was shown by model simulations with varying oxygen concentration, temperature and granule size. The optimum granule diameter for maximum N- and P-removal in the standard case operating conditions (DO 2 mg L^{-1}, 20°C) was found between 1.2 and 1.4 mm and the optimum COD loading rate was 1.9 kg COD m^{-3} day^{-1}. When all ammonia is oxidised, oxygen diffuses to the core of the granule inhibiting the denitrification process. In order to optimise the process, anoxic phases can be implemented in the SBR-cycle configuration, leading to a more efficient overall N-removal. Phosphate removal efficiency mainly depends on the sludge age; if the SRT exceeds 30 days not enough biomass is removed from the system to keep effluent phosphate concentrations low.

This chapter is submitted to Biotechnology and Bioengineering as: de Kreuk, M.K., Picioreanu, C., Hosseini, M., Xavier, J.B. and van Loosdrecht, M.C.M. "Kinetic model of a granular sludge SBR – Influences on nutrient removal".

Introduction

Aerobic granular sludge technology is a new and promising development in the field of aerobic wastewater treatment. This technology is based on sequencing batch reactors (SBR), with a cycle configuration chosen such that a strict selection occurs for fast settling aerobic granules and that these granules are oxygen limited instead of substrate limited. This leads to the growth of stable and dense granules under anaerobic/aerobic conditions (De Kreuk et al., 2005a; Dulekgurgen et al., 2003; Li et al., 2005). Compared to conventional activated sludge systems, a system based on aerobic granular sludge has several advantages, namely compactness, lower operational and construction costs and lower energy requirement. Many laboratory studies (among others Beun et al., 1999; Liu and Tay, 2004; Morgenroth et al., 1997; Tay et al., 2002) and a pilot study (De Bruin et al., 2005) showed the potential of aerobic granular sludge SBR's, and the next step is to apply this technology in practice.

Mathematical modelling has proven very useful to study complex processes, such as the aerobic granular sludge systems (Beun et al., 2001; Lübken et al., 2005). Biological processes in the granules are determined by concentration gradients of oxygen and diverse substrates. The concentration profiles are the result of many factors, e.g., diffusion coefficients, conversions rates, granule size, biomass spatial distribution and density. All of these factors tightly influence each other, thus the effect of separate factors cannot be studied experimentally. Moreover, due to the long sludge age in granule systems (usually up to 70 days, De Kreuk and Van Loosdrecht, 2004), lengthy experiments should be carried out before we can speak of a steady state reaching system. Therefore, a good computational model for the granular sludge process provides significant insight in the most important factors that affect the nutrient removal rates and in the distribution of different microbial populations in the granules. Further, the model could also be used for process optimisation and for the scale-up and design of a full-scale reactor for the new aerobic granular sludge technology, currently commercialised by DHV-Water (The Netherlands) under the name *Nereda*™.

The computational model used in this study is based on previously developed models. The SBR and granular sludge descriptions are principally as in the model of Beun et al. (2001), which described heterotrophic organisms storing acetate in a feast-famine regime in combination with autotrophic organisms for nitrogen removal. An extension of this model with more biological processes having specific kinetics and stoichiometry was needed, because the design of the Nereda™ aims at phosphate removal via the selection of slow growing phosphate accumulating organisms (PAO). Therefore, conversion processes involving PAO are included as described in the models of continuous activated sludge systems by Hao et al. (2001) and Meijer (2004). The results from the present model simulations are compared here to different laboratory scale experiments. This model will be used here for evaluation of the overall reactor performance only. A detailed modelling study of the population dynamics within aerobic granular sludge is described in Xavier et al. (In Preparation).

Materials and Methods

Laboratory scale set-up

Experiments were performed in 3 L sequencing batch reactors (internal diameter 6.25 cm) operated as bubble column (SBBC) or airlift reactor (SBAR). Air was introduced via a fine bubble aerator at the bottom of the reactors (4 L/min). Dissolved oxygen (DO) was measured as percentage of oxygen saturation and could be controlled via adding N_2 or air to the recirculation flow of the off gas through the reactor. pH was maintained at 7.0 ± 0.2 by dosing 1*M* NaOH or 1*M* HCl. Hydraulic retention time was 5.6 h and the substrate load, measured in chemical oxygen demand (COD), was 1.6 kgCOD $m^{-3}day^{-1}$.

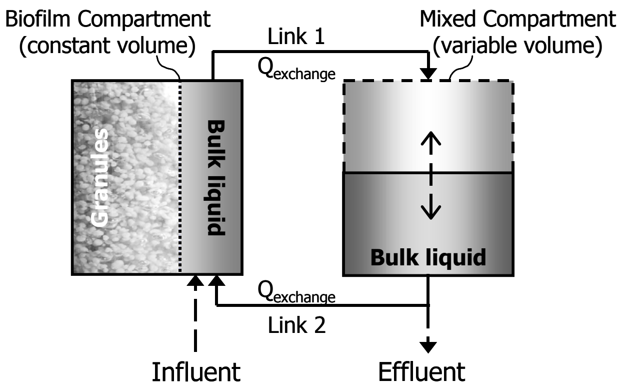


Figure 1 Schematic representation of the model set-up in AQUASIM. Each compartment contains 7 soluble components (S_{O2}, S_{Ac}, S_{NH4}, S_{NO2}, S_{NO3}, S_{PO4}, S_{N2}) and 7 particulate components (X_{PAO}, X_{NH}, X_{NO}, X_I, X_{PP}, X_{PHB}, X_{GLY}) involved in 21 metabolic processes. All soluble and particle components are found in effluent and recirculation links.

The reactors were operated in successive cycles of 3 hours: 60 minutes of anaerobic feeding from the bottom of the reactor; 112 min aeration; 3 min settling; 5 min effluent discharge.
The composition of the influent media were (A) Na acetate 63 mM, $MgSO_4.7H_2O$ 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 each medium (A and B) 0.15 L per cycle were dosed together with 1.3 L tap water.
Exact methods for reactor operation, trace element solution and measurement of the experimental data used for model calibration and comparison are described in De Kreuk et al. (2005a; 2005b).

Model description
The mathematical model describing the performance of the laboratory-scale granular sludge SBR was implemented in the well-established simulation software for environmental applications AQUASIM (Reichert, 1998). A combination of completely mixed reactor and biofilm reactor compartments (based on Wanner and Gujer, 1986), provided by AQUASIM, was used to simulate the mass transport and conversion processes occurring in the bulk liquid and in the granules of the SBR system. The biological conversion processes are described using stoichiometric and kinetic parameters from the models published by Hao et al. (2001; 2002b) and Meijer (2004).
The model was used to simulate the conversion processes that occur in a laboratory scale reactor and compare them with those obtained experimentally under the same conditions. Furthermore, it was used to predict the nutrient removal efficiencies under different circumstances. Special attention has been paid to the distribution of the different microbial species in the granules, as resulting from the model simulations. This distribution, in combination with the oxygen penetration depth, gives insight in the overall reactor performance and its sensitivities.

Conversion processes
The model accounts for biomass distribution among seven particulate components (figure 1). Three active biomass types include the phosphate-accumulating organisms (X_{PAO}) and the nitrifiers (ammonia-oxidizers, X_{NH} and nitrite-oxidizers, X_{NO}). One inactive biomass component (X_I) was also defined. Special pools are defined for internally stored biomass: polyphosphate (X_{PP}), poly-hydroxybutyrate

(X_{PHB}) and glycogen (X_{GLY}). Seven dissolved components are considered relevant for the biological conversions and process stoichiometry: oxygen (S_{O2}), acetate (S_{Ac}), ammonium (S_{NH4}), nitrite (S_{NO2}), nitrate (S_{NO3}), nitrogen (S_{N2}), and phosphate (S_{PO4}).

It was assumed that acetate would be consumed by PAO and converted to a storage polymer (polyhydroxybutyrate, PHB) during the anaerobic period. Converting glycogen and degrading poly-P generate energy needed for the active uptake of acetate. During the aerobic period, the stored PHB is used for growth, maintenance and restoring the glycogen and poly-P pools. In this period, also nitrification takes place, with ammonia converted via nitrite into nitrate. All anoxic processes can take place on nitrite and nitrate as electron acceptors, depending on their availability. Also maintenance and endogenous respiration for autotrophic organisms is included. When no PHB, glycogen nor acetate is available, PAO will decay. For simplicity and to maintain the granule structure, the decayed biomass was considered to be turned into inert material (Beun et al., 2001). To limit the calculation time for the simulation, no other heterotrophic organisms (X_H) were included in the model. This is justified by the fact that a very small amount of the incoming acetate is left at the start of the aerobic period, which could be consumed by aerobic heterotrophs and by PAO (figure 2). The lack of acetate during the aerobic period will limit the growth and will result in the total disappearance of heterotrophic organisms other than the ones capable of anaerobically storing substrate (as shown by Xavier et al., In Preparation). Therefore, omitting the heterotrophs that do not store substrate is not expected to greatly influence the long term ("steady-state") simulation results.

The stoichiometry and kinetics of biological conversions are derived from the Delft bio-P model for activated sludge (Meijer, 2004; van Veldhuizen et al., 1999) and presented with the ASM (Activated Sludge Model) notation (Henze et al., 1999) in Appendix 1, table 1. Stoichiometric and kinetic parameter values are given in Appendix 1, table 2 and Appendix 1, table 3, respectively. A few model parameters were altered in respect with the original models.

Since only one kinetic model describes all the different processes and the experimental results in both the aerobic granules and in the reactor, some parameters needed to be adjusted. The reduction factor for process rates under anoxic conditions (η) was chosen 0.3, since this described best the experimentally measured values for the nitrate production. This low reduction value is needed, because in this model denitrification can occur on both nitrite and nitrate. In other models, denitrification often only takes place on nitrate. Allowing denitrification with nitrate and nitrite simultaneously and not adding an inhibition factor for the preference of one of the two electron acceptors would double the denitrification rates, producing unrealistic results if no reduction factor (η) was used.

Part of the phosphate removal in aerobic granular sludge is removed by (biologically induced) precipitation (De Kreuk et al., 2005a). For simplicity, precipitation was not included in the model. As an alternative, the maximum fraction of poly-phosphate (f_{PP}^{max}) was increased from 0.35 (Wentzel et al., 1989) to 0.65. Consequently, the PHA storage had to be adjusted in order to generate enough PHA for the increased poly-P uptake.

Another model adjustment concerns the expressions for the reaction rates. In order to prevent the occurrence of negative values for solute concentrations, switch functions were used (Appendices 1 and 3). These continuous S-shaped functions shut down a process consuming a reactant whose concentration is nearly zero and, in addition, make the simulations numerically more stable.

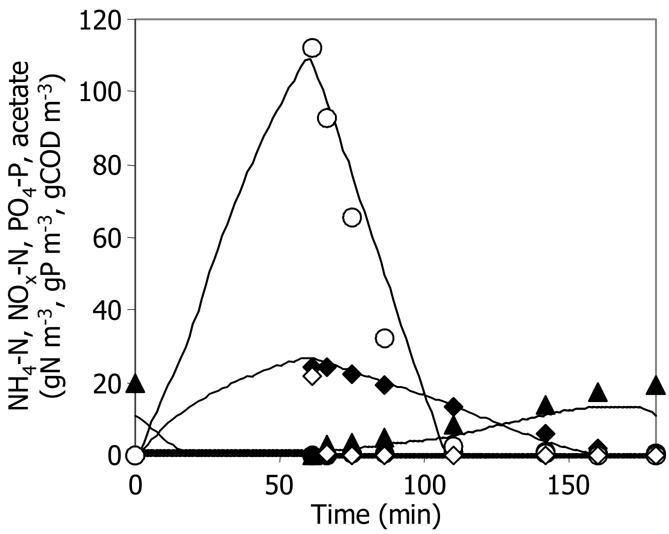


Figure 2 Measured concentrations during a cycle measurement (points; PO_4-P (○), Acetate (◇), NH_4-N (◆), NO_3-N (▲), NO_2-N (●)) and simulated results with the present model (lines), in the standard case (DO 2 mg·L^{-1}, 20 °C, granule diameter 1.1 mm).

Reactor description

Liquid circulation pattern: In the laboratory scale SBR, influent is fed from the bottom of the reactor with a flow pattern close to plug flow through the settled bed of granules (De Kreuk and Van Loosdrecht, 2004). At the end of each cycle, the effluent is discharged from a port at half of the reactor height. The AQUASIM software does not allow the volume of the bulk liquid in the biofilm compartment to vary in time. In order to circumvent this and simulate the fill and discharge process, two linked compartments have to be defined. A completely mixed liquid compartment with variable volume is connected to the biofilm compartment. A high fluid circulation rate ($Q_{exchange}$) between the two compartments ensures the same bulk liquid concentrations in both compartments (Figure 1).

We chose to represent the flow pattern in the reactor as completely mixed because the simulation in AQUASIM of different liquid circulation regimes in different phases of the SBR cycle would imply enormous difficulties. It is expected that the liquid circulation in the feeding phase affects the diffusion rate of acetate into the granule. Consequently, it also determines the stored substrate in the anoxic interior of the granules available for denitrification during the aerobic period (simultaneous nitrification/denitrification, SND). With a plug-flow feeding pattern, the substrate concentration in the lowest part of the settled granule bed is equal to the influent substrate concentration. In the model however, the influent is directly mixed with the remaining bulk liquid in the reactor, leading to a decreased concentration in the bulk liquid surrounding the granules. To compensate for this lower substrate concentration, the diffusion coefficient of acetate in the AQUASIM model during feeding was increased arbitrarily.

Cyclic SBR operation: The model biofilm compartment contains a biofilm volume, which accounts for all the granules, and bulk liquid volume (Figure 1). The volume of this compartment was fixed at 1.5 L. The completely mixed compartment contained the remainder of the liquid volume in the system, with a maximum volume of 1.6 L. The influent (1.5 L) entered the reactor in the biofilm compartment during the first 60 minutes of the cycle. In the model, aeration of the bulk liquid was switched on during the next 112 minutes (3 hour cycle) or 232 minutes (4 hour cycle). The last 8 minutes of the cycle effluent is discharged from the mixed compartment. Other parameters needed for the AQUASIM model are given in Appendix 1, table 4.

Number and size of granules: The number of granules, in combination with the granule diameter, can affect the outcome of the simulation, because these influence the overall liquid/granule interfacial area. The granules grown in the laboratory had the following characteristics: granule size distribution between 0.36 mm and 3.7 mm (average diameter 1.1 mm, standard deviation 0.45); aspect ratio 0.72; shape factor 0.74; dry weight in reactor 18 gVSS·L^{-1}; ash content 37%; SVI_8 15 mL·g^{-1} (De Kreuk et al., 2005b). The laboratory reactor contains thus a wide variation in granule sizes, which also change in time. However, the use of a system with a granule size distribution was avoided in this model, since it would significantly increase the complexity of the numerics and it is not expected to contribute to a better understanding of the system. Therefore, in the simulations the diameter chosen was 1.1 mm, which is the most representative for aerobic granules in our reactor. The number of granules was set to 600000, resulting in a comparable amount of biomass as present in the laboratory scale reactor. The sensitivity of the size and the number of granules was evaluated in several simulations. A radius-dependent biofilm area was set in AQUASIM to correctly represent the spherical symmetry of the granules.

Simulation strategy

Since the sets of experimental data measured during a cycle are different in time, depending on granule size, morphology and reactor operation, a representative set of measured concentrations was chosen to compare to a standard simulation case (Figure 2). The simulation was performed under the same operational conditions as applied during this standard operation of the SBR. The DO was controlled at 2 mg·L^{-1} during the aerobic phase, the temperature was 20°C and influent concentrations were as given in Table 4. The initial concentrations of the biomass in the granule were: PAO 50,000 gCOD·m^{-3}; ammonia oxidisers (X_{NH}) and nitrite oxidisers (X_{NO}) 5,000 gCOD·m^{-3} each; inerts (X_I) 1 gCOD·m^{-3}; glycogen concentration (X_{GLY}) 22,500 gCOD·m^{-3}; PHA concentration (X_{PHA}) 36,000 gCOD·m^{-3}; poly-P concentration (X_{PP}) 29,250 gCOD·m^{-3} (initial concentration of the storage polymers was set to 90% of the maximum fraction). These initial values were based on the overall density of aerobic granular sludge (80 gTSS·L^{-1}) and a sufficient amount of storage polymers for the conversions.

The operation of the SBR in different conditions (such as changed dissolved oxygen concentrations, the influence of granule diameter and of sludge load) was simulated for 365 days, which corresponds to about 8 hours of computing time on a Pentium 4, 2.4 GHz processor. After 200 days in the simulation the average effluent concentrations over 5 days did not fluctuate more than 1%. Inside the simulated granule, in the outer layer from radius 0.22 to 0.55mm (87.5% of the volume of the granule), also the biomass concentration did not change more than 1% per 5 days after 350 days of simulation. However, in the inner zone of the granule (radius 0 to 0.22 mm) the biomass concentrations of the autotrophic organisms still changed with less than 4% per 5 days. These results showed that, in the simulations, after 365 days the reactor effluent and the granule composition could be considered sufficiently close to a steady state. In the experiments at laboratory scale, measurements are mostly performed between 4 months and 1.5 years after an operation change, which correlates with the simulated 365 days of operation.

Results

Standard case: evaluation of the model with experimental data

The standard operation of the granule reactor was simulated first. The results of this simulation, in steady state reached after 365 days, are shown in Figure 2, together with the concentrations measured in the laboratory scale reactor during a cycle with the same operational conditions. Conversion rates measured in the experiments were compared to the ammonium and phosphate consumption and to the net nitrite and nitrate production rates obtained from the model. Conversion rates in the experiments were determined by taking the largest slope of at least 3 data points in the cycle. This was compared to the average conversion rate over the same time interval in the simulation. The conversion rates resulting from the model in this standard case did not differ with more than 30% from the values found in the experiments (Table 1, first row). Given all the uncertain parameters (e.g. exact granule size distribution and granule surface area), it was concluded that the model described the experimental data satisfactorily. Default parameters were thereby considered to be good enough not to calibrate the model any further and to start performing other simulations in different operational conditions. The parameter values for this standard case are also used to obtain all other results, unless mentioned otherwise.

Short and long term DO changes

To study the effects of short and long term dissolved oxygen (DO) changes, the DO was varied during a set of model simulations and the results were compared with data from laboratory experiments (De Kreuk et al., 2005a). For long-term

Table 1 Effect of dissolved oxygen concentration on the overall N- and P-removal rates in the SBR during short term and long-term changes

	Overall net conversion rates ($mg\text{-}N{\cdot}L^{-1}{\cdot}h^{-1}$, $mg\text{-}P{\cdot}L^{-1}{\cdot}h^{-1}$)[2, 3]			
	NH_4^+ consumption	NO_2^- production	NO_3^- production	PO_4^{3-} consumption
DO concentration 1 mg L^{-1} (one cycle change)[1]	7.2 (8.5)	0 (0)	1.5 (0)	98 (144)
DO concentration 2 mg L^{-1} (long term operation)	15.9 (18.0)	1.1 (1.1)	10.0 (14.4)	142 (156)
DO concentration 4 mg L^{-1} (long term operation and start for the one-cycle changes)	23.1 (15.1)	0 (0.2)	16.4 (2.4)	173 (199)
DO concentration 10 mg L^{-1} (one cycle change)[1]	28.8 (19.4)	1.0 (n.a.)	26.0 (20.2)	197 (267)
DO concentration 10 mg L^{-1} (long term operation)	31.9 (15.4)	1.2 (1.7)	26.1 (22.5)	193 (202)

[1] The short-term changes are simulated with the biomass distribution as developed after 365 days at DO 4mg/L.

[2] Rates are calculated from the slopes of the simulated and experimentally determined concentration profiles in the reactor during a cycle.

[3] Experimental data are shown in parentheses (De Kreuk et al., 2005a)

effects, the development of granular sludge was simulated during 365 days of reactor operation at DO concentrations of 2 $mg{\cdot}L^{-1}$, 4 $mg{\cdot}L^{-1}$ and 10 $mg{\cdot}L^{-1}$. The results of the 4 $mg{\cdot}L^{-1}$ simulation were then used to simulate short-term effects of a DO shift during one cycle to 1 mg $O_2{\cdot}L^{-1}$ and to 10 $mgO_2{\cdot}L^{-1}$, respectively. Due to the relatively short time interval during these DO shifts, the biomass distribution within the granule did not significantly change.

Conversion rates obtained experimentally and by simulation, are given in Table 1. The conversion rates for the short-term experiments show all the same trend. At an increased DO most rates are enhanced. Ammonium is consumed faster, while the net nitrate production rate increased even more, due to lower denitrification rates in the presence of high oxygen concentration. The anoxic zone inside the granules is minimized during the aeration period (Figure 3f).

The simulated biomass distribution in a granule grown at 2 and 10 mg $O_2{\cdot}L^{-1}$ and the oxygen penetration depths during the cycle are compared in Figure 3a-f. The increased oxygen penetration depth during the total aeration period (DO 10 mg/L) reduced the competition for oxygen between autotrophs and PAO in the outer layer and created the possibility for autotrophic organisms to accumulate in the inner zones of the granule. This increased concentration of autotrophs enhanced the nitrification rates at higher oxygen concentrations (Table 1), however, because an anoxic zone was lacking the total N-removal efficiency decreased. At 2 mg $O_2 \cdot L^{-1}$ the simulated total N-removal efficiency was 60% (61% in the experimental data-set), while at 10 mg $O_2 \cdot L^{-1}$ it was only 33% (10% in the experimental data-set). The ammonia consumption rate at higher oxygen concentrations is particularly overestimated in the model simulations, especially for the long-term oxygen change. These higher N-removal rates and efficiencies obtained from the model simulations compared to the experiments can be explained by the lower biomass concentration present in the reactor during the experiments at 10 mg $O_2 \cdot L^{-1}$ (16 $gTSS \cdot L^{-1}$ versus 28 $gTSS \cdot L^{-1}$ during the experiment at 2 mg $O_2 \cdot L^{-1}$, later TSS value was used for model calibration).

The phosphate removal at higher oxygen concentrations is faster than at low oxygen concentrations. This is due to the significantly faster storage of Poly-P in aerobic conditions than in anoxic conditions. At higher oxygen concentrations and thus at an increased oxygen penetration depth in the granules, more PAO will be able to store their phosphate aerobically, resulting in an increased P-removal rate. At all simulated oxygen concentrations here, the storage was high enough to have complete phosphate removal.

The influence of temperature

Variations in process temperature (20°C, 15°C and 8°C) were simulated according to experiments performed in a laboratory scale reactor. Starting with the steady state data of the standard case simulation at 20°C (2 mg $O_2 \cdot L^{-1}$), temperature was lowered to 15°C and the process was simulated for 50 more days. Subsequently, the temperature was lowered again to 8°C followed by an additional simulation of 65 days. Both simulated periods were equal to the duration of the long-term temperature change experiments (De Kreuk et al., 2005b). Short-term temperature shift experiments were also simulated by changing the temperature to 15°C and 8°C during two cycles each. Especially the simulated biomass distribution in the long-term low temperature experiments was of interest, since experiments suggested an enrichment of autotrophic organisms in deeper layers of the granules at low temperatures. Simulations indicated that this accumulation of autotrophic organisms was mainly due to a higher oxygen penetration depth, caused by decreased activity in the outer layers of the granules.

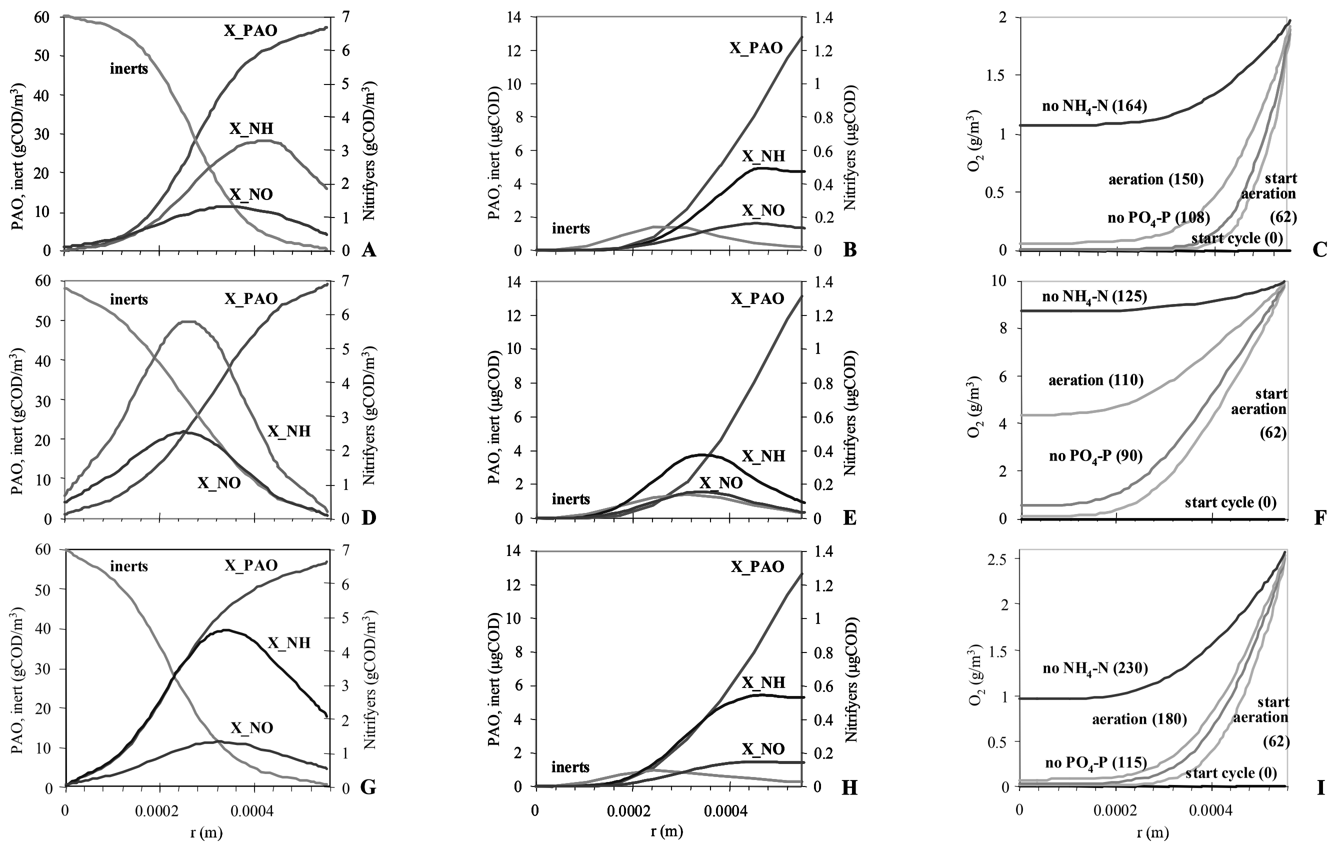
Table 2 Effect of temperature on the overall N- and P-removal rates in the SBR, during short term and long term changes.

	Overall net conversion rates ($mg\text{-}N \cdot L^{-1} \cdot h^{-1}$, $mg\text{-}P \cdot L^{-1} \cdot h^{-1}$)[2, 3]			
	NH_4^+ consumption	NO_2^- production	NO_3^- production	PO_4^{3-} consumption
DO concentration 1 mg L^{-1} (one cycle change)[1]	7.2 (8.5)	0 (0)	1.5 (0)	98 (144)
DO concentration 2 mg L^{-1} (long term operation)	15.9 (18.0)	1.1 (1.1)	10.0 (14.4)	142 (156)
DO concentration 4 mg L^{-1} (long term operation and start for the one-cycle changes)	23.1 (15.1)	0 (0.2)	16.4 (2.4)	173 (199)
DO concentration 10 mg L^{-1} (one cycle change)[1]	28.8 (19.4)	1.0 (n.a.)	26.0 (20.2)	197 (267)
DO concentration 10 mg L^{-1} (long term operation)	31.9 (15.4)	1.2 (1.7)	26.1 (22.5)	193 (202)

[1] The short term changes are simulated with the biomass distribution as developed at 20 °C.
[2] Rates are calculated from the slopes of the simulated and experimentally determined concentration profiles in the reactor during a cycle.
[3] Experimental data are shown in parentheses (partly from De Kreuk et al., 2005b).

The results of the simulations were largely according to those expected from the experiments (Table 2). Figure 3g-h shows an increased amount of ammonia oxidizing bacteria in the granules simulated at 8°C. This increase results in a higher ammonia consumption rate in the long-term simulation at 8°C compared to the short-term simulated shift. These rates are similar to the ones found in the experiments (Table 2). Consistently, the N- and P-removal rates are significantly lower after short temperature shifts, both in experiments and in simulations. However, it can be seen that the rates of 20°C and 15°C long-term simulations do not differ largely. This shows that the granules are adapting to a small temperature change if the time is long enough. The PAO population increases slightly at lower temperatures, leading to a faster phosphate consumption rate during the long-term experiment at 8°C than during the short-term experiment.
Similar to the results of simulations with different oxygen concentrations, enrichment of PAO and ammonia oxidizers can be explained by the higher oxygen penetration depth during the first period of the cycle. Increased oxygen penetration at lower temperatures is due to decreased activity of the organisms in the outer layers (Figure 3i).

Figure 3 Distribution of biomass concentration in the granules (a, d, g), the absolute quantity of biomass at different positions inside the granules (b, e, h) and the oxygen profile inside the granules at different moments in a cycle, cycle times (minutes) between brackets (c, f, i). Oxygen concentration in the bulk liquid was set at 2 mg·L^{-1} (a, b, c) and10 mg·L^{-1} (d, e, f) with a temperature of 20°C during 365 days simulation. The temperature set to 8°C with an oxygen concentration maintained at 2.6 mg·L^{-1} (DO 20% saturation) during 80 days of simulation (g, h, i)



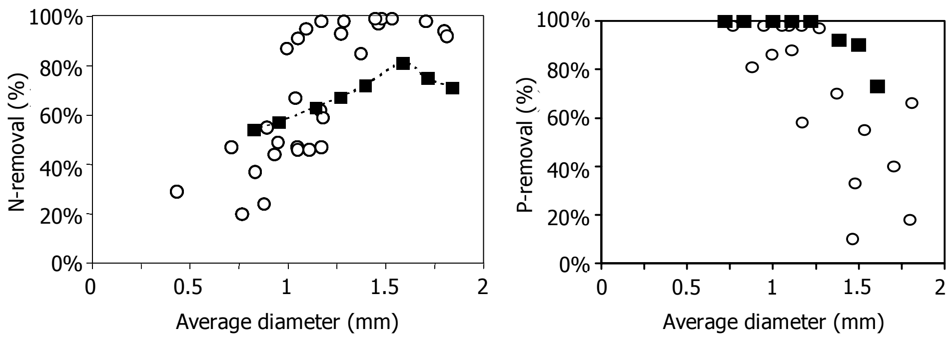


Figure 4 N-removal (a) and P-removal efficiency (b) at different average granule diameters: measured data (○,De Kreuk et al., 2005a) and data from several simulations (■)

Influence of granule diameter on nutrient removal efficiency

In the laboratory scale experiments, it was found that the average granule diameter fluctuated in time between 0.4 and 2 mm. This granule diameter is not controllable in current systems, but experimental data strongly suggests that it is correlated with nutrient removal (De Kreuk et al., 2005a and Figure 4 here). In order to evaluate how sensitive this model is for granule size, spherical particles with diameters in the range from 0.72 to 1.61 mm were used in the simulations. In order to keep the total volume of the granules the same in all simulations (0.43 L), the number of granules (NG) was varied as well (NG between 197,000 and 2,190,000 particles). Reactor operation for every diameter was simulated for 365 days, each time starting with initial conditions.

The N-removal followed in general the trends as found in the experiments. However, the effect of granule diameter on N-removal in the simulations was smaller than measured in practice (Figure 4a). Reading these figures, it should be noted that, due to the cycles in the SBR, a relatively small difference in conversion rate could result in a significant difference in removal efficiency. For example at the simulated diameter of 1.6 mm, an increased ammonia consumption rate from the present 12 to 14 mg $N{\cdot}L^{-1}{\cdot}h^{-1}$ would lead to an increased N-removal efficiency from 71% to 83%.

At small granule diameters, there is a large surface area to biomass volume ratio. Therefore, oxygen penetrates relatively deep into the granules. This leads to sufficient aerobic volume for a fast oxidation of ammonia. However, as soon as all ammonia and phosphate are removed from the system, oxygen will diffuse through the whole granule and denitrification will be inhibited. Therefore, the overall N-removal efficiency will be lower at smaller diameters. When the diameter is larger than 1.4 mm, both N- and P-removal efficiencies start decreasing. At this granule size, the surface area becomes limiting for oxygen transport and thus for the conversion processes. This leads to a decreased ammonia oxidation rate and phosphate uptake rate and to ammonia and phosphate in the effluent. Simulations and experiments reveal that the optimum diameter for nutrient removal in the standard case operating conditions will be between 1.2 and 1.4 mm.

Influence sludge load on nutrient removal

In practice wastewater treatment plants are confronted with night and day fluctuations, dry weather and storm water influent, all leading to fluctuations in the sludge loading of the aerobic granules. Also industrial wastewaters are often more concentrated than sewage. The model was further used to study the optimum COD loading for nutrient removal. Sludge loading was varied by changing the amount of granules in the system (NG from 375,000 to 1,200,000), leading to a variation in sludge loading from 17.7 to 5.5 kg $COD \cdot m_{granules}^{-3} \cdot d^{-1}$ or loading rate of 0.8 to 2.5 kg $COD \cdot m_{reactor}^{-3} \cdot d^{-1}$. Every sludge-loading rate was applied for 365 days in simulations starting with the normal initial conditions.

From the simulations (Figure 5), it can be observed that the N-removal efficiency slightly increases with an increasing load, with an optimum of 1.9 $kgCOD \cdot m^{-3} \cdot d^{-1}$, corresponding to a sludge loading rate of 13 $kgCOD \cdot m_{granules}^{-3} \cdot d^{-1}$. At higher sludge loading rates, the N-removal efficiency quickly deteriorates. At very low sludge loading rates, ammonia is quickly oxidized to nitrate. As soon as all ammonia is converted, the oxygen uptake rate decreases, resulting in a fully aerobic granule and thus in limited denitrification during the rest of the cycle. The higher the sludge loading, the longer it takes to consume all ammonia and thus the anoxic zones in the granule exist during a longer part of the cycle. This results in more N-removal via denitrification at high sludge loading rates. However, at sludge loading rates higher than 1.9 $kg \cdot m^{-3} \cdot d^{-1}$, the amount of ammonium oxidizers is too low relative to the incoming ammonium, resulting in ammonium in the effluent. This leads to a fast decrease in the N-removal efficiency and accumulation of ammonium in the system.

Phosphate removal efficiency shows the opposite trend of the ammonium removal. At loading rates lower than 1.3 $kgCOD \cdot m^{-3} \cdot d^{-1}$ (corresponding to 9 $kg \cdot m_{granules}^{-3} \cdot d^{-1}$),

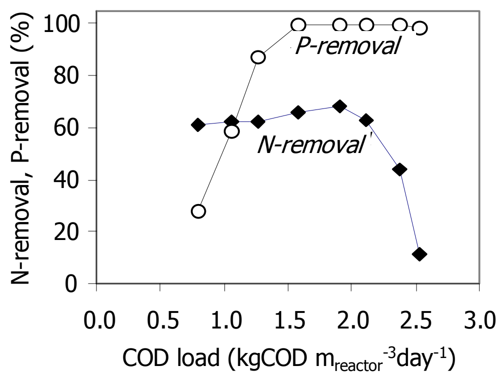


Figure 5 Influence of COD loading (in COD per m3 reactor volume per day) on the N-removal efficiency (◆) and P-removal efficiency (○)

the phosphate removal efficiency decreases. This can be explained by the fact that a decreased COD loading rate reduces the concentration of PHA accumulated in the granules (in the anaerobic phase). In turn, less PHA causes slower biomass growth rates and in consequence an increasing sludge age. For example, the solid retention time (SRT) or sludge age at sludge loading rates 11 and 9 $kg{\cdot}m_{granules}^{-3}{\cdot}d^{-1}$ are respectively 20 and 38 days. Since phosphate can only be removed with the biomass in the effluent, a long SRT results in an insufficient phosphate removal from the system. As the poly-P concentrations in the biomass increase to high values, the rates of poly-P storage will become too low to remove all phosphate from the influent, resulting finally in accumulation of phosphate in the reactor and in the effluent.

Cycle optimization; the effect of an extra denitrification period

Given the fact that ammonia and phosphate are both fully converted before the end of the SBR cycle and that nitrate accumulates in the standard operation (Figure 2), an extra anoxic phase after the aeration period could increase the total N-removal efficiency. In order to study this effect of a denitrification period at the end of the cycle, one simulation was performed with a 90 minutes aeration period, followed by an anoxic period of 22 minutes, instead of the normal aeration period of 172 minutes. Especially the denitrification capacity with the remaining stored PHA was studied.

Since all phosphate is already consumed after the first hour of aeration, the anoxic period did not negatively influence the overall phosphate removal efficiency. It did however increase the N-removal efficiency to 80%, by significantly reducing nitrate concentration in the effluent (Figure 6). This simulation showed that during the anoxic phase ammonia slightly increased due to biomass decay processes. Of the total incoming ammonium, 17% was discharged in the effluent as nitrate and 3% as ammonium. During the simulation of optimized SBR cycle, only very small changes were found in the pools of storage products, so the extra denitrification is not much affecting the PHA, glycogen, poly-P or the PAO concentrations in the granules. Therefore, it can be concluded that an extra denitrification period after the aerobic phase would be a logical step in the process optimization.

Discussion

In general, the mathematical model developed here described the experimental data well. However, an absolute prediction of the process with this model is difficult. Sensitivity analysis clearly showed that a small decrease in a conversion rate can easily lead to an increased concentration of that component in the effluent. Because the model is based on a SBR system, this component will accumulate during the next cycles, leading to effluent data incomparable to experimental results. However, the trends of the conversion rates are comparable to practice, so the model can be used to obtain insight in the process rates and in the granule structure.

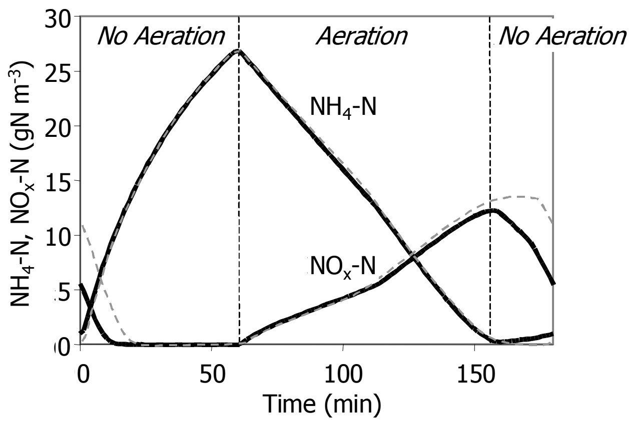


Figure 6 Simulated effect of an extra denitrification period at the end of the SBR cycle on the concentration profiles of N compounds (dotted lines: the standard cycle operation, solid lines: optimised cycle)

The main reason for the model to be less predictive is the complexity of the granular sludge itself. First of all, the granules in the model are of a certain diameter and are assumed to be perfect spheres. In reality, we are facing a wide range of granule sizes and shapes in a reactor (De Kreuk and Van Loosdrecht, 2004; Etterer and Wilderer, 2001; Toh et al., 2003). Less regularly shaped granules will lead to an increased surface to volume ratio, this influencing accordingly the oxygen transport into the granules. As can be seen from the simulations, mass transfer (especially the oxygen penetration in the granule) is decisive for the overall performance of the (simulated) system. It is possible to include several biofilm compartments with different granule diameters in AQUASIM, in order to simulate a granule size distribution, but this will make the model implementation and the interpretation of the data rather complex and will drastically increase the needed calculation time. Diffusive mass transport has never been a direct problem in activated sludge models (ASM), because in these models biomass is assumed in suspension in the bulk liquid. Consequently, nitrification and denitrification occur in different compartments, rather than simultaneously in the same reactor due to oxygen limitation in aerobic granules. This increases the ability of ASM's to predict effluent concentrations and overall system performance compared with the aerobic granular sludge systems.

When an aerobic granular sludge model must describe the behavior of this system in practice, like the ASM models, more system variables are needed due to the high complexity of the wastewater composition. In practice, the COD in the wastewater will partly consist of suspended COD. This needs to be hydrolyzed first, before organisms can use it for growth. Incorporating this substrate in the model implies the incorporation of heterotrophic biomass as well, since this relatively slow hydrolysis process will result in cell-external substrate during the aerobic period and thus to heterotrophic growth (McSwain et al., 2004; Schwarzenbeck et al., 2005). However, the exact rates and methods of conversion of suspended solids in aerobic granules are yet unknown, so more laboratory research will be needed first.

The granular sludge model was useful to obtain more insight in the biomass distribution within the granules. The increased concentration of autotrophic organisms in the outer layers of the granules and their shift to deeper layers when oxygen is available correspond to the hypothesis made from analyzing the experimental results. The trends in nutrient removal at varying granule diameter were also similar to the trends we found in practice. Simulation indicated that in the standard operating conditions the surface to volume ratio of aerobic granular sludge has its optimum at 600 to 700 m^2/m^3 reactor (corresponding to a granule diameter between 1.2 and 1.4 mm). Higher granule diameters lead to a permanent anaerobic core inside the granules and not enough autotrophic biomass in the outer aerobic layers, resulting in ammonia in the effluent. The duration of an anaerobic core existence within a SBR cycle also plays a crucial role in the N-removal at different biomass loading rates. The longer the anaerobic zone exists, the longer denitrification takes place and the higher the N-removal efficiency. As in activated sludge systems with biological phosphate removal, the phosphate removal efficiency in aerobic granules is depending on the sludge age because poly-P leaves the system incorporated in the biomass. By extrapolation, we estimate that when the SRT exceeds 30 days the amount of phosphate removed from the reactor with the biomass is less than the incoming phosphate and phosphate will be found in the effluent.

Optimization of nutrient removal efficiencies involves the cycle optimization. As it becomes clear from the simulations, the oxygen penetration depth plays a major role. The oxygen concentration in the bulk liquid could be stepwise controlled via the oxygen uptake rate. If the oxygen uptake rate decreases, it means that oxygen penetration depth increases. Lowering the oxygen concentration in the bulk when the oxygen uptake rate decreases is expected to lead to a better overall N-removal efficiency. A more simple solution is the incorporation of denitrification steps in the cycle as shown by simulations. The model could be useful to determine at which moment in the cycle the anoxic period would be most effective. Simulations suggest that at the end of the cycle the PHB pool needed for denitrification is still sufficient, increasing the possibilities of timing this denitrification period.

Conclusions

The presented model describes the performance of a laboratory scale SBR, fed with defined influent, capable of simultaneously removing COD, nitrogen and phosphate in one reactor. The model describes the experimental data from this complex system sufficiently well, as the simulated trends in nutrient removal were similar to those obtained with laboratory experiments. Simulation results underline the importance of oxygen penetration depth into the granules, and thus the ratio of anoxic and aerobic biomass, for overall nutrient removal. The model can be used for optimization of the nutrient removal efficiency and could form a starting point for models to design full-scale treatment plants.

Acknowledgements: This research was funded by the Dutch Foundation for Wat. Res. (STOWA, TNW99.262) and STW (DPC5577) within the framework of the "aerobic granule reactors" project. The project was performed in close co-operation with DHV in Amersfoort, The Netherlands. J. B. Xavier was financially supported by the FCT/MCTES, Portugal, through the Grant SFRH/BPD/11485/2002.

References

Beun JJ, Hendriks A, van Loosdrecht MCM, Morgenroth M, Wilderer PA, Heijnen JJ. (1999). Aerobic granulation in a sequencing batch reactor. *Wat. Res.* **33**(10) 2283-2290.

Beun JJ, Van Loosdrecht MCM, Heijnen JJ. (2001). N-removal in a granular sludge sequencing batch airlift reactor. *Biotechnol. Bioeng.* **75**(1) 82-92.

De Bruin LMM, Van der Roest HF, de Kreuk MK, Van Loosdrecht MCM. (2005). Promising results Pilot research aerobic granular sludge technology at WWTP Ede. In: Bathe S, de Kreuk MK, McSwain BS, Schwarzenbeck N, editors. Aerobic Granular Sludge. London, UK: IWA Publishing. p 135-142.

De Kreuk MK, Heijnen JJ, van Loosdrecht MCM. (2005a). Simultaneous COD, Nitrogen and Phosphate Removal by Aerobic Granular Sludge. *Biotechnol. Bioeng.* **90**(6) 761-769.

De Kreuk MK, Pronk M, Van Loosdrecht MCM. (2005b). Temperature effects on aerobic granulation process. *Wat. Res.* **39**(18) 4476-4484.

De Kreuk MK, Van Loosdrecht MCM. (2004). Selection of Slow Growing Organisms as a Means for Improving Aerobic Granular Sludge Stability. *Wat. Sci. Technol.* **49**(11-12) 9-19.

Dulekgurgen E, Yesiladali K, Ovez S, Tamerler C, Artan N, Orhon D. (2003). Conventional morphological and functional evaluation of the microbial populations in a sequencing batch reactor performing EBPR. *J. Env. Sci. Health Part a - Toxic/Hazardous Substances & Env. Eng.* **38**(8):1499-1515.

Etterer T, Wilderer PA. (2001). Generation and properties of aerobic granular sludge. *Wat. Sci. Technol.* **43**(3) 19-26.

Hao X, Heijnen JJ, Van Loosdrecht MCM. (2002a). Model-based evaluation of temperature and inflow variations on a partial nitrification ANAMMOX biofilm process. *Wat. Res.* **36**(19) 4839-4849.

Hao X, van Loosdrecht MCM, Meijer SCF, Heijnen JJ, Qian Y. (2001). Model-Based Evaluation of Denitrifying P Removal in a Two-Sludge System. J. Env. Eng. **127**(2):112-118.

Hao XD, Heijnen JJ, van Loosdrecht MCM. (2002b). Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process. *Biotechnol. Bioeng.* **77**(3) 266-277.

Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC, Marais GvR, Van Loosdrecht MCM. (1999). Activated Sludge Model No.2d, ASM2D. *Wat. Sci. Technol.* **39**(1) 165 - 182.

Henze M, Gujer W, Mino T, van Loosdrecht M. (2000). Activated Sludge Models; ASM1, ASM2, ASM2d and ASM3. London: IWA Publishing. 130 p.

Li XM, Yang GJ, Yang Q, Zeng GM, Liao DX, Hu MF, Wu YM. (2005). Simultaneous phosphorus and nitrogen removal by aerobic granular sludge in single SBR system. In: Bathe S, De Kreuk MK, McSwain BS, Schwarzenbeck N, editors. Aerobic Granular Sludge. London, UK: IWA. p 71-78.

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

Lübken M, Schwarzenbeck N, Wichern M, Wilderer PA. (2005). Modelling nutrient removal of an aerobic granular sludge lab-scale SBR using ASM3. In: Bathe S, De Kreuk MK, Mc Swain BS, Schwarzenbeck N, editors. Aerobic Granular Sludge. London, UK: IWA. p 103-110.

McSwain BS, Irvine RL, Wilderer PA. (2004). The effect of intermittent feeding on aerobic granule structure. *Wat. Sci. Technol.* **49**(11):19-25.

Meijer SCF. (2004). Theoretical and practical aspects of modelling activated sludge processes [PhD.]. Delft: Technical University. 204 p.

Morgenroth E, Sherden T, van Loosdrecht MCM, Heijnen JJ, Wilderer PA. (1997). Aerobic Granular Sludge in a Sequencing Batch Reactor. *Wat. Res.* **31**(12):3191 - 3194.

Murnleitner E, Kuba T, van Loosdrecht MCM, Heijnen JJ. (1997). An integrated metabolic model for the Aerobic and denitrifying Biological phosphorus removal. *Biotechnol. Bioeng.* **54**(5):434 - 450.

Reichert P. (1998). AQUASIM 2.0 - Computerprogram for the Indentification and Simulation of Aquatic Systems. Version 2.0. Dubendorf: EAWAG.

Schwarzenbeck N, Borges JM, Wilderer PA. (2005). Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. *App. Microbiol. Biotechnol.* **66**(6):711-718.

Smolders GJF, van der Meij J, Van Loosdrecht MCM, Heijnen JJ. (1994). Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. *Biotechnol. Bioeng.* **43** 461-470.

Tay JH, Liu QS, Liu Y. (2002). Aerobic granulation in sequential sludge blanket reactor. *Wat. Sci. Techn.* **46**(4-5) 13-18.

Toh SK, Tay JH, Moy BYP, Ivanov V, Tay STL. (2003). Size-effect on the physical characteristics of the aerobic granule in a SBR. *App. Microbiol. Biotechnol.* **60**(6):687-695.

van Veldhuizen HM, van Loosdrecht MCM, Heijnen JJ. (1999). Modelling biological phosphorus and nitrogen removal in a full scale activated sludge process. *Wat. Res.* **33**(16) 3459-3468.

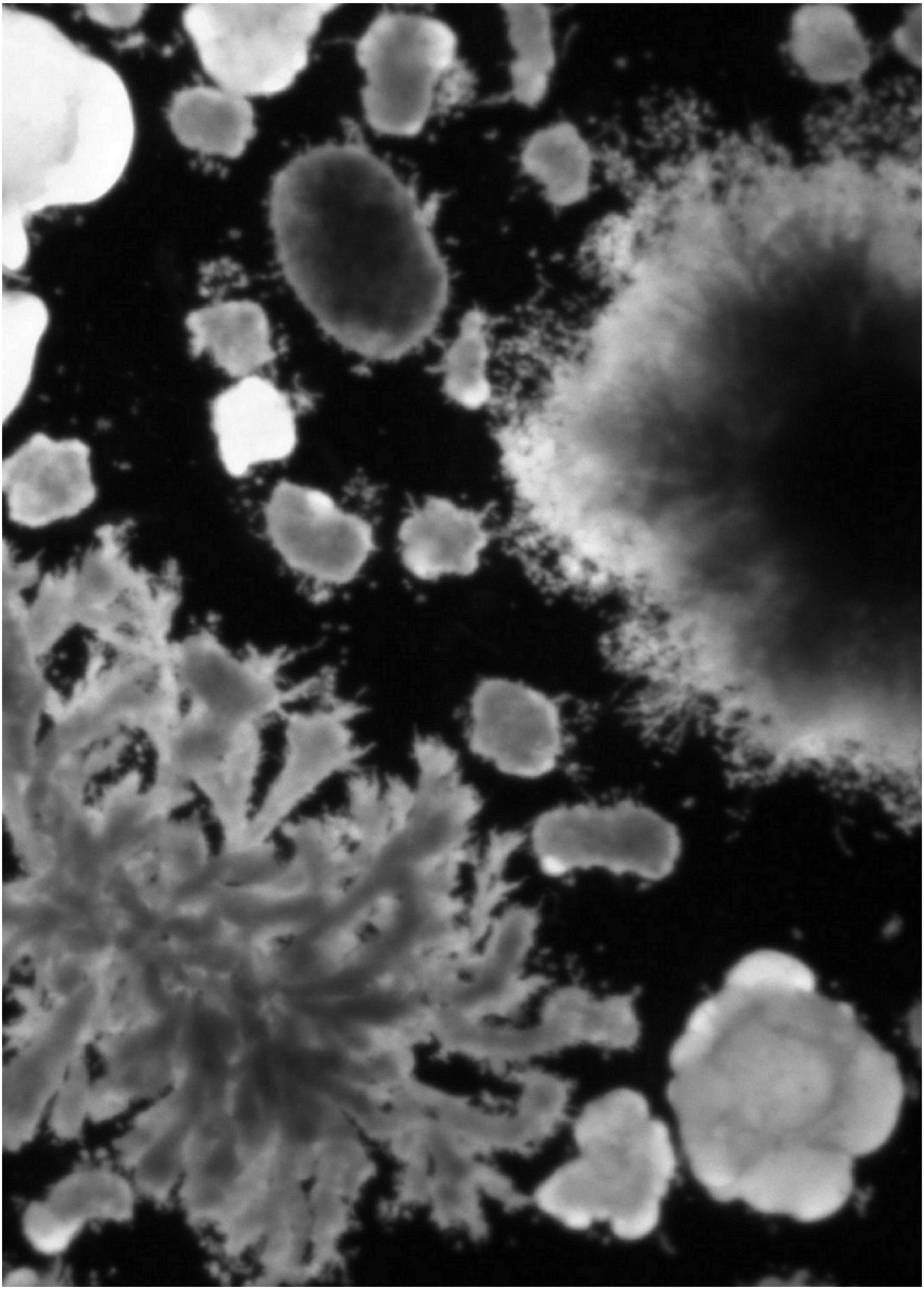
Wanner O, Gujer W. (1986). A multispecies biofilm model. *Biotechnol. Bioeng.* 28(3):314-328.

Wentzel MC, Ekama GA, Loewenthal RE, Dold PL, Marais GR. (1989). Enhanced polyphosphate organism cultures in activated sludge systems. Part II: experimental behavior. *Water SA* **15**(2) 71-88.

Wiesmann U. (1994). Biological nitrogen removal from wastewater. *Adv. Biochem. Eng./Biotechnol.* **51** 113 - 154.

Wijffels RH, Tramper J. (1995). Nitrification by immobilized cells. *Enz. Microbial Technol.* **17**(6) 482-492.

Xavier JB, de Kreuk MK, Picioreanu C, Van Loosdrecht MCM. (In Preparation). Microbial populations dynamics and nutrient conversions in aerobic granular sludge described by multi-scale modelling.



7 A feasibility study on Nereda^{TM}; an alternative for activated sludge?

Abstract

Laboratory experiments have shown that it is possible to cultivate aerobic granular sludge in sequencing batch reactors. In order to direct future research needs and the critical points for successful implementation at large scale, a full detailed design of a potential application was made. The design was based on the laboratory results and two variants of a full-scale sewage treatment plant based on Granular sludge Sequencing Batch Reactors (GSBRs), marketed as Nereda^{TM} were evaluated. As a reference a conventional treatment plant based on activated sludge technology was designed for the same case.

Based on total annual costs both Nereda^{TM} variants proved to be more attractive than the reference alternative (7-17% lower costs). From a sensitivity analysis it appeared that the Nereda^{TM} technology was less sensitive to the land price and more sensitive to a rain water flow (RWF). This means that the Nereda^{TM} becomes more attractive at lower permissible RWF/DWF ratios and higher land prices. The footprint of the Nereda^{TM} was only 25% compared to the reference. However, the Nereda^{TM} with primary treatment only, cannot meet the present effluent standards for municipal wastewater in the Netherlands, mainly because of a too high suspended solids concentration in the effluent.

A growing number of sewage treatment plants in the Netherlands is going to be faced with more stringent effluent standards. In general, activated sludge plants will have to be extended with a post treatment step (e.g. sand filtration) or being transformed into a Membrane Bio Reactor. In this case a Nereda^{TM} with primary treatment as well as post treatment can be an attractive alternative.

This chapter was published as De Bruin, L.M.M., De Kreuk, M.K., van der Roest, H.F.R., Van Loosdrecht, M.C.M. and Uijterlinde, C. (2004) "Aerobic granular sludge technology, alternative for activated sludge technology?". *Wat. Sci. Technol.49* (11-12) 1-9.

Introduction

Conventional Wastewater Treatment Plants (WWTPs) based on activated sludge technology require a large footprint. This is caused by the relatively poor settling characteristics of activated sludge, resulting in low permissible dry solids (DS) concentrations in aeration tanks and in a low maximum hydraulic load of secondary sedimentation tanks. In the nineties of last century, compact attached growth technologies in several configurations were developed (immobilised bed, fluidised bed and airlift reactors). Main feature of these, continuously operated technologies is a high volumetric load, occasionally without a separate sludge/water separation step. The process conditions in airlift reactors are simple and the area requirement is limited. Because of the large specific biofilm area, the volumetric conversion capacities can be high (Heijnen et al., 1993). Main disadvantage of these systems is the relative high investment costs. Recent research showed that it is possible to grow granular sludge in a batch wise operated system without a carrier at high dissolved oxygen (DO) concentrations (>80%) resulting in high biomass concentrations and high volumetric loads (7.5 kgCOD $(m^3.d)^{-1}$) (Beun et al., 2000, Etterer and Wilderer, 2001). Because of the outstanding settling properties and sequential operation the use of a traditional or integrated settler is not necessary. Separation of sludge and effluent occurs within the reactor during a short settling phase. No long idle times due to sludge settling are required in these Granule Sequencing Batch Reactors (GSBRs).

Presently, research is focussing on the mechanism of granulation and although the precise mechanism is not known (yet) the following process conditions are assumed to play an important role:

- A short settling time results in the selection of well settling biomass, because biomass particles with low settling velocities are washed out with the effluent. At laboratory scale, granules with settling velocities higher than 12 m h^{-1} were maintained in the reactor;
- Applying high substrate gradients, for example by means of a pulse feed or a plug flow feed through the settled bed, is essential for granulation and for simultaneous conversion processes within the granule. Substrate diffuses into the core of the granules and a fraction is converted into a storage product as poly-β-hydroxybutyrate (PHB). As a result substrate is equally distributed in the granule and the actual growth rate of the organisms will be lower, because of the growth on storage polymers. This growth rate combined with a non-

transport-limited system, results in the formation of smooth and dense granules (Picioreanu et al., 1998). Another aspect of substrate diffusion throughout the granules is that shortages and decay of bacteria are minimised, which reduces disintegration of granules and which enables simultaneous COD and N-removal;

- Formation of smooth and dense granules is, as is showed in biofilm research (Gjaltema et al., 1997, Tay et al., 2001, Kwok et al., 1998), stimulated when granules are exposed to large shear forces caused by intensive (non-mechanical) mixing in the reactor. Because of the requirement of sufficient shear forces laboratory research up to now has been carried out in an airlift reactor.

Laboratory research has shown good results concerning simultaneous COD-, N- and P-removal, when an anaerobic feeding phase was combined with a low DO (10-20 % saturation) in the aerated period. In a 3-litre laboratory scale reactor synthetic wastewater was fed as a plug-flow through the settled bed of granules, during an anaerobic period of one hour (total cycle time three hours). The composition of the synthetic wastewater based on COD (with acetate as carbon source), N_{kj} and P_{total} corresponds with sewage. The aforementioned feeding pattern proved to generate more stable granules than with a pulse feed of three minutes. This was due to the selection of relative slow growing phosphate-accumulating organisms (PAO's). These organisms store acetate as PHB during the anaerobic feeding period while releasing phosphate; while during the aerated period PAO's use the stored PHB as carbon source and take up the released phosphate again. During aeration, ammonia is converted to nitrate, which can serve as an electron acceptor for PAO's in the core of granules where oxygen is depleted. To enlarge the anoxic zone volume within granules, a low DO was applied (20%). At these conditions high COD-, N- and P-removal efficiencies were reached and amounted respectively 100%, 98% and 99%.

The effect of different hydrodynamic conditions (shear stress) was tested by comparing granulation in an airlift reactor and in a bubble column. The disadvantage of a bubble column is a lower effective shear force compared with an airlift reactor, which may influence granulation negatively. Except for a longer start-up phase, granulation occurred in the bubble column with a similar performance compared to the airlift reactor.

Table 1 Starting points costs calculations

Cost factor	Unit	Value
Electricity	€/kWh	0.054
Sludge disposal	€/tonDrySolids	320
Iron chloride (41%)	€/ton	115
Land price	€/m^2	22.7
Poly electrolyte (liquid, 50%)	€/kg active	3.6
Maintenance civil parts	% of investments	0.5
Maintenance mechanical parts	% of investments	2
Maintenance electro technical parts	% of investments	2

An experiment with presettled sewage showed good results towards granulation. Granular sludge with a sludge volume index (SVI) of 36 ml g^{-1} and an average diameter of 1.1 mm was formed in a laboratory reactor.
The complete laboratory research, which results are summarised above, will be further outlined in other publications. In this paper the feasibility of a full-scale WWTP based on aerobic granular sludge technology was evaluated by means of a comparison with a conventional WWTP based on activated sludge technology.

General assumptions

A standard Dutch wastewater composition was used, as defined by the Dutch foundation for applied research for water management (STOWA): 600 mg L^{-1} COD (216 mg L^{-1} dissolved; 384 mg L^{-1} suspended); 55 mgNkj L^{-1} (45.7 mg L^{-1} dissolved; 9.3 mg L^{-1} suspended), 9 mg L^{-1} P-total and 250 mg L^{-1} suspended solids. The average wastewater flow 160 L (p.e. d)$^{-1}$ was taken with a peak Rain Weather Flow (RWF) of 34 L (p.e. h)$^{-1}$. The effluent requirements are based on the discharge regulations for municipal wastewater in the Netherlands, meaning extensive total nitrogen en phosphate removal (N-total = 10 mg L^{-1} and P-total = 1 mg L^{-1}). Primary treatment consists of conventional sedimentation and the removal of suspended solids depends on the dosing of flocculants. Removal efficiencies for suspended solids of 50% (no flocculants) and 80% (dosing of flocculants) were taken. Metal salts were not dosed in the Nereda™ variants because of the need for the selection of PAO's.
For the calculation of the capital costs depreciation periods for civil parts and mechanical/electrical parts of respectively 30 and 15 years were used. Capital costs were calculated based on annuities with an interest rate of 6%. Operational costs

were based on main cost factors such as sludge disposal costs, power use of aerators, chemical use and maintenance costs. The power use was corrected for power production generated from biogas. In Table 1 the starting points for the calculation of the operating costs are given.

Treatment alternatives and technological starting points

Two alternatives of aerobic granular sludge technology were compared to a conventional WWTP based on activated sludge technology. The following Nereda^{TM} variants were taken into account:

- Nereda^{TM} with primary treatment including chemical dosing with extensive removal of suspended solids. Post treatment was assumed not to be required;
- Nereda^{TM} with post treatment only. The post treatment consists of removal of suspended solids from the effluent from the GSBR (see figure 1).

Figure 2 gives a global process flow diagram (PFD) of the reference variant. The calculations of the reference alternative were based on a process temperature of 10 ^{o}C, a design sludge load of the aeration tanks of 0.14 kgCOD (kgDS d)$^{-1}$ and a SVI of 150 ml g^{-1} for the design of secondary sedimentation tanks.

The design of the Nereda^{TM}-alternatives was based on biological phosphate removal, which was assumed to be possible in a full-scale reactor applying alternating anaerobic feeding periods and aerobic reaction periods. Preliminary calculations showed that chemical phosphate removal is too costly because of a higher sludge production resulting in increased sludge disposal costs and also because of a higher chemical use.

In order to be assured of plug flow conditions while feeding from the bottom of the reactor through the settled granules bed, the maximum hydraulic surface load during the feeding period was chosen at 7.5 m h^{-1}. This leads to a construction height of the Nereda^{TM}, which were assumed to be built as bubble columns, of 5-6 m. The granule sedimentation velocity was estimated at 15 m h^{-1}. The total cycle time amounts to 60 minutes, which is formed by 20 minutes anaerobic feeding period, 27 minutes aeration or reaction phase, 5 minutes sedimentation followed by 7.5 minutes decantation. The COD design load of the Nereda^{TM} was chosen at 0.3 kgCOD (kgDS d)$^{-1}$, which corresponds with a pilot research for the application of an airlift reactor treating municipal wastewater (STOWA, 1997). In this research the net sludge production was almost zero, meaning equal sludge content in influent and effluent.

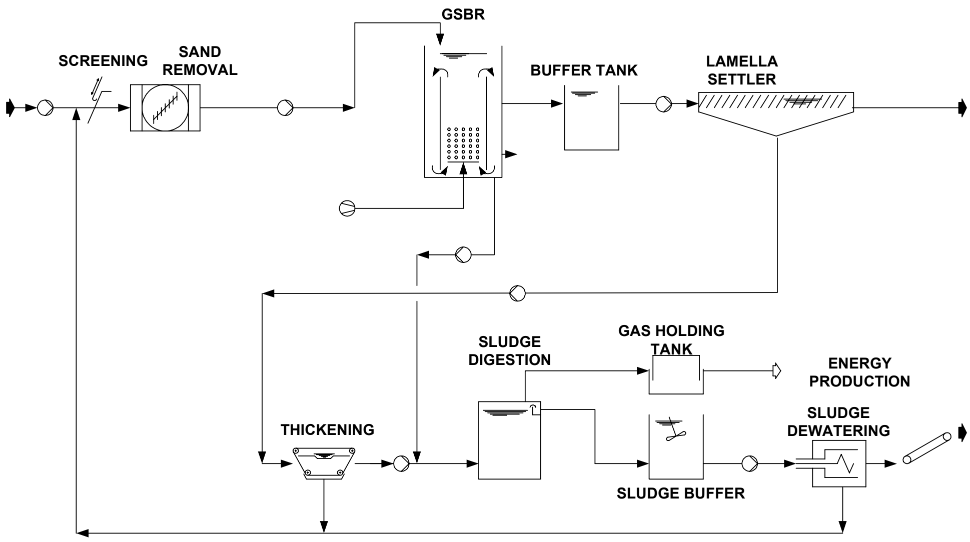


Figure 1 Process scheme for Nereda™ *with post treatment*

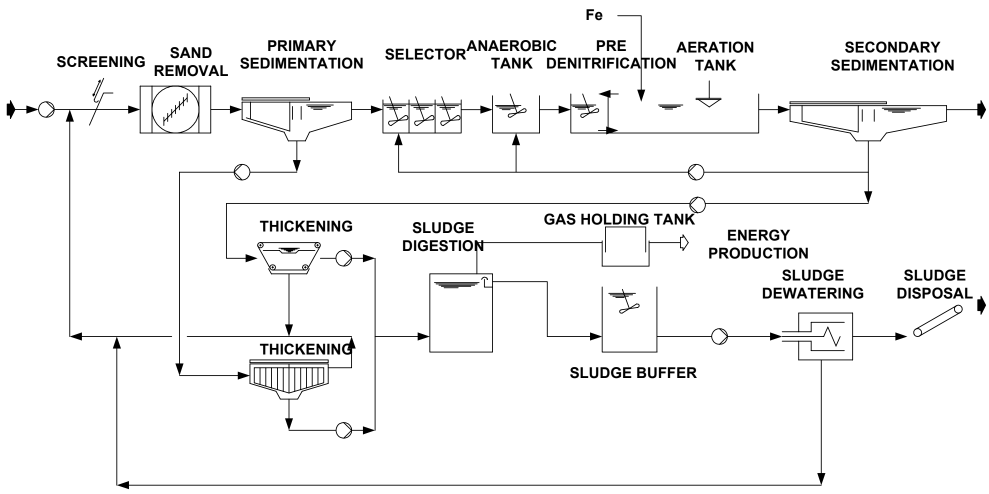


Figure 2 Process scheme for reference treatment plant

Table 2 Estimated effluent quality for Nereda^{TM} variants

Parameter	Nereda^{TM} with primary treatment	Nereda^{TM} with post treatment
COD (mg L^{-1})	80	40
Suspended solids (mg L^{-1})	50	< 10
Nkj (mgN L^{-1})	4.1	2.0
NO_3^- (mgN L^{-1})	5.9	8.0
Ptotal (mg L^{-1})	1.5	1.0

The number of parallel treatment lines was determined by the length of feeding time compared to the total cycle time. Assumed was that at most one reactor can be fed with wastewater, which would lead to three treatment lines if the filling time is a third part of total cycle time. In case of post treatment, the overflow from the Nereda^{TM} is buffered. Because effluent is discharged by gravity, the initial flow is high. In order to reduce the dimensions of the post treatment step, the effluent of the Nereda^{TM} has to be buffered.
Sludge treatment for the reference and Nereda^{TM}-alternatives consists of gravitational thickening of primary sludge, mechanical thickening of surplus sludge, digestion and dewatering.

Results

Table 2 shows the effluent qualities for both Nereda^{TM}-alternatives. Based on the influent characteristics and technological starting points the Nereda^{TM} with post treatment can meet the effluent requirements. The Nereda^{TM} with primary treatment does not meet the required effluent quality with respect to suspended solids. This is caused by an insufficient removal of suspended solids in the primary treatment and the assumption that suspended solids in a Nereda^{TM} cannot be removed. A high suspended solids effluent concentration results also in increased Nkj- and P-total concentrations.
The effluent requirements for the alternative with only primary treatment can only be met if the suspended solids concentration in the wastewater fed to the Nereda^{TM} is less than 10-30 mg L^{-1}. However, this does not mean that a Nereda^{TM} with primary treatment is not an attractive concept. If a more stringent effluent quality is required (e.g. N-total = 2.2 mg L^{-1}, P-total = 0.15 mg L^{-1}), which is in the Netherlands actual for a growing number of WWTP's discharging on sensitive surface waters, conventional activated sludge systems have to be extended with a

post treatment step (e.g. with sand filtration) or can be transformed in Membrane Bio Reactors. In this case a Nereda™ with primary treatment as well as post treatment can be an attractive alternative.

The footprint of the total treatment plant was calculated by the sum of the net surfaces of all process units and buildings, multiplied by a factor 1.3. The calculations show that the footprints of the Nereda™-alternatives were only around 25% of the footprint of the reference alternative. It can be concluded that the aerobic granular sludge technology is very compact, which is an important advantage in relation to activated sludge technology, especially in densely populated areas.

Figures 3 and 4 show respectively the investment costs and the total specific annual costs (sum of capital and operational costs). Despite the fact that a Nereda™ with primary treatment cannot comply with the effluent standards, the

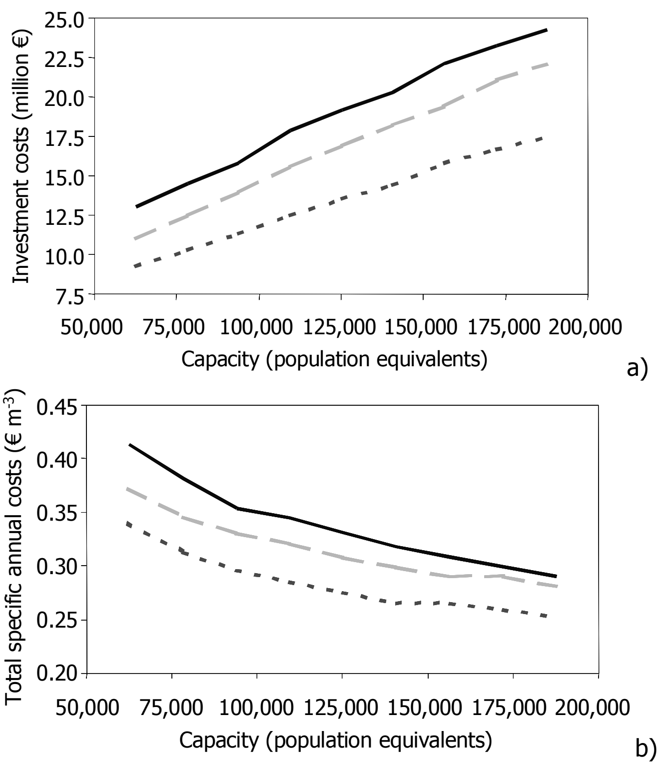


Figure 3 Investment costs (a) and total specific annual costs of the reference activated sludge system (line); the Nereda™ *with primary treatment (dashed) and the* Nereda™ *with post treatment (long dashed)*

costs for this variant are also depicted in figures 3 and 4. As can be seen from figure 3 the investments for the Nereda^{TM}-alternatives are lower compared to the reference alternative (on the average 15-30%). As can be expected, the investment costs for the Nereda^{TM} with primary treatment appear to be lower than the Nereda^{TM} with post treatment. The lower hydraulic design load for secondary treatment in comparison with primary treatment mainly causes this.

On the basis of the total specific annual costs the picture does not change (Figure 4). Again the Nereda^{TM}-alternatives prove to be the most attractive. The total annual costs of the Nereda^{TM} with primary respectively post treatment are on the average 17% and 7% lower compared to the reference alternative. The capital costs of the Nereda^{TM}-alternatives are relatively high because of the high share of the mechanical/electrical works on the investments (40-45%). In general, the part of the mechanical/electrical works for conventional activated sludge systems amounts to 25-30%.

Sensitivity analysis

The influence of the RWF/DWF ratio and the land price on the total annual costs was calculated for a Nereda^{TM} with post treatment as well for the reference, both for a capacity of 120,000 p.e. The results of the calculations are given in figure 5. The Nereda^{TM} concept appears to be less sensitive to the land price than the reference, which is logical because of the compactness of the technology. On the other hand the Nereda^{TM} technology is more sensitive to an increasing RWF

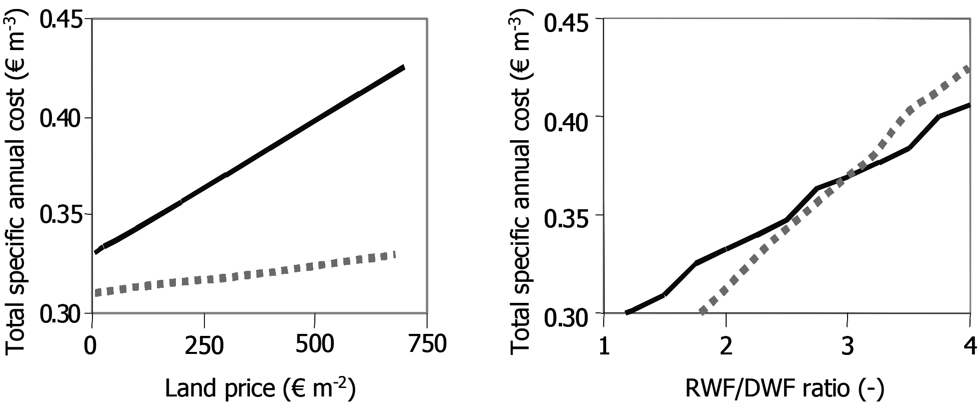


Figure 5 Sensitivity of the total annual costs of the reference activvated sludge system (line) and the Nereda^{TM} with post treatment (dashed line) for the landprice and contribution of rain weather flow

compared to activated sludge technology. The reason for this higher sensitivity was because of the large impact of the maximum batch volume on the design of the Nereda^{TM}. At higher RWF the maximum batch to be treated increases and as a result of this also the volume of the reactor increases.

Energy requirement

The Nereda^{TM} is a one-reactor technology, which means that all processes take place in the same reactor, only separated in time. In activated sludge systems the different circumstances needed for the different nutrient removal steps are separated in space. Therefore, the water/sludge mixture needs to be circulated over the plant (e.g. recirculation from pre-denitrification tank to the anaerobic tank; recirculation from the aeration tank to the pre-denitrification tank; recirculation from sludge from the settling tank to the aeration tank, etc.). Also mixing and maintaining the flow on the reactors is an energy requiring step. All mentioned processes are responsible for 15% to 30% of the total energy requirement of a conventional activated sludge system, depending on the aeration system used. If a one reactor system as Nereda^{TM} is used, these recirculation flows could be avoided, reducing the total energy consumption significantly. This energy reduction has not yet been incorporated in the annual costs presented in this feasibility study.

Conclusion

Aerobic granular sludge is a very promising technology from an engineering as well as economic point of view, and should therefore be further developed. Essentially will be the demonstration of combined N, P and COD removal at field conditions with municipal wastewater.

Based on total annual costs both Nereda^{TM} variants prove to be more attractive than the reference alternative (7-17%). Additional calculations showed a low sensitivity to the land price and a high sensitivity to rain weather flow. The footprint of the Nereda^{TM} variants is only 25% compared to a conventional activated sludge design.

A growing number of sewage treatment plants in the Netherlands are going to be faced with more stringent effluent standards. In general, activated sludge plants will have to be extended with a post treatment step (e.g. sand filtration) or will have to be transformed into a Membrane Bio Reactor. In this case a Nereda^{TM} with primary treatment as well as post treatment can be an attractive alternative.

Acknowledgements: This research was funded by the Dutch Foundation for Water Research (STOWA) within the framework of the "aerobic granule reactors" project (TNW99.262) as well as by the Dutch technology Foundation (STW) under project number DPC.5577.

References

Beun, J.J., M.C.M. van Loosdrecht and J.J. Heijnen (2000). Aerobic Granulation. *Wat. Sci. Technol.* **41** (4-5). 41-48.

Etterer, T. and P.A. Wilderer (2001). Generation and properties of aerobic granular sludge. *Wat. Sci. Technol.* **43** (3). 19-26.

Gjaltema, A., J.L. Vinke, M.C.M. Van Loosdrecht and J.J. Heijnen (1997). Abrasion of suspended biofilm pellets in airlift reactors: Importance of shape, structure and particle concentrations. *Biotechnol. Bioeng.* **53.** 88 - 99.

Heijnen, J.J., M.C.M. Loosdrecht, R. Mulder, R. Weltevrede and A. Mulder (1993). Development and scale-up of an aerobic biofilm air-lift suspension reactor. *Wat. Sci. Technol.* **27** (5-6). 253-261.

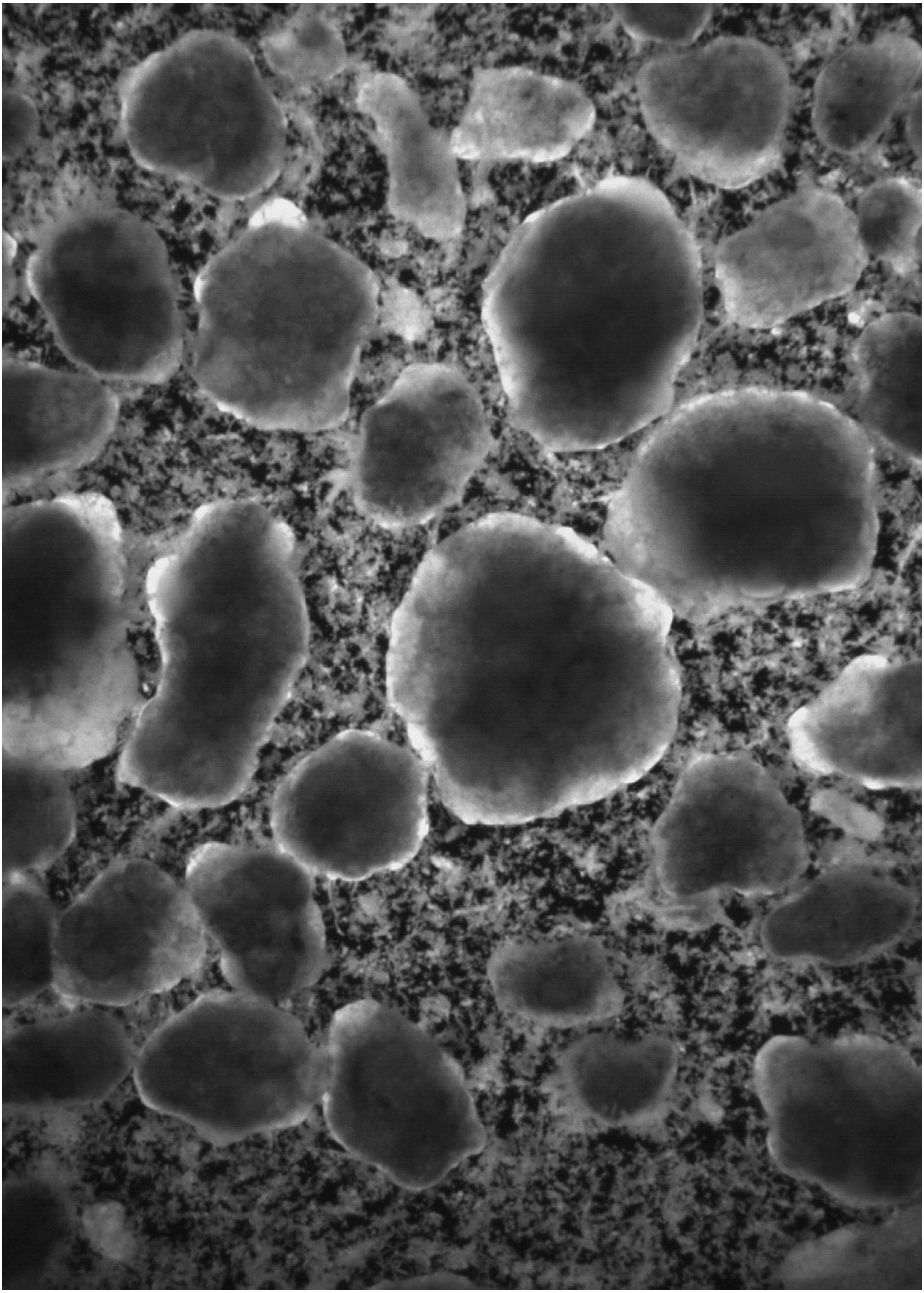
Kwok, W.K., C. Picioreanu, S.L. Ong, M.C.M. van Loosdrecht, W.J. Ng and J.J. Heijnen (1998). Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. *Biotechnol. Bioeng.* **58** (4). 400- 407.

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

STOWA (1997). Zuivering van stedelijk afvalwater in een airlift-biofilmreactor - uitontwikkeling en demonstratie. Utrecht, STOWA. 96p.

STOWA (2001). Handboek biologische fosfaatverwijdering. Utrecht, Stichting Toegepast Onderzoek Waterbeheer. 234p.

Tay, J.H., Q.S. Liu and Y. Liu (2001). The effects of shear force on the formation, structure and metabolism of aerobic granules. *Appl. Microbiol. Biotechnol.* **57**. 227 - 233.



8 Formation of Aerobic Granules with Domestic Sewage

Abstract

The use of aerobic granules in wastewater treatment can reduce the land area that is needed for the treatment of sewage. Until now granulation has been mainly studied using artificial wastewater. Studying the possibility of forming aerobic granules on domestic sewage in a sequencing batch reactor was a logical step in the scaling-up process and development of this technology. Therefore, aerobic granulation was studied using presettled sewage as influent. After 20 days of operation at high chemical oxygen demand (COD)-loading heterogeneous aerobic granular structures were observed, with a sludge volume index (SVI) after 10 minutes settling of 38 $ml \cdot g^{-1}$ and an average diameter of 1.1 mm. Applying a high COD-load was found to be a critical factor for the formation of aerobic granules on this type of influent.

The positive results of the experiment with domestic sewage subsequently led to a pilot-scale test at wastewater treatment plant (WWTP) Ede, The Netherlands. Two bubble columns of 0.6 m diameter and 6 m height (effective volume 1.5 m^3) were used. During steady-state operation, dry weight concentration was 10 g L^{-1}, of which 80% consisted of granular sludge. Sludge loading rates up to 0.212 gCOD $gTSS^{-1}$ d^{-1} were applied. The sum of ammonia and nitrate in the effluent were below 10 mg L^{-1} even at average temperatures of 13°C. Average phosphate concentrations in the effluent were 0.9 mg L^{-1} and suspended solids concentrations in the effluent were on average 10 – 20 mg L^{-1}. A simple drum filter could lower this to 4 mg L^{-1}, even during additional wash-out of suspended solids during increased influent flow rates.

This Chapter is a combination of 2 publications: Part I is accepted for publication by the Journal of Environmental Engineering (ASCE) as De Kreuk, M.K. and Van Loosdrecht, M.C.M. (2006). "Formation of aerobic granules with domestic sewage". Part II is a selection from: De Bruin, B., De Kreuk, M.K., Uijterlinde, C. and Marcelis, C. (2006). "Aëroob korrelslib klaar voor de praktijk." *H2O* 39(3), 31-34.

Introduction

Aerobic granular sludge could form the heart of a new compact wastewater treatment system based on sequencing batch reactor (SBR) technology (De Bruin et al. 2004; Heijnen and Van Loosdrecht 1998). Several studies showed that aerobic granules are stable aggregates of biomass that settle well and which can be able to convert organic substrates, nitrogen compounds and phosphate simultaneously (De Kreuk and Van Loosdrecht 2004; Dulekgurgen et al. 2003; Lin et al. 2003). Because of settling characteristics and the specific structure of aerobic granules, the land area needed for a domestic sewage treatment plant could be reduced by 80% when a granular sludge system is applied instead of conventional activated sludge systems (De Bruin et al. 2004). The state of the art knowledge of granule formation and critical process conditions were reviewed by Liu and Tay (2004) and by De Kreuk et al. (2005).

So far, granule formation and conversion processes have been studied in well-controlled laboratory set-ups using different types of synthetic influent (among others: Beun et al. 1999; De Kreuk and Van Loosdrecht 2004; Moy et al. 2002) and industrial influent (Arrojo et al. 2004; Inizan et al. 2004; Schwarzenbeck et al. 2004). Reported experiments with synthetic influent did not consider complex substrate and suspended solids and only preliminary investigations with domestic and industrial influent are described in literature. Mishima and Nakurama (1991) were the first to report aerobic granules on sewage, using a continuously operated upflow aerobic sludge reactor, with pure oxygen injection in the influent. This is costly but necessary because this enhances granule formation in their case. They reported large granular structures (2-8 mm) with a relatively high average sludge volume index (SVI; 72 ml g^{-1}).

Most recent experiments on aerobic granular sludge described in literature are performed in a discontinuously fed sequencing batch reactor (SBR), using aerated pulse feeding or an anaerobic feeding period, followed by an aeration period using pressurized air. Formation of aerobic granular sludge on domestic sewage in this type of SBR systems has not been reported so far. Domestic sewage typically has a much lower COD content than industrial wastewater and it can be expected that this will influence the granule formation process (Moy et al. 2002). Therefore, aerobic granular sludge formation was studied using pre-settled sewage in a 3-litre sequencing batch airlift reactor (SBAR; part I). This led to a pilot-scale (1.5 m^3) test at wastewater treatment plant (WWTP) Ede, The Netherlands (Part II).

PART I: Laboratory scale experiment with presettled sewage

Methodology

Reactor set-up and analysis

A double walled 3-litre SBAR was used (internal diameter of 6.25 cm, containing a riser of 90 cm high, 4 cm internal diameter and clearance 1.25 cm). Air was introduced via a fine bubble aerator at the bottom of the reactor (4 $L \cdot min^{-1}$ or superficial upflow velocity 0.025 $m \cdot s^{-1}$). Dissolved oxygen (DO) concentration and pH were measured continuously. The pH was maintained at 7.0 ±0.2 by dosing 1 M NaOH or 1 M HCl. Oxygen concentration was close to 100% of oxygen saturation during the whole aeration time (100% = 9.1 $mg \cdot L^{-1}$). Temperature was kept at 20°C. Hydraulic retention time (HRT) was 5.6 hour. Effluent was extracted from a port positioned at half the reactor height.

Initially the reactor was operated in successive cycles of 3 hours: 60 minutes feeding from the bottom of the reactor (plug-flow through the settled bed), 115 minutes minus the applied settling time for aeration, variable settling (6 to 15 minutes) and 5 minutes effluent discharge. During the second period, cycles were shortened to two hours: 30 minutes of anaerobic feeding; a 15 minutes anaerobic period; 70 minutes minus the applied settling time for aeration, variable settling (6 to 10 minutes) and 5 minutes discharge.

Particle diameter, density, dry weight (total suspended solids or TSS) and ash content of the granules and total organic carbon (TOC), volatile fatty acids (VFA) and biomass concentration in the effluent (volatile suspended solids or VSS) were measured as described in Beun et al. (2001). NH_4^+, NO_2^-, NO_3^-, total N (organically

Table 1 Composition of sewage and synthetic influent and of the effluent

Component ($mg \cdot L^{-1}$)	Dosed sewage[1]	Effluent after 2 hour cycle[2]
Volatile suspended solids	160 (*58*)	100 (*26*)
Soluble COD	280 (*62*)	130 (*38*)
Total COD	330 (*64*)	170 (*67*)
VFA (COD)	80 (*19*)	1 (*0.9)*
PO_4^{3-}-P	9 (*2*)	6 (*3.5*)
NH_4^+-N	57 (*8*)	51 (*9*)
Total-N	70 (*11*)	60 (*13*)

[1] *This Study: influent values after storage at 4°C, averages of 8 collected batches;*

[2] *Average effluent composition; standard deviations between brackets.*

and inorganically bound nitrogen), PO_4^{3-} and COD concentrations in influent and effluent were measured using test kits (Dr. Lange type LCK, Manufacturer: Hach Lange, Düsseldorf, Germany). The SVI_{10} was determined by reading the height of the bed after a settling period of 10 minutes and calculated from the settled bed volume and the dry weight in the reactor.

Selection and treatment of the influent

The influent was collected at a domestic wastewater treatment plant (WWTP) in the city of Berkel, in The Netherlands and was taken directly after the screen (125 litres per batch, divided in 5 tanks). Tanks were stored at 4°C for a maximum of 10 days. During this storage time, the suspended solids that settled were removed. Every 2 days, an influent tank was filled with the stored wastewater. This influent tank was kept at 10°C by a thermostatically controlled bath. Every cycle a volume of 1.5 litres was heated to room temperature and dosed into the reactor. The composition of the influent dosed per reactor cycle is presented in Table 1.

Results and Discussion

The main goal of this experiment was to demonstrate granule formation on sewage in a SBR system. In order to do so, the reactor was started-up with influent from WWTP Berkel in The Netherlands and inoculated with activated sludge and suspended solids (<0.2 mm) from the effluent of a laboratory scale granular sludge reactor fed with synthetic influent. A 3-hour cycle was selected according to positive results from earlier experiments (De Kreuk and Van Loosdrecht 2004). However, the COD concentration of sewage was lower than the concentration of earlier used synthetic influent (composition: 396 $mgCOD \cdot L^{-1}$; 50 mg $NH_4\text{-}N \cdot L^{-1}$; 19.6 mg $PO_4\text{-}P \cdot L^{-1}$), resulting in a lower availability of readily biodegradable COD (1.0 kg $COD \cdot m^{-3} \cdot day^{-1}$, instead of 1.6 kg $COD \cdot m^{-3} \cdot day^{-1}$ using synthetic influent). This lower load resulted in insufficient biomass increase in time (Figure 1). All particles and biomass with a settling velocity lower than 2 $m \cdot h^{-1}$ washed out of the reactor. In order to increase the COD-load, the cycle time was shortened to 2 hours.

20 Days after changing the cycle time or 36 days after start-up, granules were obtained with an average diameter of 1.1 mm. The SVI_{10} decreased to 38 $ml \cdot g^{-1}$, comparable to SVI's found with synthetic influent (De Kreuk and Van Loosdrecht 2004; Dulekgurgen et al. 2003; Lin et al. 2003). In contrast to granules grown on

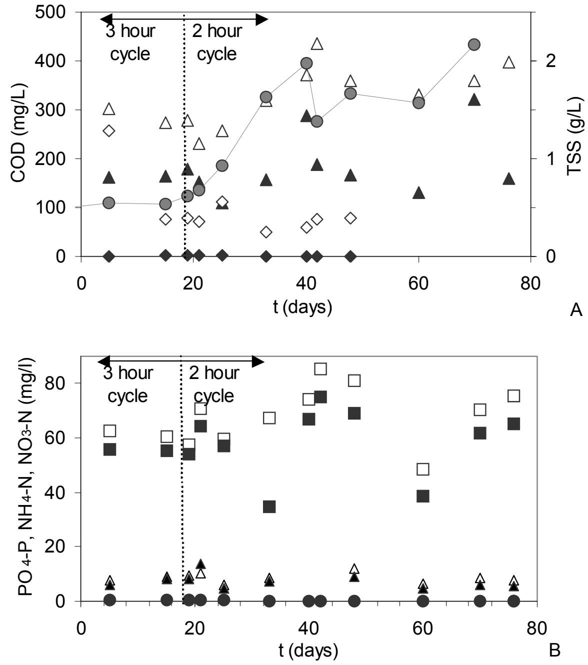
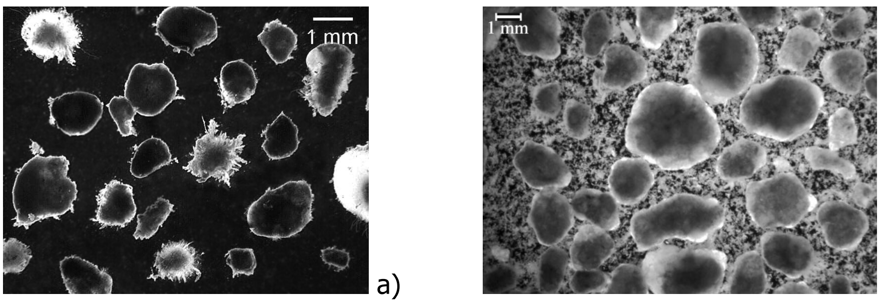


Figure 1 Suspended solid concentration in the reactor (-○-), influent and effluent concentrations during the reactor operation (A: concentrations of COD in influent (△) and effluent (▲), of which VFA in influent (◇) and effluent (◆); B: concentrations of N-total in influent (□) and effluent (■), phosphate in influent (△) and effluent (▲) and nitrate in effluent (●))

and fingertype structures (Figure 2), which can be explained by the presence of COD during the aerobic period and also by a lack of shear stress. Readily degradable substrates (e.g. acetate) are taken up during the anaerobic period, but complex substrates are still available during the aerobic period. This could favour the growth of filamentous heterotrophic organisms (Martins et al. 2004; McSwain et al. 2004). Mishima and Nakamura (1991) observed filamentous structures as well, although the granule surface remained smooth. The researchers reasoned that this was caused by the high oxygen concentration in the reactor. In order to obtain smoother and denser granules, a granular sludge reactor should be operated with the aim of maximizing COD removal during the anaerobic period, as in laboratory systems fed with synthetic influent (De Kreuk and Van Loosdrecht 2004). Applications of higher oxygen concentrations as suggested by Mishima and Nakamura (1991) would be too costly in full-scale applications.

Lack of shear stress on aerobic granules can result in irregularities or low density as well (Liu and Tay 2002; Van Loosdrecht et al. 1995). Low biomass concentrations during the experiment (Figure 1) resulted in few particle/particle collisions and therefore in low shear stress on the granules (Gjaltema et al. 1997). Hence, it can be expected that irregularity will be less common when more granules are present resulting in more collisions. This underlines again that high COD-loading (quick build up of granular biomass) is crucial during start-up, which might be hampered if the sewage is too diluted or cycle times are too long.

The goal of this experiment was to show that aerobic granular sludge formation on domestic sewage occurs in a SBR system. During the experiments, conversion processes were also monitored. However, a stable process was not obtained during the 70 days of operation. The VFA were almost totally removed (99%) during the anaerobic period, although the COD concentration in the effluent was on average 170 $mg \cdot L^{-1}$ (COD removal efficiency of 49%, Figure 1A). The removal of VFA's during the anaerobic period was associated with substantial P-release (0.11 mg P released per mg COD taken-up), however phosphate removal efficiencies fluctuated highly (Table 1, Figure 1B). This is partly due to the fact that the reactor did not reach full steady state. Nitrification was limited (Figure 1B). Nitrogen was removed by growth plus limited nitrification/denitrification (11% nitrogen removal efficiency on average, Table 1). Mishima and Nakamura (1991) reported absence of nitrification and biological phosphate removal in their system. The effluent contained suspended and colloidal material (Table 1). Clearly the removal of this COD needs further attention. However, at the laboratory scale employed these compounds are difficult to handle (clogging of tubes, etc.) and therefore this was further investigated at pilot scale.



a) b)

Figure 2 Aerobic granules grown on sewage: laboratory scale reactor (picture taken 7 days after shortening the cycle length; a) and pilot plant (June 2005; b)

Part II – Pilot scale experiments at WWTP Ede

Aims of the pilot-plant study

The first aim of the pilot plant study was the investigation of possibilities of, and which circumstances were preferable for granule formation. Aspects taken into account were pre-treatment, biological phosphate removal and the influence of shear stress. The latter was studied by using two types of reactors: an airlift reactor (high local shear forces) and a bubble column (stratification). Average shear stress in both reactors was more or less equal, depending on aeration rate.
The second part of the research aimed at the optimisation of the process. The conditions for high nitrogen and phosphate removal efficiencies were investigated. This included the stability of the granules, the effects of a dynamic feeding pattern and the need for post- or pre-treatment.

Methodology

The Pilot-plant (figure 2 and 3) was build by Logisticon Water Treatment (Groot Ammers, The Netherlands). Equipment for measurement and control was supplied by Hach Lange (Tiel, The Netherlands) and Siemens (Den Haag, The Netherlands). The pilot installation existed of two parallel reactors (height 6 m; diameter 0.6 m; effective volume 1.5m^3). The reactors could be used as airlift reactor or bubble column and could be fed with pre-treated (pre-setteled) or raw sewage. To

Figure 2 The two reactors of the pilot plant at WWTP Ede, with other equipment in the container and the tank to pre-settle incoming sewage

improve the pre-treatment, a sand-filter could be placed after the settler. In the first period, both reactors were fed with pre-treated (pre-settling and sand-filter) sewage. When granule formation was complete, reactor 1 was fed with pre-settled sewage, while reactor 2 was fed with raw sewage (January to July 2005).
The reactors were aerated by using a fine bubble aerator (bubble column, reactor 1) or a coarse bubble aerator (airlift, reactor 2). Oxygen concentrations were measured on-line and the aeration was controlled around 2 mg L^{-1} with a proportional integral derivative (PID) control connected to the compressors.

Two methods of effluent withdrawal could be used. The effluent could be withdrawn from an extraction point at 4 meters. This method was closest to the laboratory scale experiments and effluent withdrawal and feeding occurred separately. The second method was effluent withdrawal via an overflow at the top of the reactor. Effluent withdrawal and influent feeding could occur simultaneously; the incoming influent pushed the effluent out of the reactor. After the feeding, the upper 30 centimetres of the reactor content was drained to compensate for the gas hold-up. This method was used when granule formation was completed.
The total cycle consisted of the following steps: simultaneous feeding and effluent withdrawal 60-70 minutes, drain 5 minutes, aeration 100-165 minutes, denitrification 0-10 minutes (no aeration), settling 10-25 minutes.
Granule formation was monitored by SVI measurements and by determination of the granule size distribution. The latter was done by sieving a sample through a

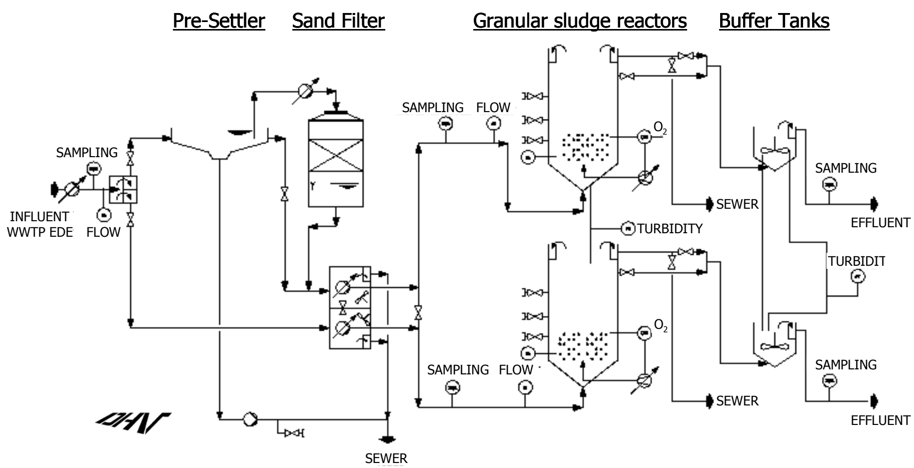


Figure 3 Process scheme of the pilot plant set-up

0.212 mm, 0.425 mm and 0.6 mm sieve and measuring the total dry weight of the biomass on the sieves. Biomass larger than 0.212 mm was considered granular sludge and the amount of granules was expressed as percentage of total dry weight in the sample. Dry weight (total suspended solids or TSS) and ash content were measured as in the laboratory scale experiments. NH_4^+, NO_2^-, NO_3^-, total N (organically and inorganically bound nitrogen), ortho- and total PO_4^{3-} and COD concentrations in influent and effluent were measured using test kits (Dr. Lange type LCK, Manufacturer: Hach Lange, Düsseldorf, Germany). The SVI_5 and SVI_{30} were determined by reading the volume of biomass in a 1 L sample, after a settling period of 5 and 30 minutes respectively and calculated with the biomass dry weight in this sample.

Results and Discussion

Granule formation

During the start-up period, nitrification was suppressed, in order to obtain fully anaerobic conditions during feeding and to create optimal conditions for the growth of phosphate accumulating organisms (PAO). The influent during the start-up was pre-treated with pre-settling and sand filtration, in order to limit the growth of protozoa during this period. When the first aerobic granular sludge appeared (around 50% granules) the selection pressure was increased, by decreasing the settling time. This led to more than 95% of granular sludge, which did not change significantly when this selection pressure was lowered again. The different shear stress in the airlift reactor and bubble column did not lead to different granule formation rates. Because of the full-scale advantages of bubble columns, the riser from reactor 2 was removed for the second part of the research, resulting in two parallel bubble columns.

From October 2004 the feeding and effluent withdrawal was changed to a simultaneous feeding and withdrawal regime. This resulted in a decreased settling pressure from 6.5 m h^{-1} to 2-3 m h^{-1}. The maximum hydraulic load during feeding was limited to 3 m h^{-1} to avoid wash-out of suspended solids with the effluent. This change did not result in changed granule stability or morphology.

The TSS concentration in the bubble columns was 9 to 10 g L^{-1} and consisted of more than 80% granular sludge, during the whole steady-state operation (in total 10 months, figure 4). The largest fraction of the granules was bigger than 0.6 mm. The average SVI_5 and SVI_{30} were 77 mL g^{-1} and 60 mL g^{-1} respectively.

Proces optimisation

After granule formation was completed, the nitrification process was stimulated. After approximately 5 weeks, complete nitrification could be observed. The N-removal efficiency based on soluble nitrogen compounds was high, independent of the nitrogen load that was applied (highest load applied 2.9 mgN $kgTSS^{-1}$ d^{-1}). This means that the nitrification capacity of the granular sludge could be considered robust and resistant against changes in sludge loading.

Total nitrogen removal (nitrification and denitrification) was sufficient (figure 5). From January 2005 till July 2005, the average value of the sum of ammonium and nitrate in reactor 1 and 2 was 9.2 and 8.9 mg L^{-1} respectively.

The biological phosphate removal was continuously high during the total period of N-removal (January to July 2005). Average orthophosphate concentrations in the effluent of reactor 1 and 2 were respectively 0.9 and 0.8 mg P L^{-1} (table 2).

The total-N, and to a lesser extent the total P, removal highly depends on the presence of suspended solids in the effluent. When the reactor is fed with a constant flow, the concentration of suspended solids in the effluent stayed low (10-20 mgTSS L^{-1}). Pretreatment did not influence this concentration, while surplus sludge was removed during the drain period. Applying a simple post-treatment could easily reduce the concentration of suspended solids in the effluent.

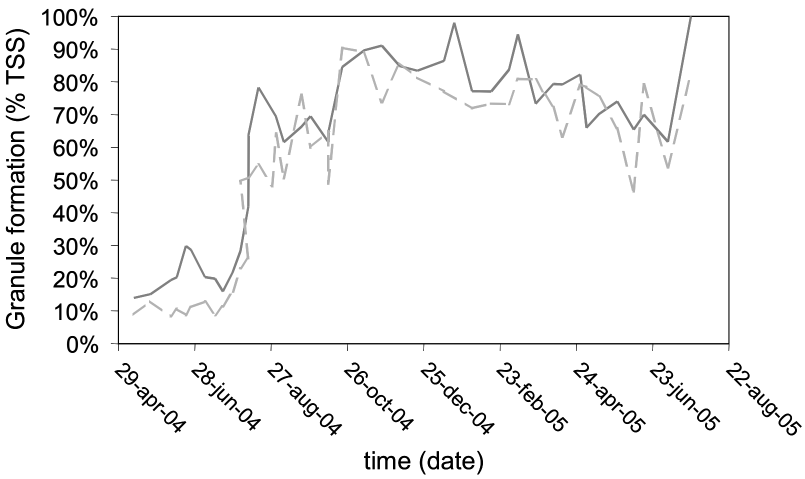


Figure 4 Development of the amount of granules (>0.212 mm) in the pilot reactors as percentage of the total dry weight (reactor 1 solid line, reactor 2 dotted line).

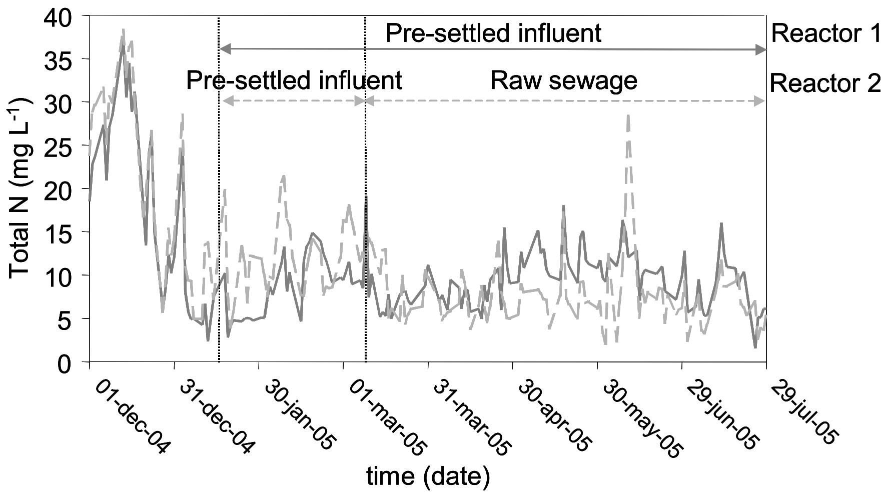


Figure 5 Nitrogen concentrations (sum of NH_4-N and NO_3-N) in the effluent of the two pilot reactors (reactor 1 solid line, reactor 2 dotted line)

Preliminary studies were carried out with the use of a drum-filter. With a mesh of 0.01 mm, the average suspended solids concentration decreased to 4 mgTSS L^{-1}. When the influent flow was varied, more wash-out of the sludge was observed. When the influent flow is kept constant during a certain period, fine material can accumulate in the reactor, which was washed out at as soon as the influent flow is increased.

Table 2a Average influent and effluent concentrations measured in pilot scale reactor 1 (Januari to July 2005).

Month	Temp (°C)	COD load[1] (g_{COD} $g_{TSS}^{-1}d^{-1}$)	Influent N_{total} (mgN L^{-1})	Influent NH_4-N (mgN L^{-1})	Influent PO_4-P (mgL^{-1})	Effluent NH_4-N (mgN L^{-1})	Effluent NO_3-N (mgN L^{-1})	Effluent PO_4-P (mgL^{-1})
Jan.	14.1	0.157	48.3	29.6	5.3	2.3	4.1	0.8
Feb.	13.5	0.153	50.0	28.3	5.1	0.6	9.7	1.1
March	12.9	0.143	55.5	32.6	5.6	0.6	8.1	07
April	13.7	0.122	53.5	32.9	5.6	0.3	7.8	0.6
May	15.4	0.149	55.5	37.6	6.5	0.4	11.7	0.9
June	19.5	0.200	63.9	39.4	7.4	1.4	9.3	0.8
July	20.6	0.223	52.2	33.5	6.2	1.1	7.0	1.0

[1] COD load is based on the aeration time

Table 2b Average influent and effluent concentrations measured in pilot scale reactor 2 (Januari to July 2005).

			Influent			Effluent		
Month	Temp	COD load[1]	N_{total}	NH_4-N	PO_4-P	NH_4-N	NO_3-N	PO_4-P
	(°C)	($g_{COD}\,g_{TSS}^{-1}d^{-1}$)	(mgN L^{-1})		(mgL^{-1})	(mgN L^{-1})		(mgL^{-1})
Jan.	14.1	0.183	48.4	28.7	5.3	5.5	5.4	0.9
Feb.	13.5	0.188	50.3	28.6	5.1	3.9	7.8	1.0
March	12.9	0.226	62.0	37.1	6.5	2.3	8.3	0.7
April	13.7	0.212	58.3	31.3	6.3	0.8	6.1	0.7
May	15.4	0.214	56.8	36.3	6.6	0.8	6.9	0.8
June	19.5	0.253	63.8	39.4	7.6	3.3	5.1	0.6
July	20.6	0.354	57.2	31.3	6.4	0.8	5.6	1.1

[1] COD load is based on the aeration time

Conclusions

Granule formation in a SBR system is achievable using a complex influent such as domestic sewage. However, a considerably longer start-up time is needed compared with that reported in previous studies using synthetic influent or more concentrated industrial effluents. The granules grown on sewage are more heterogeneous than granules found with synthetic influent. In future installations, it is expected that granules will be formed as long as a high COD load is applied. Therefore, COD-load will be an important process parameter at larger scale operation. The results of the pilot-plant study shows that aerobic granular sludge is a promising technology. When granule formation was completed, the granules showed to be robust even at changing temperatures, influent flows and loading rates. Furthermore, the stability of the granules and the effluent quality did not change when the influent was changed to raw sewage. The nitrogen removal even improved because a better COD/N ratio.
A post-treatment for the removal of suspended solids from the effluent is needed to fulfil the required effluent demands. This will also improve the total nitrogen and phosphate concentrations in the effluent.

Acknowledgements: This research was funded by the Dutch Foundation for Water Research (STOWA, TNW99.262) and STW (DPC5577) and is in co-operation with DHV, The Netherlands.

References

Arrojo, B., Mosquera-Corral, A., Garrido, J. M., and Mendez, R. (2004). "Aerobic Granulation with Industrial Wastewater in Sequencing Batch Reactors." Wat. Res., **38**(14-15), 3389-3399.

Beun, J. J., Hendriks, A., Van Loosdrecht, M. C. M., Morgenroth, M., Wilderer, P. A., and Heijnen, J. J. (1999). "Aerobic Granulation in a Sequencing Batch Reactor." *Wat. Res.*, **33**(10), 2283-2290.

Beun, J. J., Van Loosdrecht, M. C. M., and Heijnen, J. J. (2001). "N-Removal in a Granular Sludge Sequencing Batch Airlift Reactor." *Biotechnol. Bioeng.*, **75**(1), 82-92.

De Bruin, L. M. M., De Kreuk, M. K., Van Der Roest, H. F. R., Van Loosdrecht, M. C. M., and Uijterlinde, C. (2004). "Aerobic Granular Sludge Technology, Alternative for Activated Sludge Technology?" *Wat. Sci. Technol.*, **49**(11-12), 1-9.

De Kreuk, M. K., and Van Loosdrecht, M. C. M. (2004). "Selection of Slow Growing Organisms as a Means for Improving Aerobic Granular Sludge Stability." *Water Science and Technology*, **49**(11-12), 9-19.

De Kreuk, M. K., De Bruin, L. M. M., and Van Loosdrecht, M. C. M. (2005). "Aerobic Granular Sludge; from Idea to Pilot Plant." *Aerobic Granular Sludge*, S. Bathe, M. K. De Kreuk, B. S. Mc Swain, and N. Schwarzenbeck, eds., IWA, London, UK, 111-123.

Dulekgurgen, E., Ovez, S., Artan, N., and Orhon, D. (2003). "Enhanced Biological Phosphate Removal by Granular Sludge in a Sequencing Batch Reactor." *Biotechnol. Lett.*, **25**(9), 687 - 693.

Gjaltema, A., Vinke, J. L., Van Loosdrecht, M. C. M., and Heijnen, J. J. (1997). "Abrasion of Suspended Biofilm Pellets in Airlift Reactors: Importance of Shape, Structure and Particle Concentrations." *Biotechnol. Bioeng.*, **53**(1), 88 - 99.

Heijnen, J. J., and Van Loosdrecht, M. C. M. (1998). "Method for Acquiring Grain-Shaped Growth of a Microorganism in a Reactor." Patent Cooperation Treaty (PCT), Technische Universiteit Delft, US, European patent, 13p.

Inizan, M., Freval, A., Cigana, J., and Meinhold, J. "Aerobic Granulation in Sequencing Batch Reactor (Sbr) for Industrial Wastewater Treatment." *World Water Congress & Exhibition 2004*, Marrakech, Marocco.

Lin, Y. M., Liu, Y., and Tay, J. H. (2003). "Development and Characteristics of Phosphorus-Accumulating Microbial Granules in Sequencing Batch Reactors." *Appl. Microbiol. Biotechnol.*, **62**(4), 430-435.

Liu, Y., and Tay, J.-H. (2002). "The Essential Role of Hydrodynamic Shear Force in the Formation of Biofilm and Granular Sludge - *Review*." *Wat. Res.*, **36**(7), 1653-1665.

Liu, Y., and Tay, J.-H. (2004). "State of the Art of Biogranulation Technology for Wastewater Treatment." *Biotechnol. Adv.*, **22**(7), 533-563.

Martins, A. M. P., Pagilla, K., Heijnen, J. J., and Van Loosdrecht, M. C. M. (2004). "Filamentous Bulking Sludge - a Critical Review." *Wat. Res.*, **38**(4), 793-817.

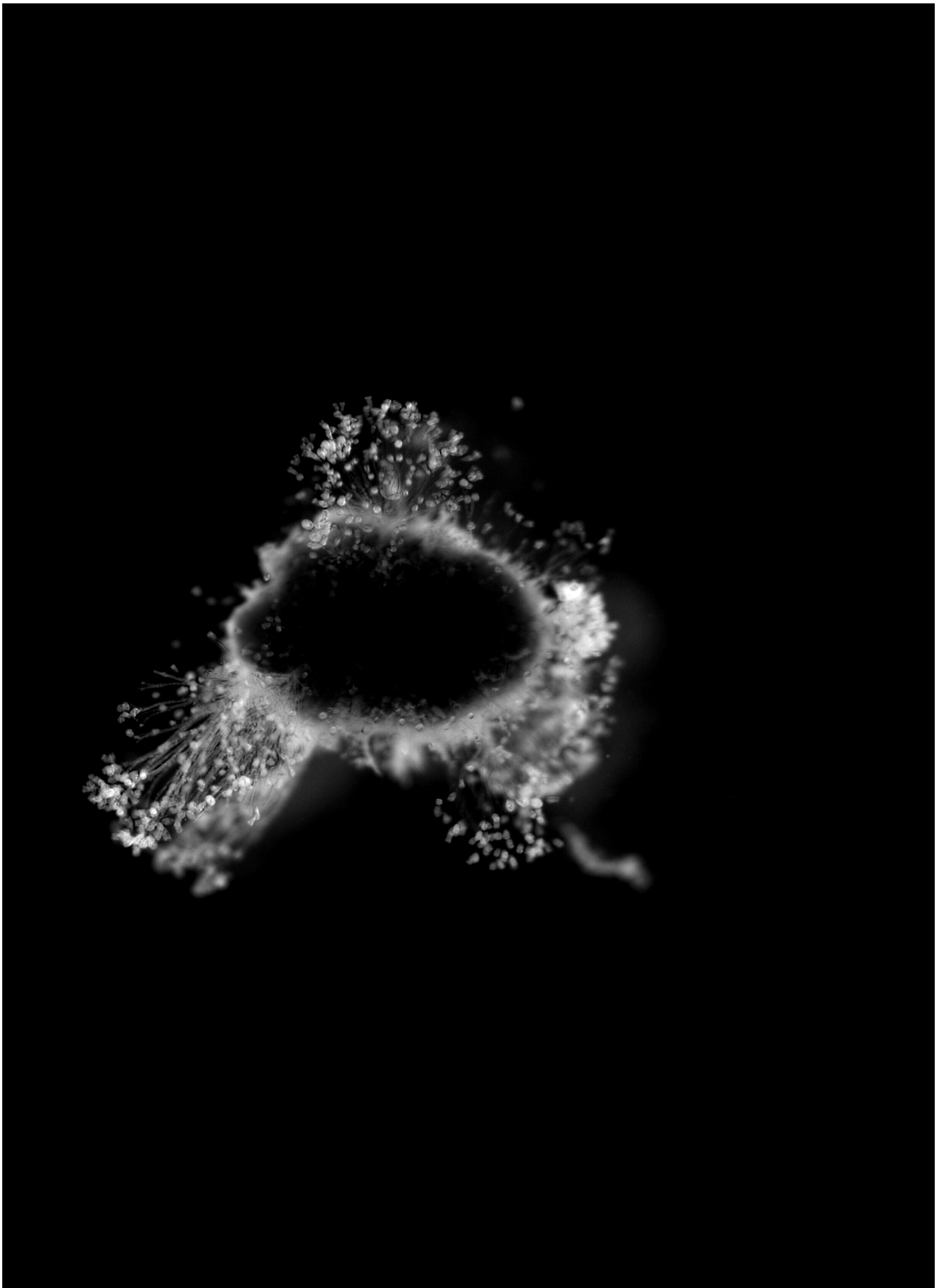
Mcswain, B. S., Irvine, R. L., and Wilderer, P. A. (2004). "The Effect of Intermittent Feeding on Aerobic Granule Structure." *Wat. Sci. Technol.*, **49**(11), 19-25.

Mishima, K., and Nakamura, M. (1991). "Self-Immobilization of Aerobic Activated Sludge - a Pilot Study of the Aerobic Upflow Sludge Blanket Process in Municipal Sewage Treatment." *Wat. Sci. Technol.*, **23**(4-6), 981 - 990.

Moy, B. Y. P., Tay, J. H., Toh, S. K., Liu, Y., and Tay, S. T. L. (2002). "High Organic Loading Influences the Physical Characteristics of Aerobic Sludge Granules." *Letters Appl. Microbiol.*, **34**(6), 407-412.

Schwarzenbeck, N., Erley, R., Mc Swain, B. S., Wilderer, P. A., and Irvine, R. L. (2004). "Treatment of Malting Wastewater in a Granular Sludge Sequencing Batch Reactor (Sbr)." *Acta Hydrochim. Hydrobiol.*, **32**(1), 16-24.

Van Loosdrecht, M. C. M., Eikelboom, D. H., Gjaltema, A., Mulder, A., Tijhuis, L., and Heijnen, J. J. (1995). "Biofilm Structures." *Wat. Sci. Technol.*, **32**(8), 35-43.



9 Evaluation and Outlook

Aim of the research

The objective of this research was to investigate the formation and structure of aerobic granular sludge and to optimize conversion processes within the granules. This was mainly done experimentally and evaluated with a granular sludge model. Besides increasing knowledge about mixed culture structures, the research was carried out in order to scale-up the first laboratory set-up as described by Beun (Beun et al., 1999), to a full-scale design and a pilot plant test.

Evaluation

The development of the aerobic granular sludge technology

For the development of the aerobic granular sludge reactor technology, a scale-up/scale-down approach was used: a full-scale wastewater treatment plant (WWTP) was designed based on initial results from laboratory scale experiments. Design and experimental research were carried out simultaneously and bottlenecks discovered in the large-scale design could be immediately studied, evaluated and/or eliminated using laboratory scale experiments. These experimental results could lead to an adjustment of the design. This approach was possible because of a close cooperation with industry (DHV, The Netherlands).

The detected bottlenecks influencing the granule formation and stability and/or reactor design, which were studied in laboratory scale experiments, were:

- The oxygen concentration during aeration: a low applied oxygen concentration restricts the energy requirement and allows optimization of simultaneous nitrification and denitrification within the granules. However, low oxygen concentrations in combination with an aerated pulse feed influenced granule stability negatively.
- Applied feeding pattern (aerated pulse feed or unaerated plug-flow feeding); the feeding pattern determines the reactor design, use of buffer tanks and the capacity of the pre- and post-treatment. A long feeding period is advantageous, since a continuous influent flow can be applied to a system of two or more SBR's. Keeping a constant filled volume in the system (i.e. simultaneous feeding and effluent withdrawal) gives a constant effluent flow to a post-treatment. Furthermore, anaerobic feeding followed by an aerated period leads to biological phosphate removal and increased granule stability.

- Adequate nutrient removal (COD, N and P): with stringent effluent standards WWTP are faced with nowadays, high nutrient removal efficiencies should be obtained to make the technology feasible for large-scale application.
- Temperature: low temperatures during winter influence the biological processes and can thus influence the cycle configuration and granule formation processes.
- Reactor configuration; use of an airlift reactor (high local shear stress and excellent mixing) or bubble column (easy construction).

Other aspects influencing reactor design, indicated in the feasibility study, were: rainwater versus dry weather flow, post- or pre-treatment, effluent suspended solids and decanting time in relation to a post-treatment step. These aspects were not investigated in laboratory experiments, but were considered in a pilot plant research.

The main conclusion that could be drawn from all experiments described in this thesis was that in order to obtain stable, dense and smooth granules at all circumstances, the actual growth rate of the organisms in the aerobic granules had to be lowered. To decrease this growth rate, easy biodegradable substrate (e.g. acetate) had to be converted to slowly degradable COD like microbial storage polymers (e.g. as Poly-β-hydroxybutyrate (PHB)).
During preliminary experiments and in many other studies (de Kreuk et al., 2004), conversion of external substrates into cell-internally stored polymers was obtained by applying a feast-famine regime. With such regime, about 60% of the dosed COD is first converted into PHB before it is used for growth (Beun et al., 2002). This feeding regime allowed formation of stable granular sludge at specific process conditions (high DO, high shear stress), but it was not sufficient to maintain stable aerobic granules at low oxygen concentration (chapter 2 and 3).
Phosphate or glycogen accumulating organisms (respectively PAO or GAO) perform the conversion step from readily biodegradable substrate to PHA most efficiently. By applying an anaerobic feeding period, PAO or GAO were able to proliferate, because of their ability to store the substrate cell-internally during an anaerobic period and grow during the subsequent aerobic period. Readily biodegradable substrate will be taken from the influent during the anaerobic period, leaving no substrate for solely aerobic fast growing heterotrophic organisms during the aeration period. Therefore, PAO or GAO will dominate, resulting in stable and smooth aerobic granular sludge (chapter 3), even under low oxygen concentrations and decreased shear stress (in a bubble column).

By the application of an anaerobic feeding period and thus the selection of PAO or GAO, many bottlenecks were solved (e.g. stable granules were obtained as well as a high nutrient removal efficiency, a bubble column could be used and that buffer tanks will not be necessary to store influent). By dosing the influent as a plug-flow through the settled bed of granules, it also becomes possible to feed the influent and withdraw the effluent simultaneously, resulting in a continuous flow to the post-treatment. These positive findings resulted in a follow-up of this research with the operation of a pilot scale reactor (chapter 8).
In this innovative development of a new process for the treatment of municipal and industrial wastewater, the combination of scientific research at a university and the practical approach of a consultant was shown to be very effective. Knowledge transfer from the university to the outside world led to the rapid application of that knowledge and its implementation in the design of the required installations. The feed back from practice in its turn helped scientific research to define its course and targets.

Bulking sludge versus Aerobic Granules
Contrary to earlier believe, this research concludes that factors as shear stress and selection by settling velocity are helpful in the formation of dense and smooth granules, but are not the main factor for granule formation. The most important aspect for good granule formation, as found in this research, shows a large analogy to activated sludge.
From bulking sludge research it was found that the specific maximum substrate uptake rates in bulking sludge are more or less equal to the specific maximum substrate uptake rates of well settling sludge ($-q_S^{max}{}_{\text{bulking sludge}} \approx -q_S^{max}{}_{\text{well settling sludge}}$). Furthermore, the specific PHB production rate of the two types of sludge is also more or less similar ($q_{PHB}^{max}{}_{\text{bulking sludge}} \approx q_{PHB}^{max}{}_{\text{well settling sludge}}$) (Martins et al., 2004). These two observations contradict the conventional kinetic selection theory and underlines that the substrate concentration in bulking sludge phenomena is important as well. Experiments show that with decreasing substrate concentration in the reactor and therefore decreasing actual substrate uptake rates, activated sludge flocs become more and more irregular. Martins et al. (2003) found that at an actual substrate uptake rate below 0.6 times the maximum substrate uptake rate, growth of filamentous bacteria and sludge bulking proliferated, while substrate consumption rates above 0.8 times the maximum uptake rate led to the formation of granular structures. The same mechanisms are valid for aerobic

granules. When sharp substrate or oxygen concentrations occur in aerobic granules (as in continuous fed systems or under low oxygen concentrations in combination with pulse feeding), granules will become unstable and wash out (Beun et al., 2000, chapter 2). This phenomenon has also been seen at low temperatures (chapter 5); when substrate was left after the anaerobic feeding period as during the start-up period, substrate was available during a large part of the aeration period, leading to heterotrophic growth (other than PAO or GAO). Combined with a low temperature, the substrate uptake rate was low as well, leading to irregular flocculated growth and biomass wash-out. In the experiments described in this thesis, instable granules and flocculated biomass were observed in all investigated cases when lower substrate uptake rates occurred and thus an important similarity between the bulking sludge and granule formation phenomena has been found. One can even ask if aerobic granular sludge is just another growth structure or morphology of activated sludge; the opposite of bulking sludge on the activated sludge morphology scale. Finally, it can be concluded that these different morphologies of microbial structures are outings of comparable phenomena and can be explained from similar basic principles; that process conditions such as substrate concentration, temperature and flow pattern are of major importance for the final structure (Van Loosdrecht et al., 2005).

Outlook

Much pioneering research is performed on the topic of aerobic granule formation during the last decade. The main topics of interest that have been published so far, have been the relations of granule size, morphology, SVI, density, hydrophobicity and conversion processes under different process conditions (as types of substrate; method substrate was dosed; shear stress (by adjusting the superficial gas velocity); dissolved oxygen concentration (by recycling the off-gas); organic load; COD/N ratio; settling velocity). An overview of these and other conditions believed to be important for aerobic granule formation (e.g. Ca2+ concentration, hydraulic retention times, seed sludge and inhibition) is given by Liu and Tay (2004) and De Kreuk et al. (2004).

Despite of the increasing number of studies on aerobic granular sludge that are carried out worldwide (chapter 1), many aspects of granule formation and importance of certain process conditions are unknown. Aerobic granular sludge technology is still a young technology, with all children's diseases when it is applied in practice and unknown aspects that have to be explored. Future research should

not be limited to the laboratory; the technology is ready to be taken to larger scale (pilot- and/or demonstration scale) as is already started at WWTP Ede (chapter 8).
Suggestions for further research, both in the laboratory and at large scale, are given in this section, of which some are taken from the first aerobic granule workshop 2004 in Munich, Germany (de Kreuk et al., 2005).

Laboratory scale research
Some aspects of the granule composition are theoretically or experimentally studied (as for example in chapter 2, 3, 5 and 6). However, other aspects related to the morphology of the granules should still be researched in detail.

Granule Size: the role of shear and strength
The most important factor for granule formation studied in this thesis was the reduction of the actual growth rate of the organisms. A side factor that was studied was the importance of shear, by applying airlift reactors and bubble columns. However, the latter has not been studied in detail (e.g. by applying different gas flow rates, changing clearance or applying mechanical stirring). The role of shear should be understood more to be able to come to a robust large-scale system. Besides a possible enhanced granule formation when optimal shear forces are applied, also the granule size might be controllable by applying increased or decreased shear stress, which can be very important for process control and nitrogen removal capacity.
In line with the influence of shear, also more knowledge about the mechanical strength of aerobics granules could influence the reactor design concerning mixing and pumping. Therefore breakage and erosion should be investigated when the granules endure known shear rates, which can be translated to methods for handling the granule/water mixture.

Granule formation, density and stability; the role of precipitates, EPS and filaments
Other factors that might enhance the granule formation or influence the stability of structure should be studied more. For example the role of precipitates, that was studied for nitrifying granules (Tsuneda et al., 2004) and observed in this study (chapter 4). The role of intracellular polymeric structures (EPS) production in granule formation and its function within the granule is unclear yet. McSwain et al. (2005) reported different composition and concentration in the different layers of aerobic granules. An overview of the different functions that polysaccharides can

have in biofilms or microbial aggregates is given by Liu et al. (2004). However, it is not clear which of these specific functions the EPS fulfills in aerobic granules. One could ask if the EPS is just a matrix in which the granules are embedded or if it is playing a different role in the catabolism, cell protection or membrane stability. For a detailed understanding about the stability of aerobic granules, the role of EPS should be understood more.

One of the theories for anaerobic granule formation is the so-called spaghetti theory, in which filamentous organisms are claimed to form the backbone structure of the granules. Aerobic granular sludge is often started with activated sludge as inoculum, in which many filamentous structures are present. Beun (1999) showed aerobic granule formation without adding an inoculum, but start up went via fungi pellets; filamentous structures as well. If the filaments play a role in the initiation of granule formation is unknown, as well as their possible existence and morphology inside the granules. Filamentous growth can lead to excessive biomass washout and system instability (examples are described in chapter 2 and 5). Therefore the role of filamentous structures inside the granules should be understood in order to be able to avoid their outgrowth outside the granule surface.

Granule structure; microbial composition and architecture

Studies on the penetration depth of oxygen, measured with micro-electrodes on granules grown with an aerobic pulse feed (Tay et al., 2002; Jang et al., 2003) and granules consisting of GAO (Meyer et al., 2003) were described in literature. Also during this research preliminary measurements with micro-sensors were performed (unpublished data). The oxygen penetration depth was measured under different circumstances (high and low PHB concentrations in the reactor, different nutrient concentrations and different oxygen levels), simulating a SBR cycle. Besides the modelling work and the FISH research, this kind of micro-electrode-experiments measuring oxygen consumption or N_2O or NO_3^- production, might clarify the position of groups of bacteria inside the granule. Comparing oxygen penetration depths under different circumstances will be a measure of the activity of the different groups of organisms and will show the dominant organism under those conditions and their position inside the granule. Also the level of heterogeneity of the aerobic granules can be shown with these experiments: the existence of micro colonies (areas with increased activity) and the existence of pores (areas without activity, which might play a role in the mass transport inside the granules).

Correlating this research to modeling results or FISH and staining techniques might increase the understanding of the morphology and structure of the aerobic granules even more.

<u>Complex substrates; the consequence of feeding sewage and industrial effluent</u>

So far, granule formation and conversion processes are studied in well-controlled laboratory set-ups using different types of synthetic influent and industrial influent. Especially research reported in literature using synthetic influent did not consider complex substrate and suspended solids. Schwarzenbeck et al. (2004a; 2004b) treated malting effluent, which is rich in particulate matter and dairy effluent (Schwarzenbeck et al., 2005). The particles of the malting effluent were removed by incorporation into the growing biofilm matrix or by high and diverse protozoa growth on the surface of matured granules. The complex substrates in the dairy wastewater caused growth of filamentous organisms at the surface of the granules due to presence of substrate in the aerobic period, or in other words, due to an extended feast phase (as described in chapter 2 and by McSwain et al., 2004).

However, fundamental research on the interaction of particles on granule formation and how particles are degraded is limited. The growth of protozoa and even higher organisms, as worms, is observed in presence of suspended solids, but their impact on morphology of the granules and on kinetics is yet unknown. Also the degradation of polymers is unknown; does the structure of the granules affect these conversion processes and how do or will the polymers influence this structure, could be a research topic of interest. Especially when the aerobic granular sludge reactor will be used in practice, more knowledge about these complex substrates is needed, in order to optimize the SBR cycles and to be able to maintain compact granules in the system.

Pilot scale research

With the knowledge obtained in the laboratory, the system is scaled up to a pilot plant (chapter 8). This pilot study gives insight in the behavior of aerobic granules in practice (fluctuating influent concentrations and composition, temperature changes and less defined circumstances). Besides investigating the most obvious aspects in a pilot plant research, as start-up of the reactor, conversion efficiencies, stability of the granules, the process and the characteristics of the effluent, the type of post and/or pretreatment, the morphology of granules in practice and their strength, the following aspects can be interesting to study.

Process control

Operating a granular sludge SBR means that all processes, including conversions, will occur in the same reactor and in one cycle. In order to be able to fulfill strict effluent demands, these processes should be controlled extensively. For example, nitrogen removal should be balanced in order to consume just all ammonia and simultaneously denitrify most nitrates. In this way, best nitrogen removal capacities will be obtained. To do so however, a well-designed control has to be incorporated, that adjusts the oxygen supply to the amount of nitrate that is formed. In that way unaerated denitrification periods could be integrated in the reaction phase or the dissolved oxygen concentration during aeration could be lowered.

Another example of important control is the batch scheduling of a treatment plant, based on aerobic granular sludge, depending on the incoming flow to the WWTP. During rainy weather, a larger flow has to be processed in the WWTP. Since the use of a buffer tank should be avoided, this increased flow the hydraulic retention time (HRT) needs to be decreased. Decreasing this HRT in order to be able to feed all incoming water, will consequently lead to shorter cycle times. When simultaneous influent feeding and effluent withdrawal is applied, fluidization of the settled aerobic granule bed has to be avoided, since fluidization will lead to mixing and thus contamination of the effluent. Therefore, the upflow velocity of the influent during feeding that can be applied is limited. Therefore, the increased flow of incoming water needs to be distributed over more reactors to increase the total surface area at that moment. These two changes due to rainfall require advanced batch scheduling and anticipation on the incoming flow.

Summarizing can be said that a one-reactor system increases the need for new and well-developed control systems, which should be studied in practice during pilot or demonstration projects.

Effluent characteristics

Beside the composition of the effluent, studying the characteristics of the effluent should also involve the origin of the suspended solids in the effluent. The suspended solids in the effluent can originate from the influent suspended solids, from the outer layers of the granules (due to erosion and sloughing), or from broken granules. The latter would mean that also organisms and precipitates from the core of the granules could be found in the effluent. When the particles that wash out would only originate from the eroded and sloughed particle fractions, the

composition of the organisms in the effluent is different from the composition in the reactor, e.g. only autotrophic organisms would be washed out, if they dominate the outer layers. On the other hand, when the effluent suspended solids mainly consist of flocculated sludge, controlling these could influence the selection for granular sludge. For the stability of the conversion processes and the selection for granular sludge, it is important to study the effluent suspended solids composition and to investigate if this can be influenced.
Another important aspect of wastewater treatment is the removal of pathogens. In activated sludge pathogens (viruses, bacteria, protozoa and helminths) are incorporated in the sludge flocs and are removed with the sludge in the sludge digestor. Because of the open structure of the activated sludge flocs, pathogens can adequately be removed from the influent. However, the dense structure of aerobic granules and their different ability of incorporating suspended solids could lead to a different removal capacity. This should be a subject of attention in pilot plant research in order to guarantee the protection of public health.

Post- and/or pretreatment systems

Correlated with effluent characteristics and with economical feasibility (chapter 8), the use of a pre- or post-treatment system should be studied. Operation with excessive pretreatment and without post-treatment would be economically most advantageous. However, pilot plant operation already showed that with only pretreatment, required effluent standards will not be reached. Another feasible option would be influent feeding without pretreatment and apply excessive post-treatment. Feeding influent without pretreatment will most certainly lead to a higher load with suspended solids, with a possible effect of extra protozoa growth. These protozoa can hinder settling, leading to higher SVI values. On the other hand, more protozoa could lead to higher COD removal efficiencies (particulate COD). These effects need to be studied with municipal sewage in a pilot plant study.
The particles that are washed-out with the effluent need to be removed to reach effluent standards. This can be done in several ways, like sand filtering with or without polyelectrolyte dosage, membrane filtration, flotation, sieving, etc. The methods should be investigated with effluent from a pilot plant, since this effluent will be most realistic. Studying the best type of post-treatment will give insight in costs and feasibility of the total process.

Mixing

Influence of mixing during feeding and aeration has to be studied and should be controlled at large scale. Simultaneous feeding and effluent withdrawal is most advantageous: this shortens the total cycle time and thus the needed reactor volume and, as the influent flow is constant when for example applying N reactors when the influent feeding duration is $1/N^{th}$ of the cycle time, the effluent flow would be constant too. In this case buffer tanks for the effluent would not be necessary and the design of a possible post-treatment would be simpler and cheaper. One of the most important aspects of simultaneous feeding and effluent withdrawal is to avoid contamination of the effluent with incoming influent. Therefore, feeding should take place in a plug flow, pushing out the effluent via the top of the reactor. According to studies by Arnz et al. (2000), 90% of the water can be exchanged without mixing in a semi-full scale SBR with biomass grown on packed support media. Since granular sludge might behave differently than this support material and at laboratory scale wall effects play an important role, mixing during feeding should be studied at pilot-scale and by modeling studies.

The beginning of a new technology

From the laboratory scale research as presented in this thesis and in other studies, the preliminary results from the pilot- and demonstration scale, the interest and investments of industry to continue the development of aerobic granular sludge research at pilot and full-scale, it is justified in the author's opinion to conclude this thesis that technologies based on aerobic granular sludge are facing a promising future. As described in this outlook, still a lot of research is needed to fulfill this expectation. However, after the first important steps were taken by Morgenroth et al. (1997), Heijnen and Van Loosdrecht (1998) and Dangcong et al. (1999), the journey that followed was thriving and the technology can be taken to a new level. The torch is handed to DHV, The Netherlands, to find applications of aerobic granular sludge technology in practice and to continue together in order to bring this journey to a successful end.

References

Arnz, P., Esterl, M.S., Nerger, C., Delgado, A. and Wilderer, P.A. (2000). Simultaneous loading and draining as a means to enhance efficacy of sequencing biofilm batch reactors. *Wat. Res.*, **34** (5), 1763 - 1766.

Beun, J.J., Hendriks, A., van Loosdrecht, M.C.M., Morgenroth, M., Wilderer, P.A. and Heijnen, J.J. (1999). Aerobic granule formation in a sequencing batch reactor. *Wat. Res.*, **33** (10), 2283-2290.

Beun, J.J., van Loosdrecht, M.C.M. and Heijnen, J.J. (2000). Aerobic granule formation. *Wat. Sci. Technol.*, **41** (4-5), 41-48.

Beun, J.J., van Loosdrecht, M.C.M. and Heijnen, J.J. (2002). Aerobic granule formation in a sequencing batch airlift reactor. *Wat. Res.*, **36** (3), 702-712.

Dangcong, P., Bernet, N., Delgenes, J.-P. and Moletta, R. (1999). Aerobic granular sludge--a case report. *Wat. Res.*, **33** (3), 890-893.

De Kreuk, M.K., De Bruin, L.M.M. and Van Loosdrecht, M.C.M. (2004). Aerobic granular sludge; from idea to pilot plant. In: Bathe, S., De Kreuk, M.K., Mc Swain, B.S. and Schwarzenbeck, N. (Eds.), *Aerobic granular sludge*. IWA: London, UK.

de Kreuk, M.K., McSwain, B.S., Bathe, S., Tay, S.T.L., Schwarzenbeck, N. and Wilderer, P.A. (2005). Discussion outcomes. In: Bathe, S., De Kreuk, M.K., Mc Swain, B.S. and Schwarzenbeck, N. (Eds.), *Aerobic granular sludge* (153-169 pp.). IWA: London, UK.

Heijnen, J.J. and Van Loosdrecht, M.C.M. (1998). Method for acquiring grain-shaped growth of a microorganism in a reactor. *Patent Cooperation Treaty (PCT)* (p. 13). Technische Universiteit Delft: US, European patent.

Jang, A., Yoon, Y.-H., Kim, I.S., Kim, K.-S. and Bishop, P.L. (2003). Characterization and evaluation of aerobic granules in sequencing batch reactor. *J. Biotechnol.*, **105** (1-2), 71-82.

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

Liu, Y.Q., Liu, Y. and Tay, J.H. (2004). The effects of extracellular polymeric substances on the formation and stability of biogranules. *Appl. Microbiol. Biotechnol.*, **65** (2), 143-148.

Martins, A.M.P., Heijnen, J.J. and van Loosdrecht, M.C.M. (2003). Effect of feeding pattern and storage on the sludge settleability under aerobic conditions. *Wat. Res.*, **37** (11), 2555-2570.

Martins, A.M.P., Pagilla, K., Heijnen, J.J. and van Loosdrecht, M.C.M. (2004). Filamentous bulking sludge - a critical review. *Wat. Res.*, **38** (4), 793-817.

McSwain, B.S., Irvine, R.L., Hausner, M. and Wilderer, P.A. (2005). Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. *Appl. Env. Microbiol.*, **71** (2), 1051-1057.

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

Meyer, R.L., Saunders, A.M., Zeng, R.J.X., Keller, J. and Blackall, L.L. (2003). Microscale structure and function of anaerobic-aerobic granules containing glycogen accumulating organisms. *FEMS Microbiol. Ecol.*, **45** (3), 253-261.

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

Schwarzenbeck, N., Borges, J.M. and Wilderer, P.A. (2005). Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. *Appl. Microbiol. Biotechnol.*, **66** (6), 711-718.

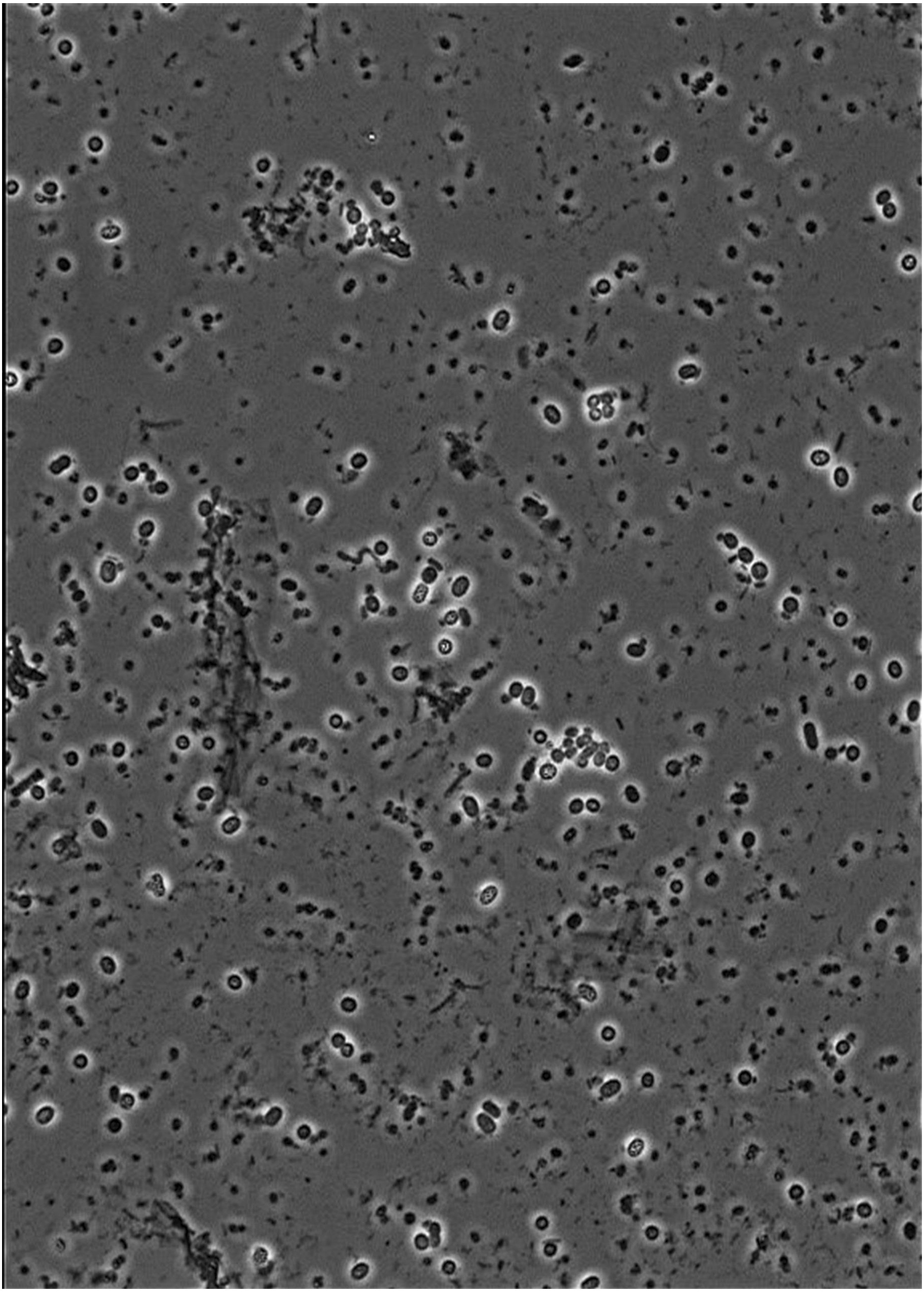
Schwarzenbeck, N., Erley, R., Mc Swain, B.S., Wilderer, P.A. and Irvine, R.L. (2004a). Treatment of malting wastewater in a granular sludge sequencing batch reactor (sbr). *Acta Hydrochim. Hydrobiol.*, **32** (1), 16-24.

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

Tay, J.H., Ivanov, V., Pan, S. and Tay, S.T.L. (2002). Specific layers in aerobically grown microbial granules. *Letters Appl. Microbiol.*, **34** (4), 254-258.

Tsuneda, S., Ejiri, Y., Nagano, T. and Hirata, A. (2004). Formation mechanism of nitrifying granules observed in an aerobic upflow fluidized bed (aufb) reactor. *Wat. Sci. Technol.*, **49** (11-12), 27-34.

Van Loosdrecht, M.C.M., de Kreuk, M.K. and Heijnen, J.J. (2005). The unity of biofilm structures. In: Bathe, S., De Kreuk, M.K., Mc Swain, B.S. and Schwarzenbeck, N. (Eds.), *Aerobic granular sludge* (1-5 pp.). IWA: London, UK.



Appendix 1
List of symbols and abbreviations

Symbols that are used in the aerobic granular sludge model (chapter 6) are given and explained in appendix 2

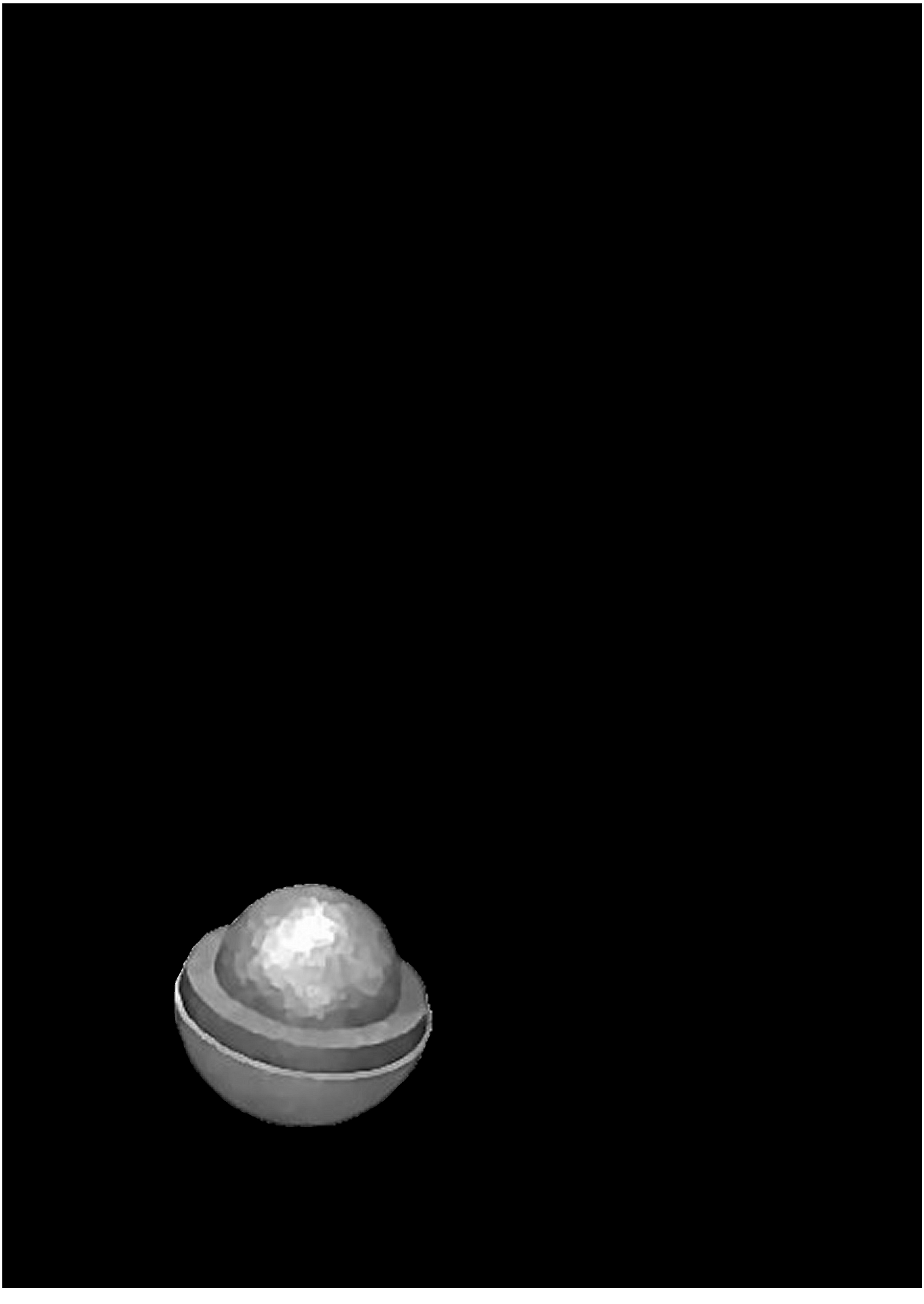
$\%N_c$	Nitrogen removal efficiency during the total cycle	[%]
$\%N_f$	Nitrogen removal efficiency during the famine period	[%]
$\%NH4_e$	Ammonium in the effluent related to influent concentration	[%]
$\%NOx_e$	Nitrate and nitrite in the effluent related to influent concentration	[%]
%V	Aerobic biomass volume relative to acetate or PHB rich biomass volume	[%]
a	Specific area of the granules	[m^2]
ASM	Activated Sludge Model	
ATU	AllylThioUrea, used for nitrification inhibition	
BASR	Biofilm Airlift Suspension Reactor	
BOD	Biological Oxygen Demand	[$g\ O_2\ m^{-3}$]
C_{NH4}	Ammonium concentration	[$gN\ m^{-3}$]
C_{NOx}	Sum of nitrite and nitrate concentration	[$gN\ m^{-3}$]
COD	Chemical Oxygen Demand	[$g\ O_2\ m^{-3}$]
C_{SO}	Concentration of soluble nutrients in the bulk	[$g\ m^{-3}$]
C_{Xm}	Maximum biomass density in the biofilm or granule	[$g\ m^{-3}$]
DO	Dissolved Oxygen	[$g\ O_2\ m^{-3}$]
D_s	Diffusion Coefficient of substance S	[$m^2\ d^{-1}$]
DWF	Dry Weather Flow	[$m^3\ d^{-1}$]
EBPR	Enhanced Biological Phosphate Removal	
EGSB	Expanded Granular Sludge Bed	
EPS	Extracellular Polymeric Structures	
FISH	Fluorescent In-Situ Hybridisation	
G	Characteristic value for gradients in a biofilm or granule	[-]
GAO	Glycogen Accumulating Organisms	
GSBR	Granular sludge Sequentially operated Batch Reactor	
HRT	Hydraulic Retention Time	[days]

IC	Internal Circulation (reactor)	
IWW	Integrale Water Wet	
k(T)	Conversion rate at temperature T	[h^{-1}]
KRW	Kader Richtlijn Water	
LSA	Lozingsbesluit WVO Stedelijk Afvalwater	
L_y	Biofilm Thickness or Granule radius	[m]
MBR	Membrane Bioreactor	
MFC	Mass Flow Controller	
MTR	Maximaal Toelaatbaar Risiconiveau	
N-removal	Removal of nitrogen compounds	
N-total	Total Nitrogen content	[g N m^{-3}]
p.e.	Population equivalents	
PAO	Phosphate Accumulating Organisms	
PHA	Poly-hydroxyalkanoates	
PHB	Poly-β-hydroxybutyrate	
poly-P	Poly-Phosphate	
P-removal	Phosphate removal	
P-total	Total Phosphate content	[g P m^{-3}]
q_{PHB}^{max}	Maximum PHB production rate	[g g^{-1} d^{-1}]
q_s^{max}	Maximum substrate uptake rate	[g g^{-1} d^{-1}]
$r_{NH4,f}$	Consumption rate for ammonium during famine period	[gN m^{-3} d^{-1}]
$r_{NOx,f}$	Consumption rate for nitrite+nitrate during famine period	[gN m^{-3} d^{-1}]
$r_{NOx,h}$	Maximum production rate for nitrite and nitrate	[gN m^{-3} d^{-1}]
RWF	Rain Water Flow	[m^3 d^{-1}]
SBAR	Sequencing Batch Airlift Reactor	
SBBC	Sequencing Batch Bubble Column	
SBR	Sequentially operated Batch Reactor	
SND	Simultaneous Nitrification and Denitrification	
SRT	Solid Retention Time or sludge age	[days]
SS	Suspended Solids	[g m^{-3}]
Stowa	Stichting Toegepast Onderzoek Waterbeheer	
STW	Stichting Technische Wetenschappen, onderdeel van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek	
SVI_n	Sludge Volume Index, measured after n minutes of settling	[mL g^{-1}]
T	Temperature	[°C]
TOC	Total Organic Carbon	[g m^{-3}]

TSS	Total Suspended Solids	[g m^{-3}]
UASB	Upflow Anaerobic Sludge Blanket	
VFA	Volatile Fatty Acids	
VSS	Volatile Suspended Solids	[g m^{-3}]
V_x	Volume occupied by biomass	[m^3]
WVO	Wet Verontreiniging Oppervlaktewater	
WWTP	WasteWater Treatment Plant	
Y	Yield	[g g^{-1}]

Greek Symbols

δ_{O2}	Oxygen penetration depth in the granule	[m^2]
μ_m	Maximum specific microbial growth rate	[d^{-1}]
θ	Temperature coefficient	[-]



Appendix 2
Aerobic granular sludge model

Table 1 Stoichiometry and rates for the bioconversion processes considered in the model

Process ➔	Component ➔	S_{O2}	S_{Ac}	S_{NH4}	S_{NO3}	S_{NO2}
		g O_2/m^3	gCOD/m^3	gN/m^3	gN/m^3	gN/m^3
	Phosphate Accumulating Organisms (PAO)					
	Anaerobic					
1	Storage of acetate		-1			
2	Maintenance					
	Aerobic					
3	Consumption of PHA	$1/Y_{PHAO}$-1		$-i_{NBM}/Y_{PHAO}$		
4	Storage of poly-P	$-1/Y_{PPO}$		i_{NBM}/Y_{PPO}		
5	Glycogen formation	$1-1/Y_{GLYO}$		i_{NBM}/Y_{GLYO}		
6	Maintenance	-1		i_{NBM}		
	Anoxic (NO3)					
7	Consumption of PHA			$-i_{NBM}/Y_{PHANO3}$	$(1/Y_{PHANO3}-1)/2.86$	
8	Storage of poly-P			i_{NBM}/Y_{PPNO3}	$-1/(Y_{PPNO3}\cdot 2.86)$	
9	Glycogen formation			i_{NBM}/Y_{GLYNO3}	$(1-1/Y_{GLYNO3})/2.86$	
10	Maintenance			i_{NBM}	-1/2.86	
	Anoxic (NO2)					
11	Consumption of PHA			$-i_{NBM}/Y_{PHANO2}$		$(1/Y_{PHANO2}-1)/1.71$
12	Storage of poly-P			i_{NBM}/Y_{PPNO2}		$-1/(Y_{PPNO2}\cdot 1.71)$
13	Glycogen formation			I_{NBM}/Y_{GLYNO2}		$(1-Y_{GLYNO2})/1.71$
14	Maintenance			i_{NBM}		-1/1.71
	Decay					
15	Decay		$1-f_{XI}$	$I_{NBM}-i_{NXI}\cdot f_{XI}$		
	Autotrophic Organisms					
	Nitrification (X_{NH})					
16	Growth	$1-3.43/Y_{NH}$		$-1/Y_{NH}-i_{NBM}$		$1/(Y_{NH})$
17	Aerobic end. resp.	$-(1-f_{XI})$		$i_{NBM}-i_{NXI}\cdot f_{XI}$		
18	Anoxic end. resp.			$i_{NBM}-i_{NXI}\cdot f_{XI}$	$-(1-f_{XI})/2.86$	
	Nitrification (X_{NO})					
19	Growth	$1-1.14/Y_{NO}$			$1/(Y_{NO})$	$-1/(Y_{NO})-i_{NBM}$
20	Aerobic end. resp.	$-(1-f_{XI})$		$i_{NBM}-i_{NXI}\cdot f_{XI}$		
21	Anoxic end. resp.			$i_{NBM}-i_{NXI}\cdot f_{XI}$	$-(1-f_{XI})/2.86$	

S_{N2}	S_{PO4}	X_{PAO}	X_{PP}	X_{PHB}	X_{GLY}	X_{NH}	X_{NO}	X_I	Rate	Process ↓
gN/m^3	gP/m^3	$gCOD/m^3$	gP/m^3	$gCOD/m^3$	$gCOD/m^3$	$gCOD/m^3$	$gCOD/m^3$	$gCOD/m^3$		
Phosphate Accumulating Organisms (PAO)										
	Y_{PO4}		$-Y_{PO4}$	Y_{PHA}	$1-Y_{PHA}$				r_{SA}^{AN}	1
	1		-1						r_{M}^{AN}	2
	$-i_{PBM}/Y_{PHAO}$	$1/Y_{PHAO}$		-1					r_{PHA}^{O}	3
	$i_{PBM}/Y_{PPO}-1$	$-1/Y_{PPO}$	1						r_{PP}^{O}	4
	i_{PBM}/Y_{GLYO}	$-1/Y_{GLYO}$			1				r_{GLY}^{O}	5
	i_{PBM}	-1							r_{M}^{O}	6
$-(1/Y_{PHANO3}-1)/2.86$	$-i_{PBM}/Y_{PHANO3}$	$1/Y_{PHANO3}$		-1					r_{PHA}^{NO3}	7
$1/(2.86 \cdot Y_{PPNO3})$	$i_{PBM}/Y_{PPNO3}-1$	$-1/Y_{PPNO3}$	1						r_{PP}^{NO3}	8
$-(1-1/Y_{GLYNO3})/2.86$	i_{PBM}/Y_{GLYNO3}	$-1/Y_{GLYNO3}$			1				r_{GLY}^{NO3}	9
1/2.86	i_{PBM}	-1							r_{M}^{NO3}	10
$-(1/Y_{PHANO2}-1)/1.71$	$-i_{PBM}/Y_{PHANO2}$	$1/Y_{PHANO2}$		-1					r_{PHA}^{NO2}	11
$1/(Y_{PPNO2} \cdot 1.71)$	$i_{PBM}/Y_{PPNO2}-1$	$-1/Y_{PPNO2}$	1						r_{PP}^{NO2}	12
$-(1-1/Y_{GLYNO2})/1.71$	i_{PBM}/Y_{GLYNO2}	$-1/Y_{GLYNO2}$			1				r_{GLY}^{NO2}	13
1/1.71	i_{PBM}	-1							r_{M}^{NO2}	14
	$i_{PBM}-i_{PXI} \cdot f_{XI}$	-1						f_{XI}	r_D	15
Autotrophic Organisms										
						1			$r_{G,NH}^{O}$	16
						-1		f_{XI}	$r_{ER,NH}^{O}$	17
$(1-f_{XI})/2.86$						-1		f_{XI}	$r_{ER,NH}^{NO3}$	18
							1		$r_{G,NO}^{O}$	19
							-1	f_{XI}	$r_{ER,NO}^{O}$	20
$(1-f_{XI})/2.86$							-1	f_{XI}	$r_{ER,NO}^{NO3}$	21

Table 1 (Continuation)

	Process		Process Rate
Phosphate Accumulating Organisms			
	Anaerobic		
1	Storage of acetate	r_{SA}^{AN}	$q_{s,max} \cdot \frac{f_{PHA,max} - f_{PHA}}{(f_{PHA,max} - f_{PHA}) + K_{PHAP}} \cdot \frac{S_A}{S_A + K_{AP}} \cdot X_{PAO}$
2	Maintenance	r_M^{AN}	$m_{An} \cdot \frac{K_{O2,P}}{S_{O2} + K_{O2,P}} \cdot \frac{K_{NO3,P}}{S_{NO3,P} + K_{NO3,P}} \cdot \frac{K_{NO2,P}}{S_{NO2} + K_{NO2,P}} \cdot X_{PAO}$
	Aerobic		
3	Consumption of PHA	r_{PHA}^{O}	$z_{O2} \cdot z_{NH4} \cdot k_{PHA} \cdot \frac{f_{PHA}}{f_{PHA} + K_{fPHA,P}} \cdot \frac{S_{PO4}}{S_{PO4} + K_{PO4}} \cdot \frac{S_{O2}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
4	Storage of poly-P	r_{PP}^{O}	$z_{O2} \cdot \frac{X_{PAO}}{X_{PP}} \cdot k_{PP} \cdot \frac{f_{PP,max} - f_{PP}}{(f_{PP,max} - f_{PP}) + K_{PP,P}} \cdot \frac{S_{PO4}}{S_{PO4} + K_{PO4,P}} \cdot \frac{S_{O2}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
5	Glycogen formation	r_{GLY}^{O}	$z_{O2} \cdot \frac{X_{PHA}}{X_{GLY}} \cdot k_{GLY} \cdot \frac{f_{GLY,max} - f_{PP}}{(f_{GLY,max} - f_{GLY}) + K_{GLY,P}} \cdot \frac{S_{O2}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
6	Maintenance	r_M^{O}	$m_{O2} \cdot \frac{S_{O2}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
	Anoxic (NO3)		
7	Consumption of PHA	r_{PHA}^{NO2}	$z_{NO3} \cdot z_{NH4} \cdot k_{PHA} \cdot \eta_{P,NO3} \cdot \frac{f_{PHA}}{f_{PHA} + K_{fPHA,P}} \cdot \frac{S_{PO4}}{S_{PO4} + K_{PO4}} \cdot \frac{S_{NO3}}{S_{NO3} + K_{NO3,P}} \cdot \frac{K_{O2,P}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
8	Storage of poly-P	r_{PP}^{NO2}	$z_{NO3} \cdot \frac{X_{PAO}}{X_{PP}} \cdot k_{PP} \cdot \eta_{P,NO3} \cdot \frac{f_{PP,max} - f_{PP}}{(f_{PP,max} - f_{PP}) + K_{PP,P}} \cdot \frac{S_{PO4}}{S_{PO4} + K_{PO4,P}} \cdot \frac{S_{NO3}}{S_{NO3} + K_{NO3,P}} \cdot \frac{K_{O2,P}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
9	Glycogen formation	r_{GLY}^{NO2}	$z_{NO3} \cdot \frac{X_{PHA}}{X_{GLY}} \cdot k_{GLY} \cdot \eta_{P,NO3} \cdot \frac{f_{GLY,max} - f_{GLY}}{(f_{GLY,max} - f_{GLY}) + K_{GLY,P}} \cdot \frac{S_{NO3}}{S_{NO3} + K_{NO3,P}} \cdot \frac{K_{O2,P}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$
10	Maintenance	r_M^{NO2}	$z_{NO3} \cdot m_{NO3} \cdot \frac{S_{NO3}}{S_{NO3} + K_{NO3,P}} \cdot \frac{K_{O2,P}}{S_{O2} + K_{O2,P}} \cdot X_{PAO}$

Table 1 (Continuation)

	Anoxic (NO2)		
11	Consumption of PHA	r_{PHA}^{NO3}	$z_{NO2} \cdot z_{NH4} \cdot k_{PHA} \cdot \eta_{P,NO2} \cdot \frac{f_{PHA}}{f_{PHA}+K_{fPHA,P}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2,P}} \cdot \frac{K_{O2,P}}{S_{O2}+K_{O2,P}} \cdot X_{PAO}$
12	Storage of poly-P	r_{PP}^{NO3}	$z_{NO2} \cdot \frac{X_{PAO}}{X_{PP}} \cdot k_{PP} \cdot \eta_{P,NO2} \cdot \frac{f_{PP,max}-f_{PP}}{(f_{PP,max}-f_{PP})+K_{PP,P}} \cdot \frac{S_{PO4}}{S_{PO4}+K_{PO4,P}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2,P}} \cdot \frac{K_{O2,P}}{S_{O2}+K_{O2,P}} \cdot X_{PAO}$
13	Glycogen formation	r_{GLY}^{NO3}	$z_{NO2} \cdot \frac{X_{PHA}}{X_{GLY}} \cdot k_{GLY} \cdot \eta_{P,NO2} \cdot \frac{f_{GLY,max}-f_{GLY}}{(f_{GLY,max}-f_{GLY})+K_{GLY,P}} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2,P}} \cdot \frac{K_{O2,P}}{S_{O2}+K_{O2,P}} \cdot X_{PAO}$
14	Maintenance	r_{M}^{NO3}	$z_{NO2} \cdot m_{NO2} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2,P}} \cdot \frac{K_{O2,P}}{S_{O2}+K_{O2,P}} \cdot X_{PAO}$
	Decay		
15	Decay	r_{D}	$b_{PAO} \cdot \frac{K_{PHA,P}}{f_{PHA}+K_{fPHA,P}} \cdot \frac{K_{GLY,P}}{f_{GLY}+K_{GLY,P}} \cdot \frac{K_{A,P}}{S_{A}+K_{A,P}} \cdot X_{PAO}$
Autotrophic Organisms			
	Nitrification (X_{NH})		
16	Growth	$r_{G,NH}^{O}$	$z_{O2} \cdot z_{NH4} \cdot \mu_{NH} \cdot \frac{S_{NH4}}{S_{NH4}+K_{NH4,NH}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2,NH}} \cdot X_{NH}$
17	Aerobic end. resp.	$r_{ER,NH}^{O}$	$z_{O2} \cdot b_{NH} \cdot \frac{S_{O2}}{S_{O2}+K_{O2,NH}} \cdot X_{NH}$
18	Anoxic end. resp.	$r_{ER,NH}^{NO3}$	$z_{NO3} \cdot b_{NH} \cdot \eta_{NH,NO3} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3,NH}} \cdot \frac{K_{O2,NH}}{S_{O2}+K_{O2,NH}} \cdot X_{NH}$
	Nitrification (X_{NO})		
19	Growth	$r_{G,NO}^{O}$	$z_{O2} \cdot z_{NO2} \cdot \mu_{NO} \cdot \frac{S_{NO2}}{S_{NO2}+K_{NO2,NO}} \cdot \frac{S_{O2}}{S_{O2}+K_{O2,NO}} \cdot X_{NO}$
20	Aerobic end. resp.	$r_{ER,NO}^{O}$	$z_{O2} \cdot b_{NO} \cdot \frac{S_{O2}}{S_{O2}+K_{O2,NO}} \cdot X_{NO}$
21	Anoxic end. resp.	$r_{ER,NO}^{NO3}$	$z_{NO3} \cdot b_{NO} \cdot \eta_{NO,NO3} \cdot \frac{S_{NO3}}{S_{NO3}+K_{NO3,NO}} \cdot \frac{K_{O2,NO}}{S_{O2}+K_{O2,NO}} \cdot X_{NH}$

Note: the functions $z_i=1/(1+e^{-200*S_i+4})+10^{-12}$ switch the reaction off for negative substrate concentrations S_i.

Table 2 Stoichiometric coefficients for bioconversion processes shown in Table 1

Symbol	Definition	Value	Unit	Ref.
Composition factors (X_{PAO}, X_{NH}, X_{NO})				
i_{NBM}	Nitrogen content of biomass	0.07	$gN \cdot gCOD_{BM}^{-1}$	(1)
i_{NXI}	Nitrogen content of inert particulate COD	0.02	$gN \cdot gCOD_{XI}^{-1}$	(1)
i_{PBM}	Phosphorus content of biomass	0.02	$gP \cdot gCOD_{BM}^{-1}$	(1)
i_{PXI}	Phosphorus content of inert particulate COD	0.01	$gP \cdot gCOD_{XI}^{-1}$	(1)
Phosphate Accumulating Organisms (X_{PAO})				
f_{XI}	Fraction of inert COD generated in decay	0.1	$gCOD_{XI} \cdot gCOD_{XPAO}^{-1}$	(1)
Y_{PO4}	Anaerobic yield for phosphate release	0.5	$gP \cdot gCOD_{SA}^{-1}$	(2)
Y_{PHA}	Anaerobic yield for PHA formation	1.5	$gCOD_{PHA} \cdot gCOD_{SA}^{-1}$	(2)
Y_{PHAO}	Aerobic yield for PHA degradation	1.39	$gCOD_{PHA} \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{PPO}	Aerobic yield for poly-P formation	4.42	$gP \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{GLYO}	Aerobic yield for glycogen formation	1.11	$gCOD_{GLY} \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{PHANO3}	Anoxic yield for PHA degradation on nitrate	1.7	$gCOD_{PHA} \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{PPNO3}	Anoxic yield for poly-P formation on nitrate	3.02	$gP \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{GLYNO3}	Anoxic yield for glycogen formation on nitrate	1.18	$gCOD_{GLY} \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{PHANO2}	Anoxic yield for PHA degradation on nitrite	1.7	$gCOD_{PHA} \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{PPNO2}	Anoxic yield for poly-P formation on nitrite	3.02	$gP \cdot gCOD_{XPAO}^{-1}$	(3)
Y_{GLYNO2}	Anoxic yield for glycogen formation on nitrite	1.18	$gCOD_{GLY} \cdot gCOD_{XPAO}^{-1}$	(3)
Autotrophic organisms: Ammonium oxidizers (X_{NH}) and Nitrite oxidizers (X_{NO})				
f_{XI}	Fraction of inert COD generated in endogenous respiration	0.1	$gCOD_{XI} \cdot gCOD_{XNH,NO}^{-1}$	(1)
Y_{NH}	Yield for growth of ammonium oxidizers	0.15	$gCOD_{XNH} \cdot gN^{-1}$	(4)
Y_{NO}	Yield for growth of nitrite oxidizers	0.041	$gCOD_{XNO} \cdot gN^{-1}$	(4)

(1) Henze et al., 1999; (2) Smolders et al., 1994; (3) Murnleitner et al., 1997; (4) Wiesmann, 1994

Table 3 Kinetic parameters for the bioconversions shown in Table 1

Symbol	Definition	Value	Unit	Ref.
Phosphate Accumulating Organisms (X_{PAO})				
b_{PAO}	Rate constant for lysis and decay	$0.4 \cdot e^{\theta_{PAO}(T-20)}$	1/d	(1)
$f_{GLY,max}$	Maximum ratio of stored glycogen and biomass	0.5	-	(2)
$f_{PHA,max}$	Maximum ratio of stored PHA and biomass	0.8	-	-
$f_{PP,max}$	Maximum ratio of stored Poly-P and biomass	0.65	-	-
k_{GLY}	Glycogen formation rate	$0.93 \cdot e^{\theta_{PAO}(T-20)}$	$gCOD_{GLY}^{2} \cdot gCOD_{PHA}^{-1} \cdot gCOD_{XPAO}^{-1} \cdot d^{-1}$	(2)
k_{PHA}	PHA degradation rate	$5.51 \cdot e^{\theta_{PAO}(T-20)}$	$gCOD \cdot gCOD^{-1} \cdot d^{-1}$	(2)
k_{PP}	Poly-P formation rate	$0.45 \cdot e^{\theta_{PAO}(T-20)}$	$gP \cdot gCOD^{-1} \cdot d^{-1}$	(3)
$K_{A,P}$	Half-saturation coefficient for acetate	4	$gCOD \cdot m^{-3}$	(4)
$K_{GLY,P}$	Half-saturation coefficient for glycogen	0.01	$gCOD \cdot m^{-3}$	(2)
$K_{NO2,P}$	Half-saturation coefficient for nitrite	1	$gN \cdot m^{-3}$	-
$K_{NO3,P}$	Half-saturation coefficient for nitrate	1	$gN \cdot m^{-3}$	-
$K_{O2,P}$	Half-saturation coefficient for oxygen	0.2	$gO_2 \cdot m^{-3}$	(1)
$K_{PHA,P}$	Half-saturation coefficient for PHA	0.01	$gCOD \cdot m^{-3}$	(1)
K_{PO4}	Half-saturation coefficient for phosphate for growth	0.001	$gP \cdot m^{-3}$	-
$K_{PO4,P}$	Half-saturation coefficient for phosphate for Poly-P formation	1	$gP \cdot m^{-3}$	(2)
$K_{PP,P}$	Half-saturation coefficient for Poly-P	0.01	$gP \cdot m^{-3}$	(4)
m_{An}	Anaerobic maintenance	$0.05 \cdot e^{\theta_{PAO}(T-20)}$	$gP \cdot gCOD^{-1} \cdot d^{-1}$	(3)
m_{NO2}	Anoxic maintenance on nitrite	$0.11 \cdot e^{\theta_{PAO}(T-20)}$	$gCOD \cdot gCOD^{-1} \cdot d^{-1}$	(3)
m_{NO3}	Anoxic maintenance on nitrate	$0.11 \cdot e^{\theta_{PAO}(T-20)}$	$gCOD \cdot gCOD^{-1} \cdot d^{-1}$	(3)
m_{O2}	Aerobic maintenance on oxygen	$0.06 \cdot e^{\theta_{PAO}(T-20)}$	$gO_2 \cdot gCOD^{-1} \cdot d^{-1}$	(3)
$q_{s,max}$	Maximum acetate consumption rate	$8 \cdot e^{\theta_{PAO}(T-20)}$	$gCOD \cdot gCOD^{-1} \cdot d^{-1}$	(2)
z_{NH4}	Switching function for ammonium	$1/(1+e^{-200 \cdot S\_NH4+4})+10^{-12}$	-	-
z_{NO2}	Switching function for nitrite	$1/(1+e^{-200 \cdot S\_NO2+4})+10^{-12}$	-	-
z_{NO3}	Switching function for nitrate	$1/(1+e^{-200 \cdot S\_NO3+4})+10^{-12}$	-	-
z_{O2}	Switching function for oxygen	$1/(1+e^{-200 \cdot S\_O2+4})+10^{-12}$	-	-
$\eta_{P,NO2}$	Reduction factor under anoxic conditions (NO_2)	0.3	-	-
$\eta_{P,NO3}$	Reduction factor under anoxic conditions (NO_3)	0.3	-	-
θ_{PAO}	Temperature coefficient	0.063	-	(8)

Table 3 (Continuation)

Symbol	Definition	Value	Unit	Ref.
Autotrophic organisms: Ammonium oxidizers (X_{NH}) and Nitrite oxidizers (X_{NO})				
b_{NH}	Lysis and decay rate coefficient (X_{NH})	$0.1 \cdot e^{\theta_{NH}(T-20)}$	d^{-1}	(6)
b_{NO}	Lysis and decay rate coefficient (X_{NO})	$0.06 \cdot e^{\theta_{NO}(T-20)}$	d^{-1}	(6)
$K_{NH4,NH}$	Half-saturation coefficient for ammonium (X_{NH})	2.4	$gN \cdot m^{-3}$	(6)
$K_{NO2,NO}$	Half-saturation coefficient for nitrite (X_{NO})	0.238	$gN \cdot m^{-3}$	(5)
$K_{NO3,NH}$	Half-saturation coefficient for nitrate inhibition of decay (X_{NH})	1.0	$gN \cdot m^{-3}$	-
$K_{NO3,NO}$	Half-saturation coefficient for nitrate inhibition of decay (X_{NO})	1.0	$gN \cdot m^{-3}$	-
$K_{O2,NH}$	Half-saturation coefficient for oxygen (X_{NH})	0.3	$gO_2 \cdot m^{-3}$	(6)
$K_{O2,NO}$	Half-saturation coefficient for oxygen (X_{NO})	0.1	$gO_2 \cdot m^{-3}$	(5)
$\eta_{NH,NO3}$	Reduction factor under anoxic conditions (X_{NH})	0.3	-	-
$\eta_{NO,NO3}$	Reduction factor under anoxic conditions (X_{NO})	0.5	-	-
$\mu_{NH,max}$	Maximum specific growth rate (X_{NH})	$0.4 \cdot e^{\theta_{NH}(T-20)}$	d^{-1}	Half the value from (6)
$\mu_{NO,max}$	Maximum specific growth rate (X_{NO})	$1.1 \cdot e^{\theta_{NO}(T-20)}$	d^{-1}	(6)
θ_{NH}	Temperature coefficient (X_{NH})	0.094	-	(7)
θ_{XO}	Temperature coefficient (X_{NO})	0.061	-	(7)

(1) Henze et al., 1999; (2) Meijer, 2004; (3) Murnleitner et al., 1997; (4) Henze et al., 1999;
(5) Wijffels and Tramper, 1995; (6) Wiesmann, 1994; (7)Hao et al., 2002; (8) Henze et al., 2000

Table 4 Other parameters used in the simulations, with values according to the experimental conditions.

Symbol	Definition	Value	Unit
D	Diffusion coefficient in the granule	0.0002/water fraction	$m^2 \cdot d^{-1}$
D_{Ac}	Diffusion coefficient of acetate in the granule in the feeding phase	5·D	$m^2 \cdot d^{-1}$
NG	Number of granules[a]	600000	-
r_{max}	Maximum radius of the granules[a]	0.0055	m
$S_{A, in}$	Influent concentration acetate	396	$gCOD \cdot m^{-3}$
$S_{NH4, in}$	Influent concentration ammonia	50	$gN \cdot m^{-3}$
$S_{PO4, in}$	Influent concentration phosphate	20	$gP \cdot m^{-3}$
t_{cycle}	Total cycle time[a]	0.125	days
$V_{reactor}$	Total reactor volume	0.003	m^3
$V_{biofilm}$	Volume of the biofilm compartment[c]	0.0015	m^3
ρ_X	Density of the biomass	350000	$gCOD \cdot m^{-3}$
ρ_{PHA}	Density of the storage polymers (PHA, glycogen and poly-P)[b]	$1 \cdot 10^8$	$gCOD \cdot m^{-3}$

[a)] This was the situation in the basic simulation of 365 days. In other simulations these data can vary according to the description in the text.
[b)] The density of the storage polymers was chosen extremely high, since the volume taken up by the polymers is already included in the volume of biomass (Beun et al., 2001)
[c)] The biofilm compartment in AQUASIM contains bulk liquid, biofilm water and biofilm biomass fractions.

Rerefences

Beun JJ, Van Loosdrecht MCM, Heijnen JJ. (2001). N-removal in a granular sludge sequencing batch airlift reactor. *Biotechn. Bioeng.* **75**(1). 82-92.

Hao XD, Heijnen JJ, van Loosdrecht MCM. 2002. Sensitivity analysis of a biofilm model describing a one-stage completely autotrophic nitrogen removal (CANON) process. *Biotechn. Bioeng.* **77**(3). 266-277.

Henze M, Gujer W, Mino T, Matsuo T, Wentzel MC, Marais GvR, Van Loosdrecht MCM. 1999. Activated Sludge Model No.2d, ASM2D. *Wat. Sci. Techn.* **39**(1). 165 - 182.

Henze M, Gujer W, Mino T, van Loosdrecht M. 2000. Activated Sludge Models; ASM1, ASM2, ASM2d and ASM3. London: IWA Publishing. 130 p.

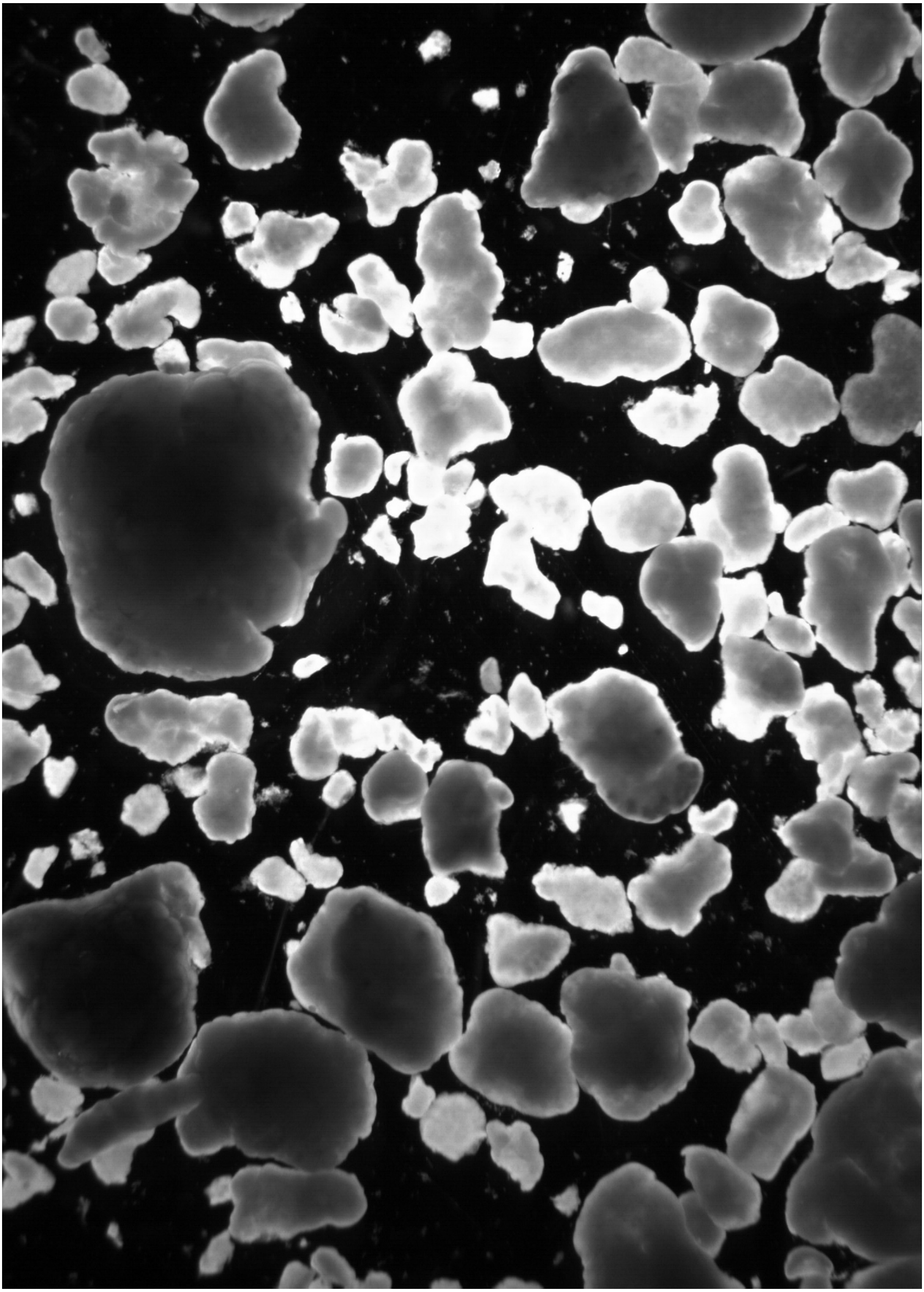
Meijer SCF. 2004. Theoretical and practical aspects of modelling activated sludge processes [PhD.]. Delft: Technical University. 204 p.

Murnleitner E, Kuba T, van Loosdrecht MCM, Heijnen JJ. 1997. An integrated metabolic model for the Aerobic and denitrifying Biological phosphorus removal. *Biotechn. Bioeng.* **54**(5). 434 - 450.

Smolders GJF, van der Meij J, Van Loosdrecht MCM, Heijnen JJ. 1994. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. *Biotechn. Bioeng.* **43**. 461-470.

Wiesmann U. 1994. Biological nitrogen removal from wastewater. *Adv. Biochem. Eng./Biotechn.* 51. 113 - 154.

Wijffels RH, Tramper J. 1995. Nitrification by immobilized cells. *Enz. Microb. Techn.* **17**(6). 482-492.



Appendix 3 – Patent Method for the treatment of waste water with sludge granules

This patent is currently owned by DHV, Amersfoort, The Netherlands

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 March 2004 (25.03.2004)

PCT

(10) International Publication Number
WO 2004/024638 A1

(51) International Patent Classification[7]: C02F 3/12, 3/26, 3/30

(21) International Application Number: PCT/NL2003/000642

(22) International Filing Date: 16 September 2003 (16.09.2003)

(25) Filing Language: Dutch

(26) Publication Language: English

(30) Priority Data:
1021466 16 September 2002 (16.09.2002) NL

(71) Applicants *(for all designated States except US)*: **TECHNISCHE UNIVERSITEIT DELFT** [NL/NL]; Julianalaan 134, NL-2628 BL Delft (NL). **STICHTING VOOR DE TECHNISCHE WETENSCHAPPEN** [NL/NL]; Van Vollenhovenlaan 661, NL-3527 JP Utrecht (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **VAN LOOSDRECHT, Marinus, Cornelis, Maria** [NL/NL]; Viool 99, NL-2678 HL De Lier (NL). **DE KREUK, Merle, Krista** [NL/NL]; Bothastraat 2, NL-2806 EM Gouda (NL).

(74) Agents: **ALTENBURG, Bernardus, Stephanus, Franciscus** et al.; Octrooibureau Los En Stigter B.V., Weteringschans 96, NL-1017 XS Amsterdam (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/024638 A1

(54) Title: METHOD FOR THE TREATMENT OF WASTE WATER WITH SLUDGE GRANULES

(57) Abstract: The invention relates to a method for the treatment of waste water comprising an organic nutrient. According to the invention, the waste water is in a first step fed to sludge granules, after the supply of the waste water to be treated the sludge granules are fluidised in the presence of an oxygen-comprising gas, and in a third step, the sludge granules are allowed to settle in a settling step. This makes it possible to effectively remove not only organic nutrients but optionally also nitrogen compounds and phosphate.

WO 2004/024638 PCT/NL2003/000642

METHOD FOR THE TREATMENT OF WASTE WATER WITH SLUDGE GRANULES

The present invention relates to a method for the treatment of waste water comprising an organic nutrient, wherein the waste water is brought into contact with microorganisms-comprising sludge particles, an oxygen-comprising gas is fed to the sludge particles, and the method further comprises the settling of the sludge particles and the discharge of organic nutrient-depleted waste water.

Such a method is known in the art, for example, from US 3,864,246. Waste water having a high rate of biological oxygen demand (BOD) is mixed with sludge flocs. The thus obtained sludge flocs-containing waste water is brought into contact with oxygen (air). The conditions chosen augment the growth of sludge flocs (that is to say biomass particles) that have improved settling properties. This reduces the time necessary for separating the microorganisms (in particular bacteria) that provide biological breakdown, from the waste water.

A drawback of the known method, despite the improved settling velocity, is that the implementation of the method requires a relatively large surface area, that is to say large-scale purification occupies an undesirable amount of space.

It is an object of the present application to improve the method, while occupying less space in comparison with the known method.

To this end the method according to the invention is characterised in that

- in a first step the waste water is fed to sludge granules,
- after the supply of the waste water to be treated an oxygen-comprising gas is introduced in a second step, with the granules being in a fluidised condition and

- in a third step, a settling step, the sludge granules are allowed to settle.

This allows the method to be carried out in a relatively limited reactor volume. This may reduce the occupation of space down to a fifth. The reaction conditions chosen promote the formation of sludge granules (as opposed to sludge flocs) with excellent settling properties. Moreover, the conditions in the first step are oxygen-depleted, and in practice they are anaerobic, since there is no oxygen added. In the first step the sludge granules take up organic nutrients from the supplied waste water, and they are stored inside the microorganisms in the form of a polymer, such as polybetahydroxybutyrate. Should oxygen be supplied in the first step, this must not be in an amount that would prevent the storage of organic nutrient. In the second step, breakdown of the stored organic nutrients occurs under aerobic conditions. In addition, this aerobic second step may effect the breakdown of possibly present ammonium into nitrate. In the second step also the interior of the sludge granules is anaerobic and this is where the stored organic nutrients are broken down utilising nitrate. This produces nitrogen gas, resulting in an effective reduction of the N-content in the waste water. For the elimination of N-compounds to be broken down, the oxygen concentration in the second step is less than 5 mg/ml, and preferably less than 2 mg/ml. In this way the use of pre-positioned or post-positioned reactors for the removal of nitrogen compounds can be avoided, or their purifying capacity can be downscaled, which means a saving in costs. The present invention also makes it possible to eliminate phosphate. To this end, in a step that is not the first step, and preferably at the end of the second step or at the beginning of the third step, sludge granules are removed. Surprisingly it so happens, that under the conditions of the present invention phosphate accumulating microorganisms are not competed out. All the microorganisms needed for the method according to the invention are found in the sludge of purification plants. They do not need to be isolated, since the conditions specified ensure that these microorganisms constitute part of the sludge granules. The conditions according to the invention give rise to the formation of sludge granules that are significantly larger and have a higher density than the sludge flocs obtained according to the conditions as known from US 3,864,246 (see Fig. 1), having a settling velocity >10 m/h (as opposed to approximately 1 m/h for the known sludge flocs) and a sludge volume index <35 ml/g. The sludge volume index is the volume taken up by 1 gram of biomass after 1 hour's settling. For the purification of a subsequent portion of waste water the steps 1 to 3 (one cycle) are repeated. The invention is very suitable for the treatment of sewage water.

In the first step the waste water is preferably fed to a bed of sludge granules, and the sludge granules settle in the third step, forming a bed of sludge granules.

This allows the microorganisms to be exposed to a higher concentration of organic nutrient, which promotes granular growth.

According to a preferred embodiment, the waste water is fed to the bed of sludge granules at a rate such as to avoid fluidisation of the bed.

Since it is to a large extent avoided that present already treated waste water mixes with waste water to be treated, this allows the microorganism to be exposed to the highest possible concentration of nutrient which, as already mentioned, promotes granular growth. The term "to avoid fluidisation" is understood to mean that the bed does not fluidise, and/or that as a result of introducing the waste water, mixing occurs at most in up to 25% of the height of the bed. The waste water may, for example, be sprayed onto the bed directly or by using means for limiting the force with which the waste water can disturb the bed surface. In any case, mixing will occur at most in up to 25%, preferably in less than 15% of the height of the bed. Instead of introduction from the top side of the bed of sludge granules, the waste water may preferably be introduced from below. Especially in the latter case, the feed rate will be limited such that no fluidisation of the bed occurs. In both cases it is possible to displace and discharge purified water still present between the sludge granules from the bed in an effective manner, i.e. with little or no mixing of waste water and purified (nutrient-depleted) waste water, as will be discussed below. In principle it is also possible to introduce the waste water into the bed of sludge granules via pipes.

According to a preferred embodiment, at least a part of the nutrient-depleted waste water is discharged in the third step, after at least partial settling.

The removal of nutrient-depleted waste water prior to the addition of fresh waste water to be treated means that a smaller reactor volume is needed, and that the microorganism-comprising sludge granules come into contact with a highest possible concentration of nutrients. This is favourable for the formation of sludge granules. The height of liquid in the reactor is for example twice, and preferably 1.5 times or less, such as 1.2 times the height of the bed of settled sludge granules.

According to a preferred embodiment, at least a part of the nutrient-depleted waste water is discharged during the feeding of waste water to the bed of sludge granules in the first step.

In that case, the discharge of nutrient-depleted waste water is preferably the consequence of displacement due to waste water being fed to the bed of sludge granules.

Thus with one single action both the addition of fresh waste water, and the discharge of treated waste water is realised. This can be accomplished at a low capital outlay. Further savings are possible on control technology (fewer measurements are required) and operating costs. Furthermore, mixing of treated waste water with waste water to be treated is avoided, so that the concentration of nutrients to which microorganisms in the sludge granules are exposed is as high as possible, providing the previously mentioned advantage of growth in the form of sludge granules. The displaced treated waste water is preferably discharged at the top side of the bed. Due to the displacement, any flocs that may be formed are flushed out of the reactor. Therefore, the waste water is advantageously introduced via the bottom of the bed.

An important embodiment is one wherein the waste water is introduced in an amount of 50 to 110%, preferably 80 to 105% and most preferably 90 to 100% of the void volume of the bed.

In this way the biomass in the form of sludge granules is utilised optimally, at the smallest possible reactor volume.

The introduction of the waste water is preferably followed by an interval before commencing the second step.

This promotes the uptake of nutrients from the waste water, and contributes to the formation of sludge granules with good settling qualities. If desired, mixing may take place during the interval.

The interval is preferably sufficiently long for the removal of at least 50%, preferably at least 75% and most preferably at least 90% of the organic nutrient from the waste water.

This contributes the most to the formation of sludge granules with good settling qualities, while the purification of the waste water is optimal.

It is preferred for the waste water to be introduced in the third step, wherein sludge granules that settle more slowly are discharged from the reactor and sludge granules that settle more quickly remain in the reactor.

This further increases the pressure to select for granular growth. The introduction of waste water may be performed at a low flow rate during settling of the sludge granules, preferably after at least part the sludge granules have formed a granular bed but, as explained elsewhere, most preferably after the granular bed has formed. In the first two methods there is overlap between the first and third step. In the second and especially in the third method, light sludge flocs that have settled on the bed, or that would have the tendency to do so, are carried away by the flow of nutrient-depleted water displaced by waste water. As a consequence there is a pressure of selection resulting in maintaining the characteristics of the sludge in the form of granules. It is preferred for the discharge to take place in the third step via a discharge opening just above the final bed.

The invention will now be elucidated with reference to the following exemplary embodiment wherein

Fig. 1 shows a graph of the acetate, phosphate, ammonium, and $NO_3^- + NO_2^-$ concentration during a cycle of the method according to the invention.

Figs. 2a and b show sludge flocs according to the prior art and sludge granules according to the present invention, respectively.

An air lift reactor (3 litre, height/diameter 20) was fed with 1.5 litres of waste water per cycle, which waste water represents an apropriate model for a domestic waste water. The composition was 6.3 mM sodium acetate; 3.6 mM ammonium chloride, 0.6 mM potassium phosphate, 0.37 mM magnesium sulphate, 0.48 mM potassium chloride and 0.9 ml/l standard solution of trace elements. The reactor was seeded with aerobic active sludge from a domestic wastewater purification plant. The reactor was operated in successive batch cycles. One cycle consisted of the following steps:

i) The introduction of 1.5 litres of model waste water at the bottom side of the reactor, for 60 minutes, so that there is a plug flow regime of waste water through the settled granular bed.
ii) Aeration for 111 minutes at a flow rate of 4 litres of air per minute.

iii) Settling of the granular sludge for 3 minutes after the termination of the aeration.

iv) Discharging the treated model waste water from the effluent outlet point at half the reactor height. Any biomass present at this moment above the effluent outlet point was removed from the reactor together with the treated waste water.

v) 1 minute interval, after which feeding with model waste water was recommenced.

By adding a base or acid, the pH in the reactor was maintained at 6.5 to 7.5 and the temperature was kept at 20°C. During the aerated phase ii) the concentration of dissolved oxygen was maintained at approximately 1.8 mg/ml. On the one hand this keeps the oxygen concentration sufficiently high for aerobic breakdown of nutrient in the external part of the sludge granules, and on the other hand only a low pumping capacity is required for the addition of air. After all, under these conditions, the transfer of oxygen from the air is very efficient. Consequently, there is also little energy required for the supply of oxygen. The breakdown of nitrogen compounds was shown to be optimal at these oxygen concentrations, with only minimal amounts of nitrate being found in the treated waste water.

In Table 1 the mean concentrations of the model waste water and the treated water are shown. The mean purification result is also given. Figure 1 shows the plot of the acetate (o), phosphate (Δ), ammonium (black diamond) and the sum of the nitrate and nitrite (open diamond) concentration during one cycle. Fig. 2b shows a photograph of the sludge granules obtained by the method. The obtained sludge granules were stable for at least 300 days, after which this experiment was stopped. The method according to the invention thus makes a reliable control of the operation possible. Fig. 2a shows typical sludge flocs having a settling rate as described in US 3,864,246. Although US 3,864,246 successfully combats the growth of filamentous organisms, which form so-called light sludge, the sludge flocs formed have a settling velocity of at best 1 m/h. In contrast, the sludge granules according to the present invention have very high settling velocities (>10 m/h), while the distance over which settling takes place may be relatively short.

Table 1 Concentrations of the untreated and treated model waste water

Mean values	Model waste water	Treated waste water	Removal Efficiency
Acetate (mM)	6.3	0	100%
NH_4^+ (mM)	3.6	0	97%
NO_3^- (mM)	0	0.1	
NO_2^- (mM)	0	0	
PO_4 (mM)	0.6	0.04	94%

One of the factors contributing to granular growth is feeding waste water with a highest possible nutrient concentration to the sludge granules. For this reason it is expedient to avoid mingling between treated waste water in the reactor and freshly supplied waste water. In those cases where a low nutrient concentration in the waste water prevails for many cycles, e.g. more than 10, nutrient may be added to the waste water if necessary. One option would be using liquid manure.

The present invention may be implemented in numerous ways. For example, instead of using one reactor it is propitious to use three reactors, the three reactors being operated out of phase. That is to say, while waste water is fed to one reactor, the aeration step is carried out in a second reactor, while in a third reactor settling takes place and possibly discharge of purified water. This keeps the capital outlay for pumps, especially with regard to their required maximum capacity, within limits. Treated waste water is released gradually and this is advantageous if this waste water needs to undergo a further treatment, since then also a smaller reactor for post-treatment suffices. Since compared with the above described experiment, reactors will in practice be relatively higher, settling will take longer. This means that feeding may take one third of the time, aeration and settling together two thirds of the time. A buffer tank for temporary storage of waste water to be treated is thus avoided and the three batch-operated reactors make continuous operation possible. The invention is illustrated by way of an airlift reactor, but the invention may be embodied with any other type of reactor, such as a bubble column reactor.

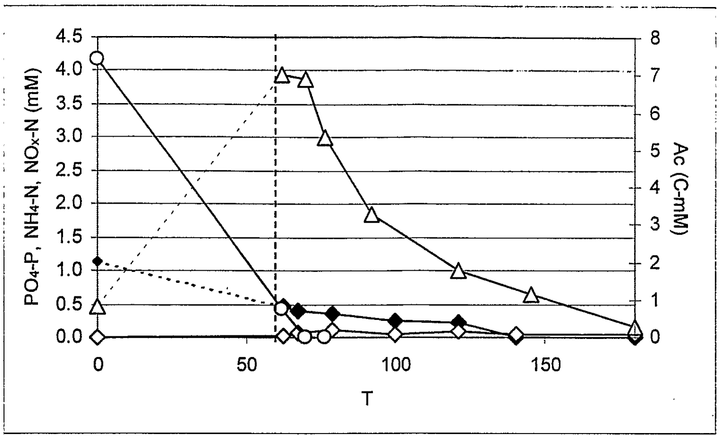


Fig. 1

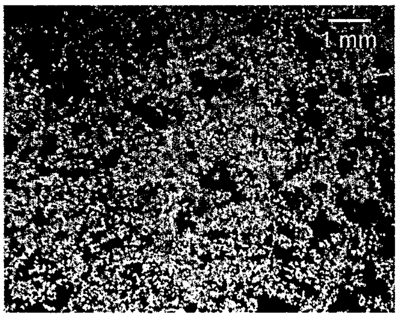


Fig. 2a

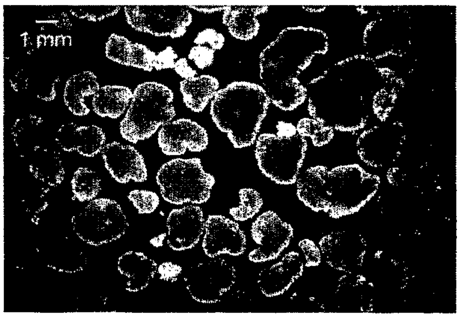


Fig. 2b

CLAIMS

1. A method for the treatment of waste water comprising an organic nutrient, wherein the waste water is brought into contact with microorganisms-comprising sludge particles, an oxygen-comprising gas is fed to the sludge particles, and the method further comprises the settling of the sludge particles and the discharge of organic nutrient-depleted waste water, **characterised** in that
- in a first step the waste water is fed to sludge granules,
- after the supply of the waste water to be treated an oxygen-comprising gas is introduced in a second step, with the granules being in a fluidised condition and
- in a third step, a settling step, the sludge granules are allowed to settle.

2. A method according to claim 1, **characterised** in that in the first step the waste water is fed to a bed of sludge granules, and the sludge granules settle in the third step, forming a bed of sludge granules.

3. A method according to claim 2, **characterised** in that the waste water is fed to the bed of sludge granules at a rate such as to avoid fluidisation of the bed.

4. A method according to one of the preceding claims, **characterised** in that at least a part of the nutrient-depleted waste water is discharged in the third step, after at least partial settling.

5. A method according to one of the preceding claims, **characterised** in that at least a part of the nutrient-depleted waste water is discharged during the feeding of waste water to the bed of sludge granules in the first step.

6. A method according to one of the preceding claims, **characterised** in that the discharge of nutrient-depleted waste water is the consequence of displacement due to waste water being fed to the bed of sludge granules.

7. A method according to one of the preceding claims, **characterised** in that the waste water is introduced in an amount of 50 to 110%, preferably 80 to 105% and most preferably 90 to 100% of the void volume of the bed.

8. A method according to one of the preceding claims, **characterised** in that the introduction of the waste water is followed by an interval before commencing the second step.

9. A method according to claim 8, **characterised** in that the interval is sufficiently long for the removal of at least 50%, preferably at least 75% and most preferably at least 90% of the organic nutrient from the waste water.

10. A method according to one of the preceding claims, **characterised** in that a selection takes place in the third step, wherein sludge granules that settle more slowly are discharged from the reactor and sludge granules that settle more quickly remain in the reactor.

Personalia

Merle Krista de Kreuk, auteur van dit proefschrift, werd geboren op 16 juli 1973 te Reeuwijk. Tijdens de middelbare school is zij via werkzaamheden bij MTI Milieutechnologie al vroeg in aanraking gekomen met de milieutechnologie. Na het voltooien van de HAVO in 1990 en het VWO in 1992, beiden aan de Nijmeegse Scholengemeenschap Groenwoud, is zij dan ook begonnen met haar studie Milieuhygiëne aan de Landbouw Universiteit te Wageningen. Deze studie heeft zij in 1997 afgerond met het behalen van een ingenieursdiploma. Het afstuderen bestond uit een internationale stage bij Umgeni water te Durban, Zuid-Afrika en twee afstudeervakken. Deze afstudeervakken bestonden uit het onderzoek naar de invloed van sporenelementen als kobalt, nikkel en ijzer op de methanogenese in anaëroob korrelslib (vakgroep milieutechnologie) en het meten en modelleren van natuurlijke convectie in compostbedden (vakgroep agrotechniek en –fysica). Naast de studie is zij actief geweest binnen studievereniging "Aktief Slip" (voorzitterschap en diverse commissies), studentenvereniging KSV st. Franciscus Xaverius (diverse commissies en voorzitterschap toneelondervereniging "de zingende Tractor") en was zij werkzaam op de administratie van ziekenhuis de Gelderse Vallei.

Na haar studie is Merle in september 1997 begonnen als junior projectleider bij MTI Holland BV., Kinderdijk. Werkzaamheden bestonden uit bepaling van bodem eigenschappen en advisering over baggerwerktuigen; studies naar de behandeling van verontreinigde waterbodems; studies naar de behandeling en eigenschappen van boorvloeistoffen voor grote diameter tunnels; projecten voor bagger-, zand en grindwinning en mijnbouw.

In 2000 is Merle de Kreuk begonnen aan haar promotieonderzoek bij de Technische Universiteit Delft. Het onderzoek betrof de opschaling en ontwikkeling van aëroob korrelslib technologie en werd uitgevoerd aan de vakgroep Biotechnologie van de faculteit Technische Natuurwetenschappen. Het onderzoek heeft plaatsgevonden in samenwerking met de ingenieurs van DHV, Amersfoort, Nederland en is beschreven in dit proefschrift. Het totale onderzoeks- en ontwikkelingsproject naar aëroob korrelslib is momenteel nog niet afgerond en Merle de Kreuk zal in vervolgprojecten nog verder gaan met de uitontwikkeling naar praktijkschaal.

List of Publications

Book

De Kreuk, M.K. and de Bruin, L.M.M. (2004). Aerobic Granule Reactor Technology. The title belongs to Water and Wastewater Practitioner Series. London: IWA Publishing. 64 p. ISBN: 1-84339-067-1

Patent

Van Loosdrecht, Marinus Cornelis Maria; De Kreuk, Merle Krista (2004). Method for the treatment of waste water with sludge granules. Patent: NL1021466C; WO2004024638.

Journal Articles and Conference Proceedings

De Kreuk, M.K., De Bruin, L.M.M., Kraan, M.W., Svecenco, A., Milia, S. and Van Loosdrecht, M.C.M. (Submitted). Nutrient removal by aerobic granular sludge; micro-electrode measurements and modeling. *Water Science and Technology*.

De Kreuk, M.K., Picioreanu, C. and Van Loosdrecht, M.C.M. (Submitted). Modelling nutrient removal in a granular sludge SBR. *Biotechnology and Bioengineering*.

Xavier, J.B., de Kreuk, M.K., Picioreanu, C. and Van Loosdrecht, M.C.M. (In Preparation). Microbial populations dynamics and nutrient conversions in aerobic granular sludge described by multi-scale modeling.

De Kreuk, M.K. and Van Loosdrecht, M.C.M. (2006). Formation of aerobic granules with Domestic Sewage. *Journal of Environmental Engineering – ASCE* **132**(6).

De Bruin, B., De Kreuk, M., Uijterlinde, C. and Marcelis, C. (2006). Aëroob korrelslib klaar voor de praktijk. *H_2O* **39**(3). 31-34.

De Kreuk, M.K., Pronk, M. and Van Loosdrecht, M.C.M. (2005). Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures. *Water Research* **39**(18). 4476-4484.

De Kreuk, M.K., Heijnen, J.J. and Van Loosdrecht, M.C.M. (2005). Simultaneous COD, Nitrogen and Phosphate Removal by Aerobic Granular Sludge. *Biotechnolology and Bioengineering* **90**(6) 761-769.

Mosquera-Corral, A., De Kreuk, M.K., Heijnen, J.J. and Van Loosdrecht, M.C.M. (2005). Effects of dissolved oxygen on N-removal in an aerobic granular sludge reactor. *Water Research* **39**(12). 2676-2686.

Bathe, S., De Kreuk, M.K., McSwain, B.S. and Schwarzenbeck, N. (Editors). Aerobic granular sludge (2005). Water and Environmental Management Series (WEMS). IWA Publishing, London, UK. 169p. ISBN:1-84339-509-6; ISSN:1476-1785.

The following contributions were included in this book:

- Van Loosdrecht, M.C.M., De Kreuk, M.K. and Heijnen, J.J. (2005). The unity of biofilm structures. pp. 1-5
- De Kreuk, M.K., De Bruin, L.M.M. and Van Loosdrecht, M.C.M. (2005). Aerobic granular sludge; from idea to pilot plant. pp. 111-123
- De Bruin, L.M.M., Van Der Roest, H.F., De Kreuk, M.K. and Van Loosdrecht, M.C.M. (2005). Promising results pilot research aerobic granular sludge technology at WWTP Ede. pp. 135-142
- De Kreuk, M.K., McSwain, B.S., Bathe, S., Tay, S.T.L., Schwarzenbeck, N. and Wilderer, P.A. (2005). Discussion outcomes. pp. 153-169

De Kreuk, M.K., De Bruin, L.M.M. and Van Loosdrecht, M.C.M. (2004). Process intensification of municipal wastewater treatment by aerobic granular sludge systems. Proceedings of the European symposium of environmental biotechnology ESEB 2004, Verstreate W. (ed.) Oostende, Belgium.

De Kreuk, M.K. and Van Loosdrecht, M.C.M. (2004). Selection of Slow Growing Organisms as a Means for Improving Aerobic Granular Sludge Stability. *Water Science and Technology* **49** (11-12). 9-19.

De Bruin, L.M.M., De Kreuk, M.K., Van Der Roest, H.F.R., Uijterlinde, C. and Van Loosdrecht, M.C.M. (2004). Aerobic Granular Sludge Technology; Alternative for Activated Sludge? *Water Science and Technology* **49** (11-12). 1-9.

De Bruin, B., De Kreuk, M. and Uijterlinde, C. (2003). Aëroob Korrelslib Technologie alternatief voor het actiefslibproces? *H_2O* **36**(14/15). 26-28.

Van Loosdrecht, M.C.M., De Kreuk, M.K., Picioreanu, C. and Heijnen, J.J. (2002). Aerobic Granular Sludge Formation. In: Lier, J van, Lexmond, M & Vos, H (Ed.), Granulation and auto-immobilisation processes in wastewater treatment. Papers of the Farewell Seminar of Dr.ir. Look Hulshoff Pol. (pp. 81-96). Wageningen, The Netherlands.

De Kreuk, M.K., van Muijen, H., De Kreuk, J.F. (2001). Higher separation Efficiency for Contaminated Soil, Sieve sand and Dredge Spoil by Pulsating Bed Separation. *Aufbereitungstechnik* 42 (5), 223 – 235.

Van Muijen, H. and De Kreuk, M.K. (1998). Ontwikkelingen in de natte mijnbouw. In: M.Ph. Hillen (Eds.). Exclusieve Economische Zones in zee; een groene export markt. SMO, The Hague, The Netherlands.

Van Loon, W.K.P., De Kreuk, M.K., Hamelers, H.V.M. and Bot, G.P.A. (1998). Water regulated air flow controller for permeability measurements. Second Int. Workshop on Control Applications in Post-harvest and Processing Technology. Farkas I. (ed.), 47-51.

Dankwoord

Af en toe heb ik gefilosofeerd of een dankwoord niet vervangen dient te worden door een "ondanks"woord. Dit ging vooral om de momenten dat er roofbouw gepleegd werd op de zorgvuldig verzamelde voor mijn onderzoek noodzakelijke attributen of als ik mij weer eens liet af leiden op sociaal vlak. Voornamelijk de laatste categorie verdient toch een grote dank op deze plek, want zonder al het plezier dat ik op het lab en daarnaast met mijn vrienden, familie en collega's heb beleefd zou ik de eindstreep nooit gehaald hebben.

Allereerst wil ik mijn promotoren Mark van Loosdrecht en Sef Heijnen bedanken voor alle raad, kennis en stroom van ideeën. De gesprekken met Mark resulteerden altijd in veel extra werk, maar dat heeft uiteindelijk wel tot een mooi onderzoek en een nieuwe zeer bruikbare technologie geleid. Mark, vooral je toegankelijkheid en de "binnenwandeltijd" heb ik altijd zeer gewaardeerd en bewonderd. Zo kon altijd een korte vraag beantwoord of iets geregeld worden. Het idee dat je vertrouwen in de goede afloop had, door mij ruimte in het onderzoek te geven, heeft mij zelfvertrouwen gegeven. Verder wil ik je bedanken voor de mogelijkheid die je mij hebt geboden om ook de toepassingen van het aëroob korrelslib actief mee te maken in de eerste aanloop van de commercialiseringfase.
Sef, jou heb ik vooral in het begin van het onderzoek veel gesproken, maar toen de eerste start eraf was ben je wat op de achtergrond getreden. Dit nam niet weg dat er een grote interesse voor de korrels bleef en ook het pushen tot schrijven heeft mij vaak dat extra duwtje gegeven om weer achter de computer te kruipen.

Verder is er natuurlijk een onophoudende stroom collega's en studenten geweest, die hebben meegewerkt en meegedacht in mijn onderzoek. Natuurlijk wil ik hier als eerste Mario Pronk, mijn paranimf, bedanken. Mario, dank je wel voor je enthousiasme voor de korrels, de experimenten, de dingetjes die je wel even regelde, de micro-elektrodes, de gezelligheid, steun en computerinfo.
Cristi, ons begin samen op de kamer was geen onverdeeld succes, maar de laatste jaren is dat zeker goed gemaakt; zeker toen ik getuige bij jou en Viorika mocht zijn. Door jou is er toch een klein modelleervuurtje gaan branden en ik hoop dat het vuur nog eens echt oplaait.

Anuska, the acceptance of chapter 2 took longer than our cooperation, but we shed tears and laughter during our cooperation. It was fun to have you around and every time we meet, I know I won't be bored.
Er is wel eens met afgunst gereageerd door mijn collegae promovendi als er weer een nieuwe student bij mij kwam werken, maar de samenwerking met de lange en internationale rij twaio's en studenten heeft mijn promotietijd nog veel interessanter gemaakt dan het onderzoek had gekund. Tania Georgieva and Maryam Hosseini, my two wonderful Twaio-ladies, you helped me with two difficult parts of my project; SBR design/flow and modeling. I know the project sometimes drove you mad, but for me it was joyful and interesting. Furthermore, Marta, Mattia, Ruud, Celine, Elodie, Montse, Stefano, Ana, Aznah, Peter, Daniele, Rocio and Nao-Hiro thank you so much for the work, the reactor maintenance and the results! Stefano, really great that you started your own PhD project in aerobic granular sludge after you left Delft.

In dit proefschrift is duidelijk naar voren gekomen dat dit onderzoek is gedaan in samenwerking met DHV Water. Deze samenwerking is op moment van schrijven gelukkig nog niet afgelopen maar juist geïntensiveerd. Omdat dit de plek is om mijn "projectcollega's" bij DHV te bedanken voor de goede discussies en ideeën, doe ik dat niet alleen voor wat is geweest, maar ook voor wat nog komen gaat. Bart de Bruin, Helle van der Roest, Robin Kraan en Jaap Verkuijlen, ik ben blij dat ik een plaats heb in de pilotteams en dat er nog maar veel goede besprekingen en goede korrels mogen komen. Verder wil ik Andreas Giessen, Robbert van der Kuy, Arnold Zilvertant en Ronald Niermans bedanken dat jullie mij steeds hebben betrokken bij alle stappen die tijdens het commercialiseringtraject van Nereda^{TM} bij DHV genomen zijn. Ik hoop dan ook dat ik hieraan kan blijven bijdragen.
Verder bedank ik Cora Uijterlinde/Paul Roeleveld (Stowa) en Chris Marcelis (STW) voor de financiering van dit project. De begeleidingscommissie/gebruikerscommissie is onlosmakelijk verbonden met Stowa en STW: Tom Wouda, Hardy Temmink, Frank Brandse, Jan Willem Mulder, Ronald Mulder, Joost van der Pluijm en Wim Wiegant, bedankt voor jullie halfjaarlijkse kritische noot en het vertrouwen in het project, zelfs toen de goede pilot-resultaten wat op zich lieten wachten.

Binnen het lab zijn er ook nog vele collega's, die zich hebben ingezet om het project tot een goed einde te brengen. Allereerst Rob, Dirk en Susan, bedankt voor de hulp bij falende ADI's, Biodacs, pompen en dergelijke. Verder natuurlijk 'de mannen van de instrumentatiedienst', die prachtige reactoren hebben gemaakt en

acute reparaties altijd direct uitvoerden. Rob, bedankt voor de lessen in opschaling. Carla, Jenny, Miranda, Elly, Marcel, Hans, Jos, Astrid, Stef, Johan, Cor, Max, Gert, Herman, Robert en Wim: bedankt voor alle ondersteuning en beantwoorde vragen. Sjaak, gewoon bedankt voor alles.
Iedereen binnen de EBT-groep natuurlijk bedankt voor alle raad, daad en gezelligheid. In het bijzonder Udo: thanx for the fish. Al mijn kamergenoten door de jaren heen bedankt voor de koffie, gezelligheid en discussies; Alessandro, Antonio, Anuska, Arjan, Bas, Cao, Claudia, Cristian, Gundula, Hao, Joao, Leon, Marlies, Mario, Raymon, Sirous en Wouter, thank you for all the nice moments.
Buiten het werk, maar op het lab, heb ik met veel leuke clubjes te maken gehad. Beide biotour commissies en de biotour deelnemers: het waren gezellige vergaderingen en dankzij jullie allemaal uiteindelijk hebben we een mooie reis naar Canada beleefd! Feestcommissie, bedankt voor de feestjes en de vele keren samen afwassen. Praag-ladies, ik hoop dat we in de toekomst meer reisjes gaan maken.

Verder wil ik natuurlijk al mijn lieve vrienden bedanken voor alle nodige afleiding, op gezellige etentjes, feestjes, weekendjes en zelfs vakanties. Zonder deze ontspanning tussendoor was het natuurlijk helemaal niets geworden met dit werk. De dames van Eau Contraire verdienen een speciale plek, omdat jullie sinds Wageningen al een soort tweede familie vormen en Maret in het speciaal omdat je mij de extra morele steun geeft als paranimf.

Mijn familie wil ik bedanken voor alle steun tijdens de, niet altijd even ontspannen, periode van schrijven. Vooral de extra zorg voor Bart van mama en papa, Peter en Heleen en Inez tijdens de laatste fase, heeft de eindspurt mogelijk gemaakt. Pap en Mam, vooral bedankt voor alle kansen die jullie mij hebben geboden en al het vertrouwen dat jullie hebben in mij.

Als laatste, maar zeker als belangrijkste personen, bedank ik Martijn en Bart, voor al het geduld en steun tijdens dit promotietraject en des te meer voor alle momenten die we samen delen!