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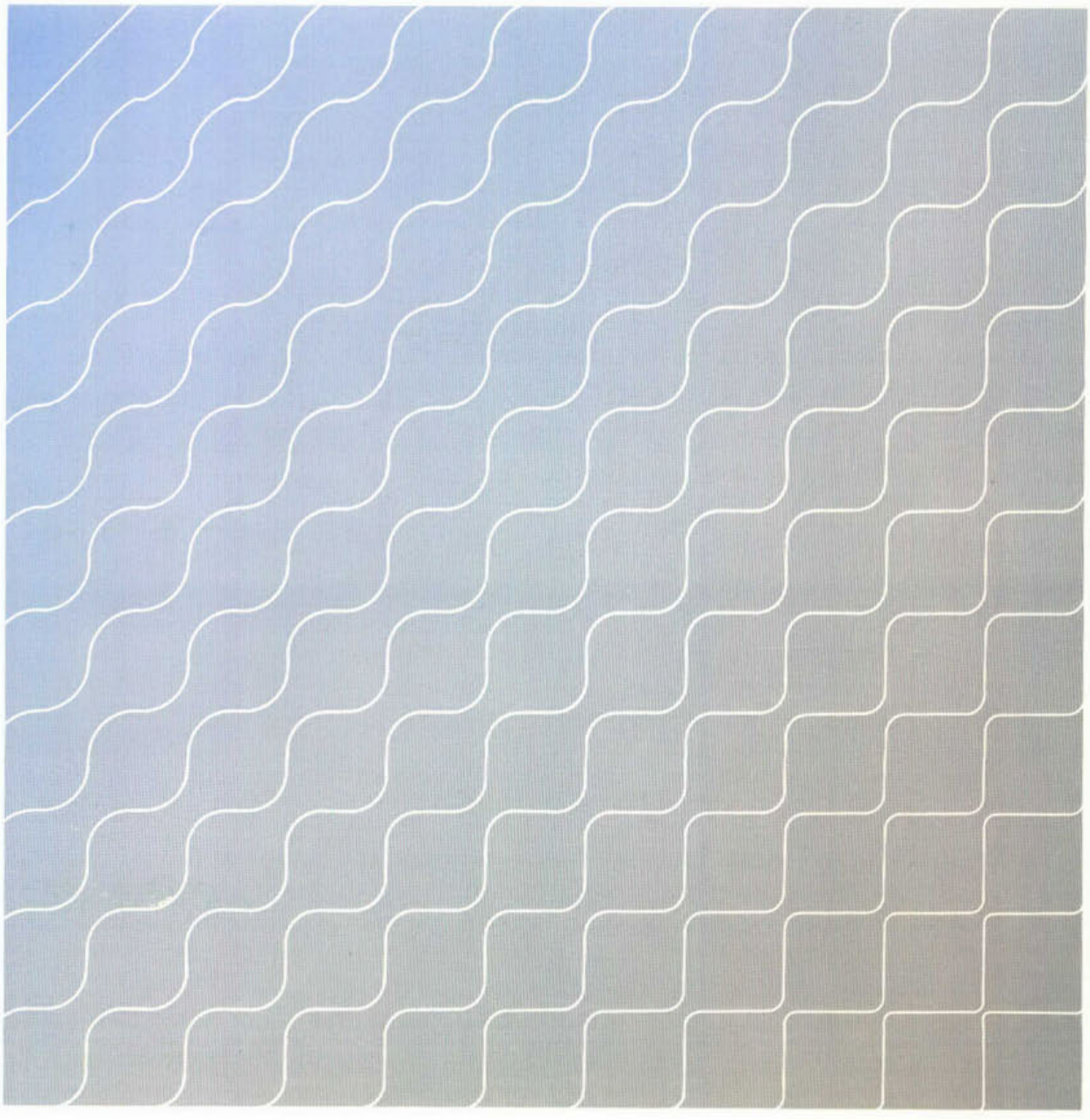
BIOLOGICAL PHOSPHATE REMOVAL UNDER DENITRIFYING CONDITIONS



Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer

Directoraat-Generaal Milieubeheer
Postbus 30945, 2500 GX Den Haag

Ministerie van Landbouw, Natuurbeheer en Visserij

Postbus 20401, 2500 EK Den Haag

RIZA

Rijkswaterstaat
Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling

Postbus 17, 8200 AA Lelystad

stowa

Stichting Toegepast Onderzoek Waterbeheer

Postbus 8090, 3503 RB Utrecht

Toekomstige generatie rioolwaterzuiveringsinrichtingen RWZI 2000

projectleiding en secretariaat: postbus 17, 8200 AA Lelystad 03200 - 70411

BIOLOGICAL PHOSPHATE REMOVAL UNDER DENITRIFYING CONDITIONS

auteur(s):

TU-Delft,vakgroep
Bioprocestechnologie:

T. Kuba Ph.D.
dr.ir. M.C.M. van Loosdrecht
prof.dr.ir. J.J. Heijnen

RWZI 2000 94-12

Het onderzoek "Toekomstige generatie rioolwaterzuiveringsinrichtingen RWZI 2000"
is een samenwerkingsverband van de STOWA en Rijkswaterstaat (RIZA).

CONTENTS

VOORWOORD

In het project "*Anaërobe/Denitrificerende defosfatering (Biological Dephosphatation under anaerobic/denitrifying conditions)*" is de mogelijkheid van een volledige integratie van biologische stikstof- en fosfaatverwijdering onderzocht. Deze integratie is gebaseerd op microorganismen die onder denitrificerende condities fosfaat kunnen opnemen. Het belangrijkste voordeel van dit proces is dat er minder CZV nodig is voor de nutriënteneliminatie; daarnaast zijn het energieverbruik (voor beluchting) en de slibproductie lager.

Het project is in 1993 gestart en was gebaseerd op eerder onderzoek naar de mogelijkheid van denitrificerende defosfatering (NOVEM 51120/1610). Dit project had tot doel om, naast het verwerven van kennis betreffende de stoichiometrie en kinetiek van denitrificerende defosfateerders, ook de mogelijkheden te onderzoeken voor diverse uitvoeringsvormen van zuiveringsprocessen, waarin deze organismen optimaal worden gebruikt.

Het onderzoek is hoofdzakelijk in laboratoriumopstellingen (SBR reactoren met synthetisch afvalwater) van de vakgroep Bioprocestechnologie van de TU-Delft uitgevoerd. Ook zijn twee r.w.z.i's in beschouwing genomen om het optreden van denitrificerende defosfatering in de praktijk te bestuderen. Dit laatste is in samenwerking met het Zuiveringschap West-Overijssel uitgevoerd.

In het kader van dit project is in samenwerking met ingenieursbureau Haskoning een feasibilitystudie uitgevoerd naar de mogelijkheden van gescheiden slibsystemen waarin denitrificerende defosfatering wordt gebruikt (NOVEM project 351230/1710).

Dit project "*Anaërobe/denitrificerende defosfatering*" is gefinancierd door STOWA/RIZA (RWZI 2000-3234/5), NOVEM (351230/1110), en de TUD (STM92.024).

Het onderzoek is uitgevoerd door een Japanse gastmedewerker bij de vakgroep Bioprocestechnologie van de TU-Delft (Kuba, T. PhD). Begeleiding vondt plaats binnen de vakgroep door dr.ir. M.C.M. van Loosdrecht en prof.dr.ir. J.J. Heijnen alsmede door een externe commissie bestaande uit ir. S.S.J. Houtman en ir. J.J.D. van der Steen (NOVEM voorzitter), ing. F.A. Brandse (Zuiveringschap West-Overijssel), dr. M.M.A. Ferdinandy (RIZA), ing. G.A.P. van Geest (Hoogheemraadschap van Rijnland), dr.ir. G.J. Kortstee (LU-Wageningen) en ir. P.C. Stamperius (STOWA).

De verslaglegging van de onderzoeksresultaten heeft plaatsgevonden in het engels omdat het onderzoek en de rapportage is gerealiseerd door de heer T. Kuba. Een uitgebreide nederlandstalige samenvatting met enkele belangrijke aspecten voor de praktijk is toegevoegd.

Lelystad, September 1995

Voor de stuurgroep RWZI 2000

prof.dr. J. de Jong
(voorzitter)

UITGEBREIDE SAMENVATTING

Introductie

Bij conventionele fosfaat- en stikstofverwijdering uit rioolwater worden stikstof en fosfaat door twee verschillende groepen microorganismen uit het water verwijderd. Voor beide processen is CZV nodig voor de groei van de betrokken bacteriën. Als complicerende factor kan nitraat (bij een onjuiste procesvoering) storend werken op de biologische fosfaatverwijdering. Minimalisatie van de CZV-behoefte kan worden verkregen indien organismen worden gebruikt die nitraat i.p.v. zuurstof gebruiken tijdens de fosfaatophoping. Denitrificatie vergt ongeveer 4 g CZV per g N verwijderd, terwijl P-eliminatie globaal 20 g CZV per g P vereist. Stel dat uit een afvalwater 40 g N en 4 g P dient te worden verwijderd dan is daarvoor (4*40 + 20*4 =) 240 mg CZV nodig, een optimale combinatie van beide processen in één organisme leidt tot een CZV behoefte van slechts 160 mg CZV. Dit betekent in dit voorbeeld een 30 % lagere behoefte. Globaal kan gesteld worden dat iedere g P die onder anoxische condities wordt verwijderd een besparing op de CZV-behoefte oplevert van 20 g.

In diverse recente onderzoeken zijn denitrificerende P-verwijderende bacteriën (DPB's) in actief slib systemen aangetoond (Vlekke *et al.* 1988, Pokethitiyook *et al.* 1990, Wanner *et al.* 1992, and Kern-Jespersen *et al.* 1993). Uit onderzoek voorafgaand aan het in dit rapport beschreven onderzoek bleek dat P-eliminatie onder denitrificerende condities dezelfde mogelijkheden heeft als onder aërobe condities (Van Loosdrecht *et al.* 1992, Kuba *et al.* 1992, 1993a). Het belangrijkste voordeel van het gebruik van DPB's is de besparing van CZV en beluchtingsenergie, omdat het fosfaat door deze organismen onder denitrificerende condities kan worden opgenomen.

Voor het verkrijgen van nitraat als elektron-acceptor voor DPB's dient nitrificatie plaats te vinden. Dit kan worden verkregen in een gemengd slib-systeem (DPB's en nitrificeerders samen) of in een twee-slibsysteem. In het eerste geval ondergaan alle microorganismen anaërobe, anoxische en aërobe condities. In het tweede systeem worden de nitrificeerders onder aërobe condities gehouden, terwijl de DPB's zo min mogelijk aërobe condities ondergaan. In een twee-slibsysteem kunnen de nitrificeerders worden gekweekt in b.v. een biofilm reactor.

Doel van het verrichte onderzoek was het vaststellen van fundamentele stoichiometrische en kinetische parameters van DPB's, en de evaluatie van diverse procesopties waarin gebruik wordt gemaakt van de eigenschappen van deze organismen. Het hiertoe uitgevoerde onderzoek bestond uit een aantal deelonderzoeken:

I Evaluatie van de stoichiometrie en kinetiek van de denitrificerende defosfatering t.b.v. procesmodellering
II Effect van simultane aanwezigheid van CZV en nitraat op de fosfaat afgifte
III Interpretatie van P-afgifte of P-opname testen
IV Het effect van zuurstof op denitrificerende defosfateerders in post- en predenitrificatiesystemen
V Evaluatie van het optreden van denitrificerende defosfatering in de praktijk, gebaseerd op ontwikkelde batchtesten
VI Evaluatie van de mogelijkheden om denitrificerende defosfatering toe te passen. Het betreft hier 1-slibsystemen met post- of pre-denitrificatie, of een 2-slibsysteem met een gescheiden nitrificatie en defosfateringsslib

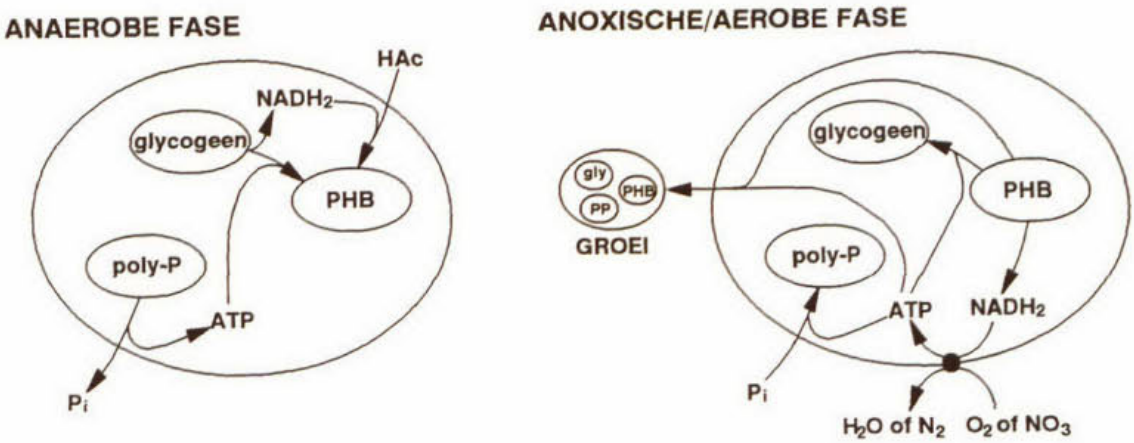
Laboratorium Anaërobe-Anoxische Sequencing Batch Reactor

Gedurende het gehele onderzoek is een laboratorium schaal (2,5 *l*) anaërobe-anoxische (A2) "sequencing batch reactor" (SBR) bedreven. De condities waren: slibleeftijd 9-20 dagen, pH 7, kamertemperatuur. Het hierin gekweekte DPB-slib werd gebruikt voor onderzoek naar kinetische parameters van DPB's. Tevens diende het als entslib voor de andere systemen. De A2-SBR werd bedreven met een 6 uurs cyclus, omdat dit praktisch gezien het meest eenvoudigst was (4 cycli per dag). Een cyclus bestond uit drie fases: 2 uur anaërobie, 3,5 uur anoxie en 0,5 bezinking. Aan het begin van de anaërobe fase werd steeds synthetisch afvalwater gedoseerd (400 mg HAc-CZV/*l*; 15 mg P/*l*; 60-120 mg NH_4-N/*l*). Gedurende de anoxische fase werd nitraat toegevoerd.

Resultaten

I Kinetiek en stoichiometrie van P-eliminatie onder denitrificerende condities

In figuur is een schematisch overzicht gegeven van het metabolisme van defosfaterende microorganismen onder anaërobe en aërobe of anoxische condities. Het metabolisme van DPB's is vrijwel identiek aan dat van aërobe defosfateerders, alleen wordt nitraat i.p.v. zuurstof gebruikt om CZV (in dit geval PHB) te oxyderen. Het is zelfs de vraag of het wel twee verschillende organismen zijn of dat slechts één organisme actief is. Hierover kan geen eenduidige uitspraak worden gedaan. Het staat echter wel vast dat alle organismen die kunnen denitrificeren ook onder aërobe condities actief zijn. Dat wil zeggen dat DPB's zowel onder anoxische als aërobe condities fosfaat op kunnen nemen.



Defosfaterende microorganismen bezitten in de cel drie soorten opslagstoffen. Polyhydroxybutyraat (PHB) wordt onder anaërobe condities gevormd uit in het afvalwater aanwezig CZV. Hiervoor zijn de andere twee opgeslagen verbindingen nodig nl. glycogeen en polyfosfaat. Onder aërobe of anoxische condities worden deze twee verbindingen weer uit PHB gevormd.

Onder anaërobe condities wordt acetaat opgenomen en omgezet naar poly-hydroxybutyraat (PHB). Hiervoor is NADH2 (een intracellulaire verbinding) nodig, dit wordt door het organisme gevormd door opgeslagen glycogeen om te zetten in PHB. De benodigde energie (in de vorm van ATP) wordt gewonnen uit de omzetting van glycogeen en hydrolyse van polyfosfaat (Mino *et al.* 1987). De pH heeft een sterk effect op de verhouding tussen acetaat-opname en fosfaat-afgifte onder anaërobe condities. De maximale P-afgifte wordt meestal bepaald door een limitatie in de hoeveelheid glycogeen in de cellen. Eigenlijk moet dus worden gesproken van een maximale acetaat-opname i.p.v. maximale P-afgifte. Deze mogelijke limitatie van glycogeen zal in de praktijk

niet snel van belang zijn. Alleen onder extreme condities of in apart uitgevoerde fosfaat-afgiftetesten met acetaatdosering zal glycogeen limitatie optreden.

Onder aërobe of anoxische condities wordt PHB geoxideerd tot CO_2 waarbij $NADH_2$ vrijkomt. Deze verbinding kan worden omgezet in ATP. De energie van ATP gebruikt het organisme om te groeien, fosfaat op te slaan en glycogeen te vormen. Het grote verschil tussen aërobe en anoxische omzettingen is dat bij de ATP vorming uit $NADH_2$ zuurstof of nitraat wordt gebruikt. De rest van het metabolisme is nagenoeg identiek. De gemeten verhouding ATP/$NADH_2$ met nitraat was ongeveer 1.0 mol ATP/mol $NADH_2$, dit is ongeveer 40 % lager dan de ATP produktie onder aërobe condities. Dit lagere ATP/$NADH_2$ verhouding verklaart de lagere biomassaproductie onder anoxische condities.

Aangezien de ATP/$NADH_2$ ratio in feite het enige verschil tussen denitrificerende en aërobe defosfaterende bacteriën is kan het in eerder onderzoek gemaakte metabole model voor aërobe P-opname (Smolders *et al.* (1994a,b, 1995a,b)) worden gebruikt voor de beschrijving van de denitrificerende fosfaatophoping. Het model was inderdaad goed in staat om het concentratieverloop van de diverse extra- en intracellulaire stoffen gedurende een cyclus in de SBR te beschrijven. Het bleek echter dat enkele kinetische parameters voor denitrificerende condities een andere waarde hadden dan voor aërobe P-opname. Aan het slot van deze samenvatting is het verkregen anoxische model in IAWQ format weergegeven.

Om het effect van snelle dynamische wisseling van anoxische/anaërobe/aërobe condities zoals die optreden in systemen met grote interne recirculatiestromen uit te testen is een SBR bedreven met meerdere anaërobe/anoxische fasen per 6 uurs cyclus. Dit leidt tot een gemiddeld lager PHB-gehalte in de cellen. Een gevolg was een niet stabiele fosfaat verwijdering. Door het lagere PHB-gehalte daalt de denitrificatie-activiteit zodat regelmatig nitraat in de anaërobe fase terecht kwam. Dit leidt automatisch tot een verslechtering van de P-eliminatie door groei van normale denitrificeerders. Omdat een conventionele anaërobe-aërobe SBR met snelle wisselingen wel een goede fosfaatverwijdering te zien geeft kan geconcludeerd worden dat vnl. de slecht gecontroleerde introductie van nitraat in de anaërobe fase de instabiliteit veroorzaakte. Deze experimenten lieten nogmaals zien dat nitraat indien het in de anaërobe fase terecht komt de fosfaatverwijdering kan verstoren. Voor de praktijk kan dit probleem waarschijnlijk worden opgelost door een adequate sturing van de nitraat concentratie (b.v. middels een redox-meting).

II Effect van nitraat in de anaërobe fase op fosfaatafgifte

In de praktijk komt het vaak voor dat nitraat via recyclestromen in de anaërobe fase terecht komt. Deze introductie van nitraat in de anaërobe fase van een biologisch P-verwijderingsproces kan leiden tot procesverstoringen, met de volgende twee effecten:

Op de lange termijn zal de introductie van nitraat in de anaërobe zone leiden tot de ingroei van normale denitrificeerders. Deze gaan efficiënter met het substraat om en hebben in het geval van simultane aanwezigheid van CZV en nitraat in het water een duidelijk competitief voordeel.

Op de korte termijn (b.v. tijdens testen om de slibactiviteit te meten) kan echter óók een verlaging van de P-afgifte optreden omdat een deel van het acetaat wordt gebruikt voor denitrificatie. Experimenteel is gevonden dat in puur DPB-slib wanneer simultaan nitraat en acetaat aanwezig zijn PHB wordt gevormd en fosfaat wordt afgegeven (het laatste in geringere mate dan in afwezigheid van nitraat). In de $NADH_2$ behoefte voor PHB-vorming wordt nu voorzien door acetaat oxydatie met nitraat terwijl polyfosfaat degradatie in de energiebehoefte voorziet. Het bleek mogelijk om ook voor deze condities het eerder opgestelde metabole model toe te

passen.

De betekenis van deze waarnemingen is enerzijds dat nogmaals onderlijnd wordt dat introductie van nitraat in de anaërobe zone dient te worden voorkomen. Anderzijds wordt duidelijk dat de vaak waargenomen verminderde P-afgifte onder anoxische condities samenhangt met de activiteit van denitrificerende defosfateerders. Dit zou er op wijzen dat dit type organismen in vrijwel elk slib reeds voorkomt. De enige andere verklaring voor een verminderde afgifte is de toxische werking van NO op defosfateerders (Appeldoorn, 1993). Deze verbinding wordt mogelijk tijdens denitrificatie gevormd, maar de concentraties nodig om remming van de P-afgifte te bewerkstelligen zijn echter veel hoger dan NO-concentraties die tijdens denitrificatie onder praktijkcondities worden gemeten.

III Interpretatie van P-afgifte en P-opname testen

In actief-slib uit rwzi's zullen twee soorten defosfateerders aanwezig kunnen zijn: (i) organismen die alleen zuurstof kunnen gebruiken en (ii) organismen die zuurstof en nitraat kunnen gebruiken. Daarnaast hoeven denitrificerende organismen niet over hun volledige capaciteit te beschikken omdat de enzymen die nodig zijn om nitraat in stikstof om te zetten niet altijd volledig beschikbaar zijn. Deze situatie maakt de interpretatie van P-afgifte en P-opname gegevens lastig. Deze interpretatie is echter van groot praktisch belang i.v.m. het meten van de hoeveelheid aërobe en denitrificerende P-organismen in actief slib.

P-afgifte. Uit eerder onderzoek is gebleken dat beide groepen defosfateerders onder anaërobe condities met ongeveer dezelfde snelheid fosfaat afgeven of acetaat opnemen.

Onder anoxische condities met acetaat aanwezig zullen de aërobe defosfateerders zich hetzelfde gedragen als onder anaërobe condities, terwijl de denitrificerende defosfateerders geen of weinig fosfaat afgeven maar wel acetaat opnemen. Complicerende factor hierbij is dat de denitrificatiesnelheid van bacteriën sterk beïnvloed kan worden door de condities waaronder het slib is opgekweekt. Het is dan ook niet mogelijk om op basis van P-afgifte tests uitspraken te doen over de samenstelling van het slib.

P-opname Onder aërobe condities zullen beide groepen defosfateerders fosfaat opnemen. Eerder onderzoek heeft laten zien dat er geen verschil is in de fosfaatopnamesnelheid tussen beide groepen. Bovendien is vanuit de microbiologie bekend dat vrijwel alle denitrificerende bacteriën ook onder aërobe condities actief zijn.

Onder anoxische condities (zonder extern substraat) zullen alleen de denitrificerende defosfateerders substraat opnemen. Fosfaatopname experimenten zijn dus in principe geschikt om onderscheid te maken tussen beide activiteiten. Echte quantificering van het aandeel denitrificerende defosfateerders is echter lastig omdat, zoals hiervoor reeds vermeld, de denitrificatiesnelheid per bacterie niet op voorhand te voorspellen is.

Gezien het belang van een goede slibkarakterisering voor de modellering van actief-slibprocessen, zal in vervolgonderzoek hier speciale aandacht aan dienen te worden gegeven.

IV Het effect van zuurstof op denitrificerende defosfateerders in 1-slib post- en pre-denitrificatiesystemen

In de meeste gangbare rwzi's gaat het slib door anaërobe, anoxische en aërobe periodes.

Hierbij moeten twee situaties worden onderscheiden: post-denitrificatie en pre-denitrificatie. In beide systemen komen de denitrificerende bacteriën voor of na de anoxische fase bloot te staan aan aërobe condities. Om de potentie van denitrificerende defosfateerders goed te kunnen inschatten is onderzocht of regelmatige blootstelling aan zuurstof leidt tot een verminderde denitrificerende P-opname capaciteit.

Nadat slib in een SBR onder anaërobe-anoxische condities was gekweekt is een aërobe fase in het systeem geïntroduceerd, vergelijkbaar met een post-denitrificatieproces. De experimenten in de anaëroob-aëroob-anoxische (AOA) SBR toonden aan dat zuurstof geen direct verstorend effect heeft op de denitrificerende P-eliminatie activiteit. In dit soort post-denitrificatie-processen wordt echter een groot deel van het fosfaat reeds in de voorliggende aërobe fase opgenomen, daarnaast wordt de denitrificatie snelheid vertraagd door het verlaagde PHB gehalte in de anoxische fase. Het PHB is namelijk al voor een groot deel geoxideerd onder aërobe condities. Het experiment toonde duidelijk aan dat voor een optimale benutting van de activiteit van DPB's een pre-denitrificatie proces de voorkeur heeft. Het retourslib (met vaak nitraat daarin) dient dan niet vooraan in het proces te worden ingevoerd maar in de anoxische fase. Deze procesvoering wordt in feite toegepast in UCT-achtige processen. Doordat in deze processen een recycle bestaat tussen anoxische en anaërobe zone, worden zeer goede condities voor de ophoping van denitrificerende defosfaterende bacteriën aangelegd. De rwzi Holten is globaal een UCT proces waar door het zuiveringschap modificaties zijn aangebracht. Metingen aan slib uit deze installatie lieten inderdaad een grote opname van fosfaat onder denitrificerende condities zien (zie verder).

In één-slibsystemen wordt de lengte van de aërobe fase bepaald door de nitrificatie-activiteit. Dit betekend relatief lange aërobe periodes en altijd een significant deel van de P-opname en PHB (of CZV) oxydatie onder aërobe condities. Vanuit dit gezichtspunt dient voor een optimale benutting van de DPB activiteit gebruik gemaakt te worden van twee-slibsystemen.

V *Toepassing van denitrificerende defosfateerders in een twee-slibsoortensysteem voor de P en N eliminatie*

Omdat bij een 1-slibsysteem altijd een deel van de P-opname (en CZV-eliminatie) plaats heeft onder aërobe condities is geëvalueerd of een strikte scheiding tussen nitrificerend en P-eliminerend slib mogelijk is. Op lab schaal is een dergelijk 2-slibsoortensysteem bedreven door een nitrificatie SBR te koppelen aan een anaërobe/anoxische SBR (A2-SBR). Alleen het supernatant (na bezinking) werd tussen beide systemen uitgewisseld. Het afvalwater ondergaat eerst een behandeling in de A2-SBR, voor P-afgifte en acetaatopname. Daarna wordt het bovenstaande water genitrificeerd in de nitrificatie-SBR. Tenslotte wordt het genitrificeerde water weer in de A2-SBR gebracht voor denitrificatie en P-opname.

Het systeem kon stabiel bedreven worden met een goede fosfaat en stikstofverwijdering (98 en 89 % respectievelijk). De mogelijkheden voor dit systeem zijn sterk afhankelijk van de ammonium-fosfaat verhouding van het influent. Het optimale werkgebied is daarnaast mede afhankelijk van de verkregen SVI. Dit bepaalt namelijk de fractie van het ammonium in het influent dat kan worden genitrificeerd. Het overgebleven ammonium dient voor slibgroei te worden aangewend.

Uit het onderzoek kwam naar voren dat voor een optimale denitrificerende defosfatering een CZV/N-ratio groter dan 3,4 nodig is. Bij hogere verhoudingen dient de rest-CZV aëroob verwijderd te worden. In het hier onderzochte systeem was 400 mg CZV nodig om 15 mg P en 110 mg N te verwijderen. In een conventioneel proces zou meer dan 600 mg CZV noodzakelijk zijn geweest.

Op basis van gegevens verkregen uit het laboratorium onderzoek is gezamenlijk met Haskoning B.V. een bureaustudie uitgevoerd naar de mogelijkheden voor het voorgestelde 2-slibsoortensysteem (NOVEM rapport no 351230/1710). Het systeem is vergeleken met conventionele chemische defosfatering en een UCT-proces. Het bleek dat het 2-slibsoortensysteem voordelen had op het gebied van slibproductie en chemicaliënverbruik. De introductie van extra processtappen leidt echter tot een aanzienlijke extra investering. Dit maakt dat het 2-slibsoortensysteem onevenredig duur uitvalt t.o.v. het UCT proces.

VI Evaluatie van het optreden van denitrificerende defosfatering in de praktijk

Naar aanleiding van de conclusie dat UCT-achtige processen een goede mogelijkheid bieden tot het ophopen van denitrificerende defosfateerders is onderzoek gedaan op twee praktijk rwzi's. Het betreft hier de rwzi's Holten en Genemuiden. Beide systemen zijn (door het waterschap) gemodificeerde UCT installaties, aangeduid als BCFS-proces. BCFS staat voor biologisch-chemisch fosfaatstripproces. In het proces wordt het deel van het fosfaat dat niet biologisch middels het spuislib kan worden verwijderd middels een in de waterlijn ingebouwde striptank verwijderd en chemisch geprecipiteerd.

Middels batch tests zijn de activiteiten van defosfaterende microorganismen onderzocht. Hieronder volgt een overzicht van de voornaamste resultaten:

	rwzi Genemuiden	rwzi Holten
Maximale P-afgifte (mg P/g org.stof)	15	30
Fractie P-organismen in slib (aëroob en denitrificerend)	0.1-0.2	0.40
Fractie DPB van de P-organismen	0.2-0.4	0.45

Uitgebreide metingen aan de rwzi Holten hebben laten zien dat een significant deel van het fosfaat in de anoxische zone van de installatie wordt opgenomen.

Het verschil tussen de fracties defosfateerders in beide installaties wordt veroorzaakt doordat de condities in de rwzi Genemuiden minder gunstig zijn. De belangrijkste verschillen zijn:

het influent van de rwzi Holten komt voornamelijk uit een persleiding met een zeer lange verblijftijd. Dit betekent dat het influent van de rwzi Holten een grotere fractie vluchtige vetzuren bevat dan dat van de rwzi Genemuiden.

de procescontrole van de rwzi Holten is geoptimaliseerd op het voorkomen van introductie van nitraat in de anaërobe fase. De procescontrole van de rwzi Genemuiden is gericht op het beschikbaar houden van voldoende aëratiecapaciteit t.b.v. de nitrificatie. Het gevolg is dat juist wanneer er influent wordt aangevoerd de anoxische fase wordt belucht. Hierdoor zal ook zuurstof in de "anaërobe" fase terecht komen. Deze regeling leidt tot een verminderd substraataanbod voor de defosfateerders en dientengevolge lagere defosfateringscapaciteit. Het niet mogelijk zijn van een betere regeling m.b.t. de defosfatering ligt met name aan het feit dat oorspronkelijk de installatie voor een andere procesvoering is uitgelegd.

De metingen laten zien dat denitrificerende defosfateerders een significante bijdrage kunnen leveren aan de omzettingen in bestaande rwzi's. Bij het evalueren (en modelleren) van biologische defosfatering kan deze activiteit niet worden verwaarloosd.

In de rwzi Holten vindt voorbezinking plaats. Omdat de denitrificatie niet volledig verliep is deze voorbezinking tijdelijk buiten werking gezet. Gedurende deze periode was de belasting van

het systeem dus hoger (voornamelijk door de toevoer gesuspendeerd materiaal) en dientengevolge was de slibleeftijd korter. Uit activiteitsmetingen bleek dat de fractie defosfateerders in het slib was afgenomen. De totale produktie aan biologische defosfateerders bleef echter constant. Ook bleef het aandeel denitrificeerders van de defosfateerders gelijk. Geconcludeerd kon dus worden dat het al dan niet toevoeren van gesuspendeerd materiaal in de rwzi Holten niet veel effect heeft op de defosfateringscapaciteit. Indien echter veel langere anaërobe verblijftijden worden toegepast kan er wel degelijk een effect optreden.

Conclusies

Dit onderzoek heeft aangetoond dat denitrificerende fosfaatverwijderende bacteriën goede mogelijkheden bieden voor de optimalisatie van geintegreerde N en P eliminatie processen, zeker indien er een efficiënt gebruik van CZV nodig is. In verscheidene rwzi's zijn dit soort organismen reeds in ruime mate aanwezig. Die aanwezigheid is niet beperkt tot de hier bestudeerde UCT-achtige processen. Bij de evaluatie van biologische defosfateringsprocessen en eventuele modelleringsstudies kan deze groep bacteriën dus niet verwaarloosd worden.

Op basis van denitrificerende defosfateerders is het mogelijk om een 2-slibproces te ontwerpen waarbij nitrificatie wordt gescheiden van P-eliminatie en denitrificatie. Het toepassen van 2-slibprocessen leidt tot een optimaal gebruik van het aanwezige CZV voor denitrificatie en P-eliminatie, de kosten van het introduceren van extra procesonderdelen zijn echter relatief hoog. In het BCFS proces zoals onderzocht in de rwzi Holten wordt ook reeds efficiënt met de beschikbare CZV omgegaan. Daarom lijkt het meest voor de handliggend om gemodificeerde (1-slibs) UCT processen te optimaliseren.

Overige conclusies:

* Denitrificerende defosfatering is analoog aan aërobe defosfatering.
* Optimaal gebruik van denitrificerende defosfatering leidt tot een verminderde CZV behoefte van ongeveer 30 %. Per gram fosfaat die anoxisch wordt verwijderd wordt globaal 20 gram CZV bespaard.
* Onder anaërobe condities is glycogeen de limiterende factor voor P-afgifte en acetaat-opname.
* Reductie van de P-afgifte door nitraat in batch tests is het gevolg van de activiteit van denitrificerende defosfateerders.
* P-opname tests geven het meest betrouwbare beeld over het aandeel denitrificeerders in de totale defosfateringspopulatie.
* Het ondergaan van aërobe periodes heeft geen grote negatieve effecten op de denitrificerende defosfateerders.
* Om N- en P-eliminatie in een 1-slibsproces optimaal te bedrijven dient een predenitrificatieproces te worden gebruikt.
* Het metabole model voor de biologische defosfatering zoals opgesteld voor aërobe defosfatering bleek goed toepasbaar op de denitrificerende defosfatering. Om het model te kunnen gebruiken indien beide soorten organismen in het systeem voorkomen dient echter nog het nodige onderzoek te worden verricht.
* Toevoer van gesuspendeerd CZV heeft geen effect op de biologische defosfateringscapaciteit in de rwzi Holten.

Bijlage bij de uitgebreide samenvatting.

IAWQ format voor het metabole model van denitrificerende defosfateerders

alles uitgedrukt in gram P of CZV

Stoichiometrie Matrix							
Proces	S_{NO3}	S_A	S_{PO4}	X_{DPB}	X_{PP}	X_{PHA}	X_{GL}
1 Opname van S_A		-1	0.35		-0.35	1.5	-0.5
2 Anoxische groei	-0.63		-0.013	1		-1.63	
3 Opslag van X_{PP}	-0.53		-1		1	-0.53	
4 Opslag van X_{GL}	-0.43					-1.43	1
5 Anaërobe maintenance			1		-1		
6 Anoxische maintenance	-1					-1	

Kinetiek vergelijkingen	
1 Opname van S_A	$q_{PHA} \cdot \frac{S_A}{K_A + S_A} \cdot X_{DPB} \cdot \left(1 - \frac{S_{NO3}}{K_{NO3} + S_{NO3}}\right)$
2 Aërobe groei	$k_{XDPB} \cdot \left(\frac{X_{PHA}}{X_{DPB}}\right) \cdot X_{DPB} \cdot \left(\frac{S_{NO3}}{K_{NO3} + S_{NO3}}\right)$
3 Opslag van X_{PP}	$k_{PP} \cdot \left(\frac{S_p}{K_p + S_p}\right) \cdot \left(1 - \frac{X_{PP}/X_{DPB}}{f_{PP}^{max}}\right) \cdot \left(\frac{X_{PHA}}{X_{DPB}}\right)^{1/3} \cdot X_{DPB} \cdot \left(\frac{S_{NO3}}{K_{NO3} + S_{NO3}}\right)$
4 Opslag van X_{GL}	$k_{GL} \cdot \left(f_{GL}^{MAX} - \frac{X_{GL}}{X_{DPB}}\right) \cdot X_{DPB} \cdot \left(\frac{S_{NO3}}{K_{NO3} + S_{NO3}}\right)$
5 Anaërobe maintenance	$m_{an} \cdot X_{DPB} \cdot \left(1 - \frac{S_{NO3}}{K_{NO3} + S_{NO3}}\right)$
6 Aërobe maintenance	$m_{anox} \cdot X_{PAO} \cdot \left(\frac{S_{NO3}}{K_{NO3} + S_{NO3}}\right)$

Definitie van de opgeloste en particulaire verbindingen

Component	Definitie	eenheden
S_A	Fermentatie produkten, beschouwd als acetaat	gCZV/*l*
S_{no3}	Nitraat	g/*l*
S_{PO4}	Anorganisch opgelost fosfaat	g/*l*
X_{DPB}	Denitrificerende defosfateerders	gCZV/*l*
X_{PP}	Polyfosfaat	gCZV/*l*
X_{PHA}	Opgeslagen PHB en PHV	gCZV/*l*
X_{GL}	Opgeslagen Glycogeen	gCZV/*l*

Definitie van de waarden voor de kinetische coëfficiënten

Coëfficiënt	Waarde	eenheid
q_{PHA}	0.2	mol-C/mol-C.h
k_X	0.05	mol-C/mol-C.h
k_{PP}	0.1	mmol/mol-C.h
k_{GL}	0.8	mmol-C/mol-C.h
m_{an}	$2.5 \cdot 10^{-3}$	mol-P/mol-C.h
m_{anox}	$3.64 \cdot 10^{-3}$	mol-C/mol-C.h
K_A	1	mmol-C/*l*
K_P	0.1	mmol/*l*
f_{PP}^{MAX}	0.3	mol/mol-C
f_{GL}^{MAX}	0.3	mol-C/mol-C
K_{PP} switch factor	$1 \cdot 10^{-3}$	gPP/gCZV-DPB
K_{N03}	0.05	mmol/*l*

Conversie factoren

Component		Massa (g)	CZV (g)
Acetaat	1 C-mol	30	32
PHB	1 C-mol	21.5	36
Biomassa	1 C-mol	26	36
Glycogeen	1 C-mol	27	32
Fosfaat	1 P-mol	31	0

ENGLISH SUMMARY

Denitrifying dephosphatation

Phosphorus and nitrogen removal from waste water are the key factor in the prevention of eutrophication of closed water areas. Therefore biological phosphorus and nitrogen (nitrification and denitrification) removal are usually integrated in waste water treatment processes. The intermediate product in the nitrogen removal process, nitrate, is often reported as an inhibitory factor for the phosphorus removal process. On the other hand, from a theoretical microbiological point of view there is no restriction against the use of nitrate as an electron acceptor for phosphorus removal, instead of oxygen, i.e., denitrifying dephosphatation. In fact, recently several publications have shown the occurrence of denitrifying phosphorus removing bacteria (DPB) in the activated sludge. And also it has shown that the capacity of dephosphatation by DPB is similar as for the conventional aerobic phosphorus removing organisms. The main advantage of applying DPB is the possible saving of COD and energy (aeration). Dephosphatation and denitrification require both separately COD in conventional treatment processes. For denitrifying phosphorus removal the same COD removes phosphorus and nitrogen, moreover the dephosphatation process does not require aeration.

To obtain nitrate as an electron acceptor for DPB, nitrification should be introduced into the systems. This can be achieved in a single-sludge or two-sludge system. In the single-sludge system, DPB coexist with nitrifiers, and the mixed sludge goes through all stages such as anaerobic, aerobic and anoxic phases. In the two-sludge system, nitrifiers are separated from DPB, e.g., in a nitrification biofilm reactor or a nitrification SBR (sequencing batch reactor). Thus DPB are not exposed to oxygen and nitrifiers are not exposed to anaerobic conditions.

The purpose of this project is to evaluate new integrated phosphorus and nitrogen removal systems based on denitrifying dephosphatation, and to provide fundamental information on the kinetics and stoichiometry of the DPB.

Anaerobic-anoxic sequencing batch reactor

A lab-scale anaerobic-anoxic (A2) SBR has been operated in the whole period of this project, and the enriched DPB sludge from the A2 SBR was utilized for batch tests and as an inoculum for the other experimental systems. The A2 SBR was operated in a cycle of 6 hours. The cycle consisted of three phases; a 2.0 hours anaerobic phase, a 3.5 hours anoxic phase, and a 0.5 hours settling period. At the beginning of the anaerobic phase, synthetic waste water containing HAc (acetic acid, 400 mg-COD/*l*) and phosphorus (15 mg-P/*l*) was pumped into the SBR. During the anoxic phase, nitrate was added. This replaces the aeration of conventional anaerobic-aerobic phosphorus removal systems. After 0.25 hours settlement, 50% of the reactor contents were removed as treated effluent from the SBR.

Modified UCT-type waste water treatment plant for phosphorus and nitrogen removal

Activated sludge from waste water treatment plants (Genemuiden and Holten) was tested to examine the occurrence of denitrifying dephosphatation and the contribution of DPB for the phosphorus removal. These waste water treatment plants have modified UCT-type configurations (anaerobic-anoxic-aerobic process using pre-denitrification). In the treatment plants, the mixed liquor at the end of the anoxic zone is recirculated to the beginning of the anaerobic zone, leading to a significant accumulation of DPB in the treatment plant. The return sludge and the nitrified

liquid at the end of the aerobic zone is recirculated into the beginning of the anoxic zone.

Results

There are three main lines of research in this project: (i)kinetics and stoichiometry of denitrifying dephosphatation, (ii)single-sludge systems, and (iii)two-sludge systems.

Kinetics and stoichiometry of denitrifying dephosphatation. The anaerobic metabolism of DPB is identical to conventional anaerobic-aerobic phosphorus removing organisms. In the enriched DPB sludge, intracellular glycogen is utilized under anaerobic conditions, probably as reducing power ($NADH_2$) for PHB (poly-β-hydroxybutyrate) synthesis from HAc. Clearly DPB have a glycogen metabolism. In the maximum HAc uptake tests with excess HAc under anaerobic conditions, it was shown that the amounts of intracellular glycogen limit the maximum HAc uptake and maximum phosphorus release.

The main difference between the anoxic metabolism by DPB and the conventional aerobic metabolism is the electron transport phosphorylation with nitrate or oxygen. The measured P/$NADH_2$ ratio in the electron transport phosphorylation with nitrate was approximately 1.0 mol-ATP/mol-$NADH_2$, which indicated that the anoxic energy production efficiency is approximately 40% less than the aerobic efficiency. Based on this P/$NADH_2$ ratio with nitrate, a metabolic model proposed for the conventional anaerobic-aerobic phosphorus removal process, can be modified for denitrifying dephosphatation. The modified model could predict the cycle behaviour of each extra- and intracellular substance in the A2 SBR.

A reduction of phosphorus release by nitrate in biological phosphorus removal systems is partly due to presence of DPB, because if nitrate is present DPB utilize COD (HAc) for denitrification, not for phosphorus release. When nitrate and HAc were simultaneously present, PHB was produced and phosphorus was released by the enriched DPB sludge. The reducing power ($NADH_2$) and the energy (ATP) for HAc conversion into PHB seems to be obtained from HAc oxidation by nitrate as well as from polyphosphate degradation.

A SBR was operated under repetitive anaerobic and anoxic (or aerobic) phases in a short cycle, which leads to a shift of operations from the SBR-type (plug flow-type) process to the process with large recycle flows (like the carousel). The performance was compared under the short cycle A2 operation and the ordinary A2 operation. The sludge retention time (SRT), loading rates and the cycle time were identical, and the difference was only the number of short cycles (recycle ratio) in the main 6 hours cycle. Under the short cycle A2 operation phosphorus removal was unstable, while a stable complete phosphorus removal was achieved under the ordinary A2 operation. A reason is that it is difficult to control the optimal nitrate loading rate under the short cycle operation. This resulted in regular nitrate introduction into the anaerobic phase. The comparison of both types of processes indicates that there is not a unique relationship between SRT (growth rate) and intracellular PHB level.

Single-sludge systems. After enrichment of DPB in an A2 SBR, an aerobic phase was introduced between the anaerobic and anoxic phases. The anaerobic-aerobic-anoxic (AOA) SBR operation indicated that oxygen has no direct detrimental effect on denitrifying dephosphatation activities. The maximum phosphorus uptake rate by the enriched DPB sludge is almost equal for anoxic and aerobic conditions. PHB (COD) is aerobically oxidised for phosphorus uptake (without denitrification) in the aerobic phase of post-denitrification processes (like the AOA SBR), which means an inefficient coupling of COD to nitrate reduction. Therefore from the AOA SBR operation, it followed that the advantage of applying DPB with respect of COD efficiency for nitrogen and phosphorus removal can only be obtained in a pre-denitrification process like the UCT-type of process.

Batch tests using the activated sludge from actual waste water treatment plant, showed that

the UCT-type configuration as applied at the waste water treatment plant in Holten (pre-denitrification processes) leads to significant accumulation of DPB in the systems. In the activated sludge, the anoxic phosphorus uptake activity was approximately 50% of the aerobic phosphorus uptake capacity.

Two-sludge systems. To achieve more efficient use of COD for nitrogen and phosphorus removal, a two-sludge system was evaluated, in which DPB and nitrifiers were completely separated in two lab-scale SBRs. One SBR was operated under anaerobic-anoxic conditions for denitrifying dephosphatation, and another was operated under aerobic conditions for nitrification. Only the supernatant was exchanged between two SBRs through a "nitrate exchange vessel". A bottle-neck for nitrogen removal in the two-sludge systems is the NH_4^+ residue in the DPB sludge stream which does not go through the nitrification step, thus is not nitrified. However this will be improved by increase of the volume exchange ratio of the supernatant between two SBRs, which can be possible because of high sludge settleability of the DPB sludge.

The proposed two-sludge system showed very stable phosphorus and nitrogen removal. The average removal efficiency was 98% and 89%, respectively. The two-sludge system was suggested to have higher flexibilities on the operations for simultaneous phosphorus and nitrogen removal, rather than the single-sludge system. It was concluded that the nitrification step can be separated from the denitrifying dephosphatation step which leads to an optimal system operation.

Conclusions

From the research in this project, it has been shown that DPB have a high potential for integrated phosphorus and nitrogen removal from waste water. In practice, it was verified that significant amounts of DPB are accumulated in actual waste water treatment plants, especially those with a modified UCT-type configuration. Although further research is needed especially in practical aspects, two-sludge systems will result in significant decrease of COD and energy use for phosphorus and nitrogen removal.

We could, therefore, reasonably conclude that one should introduce denitrifying dephosphatation in the process design, which obviously leads to the saving of COD and energy for overall phosphorus and nitrogen removal process.

1 GENERAL INTRODUCTION

1.1 Backgrounds

Phosphorus removal has to be introduced into waste water treatment processes, because phosphorus as well as nitrogen induces eutrophication of closed water areas. Phosphorus removal can be achieved by chemical methods such as addition of efficient flocculating agents or crystallization, and biological methods using activated sludge. From the present methods, biological phosphorus removal seems to be the most appropriate because it has of less running costs and less sludge production than chemical methods.

Almost all biological phosphorus removal processes which have been studied and proposed for several decades, are fundamentally based on the circulation of activated sludge through anaerobic and aerobic zones. In the anaerobic zone phosphorus removing organisms store lower fatty acids, e.g. acetic acid, inside the cells as PHB (poly-β-hydroxybutyrate) or PHV (poly-β-hydroxyvalerate). The energy for this process is most probably derived from the stored polyphosphate by hydrolysis and excretion of phosphate (Groenestijn *et al.*, 1987 and 1989; van Veen *et al.*, 1993). In the aerobic zone oxygen is utilized as an electron acceptor for the oxidation of stored carbon, which leads to growth and polyphosphate accumulation by phosphorus removing organisms.

Phosphorus removal is usually applied with nitrogen removal (nitrification and denitrification) in waste water treatment processes. The nitrogen removal process is finalized by denitrification. Both denitrification and dephosphatation require organic substances (COD), i.e., COD is separately necessary for phosphorus and nitrogen removal in conventional systems, see Fig. 1(a). Thus, a problem in the conventional system is the competition for COD between phosphorus and nitrogen removing organisms, because organic substances in municipal waste water are often limiting for phosphorus and nitrogen removal. A more efficient use of the COD can be made if it's possible to remove phosphorus with nitrate in an anoxic zone which is preceded by an anaerobic zone, see Fig. 1(b). From a theoretical microbiological point of view there is no restriction against the use of nitrate instead of oxygen as an electron acceptor by phosphorus removing organisms. In fact, recently several publications have shown the occurrence of denitrifying phosphorus removing bacteria in the activated sludge (Vlekke *et al.*, 1988; Pokethitiyook *et al.*, 1990; Kuba *et al.*, 1992;

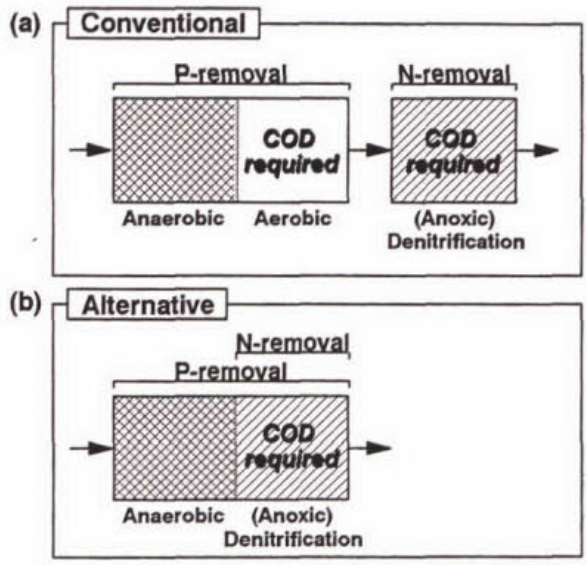


Fig. 1 Comparison of required COD in (a)conventional and (b)alternative systems for phosphorus and nitrogen removal.

Shin *et al.*, 1992; Wanner *et al.*, 1992; Kerrn-Jespersen and Henze, 1993). In this alternative system nitrate is actively utilized as sole electron acceptor for dephosphatation, and nitrate is simultaneously converted into dinitrogen gas. The required COD in this alternative system is estimated approximately 40% less than the conventional system on calculation (Kuba *et al.*, 1996b). Added to this, the energy/costs for aeration will be saved, because the aeration is needed mainly for nitrification, not for phosphorus removal.

1.2 Previously obtained results in our laboratory

The research on denitrifying dephosphatation in our laboratory was started in 1991. A lab-scale anaerobic-anoxic (A2) sequencing batch reactor (SBR) was used in order to investigate the possibility of dephosphatation with nitrate instead of oxygen as an electron acceptor, see Fig. 2(a). The anoxic phase was arranged similar to the aerobic phase in conventional anaerobic-aerobic (A/O) SBRs. An A/O SBR was also operated to compare of phosphorus removal activities with the A2 SBR, see Fig. 2(b). The SBRs were supplied with synthetic waste water containing acetic acid (HAc, 400 mg-COD/*l*) and phosphorus (15 mg-P/*l*). The objectives in the previous research were (i)to examine feasibility and stability of phosphorus removal under denitrifying conditions, (ii)to compare kinetics and stoichiometry of denitrifying dephosphatation with those in the conventional phosphorus removal system, and (iii)to propose denitrifying dephosphatation processes incorporating a nitrification step.

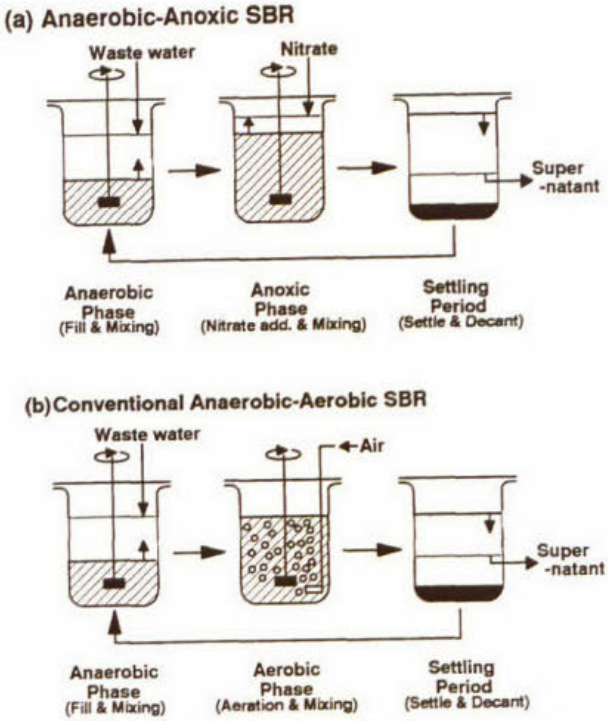


Fig. 2 Schematic diagram of (a)new anaerobic-anoxic and (b)conventional anaerobic-aerobic SBRs.

The A2 SBR operation resulted in stable phosphorus removal and easy accumulation of DPB (denitrifying phosphorus removing bacteria). Immediately after using an inoculum from a phosphorus removal plant (Renpho system) phosphorus uptake was observed in the anoxic phase, indicating that DPB were already present in the sludge of the conventional phosphorus removing plant. Approximately one week later, steady states conditions were reached in the A2 SBR. In steady state conditions all organic substance (HAc) was consumed during the anaerobic phase and released phosphorus concentrations reached to 90 ~ 120 mg-P/*l*. Almost 100% phosphorus removal efficiency was achieved under appropriate operations. As shown in Table 1, the comparison of

kinetics from both SBRs showed no great differences between phosphorus removal with nitrate or oxygen as an electron acceptor. The comparison of stoichiometry showed slight differences, but they corresponded to general differences between denitrification and aerobic oxidation. The results showed that denitrifying dephosphatation is as feasible as aerobic phosphorus removal.

Table 1 Summary of kinetics and stoichiometry in the new anaerobic-anoxic (A2) and the conventional anaerobic-aerobic (A/O) SBRs.

			New A2 SBR	Conventional A/O SBR
Anaerobic phase	Released-P/consumed-HAc	[g-P/g-C]	1.2	1.3
	P release rate	[g-P/g-SS.h]	0.03	0.04
	HAc consumption rate	[g-COD/g-SS.h]	0.06	0.08
Anoxic or Aerobic phase	Removed-P/utilized-NO_3^-	[g-P/g-NO_3^-] [mol-P/mol-e^-]	0.47 0.19	
	Removed-P/utilized-O_2	[g-P/g-O_2] [mol-P/mol-e^-]		0.91 0.23
	P-uptake rate	[g-P/g-SS.h]	0.03	0.03
	NO_3^- utilization rate	[g-NO_3^-/g-SS.h]	0.06	
	O_2 utilization rate	[g-O_2/g-SS.h]		0.03
Overall	Growth yield	[g-SS/g-COD]	0.25	0.35
	VSS/SS	[g-VSS/g-SS]	0.7	0.7
	P content	[g-P/g-SS]	0.17	0.10
	Maximum releasable P	[g-P/g-SS]	0.06	0.07
	SVI	[ml/g-SS]	50	100

Several possible process types based on denitrifying dephosphatation were proposed in the previous research. To obtain nitrate as an electron acceptor for DPB, nitrification should be introduced into the systems. The systems incorporating the nitrification step can be divided into two types; single-sludge systems and two-sludge systems (Fig. 3). In the single-sludge system, DPB coexist with nitrifiers, and the mixed sludge goes through all stages such as anaerobic, anoxic and aerobic phases. The single-sludge system can be achieved by e.g. a SBR or oxidation ditch, and the operations are relatively simple. However, due to low nitrification activities of the mixed sludge, it is expected that a long aerobic phase or long aerobic sludge retention time is required for nitrification. In the two-sludge system, nitrifiers are separated from DPB, e.g., in a nitrification biofilm reactor (Bortone *et al.*, 1994) or a nitrification SBR. This leads to strong reduction of aerobic retention times for nitrification, and there can be more possibilities for optimal process design and control in phosphorus and nitrogen removal.

The results from the previous research were summarised in two reports (Kuba *et al.*, 1992; van der Velde, 1992) and two scientific papers (van Loosdrecht *et al.*, 1992; Kuba *et al.*, 1993a), and were presented at the *International Conference on Sewage into 2000* (August 31 ~ September 4, 1992, RAI Amsterdam).

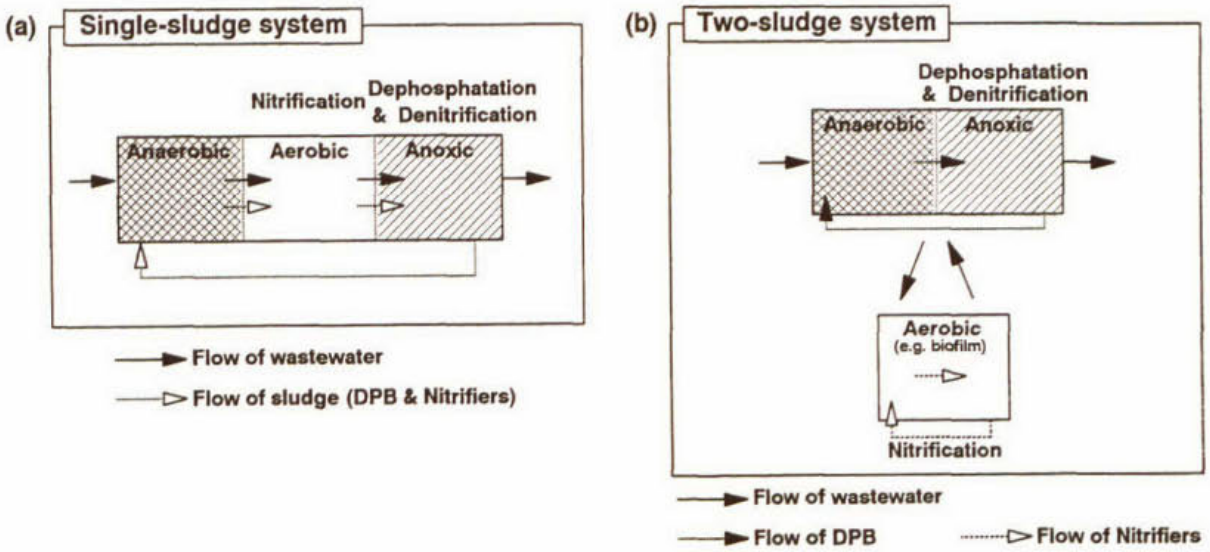


Fig. 3 Schematic diagram of denitrifying dephosphatation systems incorporating a nitrification step; (a)single-sludge and (b)two-sludge systems.

1.3 Aims and scope of this project

Based on the satisfying results from the first research period on denitrifying dephosphatation and the high potential for practical application of DPB, this project "*Anaërobe/Denitrificerende Biologische Defosfatering* (*Biological Dephosphatation under Anaerobic/Denitrifying Conditions*)" was started in 1993.

This project aims to evaluate new systems in which denitrifying dephosphatation is utilized for simultaneous removal of phosphorus and nitrogen. In this project use is made of lab-scale SBR systems or activity tests on sludge from actual waste water treatment plants. Fig. 4 shows the framework of this project. There are three main lines of research in this 2.5 years project (March 1993 ~ September 1995).

The first line is the research on kinetics and stoichiometry of denitrifying dephosphatation. The research has been done in lab-scale A2 SBR with standard cycle (Chapter 3.1 ~ 3) and short cycle A2 and A/O SBRs (Chapter 3.4). In Chapter 3.1 ~ 3 of this project report (i)effect of pH on the anaerobic phosphorus release, (ii)effect of nitrate on phosphorus release with HAc, and (iii)a limiting factor for the anaerobic HAc uptake, are described.

In Chapter 3.4, results of the short cycle A2 and A/O SBR are given showing the effect of short cycle time for phosphorus removal processes. The subject was conducted under repetitive anaerobic and anoxic (or aerobic) phases in a short cycle. The aim was to simulate the effect of high internal recycle ratios on the phosphorus removal process.

Another aspect of the first line is to obtain kinetics and stoichiometry which are fundamental data for modelling of denitrifying dephosphatation. Smolders *et al.* (1994a, b, 1995a, b) proposed a metabolic model of phosphorus removing organisms under anaerobic-aerobic conditions. In Chapter 3.5, the metabolic model is extended and applied for denitrifying dephosphatation processes.

The second line is the research on the single-sludge systems in which DPB are mixed with nitrifiers. The first part of this research has been done with an AOA (anaerobic-aerobic-anoxic) or A2O (anaerobic-anoxic-aerobic) lab-scale SBR where the sludge is subjected to a cycle of conditions. The AOA system is a post-denitrification and the A2O system is a pre-denitrification. The purpose of this subject is to examine (i)the effect of cyclic oxygen exposure on the activity of DPB and (ii)nitrification activities in the mixed sludge of nitrifiers and DPB (Chapter 4.1). Based on these results, general requirements and problems in

single-sludge phosphorus and nitrogen removal systems can be discussed.

The second part of this research line has been done with batch tests using sludge from waste water treatment plants in Genemuiden and Holten, the Netherlands. These plants are run in a mode close to the A2O system which is called a modified UCT-type of system. The batch tests were conducted (i)to evaluate the anoxic phosphorus removal activities in the actual waste water treatment plant (Chapter 4.2), and (ii)to examine effect of presettling of sewage on phosphorus and nitrogen removal in the modified UCT-type waste water treatment plant (Chapter 4.3).

The third line is the research on the two-sludge system. A simple lab-scale two-sludge system was proposed, which consists of two SBRs. In the proposed two-sludge system, DPB and nitrifiers are completely separated in the SBRs and only the supernatant is exchanged between the SBRs. In Chapter 5, the performance of the simultaneous phosphorus and nitrogen removal process was examined in the two-sludge system, and the flexibility and stability of the operation were evaluated in comparison with single-sludge systems. Added to this, we will discuss the operational strategies of two-sludge systems for optimal denitrifying dephosphatation, with reference to the effect of the applied sludge retention time of the DPB sludge and the influent COD/N ratio on phosphorus and nitrogen removal.

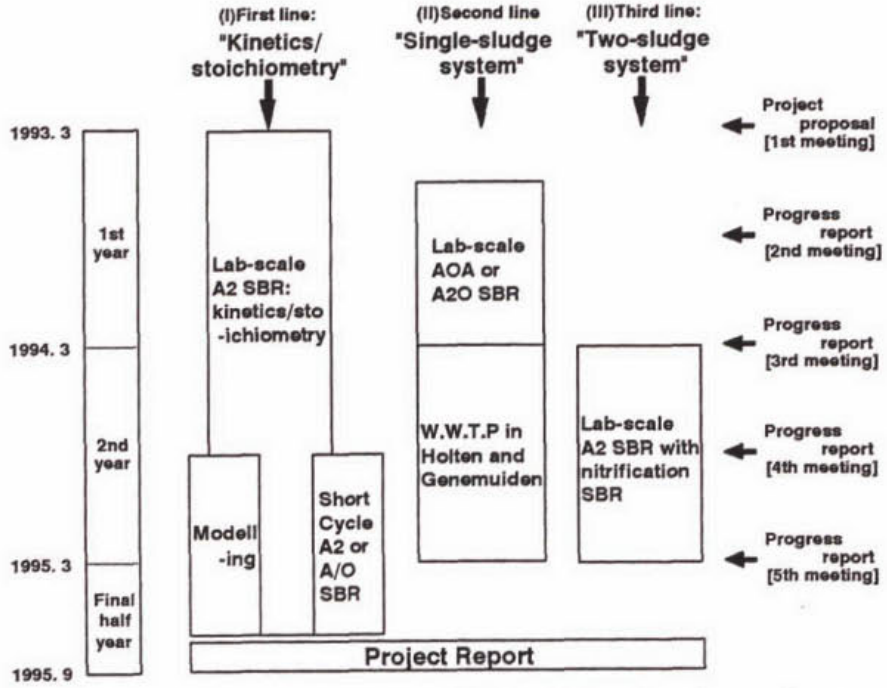


Fig. 4 The framework of the denitrifying dephosphatation project.

For further details of each subject, experimental methods and description of the modelling which are not mentioned in this project report, the following reports or scientific papers can be consulted;

Every six months, a progress report has been submitted to the project committee (see Fig. 4: Kuba *et al.*, 1993b, 1994a, b, 1995a). Three experiment summaries of the batch tests using the sludge from the waste water treatment plants of Holten and Genemuiden were prepared (Kuba *et al.*, 1994c, d, 1995b). Two reports about kinetics/stoichiometry and modelling of denitrifying dephosphatation were written by two Erasmus students in this project (Wachtmeister, 1993; Murnleitner, 1995). Finally a scientific paper with reference to this project is published (Kuba *et al.*, 1994e), 4 are submitted (Kuba *et al.*, 1996a~c; Wachtmeister *et al.*, 1996), and 5 are in preparation (Kuba *et al.*, 1996d~f; van Loosdrecht *et al.*, 1996; Murnleitner *et al.*, 1996).

2 MATERIALS AND METHODS

2.1 Apparatus and operation

A lab-scale anaerobic-anoxic (A2) sequencing batch reactor (3.5 ~ 3.6*l* working volume) was operated at room temperature (20 ~ 25°C), in the whole period of this project (Fig. 2(a)). The cycle time in the A2 sequencing batch reactor (SBR) was 6 hours. The cycle consisted of three phases: a 2.0 hours anaerobic phase, a 3.5 hours anoxic phase and a 0.5 hours settling period. 1.75*l* synthetic waste water containing acetic acid (HAc) and phosphorus was pumped into the SBR during the first 10 minutes of the anaerobic phase. Nitrate (117 mmol/*l*) was pumped into the reactor with constant flow rate (1 m*l*/min), during the fist 100 ~ 105 minutes of the anoxic phase. After the (0.25 hours) settlement approximately 1.85*l* supernatant was retrieved from the SBR. The overall hydraulic retention time was 0.5 days, and overall sludge retention time was controlled at 7 or 14 days. pH was strictly controlled at 7.0±0.05 by addition of 1N HCl or NaOH, to avoid phosphate precipitation (see Chapter 3.1).

Synthetic waste water was used containing per litre: 0.375 g HAc as carbon source (400 mg-COD/*l*), 0.049 g K_2HPO_4 and 0.028 g KH_2PO_4 as phosphorus source (15 mg-P/*l*), 0.6 g $MgSO_4 \cdot 7H_2O$, 0.07 g $CaCl_2 \cdot 2H_2O$, 0.227 g NH_4Cl, 0.01 g EDTA, 2 m*l* trace mineral solution. The trace mineral solution contained per litre: 1.5 g $FeCl_3 \cdot 6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4 \cdot 5H_2O$, 0.03 g KI, 0.12 g $MnCl_2 \cdot 4H_2O$, 0.06 g $Na_2MoO_4 \cdot 2H_2O$, 0.12 g $ZnSO_4 \cdot 7H_2O$, 0.15 g $CoCl_2 \cdot 6H_2O$. Substrate loading rates were approximately 0.8 g-COD/*l*.d.

The inoculation sludge for the A2 SBR was obtained from a phosphorus and nitrogen removal process operating on municipal waste water (Renpho-process) at Wageningen Agricultural University, the Netherlands. The enriched A2 sludge in this SBR was utilized for batch tests or for the other SBR as inoculum. The experimental methods are briefly mentioned in each chapter.

2.2 Analytical methods

The analyses of phosphorus, TOC, HAc, nitrate and nitrite were performed in accordance with Standard Methods (APHA, 1985). NH_4^+ was measured with an ammonia-selective gas electrode (METROHM, 60506010). MLSS and MLVSS were determined using glassfibre filters (Whatman GF/C). The extractions and analyses of intracellular PHB and glycogen were carried out as described by Smolders *et al.* (1994a, b).

3 KINETICS AND STOICHIOMETRY OF DENITRIFYING DEPHOSPHATATION

3.1 Effect of pH for denitrifying phosphorus removing bacteria

Abstract ——— The effect of pH on phosphorus release under anaerobic conditions was examined for denitrifying phosphorus removing bacteria (DPB). After HAc addition, enriched DPB sludge was maintained under anaerobic conditions at 5 different pH conditions (6.0, 6.5, 7.0, 7.5 and 8.0), and released phosphorus and consumed HAc concentrations were measured periodically. When the biomass concentration was around 2.7 g-VSS/*l*, the observed P/C (released-P/consumed-HAc) ratios were 0.7, 1.1 and 1.2 g-P/g-C at pH=6, 7 and 8, respectively. At 4.2 g-VSS/*l*, the observed P/C ratios were 0.9, 1.3 and 1.2 g-P/g-C, respectively. The difference between the two experiments resulted from the endogenous phosphorus release. The same pH effect as observed for conventional anaerobic-aerobic SBR sludge, was obtained for the DPB sludge in the range of pH=6.0~7.5. However, due to precipitates formation at pH=8.0, the apparent P/C ratio was approximately 20% less than the ratio calculated from the biological released phosphorus concentration by DPB.

3.1.1 Introduction

Smolders *et al.* (1994a, 1995b) observed by using conventional anaerobic-aerobic (A/O) sequencing batch reactor (SBR) sludge that phosphorus release in the anaerobic phase was strongly influenced by pH. They reported that the P/C ratios were 0.6 to 1.9 g-P/g-C in a range of pH=5.5~8.5. It was hypothesised that an explanation for the effect of pH on the energy needed for HAc uptake was due to the increasing electrical potential difference across the membrane of the cell with increasing pH. Therefore, more work must be done to take up a negatively charged ion, like acetate, against the negative electric potential of the cells. This leads to more energy production requirement from polyphosphate by hydrolysis and phosphate excretion.

In this chapter, it is discussed whether the same pH influence also applies for the enriched DPB sludge. Concurrently we will discuss "endogenous" ("secondary") phosphorus release in the anaerobic phase.

3.1.2 Experiments

The pH effect for the DPB sludge was examined in the anaerobic phase of the A2 SBR (see Chapter 2). At the beginning of a cycle, pH was changed from 7.0 to a set point (6.0, 6.5, 7.0, 7.5 or 8.0), and pH was returned to 7.0 before the start of the anoxic phase. The ratio of released phosphorus to consumed HAc (P/C ratio) in the anaerobic phase was investigated as the pH effect for DPB. Two sets of the pH effect experiments were conducted under different MLVSS conditions (2.4~3.0 g-VSS/*l* or 4.0~4.3 g-VSS/*l*).

3.1.3 Results and discussion

Effect of pH on the anaerobic phosphorus release by DPB. Figure 5 shows phosphorus and HAc concentrations under anaerobic conditions at various pH values (MLVSS = 4.0~4.3 g/*l*). As expected there was little effect of pH on the HAc consumption rate. In contrast the anaerobic phosphorus release depended strongly on the pH. Fig. 6 shows the relationship between pH and the maximum released phosphorus concentration, or the P/C ratio. The relationship in the case of 2.4~3.0 g-VSS/*l* is also shown in this figure. The same tendency as observed by Smolders *et al.*

(1994a, 1995b) for conventional anaerobic-aerobic SBR sludge was obtained for pH < 7.5, i.e., the P/HAc ratio increases with increasing pH. However the P/C ratio deceased at higher pH. It seemed reasonable to suppose that the lower P/HAc ratio at higher pH was caused by phosphate precipitates. In order to confirm the presence of precipitates, after the phosphorus release (91 mg-P/*l*) with complete HAc consumption at pH=8.0, a mixed liquor sample was taken from the A2 SBR (in the case of 2.4~3.0 g-VSS/*l*). Then the soluble phosphorus concentration was measured after incubating the mixed liquor at low pH (=2) for 10 minutes. The measured soluble phosphorus concentration was 116 mg-P/*l*, see Fig. 6(a). It was obvious that precipitates were formed at pH=8.0, and approximately 25 mg-P/*l* was present as precipitates. The same examination had been done by using the mixed liquor at pH=7.0 in the previous research (van Loosdrecht *et al.*, 1992; Kuba *et al.*, 1992, 1993a), but precipitates were not formed around this pH value. These observations indicate again that pH should be controlled carefully in the biological research of denitrifying dephosphatation, because the denitrification process might increase pH which leads to chemical precipitates formation. On the other hand it points to the fact that in practical cases always some phosphate is present in precipitated form.

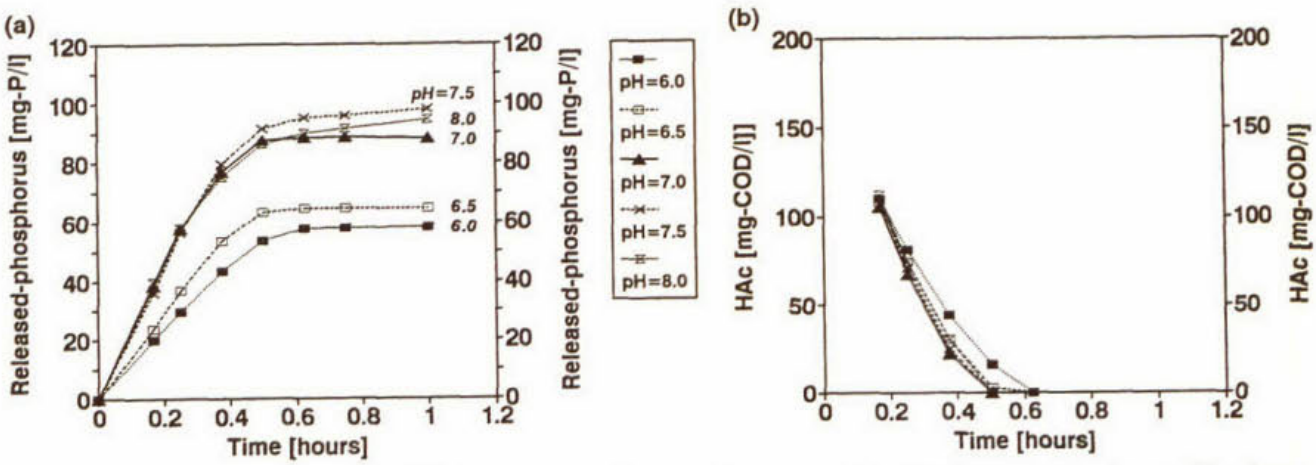


Fig. 5 (a)Phosphorus and (b)HAc concentrations under anaerobic conditions at various pH values (MLVSS = 4.0~4.3 g/*l*).

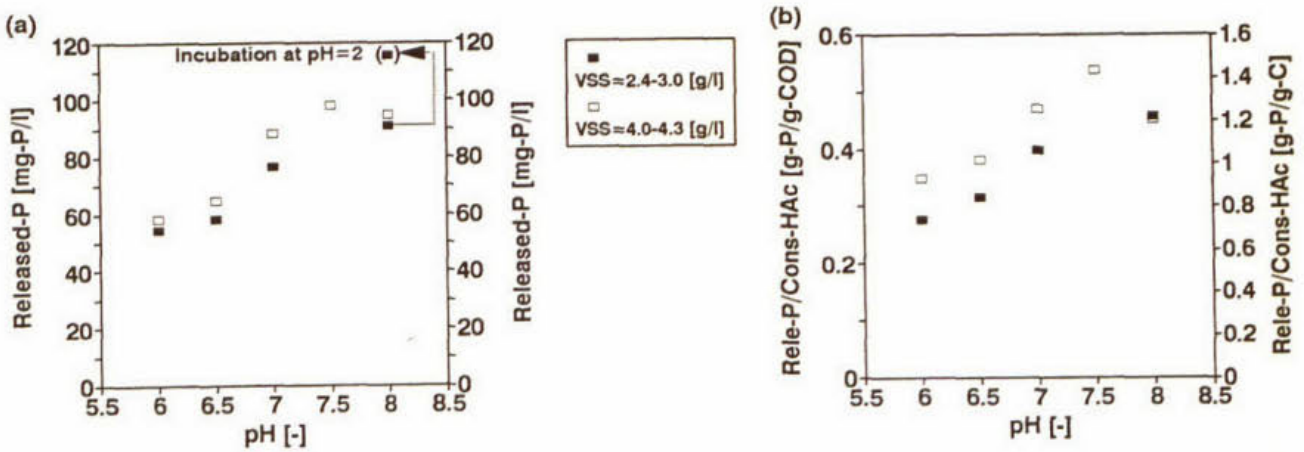


Fig. 6 (a)Relationship between pH and the maximum released phosphorus concentration, and (b)relationship between pH and the P/HAc ratio.

"Endogenous" phosphorus release. Another point from the pH effect experiments is that the P/C ratio seems to depend on MLVSS concentration (Fig. 6). This can be also seen from Fig. 7 which

shows a relationship between released phosphorus and MLVSS concentrations in the A2 SBR at pH=7.0. Although the same quantity of HAc was added into the SBR at the beginning of the anaerobic phase, the released phosphorus concentration increased with increment of MLVSS. An explanation for this is that polyphosphate is also used for maintenance requirements. From Fig. 7, the "endogenous" phosphorus release was estimated to be 3～4 mg-P/g-VSS.h. In the previous research (Kuba *et al.*, 1992, 1993a, 1996b), "secondary" phosphorus release occurred at the end of the cycle, after nitrate was gone. The "secondary" phosphorus release rate was approximately 2 mg-P/g-VSS.h. Also at the end of the anaerobic phase phosphorus release occurred after complete HAc consumption. These phosphorus release phenomena without electron acceptors (nitrate) and donors (HAc) might result from energy production for maintenance due to polyphosphate degradation. The previously obtained "secondary" phosphorus release rate was comparable to the above-mentioned "endogenous" phosphorus release rate. A similar "endogenous" phosphorus release rate was reported in sludge cultivated under conventional anaerobic-aerobic conditions (Smolders, 1995b).

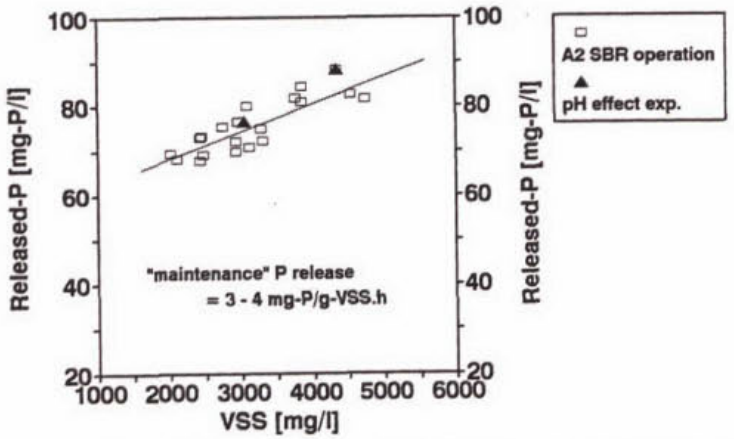


Fig. 7 Relationship between released phosphorus and MLVSS concentrations under the A2 SBR operation.

From the experiments of the pH effect for the enriched DPB sludge, the following conclusions have been drawn:

(1)The same pH effect as observed for conventional anaerobic-aerobic SBR sludge, was obtained for the DPB sludge at lower pH (6.0～7.5). However, due to precipitates formation at pH=8.0, the apparent P/HAc ratio is approximately 20% less than the ratio calculated from the biological released phosphorus concentration by DPB,

(2)The experiments point to some phosphorus release possibly due to maintenance. This "secondary" or "endogenous" or "maintenance" phosphorus release amounts 2～4 mg-P/g-VSS.h.

3.2 Effect of the presence of nitrate in the anaerobic phase on phosphorus release in biological phosphorus removal systems*

Abstract ——— The effect of the presence of nitrate in the anaerobic phase on phosphorus release by biological phosphorus removing organisms has been studied. Denitrifying (DPB) or aerobic phosphorus removing bacteria were enriched in an anaerobic-anoxic (A2) or anaerobic-aerobic (A/O) sequencing batch reactor (SBR). The two types of the enrichment sludge were used in batch tests, in which the effect of simultaneous presence of substrate (HAc) and nitrate in the anaerobic phase on the phosphorus release was studied. It could be concluded that a reduction of the phosphorus release by nitrate in biological phosphorus removal systems is partly due to the presence of DPB, which utilize HAc for denitrification, not for phosphorus release. PHB (poly-β-hydroxybutyrate) was always produced from HAc and phosphorus was released by DPB sludge when nitrate and HAc were simultaneously present. The reducing power ($NADH_2$) and the energy (ATP) for PHB production seemed to be obtained from HAc oxidation by nitrate as well as from polyphosphate degradation. After removal of the HAc, PHB degradation and phosphorus uptake occurred as usual in the presence of nitrate. Concurrently it was confirmed in the standard A2 cycle that DPB have a glycogen metabolism according to Mino model. Also an estimation method for the proportion of DPB in phosphorus removing organisms was discussed in this chapter.

3.2.1 Introduction

In conventional anaerobic-aerobic phosphorus removal systems with long aerobic retention time, nitrate is formed in the aerobic zone by nitrification. Many researchers have reported that the transfer of nitrate into the anaerobic zone inhibits the phosphorus release (Comeau *et al.*, 1990; Jenkins and Tandoi, 1991). One of the possible reasons for the reduction of phosphorus release by nitrate is simultaneous phosphorus uptake by polyphosphate accumulating denitrifying bacteria.

For ideal phosphorus removal systems, electron donors (COD) and acceptors (oxygen or nitrate) should not be present simultaneously. Disturbances occur when nitrate (or oxygen) and electron donors (COD) like HAc, are both present in the "anaerobic" phase of the process. In long term ordinary (non-polyphosphate accumulating) denitrifiers will be enriched, but nitrate has also a direct short term effect on the phosphorus release. In practice biological phosphorus removal processes are often combined with (de)nitrification. Therefore, this can easily lead to uncontrolled introduction of nitrate in the "anaerobic" phase. The purpose of this study is to examine the effect of nitrate on phosphorus release, especially a possible role of DPB. Furthermore, an estimation method for the proportion of DPB in phosphorus removing organisms is discussed.

3.2.2 Experiments

An anaerobic-anoxic (A2) SBR was operated over 150 days before the start of this study (see Chapter 2), under 14 days sludge retention time (SRT). The enriched DPB sludge from the A2 SBR was used for batch tests. 300 m*l* sludge was taken from the A2 SBR at the end of the anoxic or anaerobic phase, and put into a 1*l* laboratory fermenter. HAc and phosphorus concentrations were monitored in batch tests with and without continuous nitrate addition. Also sludge from a conventional anaerobic-aerobic (A/O) SBR (Fig. 2(b)) which had been operated under identical conditions (but SRT was approximately 5 days) to the A2 SBR, was employed for the same batch

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*: *This subject was studied by Alexandra Wachtmeister who is an Erasmus-student from Sweden (Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm).*

tests.

Another object of the batch tests is to evaluate the possibility of estimation for the proportion of DPB in phosphorus removing sludge. If the reduction of phosphorus release by nitrate in the anaerobic phase is due to the DPB which take up a part of the released phosphorus, the batch test of phosphorus release in simultaneous presence of HAc and nitrate could be useful of the evaluation of the DPB proportion in phosphorus removing organisms. The same batch test was conducted by using known mixtures of the A2 and A/O sludge in order to validate the method. The ratios of the mixture between A2 and A/O sludge were 50:50% and 33:67% on the basis of volume (62:38% and 49:51% on the basis of MLVSS).

3.2.3 Results and discussion

Cycle behaviour in the A2 SBR. Phosphorus concentrations increased with HAc consumption under anaerobic conditions, and phosphorus was biologically removed under anoxic conditions, see Fig. 8. PHB concentrations increased in the anaerobic phase, and decreased in the anoxic phase. The variation of glycogen concentration was in reverse. Not shown here, but Mg^{2+} and K^{+} concentrations in bulk liquid correlated with the phosphorus concentration. Molar ratios were $Mg^{2+}/P=0.42$ and $K^{+}/P=0.34$ (van der Velde, 1992). The molar ratios were similar to reported values in the conventional phosphorus removal sludge (Arvin and Kristensen, 1985; Comeau *et al.*, 1986; Rickard and McClintock 1992). Ca^{2+} and Na^{+} concentrations were constant during a cycle. The A2 SBR, therefore, showed similar variations of HAc, phosphorus, PHB and glycogen as reported for A/O processes (Mino *et al.*, 1987; Smolders *et al.*, 1994a).

The cycle behaviour of glycogen in the A2 SBR clearly shows that a glycogen metabolism (Mino *et al.*, 1987; Arun *et al.*, 1988 and 1989) occurred. Also the measured ratio of produced PHB to consumed HAc in the anaerobic phase was similar to the Mino model rather than the Comeau/Wentzel model, as shown in Table 2.

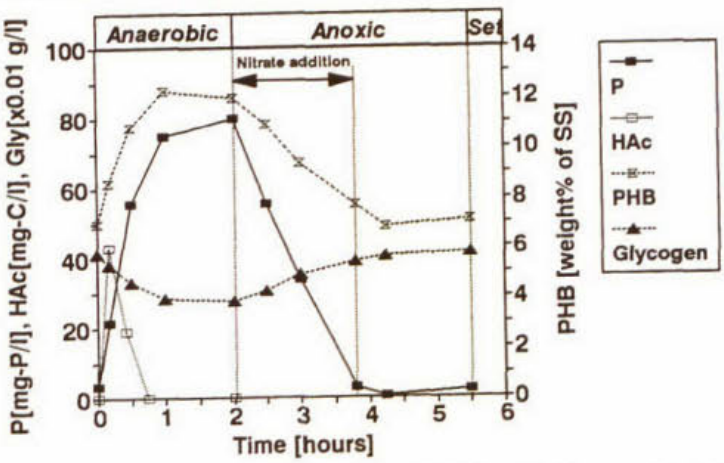

Fig. 8 Cycle behaviour of phosphorus, HAc, PHB and glycogen in the A2 SBR.

Table 2 Conversion ratios of PHB and glycogen under anaerobic conditions in the A2 SBR.

Ratio	A2 SBR	Mino model*1	Comeau/Wentzel model*2
(+)PHB/(-)HAc [mol-C/mol-C]	1.2	1.33	0.87
(-)glycogen/(-)HAc [mol-C/mol-C]	0.8	0.50	——

*1 Arun *et al.*, 1988. *2 Comeau *et al.*, 1987.

Effect of nitrate on phosphorus release by enriched A2 sludge. Figure 9(a) and (b) show results of batch tests with/without nitrate addition using sludge from the A2 and A/O SBR, respectively. The sludge for the batch test was taken from the SBR at the end of the anoxic or aerobic phase. A similar experiment with sludge taken from the A2 SBR at the end of the anaerobic phase, is shown in Fig. 10. The specific HAc consumption rates, specific phosphorus release rates and anoxic phosphorus uptake rates are summarized in Table 3. In the batch tests with nitrate, the sludge was fully loaded with nitrate, and the nitrate was always present in the bulk liquid.

The HAc consumption rate increased due to nitrate addition because of denitrification. Despite the presence of nitrate, the DPB sludge released phosphorus as long as HAc was present, and PHB was produced from HAc (Fig. 10). This is the expected pattern as observed in the absence of nitrate. The P/C ratio (a ratio of the released phosphorus to the consumed HAc) in the presence of nitrate is, however, less than in its absence (Table 3). Moreover, the measured ratio of produced PHB to consumed HAc with nitrate was approximately 0.8 mol-C/mol-C (Fig. 10) and is clearly lower than the Mino model under anaerobic conditions (1.33 mol-C/ mol-C, Table 2). Apparently part of the HAc is directly oxidised by nitrate and is not converted into PHB.

It seems that the reducing power ($NADH_2$) for the PHB formation is supplied through the TCA cycle, and that the energy (ATP) is produced by the denitrification of HAc (through the TCA cycle and from $FADH_2/NADH_2$ oxidation with nitrate) and polyphosphate degradation. From the calculation (Kuba *et al.*, 1994e), it appears that approximately 40% of the required ATP for the PHB production is supplied by the polyphosphate degradation. In the A2 sludge, the reduction of released phosphorus by nitrate is due to the fact that part of the HAc is utilized for denitrification ($NADH_2$ and ATP production through TCA cycle and by $FADH_2/NADH_2$ oxidation with nitrate). Therefore less polyphosphate hydrolysis and its related phosphorus release is required.

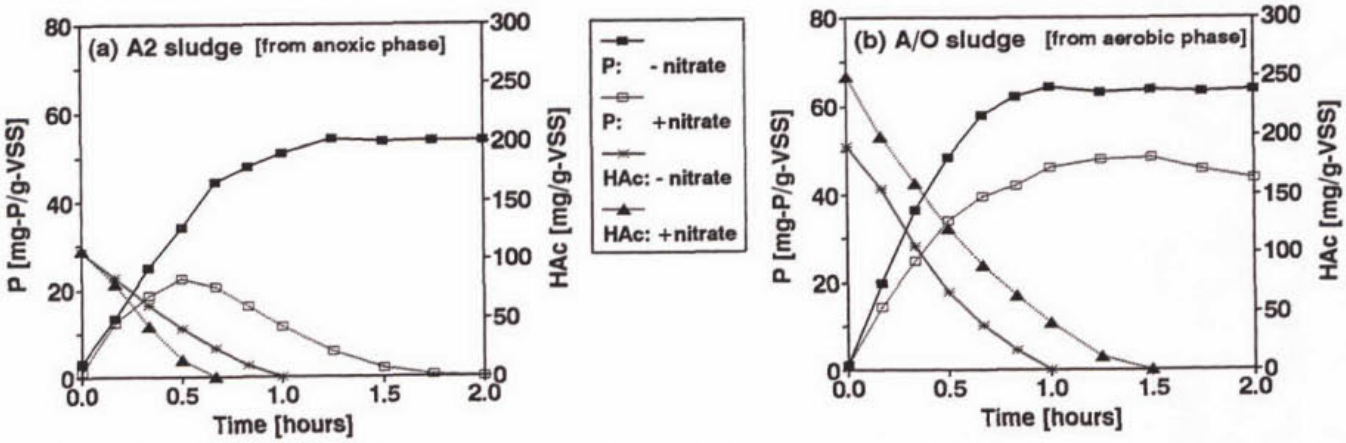

Fig. 9 Phosphorus and HAc concentrations in batch tests with/without nitrate addition, using sludge from (a)the A2 and (b)A/O SBR. The sludge was taken from the SBR at the end of the anoxic or aerobic phase.

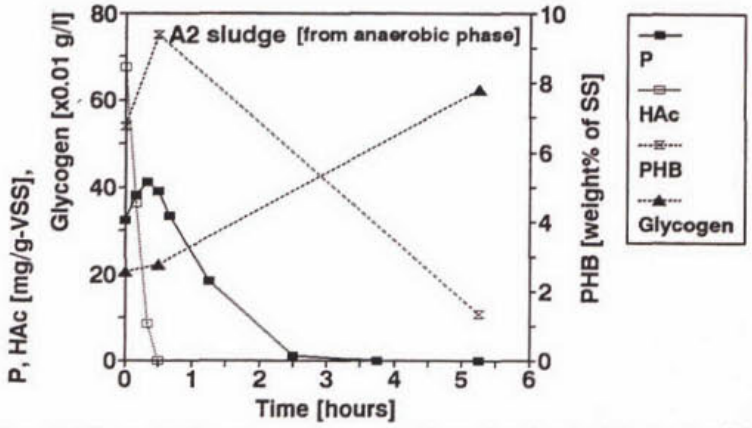

Fig. 10 Phosphorus, HAc, PHB and glycogen concentrations in the batch test with nitrate, using sludge taken from the A2 SBR at the end of the anaerobic phase.

Table 3 Summary of stoichiometry and kinetics in batch tests using the A2 and A/O sludge.

	with/without nitrate	HAc consumption rate [mg/mg-VSS.h]	P release rate [mg-P/ g-VSS.h]	Anoxic P uptake rate*1 [mg-P/ g-VSS.h]	P/C ratio*2 [mol-P/ mol-C]
A2 sludge [end anoxic phase]	$+NO_3^-$	192	52	24	0.24
	$-NO_3^-$	125	62	—	0.45
A2 sludge [end anaerobic phase]	$+NO_3^-$	177	35	27	0.24
A/O sludge	$+NO_3^-$	253	68	10	0.20
	$-NO_3^-$	236	106	—	0.35

*1 After HAc was consumed. *2 Ratio of the released phosphorus to utilized HAc.

It may be worth pointing out, in passing, that the measured PHB/HAc ratio is similar to the Comeau/Wentzel model (0.87 mol-C/mol-C, Table 2), in which the reducing power ($NADH_2$) for PHB production is supplied through the TCA cycle operating anaerobically. This indicates that part of the HAc is directly oxidised by nitrate through the TCA cycle to supply $NADH_2$ for the formation of PHB. In fact the TCA cycle replaces now the glycogen conversion (Fig. 10) which supplies $NADH_2$ in the absence of nitrate. In conclusion it appears that the glycogen catabolism into $NADH_2$, ATP and PHB is replaced by the nitrate-driven HAc oxidation.

Effect of nitrate on phosphorus release by enriched A/O sludge. Sludge with a low aerobic SRT (about 2 days) was used to assure that nitrification was not present in the A/O sludge. No difference in phosphorus release was expected in the anaerobic phase with nitrate present or absent, since it was expected that the A/O sludge did not contain any denitrifying bacteria or any denitrifying dephosphatation activity. However less phosphorus release was observed in the presence of nitrate (Fig. 9(b)). Although the difference in HAc consumption rate was small, the phosphorus release process itself was strongly inhibited by nitrate addition. One reason for the lower phosphorus release might be due to existence of DPB in the A/O sludge. Since DPB can utilize oxygen as an electron acceptor for phosphorus uptake (van der Velde, 1992; Kerrn-Jespersen and Henze, 1993; Chapter 4.1), they can occur in the A/O sludge without nitrate. Although induction of enzymes for denitrification may need time when a process is switched over from aerobic to denitrifying conditions, denitrifying phosphorus uptake activity (10 mg-P/g-VSS.h) was constitutively present in the A/O sludge (□ in Fig. 9(b) shows decrease in phosphorus after 1.5 hours).

The regular aerobic specific phosphorus uptake rate in the A/O SBR was approximately 70 mg-P/g-VSS.h. Since the uptake rates of DPB under aerobic and anoxic conditions are almost equal (van der Velde, 1992; Kuba *et al.*, 1996a; see Chapter 4.1), a proportion of the DPB in the A/O sludge is estimated to be at least 15% (see below). Calculations from the results of batch tests using the A2 sludge, show that these 15% DPB can bring about approximately 20% ~ 30% reduction to the anaerobic phosphorus release, by nitrate addition. However the reduction by nitrate was approximately 35 ~ 40%, and it seems problematic to explain the reduction by DPB only. The reason of the additional reduction on phosphorus release in the A/O sludge is not yet known, but intermediate denitrification products (such as nitric oxide) (Appeldoorn, 1993) by DPB have been shown to inhibit the phosphorus release process of the aerobic phosphorus removing bacteria. However the required nitric oxide levels for inhibition which was tested by Appeldoorn, are much higher than the nitric oxide levels observed in treatment plants (von Schulthess *et al.*, 1994).

Estimation methods of proportion of DPB in phosphorus removing sludge. The same phosphorus

release tests using different mixtures of the A2 and A/O sludge were conducted to verify the usefulness as the evaluation method for the proportion of DPB in phosphorus removing organisms. Fig. 11 shows phosphorus concentrations in the batch tests with continuous nitrate addition. Results from "100% A2" sludge and "0% A2" (100% A/O) sludge are the same as data in Fig. 9(a) and (b) (with nitrate).

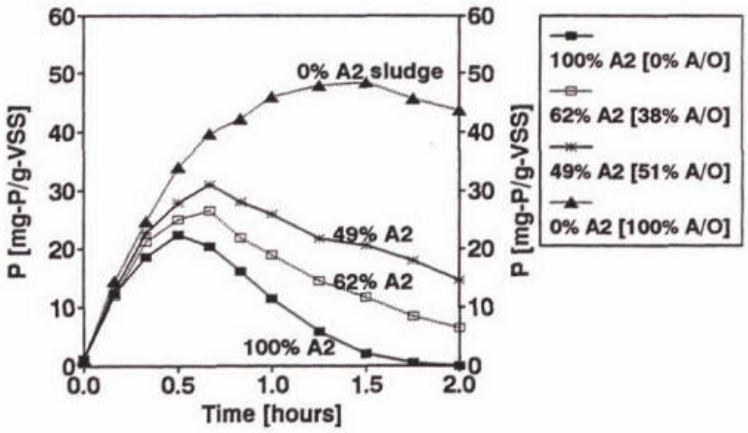

Fig. 11 Phosphorus release with nitrate in various proportions of the A2 and A/O sludge. The proportions are based on MLVSS.

The specific phosphorus release rate and the maximum released phosphorus concentration decreased with increase of the proportion of DPB. It is, however, too complex to estimate the proportion of DPB from this type of batch tests, because; (i)the DPB release phosphorus in simultaneous presence of HAc and nitrate, and (ii)phosphorus release process itself of the aerobic phosphorus removing bacteria may be inhibited by intermediates of denitrification, as stated above.

For the estimation of the proportion of DPB in phosphorus removing organisms, simple phosphorus uptake rate measurements under aerobic or anoxic condition is advised rather than batch tests on phosphorus release. This is suggested from the phosphorus uptake when HAc was gone in Fig. 11, because the phosphorus uptake rate under anoxic conditions proportionally increased with increase of the proportion of DPB. As shown in Fig. 12, two batch tests to the same phosphorus removing sludge are needed under an anaerobic-aerobic or anaerobic-anoxic condition for the estimation of DPB. HAc is added in the anaerobic phase of each batch test. After HAc is gone, each sludge is exposed under aerobic or anoxic condition. A ratio of the anoxic phosphorus uptake

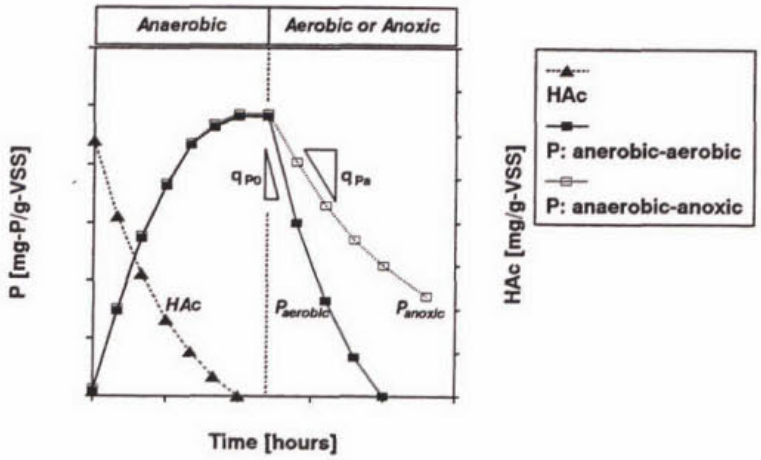

Fig. 12 Schematic diagram of the estimation method for the proportion of DPB in phosphorus removing organisms.

(q_{Pa}) to the aerobic uptake (q_{Po}) simply gives the proportion of DPB in the phosphorus removing organisms, assuming that DPB have similar specific phosphorus uptake rates in both aerobic and anoxic conditions (see Chapter 4.1), and similar specific aerobic phosphorus uptake rates to the aerobic phosphorus removing bacteria (van der Velde, 1992; Kuba *et al.*, 1993a) under the same SRT conditions.

The required experimental conditions are;

-Electron donors (HAc) are not simultaneously present with electron acceptors (nitrate, oxygen),
-The phosphorus uptake is not limited by oxygen or nitrate supply,
-Intracellular biopolymer (PHB, glycogen, polyphosphate) concentrations at the end of the anaerobic phase are similar in both tests,
-Nitrite does not accumulate under the anoxic condition (Chapter 4.1).

As above-mentioned, even in the A/O SBR without anoxic conditions denitrifying dephosphatation activity occurred. The anoxic phosphorus uptake activity was approximately 15% of the aerobic activity, thus the proportion of DPB was estimated to be 15% of phosphorus removing organisms. However this might be an underestimated proportion of DPB, since it is unlikely that under this condition full denitrification enzyme activity is induced. In the A/O sludge without anoxic conditions, this will be the proportion of denitrification enzyme activity, rather than the proportion of DPB cells. In waste water treatment plants for phosphorus and nitrogen removal, activated sludge is periodically exposed to anoxic conditions, which might lead that the measured ratio of enzyme activity is similar to the proportion of DPB in phosphorus removing organisms.

From the batch tests using the enriched A2 and A/O sludge, the following conclusions have been drawn:

(1)DPB have a metabolism according to the Mino model,
(2)Nitrate does not block phosphorus release in the DPB sludge, but HAc uptake increases and the P/C ratio decreases. A reduction of phosphorus release by nitrate is due to HAc utilization for the ATP formation in the TCA cycle and the oxidation of $NADH_2/FADH_2$ with nitrate,
(3)DPB occur even in A/O SBRs where nitrate is not present. The presence of these organisms can partly explain the decrease in phosphorus release when nitrate is present,
(4)A ratio of the anoxic phosphorus uptake rate to the aerobic phosphorus uptake rate which is obtained from two batch tests under an anaerobic-aerobic or anaerobic-anoxic condition, can give the proportion of the denitrifying dephosphatation activity in phosphorus removing organisms. Phosphorus release tests are not suitable for determining the relative proportion of DPB and aerobic phosphorus removing bacteria.

3.3 The limiting factor for the maximum HAc uptake under anaerobic conditions*

Abstract ——— With non-limitation of electron donors (HAc), maximum HAc uptake batch tests under anaerobic conditions were conducted in order to find the limiting factor of HAc uptake. It was concluded that the amount of intracellular glycogen limits the maximum HAc uptake. Neither the capacity for PHB (poly-β-hydroxybutyrate) synthesis nor the achievement of complete polyphosphate degradation limits the maximum HAc uptake. This shows the importance of maintaining the glycogen storage.

3.3.1 Introduction

Phosphorus removing organisms accumulate organic substances (COD) like fatty acids under anaerobic conditions, which are stored as intracellular PHB. The energy (ATP) for this process is obtained from intracellular polyphosphate degradation resulting in phosphorus release into the bulk liquid. The required reducing power ($NADH_2$) is obtained from glycogen degradation (Mino *et al.*, 1987; Arun *et al.*, 1988, 1989; Smolders *et al.*, 1994a; see Chapter 3.2). Comeau *et al.* (1990) proposed an indirect biological method to quantify the amount of polyphosphate in activated sludge. The method is based on the maximum released phosphorus concentration, in the presence of excess HAc under anaerobic conditions. To put it the other way round, they assumed that the content of intracellular polyphosphate limits the maximum HAc uptake. However, the limiting factor for the maximum anaerobic HAc uptake might be due to (i)complete degradation of intracellular polyphosphate, (ii)too high level of intracellular PHB content, or (iii)the lack of availability of glycogen.

The limiting factor for the maximum HAc uptake is probably important on decision of the COD loading rate in waste water treatment plants and on modelling of the phosphorus removal process. In this chapter, the limiting factor for the maximum HAc uptake under anaerobic conditions was studied by batch tests using enriched denitrifying phosphorus removing sludge.

3.3.2 Experiments

Denitrifying phosphorus removing (DPB) sludge from an anaerobic-anoxic (A2) SBR (see Chapter 2) operated under 14 days SRT, was used for batch tests. The enriched DPB sludge was taken from the A2 SBR at the end of the anoxic phase, and put into a laboratory fermenter. The batch test consisted of three phases; an anaerobic, an aerobic and a second anaerobic phase. Initially excess HAc (the final concentration was 900 mg-COD/*l*) was added to the enriched DPB sludge. After 3 hours incubation under anaerobic conditions, the mixing was stopped. When the biomass had settled, the supernatant was removed and replaced with medium (Chapter 2) without HAc and phosphorus. This procedure for removing released phosphorus and residual HAc was repeated three times. Thereafter an aerobic period (3 or 22 hours) followed to allow intracellular PHB oxidation, intracellular glycogen synthesis and biomass growth, but no polyphosphate synthesis. The batch test ended with the second anaerobic phase, where again excess HAc was added and the subsequent phosphorus release was examined.

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*: *This subject was studied by Alexandra Wachtmeister who is an Erasmus-student from Sweden (Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm).*

3.3.3 Results and discussion

In the batch tests using the A2 sludge, PHB and glycogen concentrations were measured in the presence of excess HAc under anaerobic conditions. The batch tests were conducted twice. The difference was the duration of the aerobic period (22 or 3 hours). Because it was suspected that the 22 hours aeration had been too long and this had led to lack of glycogen due to the oxidation of glycogen after PHB was oxidised. Although the results are given below, this was not true and the difference of the aerobic period did not bring about different results. The phosphorus, HAc, PHB and glycogen concentrations in the batch tests are shown in Fig. 13 (22 hours aeration) and Fig. 14 (3 hours aeration).

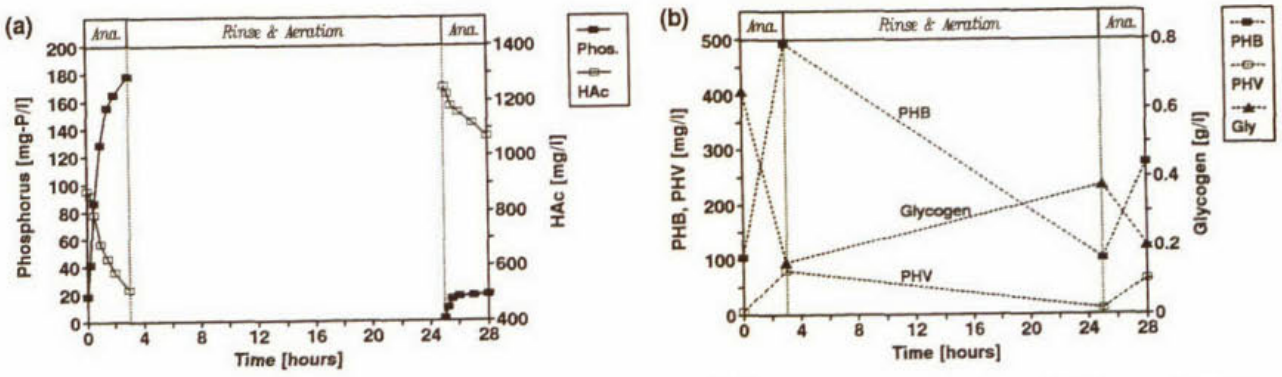

Fig. 13 (a)Phosphorus and HAc, and (b)PHB and glycogen concentrations in the maximum phosphorus release test (22 hours aeration).

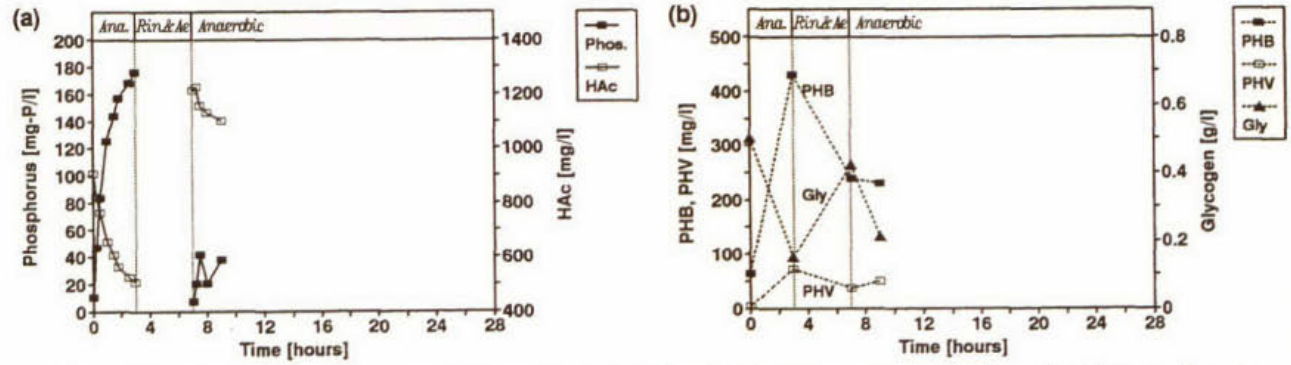

Fig. 14 (a)Phosphorus and HAc, and (b)PHB and glycogen concentrations in the maximum phosphorus release test (3 hours aeration).

After washing of the sludge and aeration, the A2 sludge released phosphorus again in the second anaerobic phase with the HAc addition. This showed that in the previous anaerobic phase polyphosphate content was not limiting the HAc uptake. The PHB concentration at the end of the second anaerobic phase was lower than at the end of the first anaerobic period. This indicates that there was still capacity available for the PHB formation inside the biomass.

The glycogen content inside the biomass seemed to be the limiting factor for the maximum HAc uptake. At the end of the both anaerobic phases, the glycogen concentrations were approximately $0.15 \sim 0.2$ g/*l*. On the glycogen extraction from the sludge samples (Chapter 2), not only intracellular glycogen but also substances in the cell walls of the bacteria are counted as glycogen. The $0.15 \sim 0.2$ g/*l* glycogen could be the normal sugar content of the bacterial polymers.

Comeau *et al.* (1990) proposed an indirect biological method to quantify the amount of polyphosphate in activated sludge, in which it was assumed that the content of intracellular ploy phosphate limits the maximum HAc uptake. However, the experiments in this chapter showed that complete polyphosphate degradation does not occur anaerobically even enough HAc is added to the sludge, because HAc uptake is stopped when probably the storage of glycogen is emptied.

It might be an error to conclude the polyphosphate content in the activated sludge, from this type of batch tests with excess HAc.

In conclusion, the amount of intracellular glycogen probably limits the maximum HAc uptake under anaerobic conditions, not the capacity of PHB synthesis nor the achievement of complete polyphosphate degradation.

3.4 Performance of short cycle SBRs

Abstract ——— A SBR was operated under repetitive anaerobic and anoxic phases in a short cycle, which simulates more the completely mixed (or large recycles)-type of phosphorus removal processes. The performance in the short cycle SBR was compared with the ordinary SBR operation under the same SRT. Under the short cycle operation phosphorus removal was unstable, while a stable complete phosphorus removal was achieved under the ordinary operation. One reason is the difficulty to control the optimal nitrate loading rate under short cycle operation, and mostly nitrate shortage or excess nitrate addition occurred which has adverse effects on phosphorus uptake. The comparison of both types of processes indicates that there is not a unique relationship between SRT and intracellular PHB level.

3.4.1 Introduction

In many waste water treatment processes (e.g. carrousels) large flow recycles (Fig. 15) are applied which causes the cyclic exposure of micro-organisms to anaerobic-aerobic or anaerobic-anoxic conditions with a cycle time of minutes. The behaviour of phosphorus removing organisms under such conditions is not known. Therefore an anaerobic-anoxic (A2) or anaerobic-aerobic (A/O) SBR has been operated under two different types of operations. One type was the ordinary operation as mentioned in Chapter 2; one large anaerobic-anoxic cycle in a main 6 hours cycle (Fig. 16(a)). Another type was the short cycle operation; three short A2 (or 3 ~ 12 A/O) cycles in a main 6 hours cycle (Fig. 16(b)). The cycle time, the time-ratio of anaerobic/anoxic conditions, and overall volumetric HAc and nitrate loading rates were identical in both operations. The difference was only the number of short cycles in a main cycle. The operation was shifted from plug flow-type (Fig. 15(a)) to processes with large internal recycles (Fig. 15(b)) (e.g. in carrousels), with increasing the number of short cycles. In this way, the difference of phosphorus removal between these two types of processes can be discussed.

In this chapter, the effect of the short cycle operation on phosphorus removal is examined. Especially, the main attention focuses on the behaviour of intracellular PHB which can be a key factor for phosphorus removal kinetics.

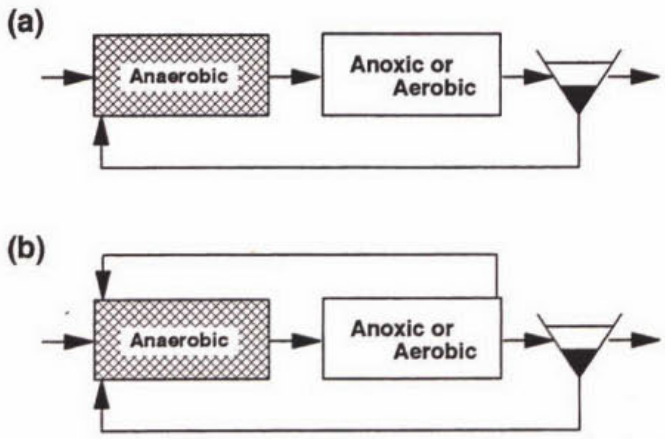

Fig. 15 Schematic diagram of (a)ordinary operations and (b)processes with internal recycles.

3.4.2 Experiments

An A2 or A/O SBR has been operated under ordinary operations or short cycle operations under 14 days SRT. Fig. 16 shows a schematic diagram of the ordinary A2 operation and the three

short A2 cycle operation. The ordinary operations consisted of one large anaerobic-anoxic cycle and a settling period, as used throughout this project. The three short cycle operations consisted of three short A2 cycles and a settling period, in the cycle time of 6 hours. Synthetic waste water containing HAc and phosphorus (Chapter 2) was added at the beginning of each anaerobic phase. The total volume of added waste water in a main cycle was the same in both operations. The mixed liquor volume increased stepwise by the addition of waste water in the short cycle operation, see Fig. 16(b). Thus overall volumetric HAc loading rates were similar in both operations. Nitrate solution was added in each anoxic phase (or aeration was done in each aerobic phase), and the total dosed amount was similar in both operations. The volume of added nitrate was adjusted, to minimise effluent phosphorus concentrations, and without detecting nitrate in effluent. The operational conditions are summarised in Fig. 17.

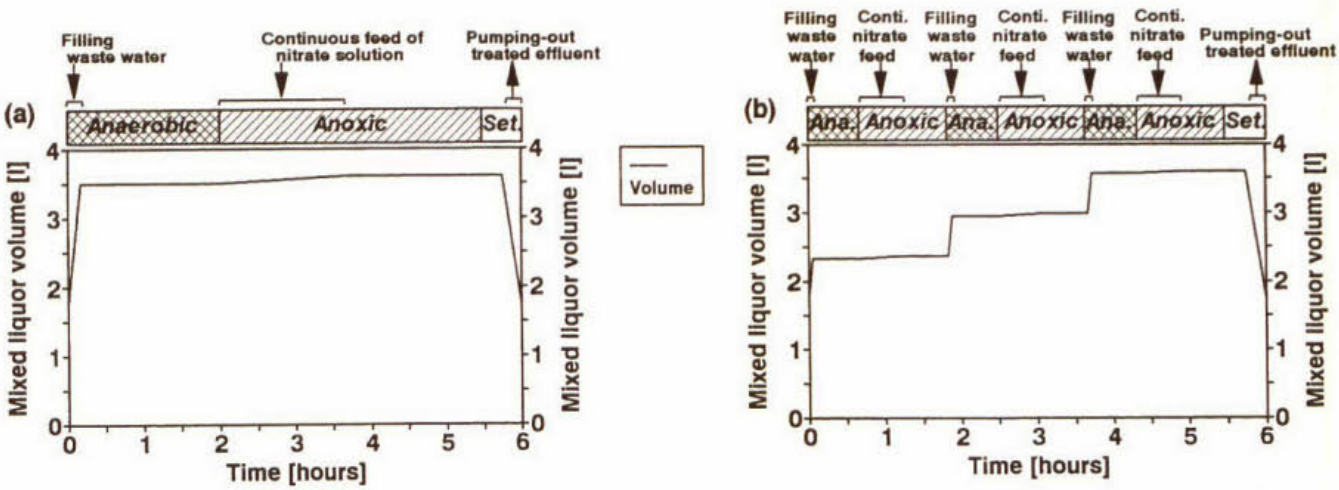

Fig. 16 Schematic diagram of (a)the ordinary A2 operation and (b)the short A2 cycle operation. Solid lines indicate that cycle behaviour of the mixed liquor volume in a main cycle.

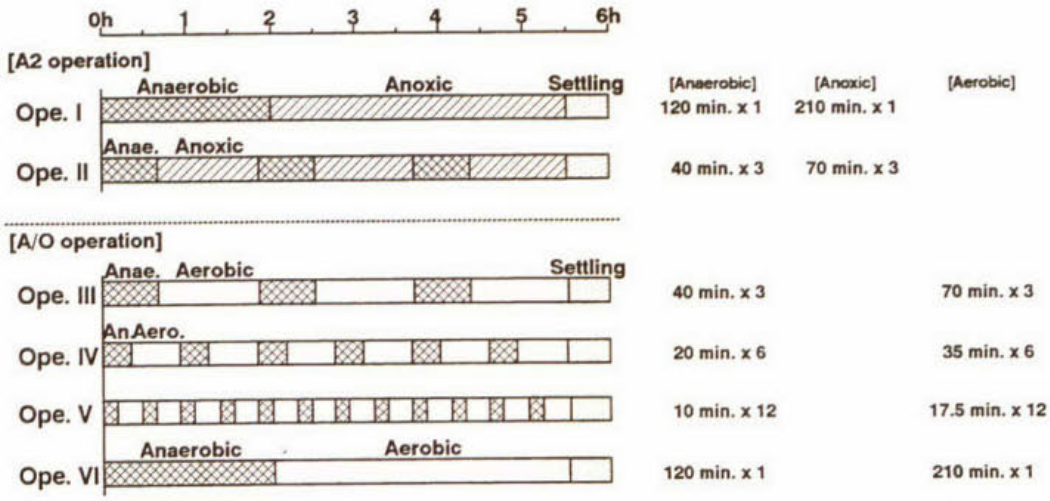

Fig. 17 Operational conditions in the A2 or A/O SBR. At the beginning of each short anaerobic phase, synthetic waste water containing HAc and phosphorus was added. The total volume of added waste water in a main 6 hours cycle was the same in each operation.

3.4.3 Results and discussion

Performance of short cycle SBRs on phosphorus removal: Before the short cycle operation, the A2 SBR had been operated under the ordinary operation (Operation I in Fig. 17). At day 79, the

short cycle operation started. There were three repetitive anaerobic-anoxic short cycles in a main 6 hours cycle (Operation II). As shown in Fig. 18(a) (□), phosphorus removal was not complete and very unstable, under the short A2 cycle operation (day 79～219). One reason for the incomplete phosphorus removal was shortage of nitrate. Based on the ORP (redox potential) profile, the flow rate of the nitrate pump was slightly modified several times, to achieve complete nitrate removal and maximal phosphorus removal. If nitrate addition was too small, phosphorus was not removed completely, though DPB might have enough capacity to accumulate more phosphorus. Another reason for the incomplete phosphorus removal was excess nitrate addition. In this case, nitrate was transferred into the anaerobic phase, which disturbed phosphorus release in the anaerobic phase (Chapter 3.2). Also normal denitrifiers can be enriched in the systems. Since too much nitrate was transferred into the anaerobic phase around day 89, the released phosphorus concentration dropped drastically (▲ in Fig. 18(a)).

Although incomplete phosphorus uptake due to these two reasons can also occur under ordinary operations, it is relatively easy to adjust the nitrate addition for complete nitrate removal and maximal phosphorus removal in the ordinary operation. However, it was quite difficult to find the optimal nitrate addition under the short cycle operation. The difficulty led to unstable phosphorus removal under the short cycle operation. After the SBR was returned to the ordinary operation (Operation I) at day 219 (until day 323), stable complete phosphorus removal was achieved (□ in Fig. 18(a)). Apparently an extensive control scheme for nitrate addition is required in the short cycle operation.

At day 323, the ordinary A2 SBR operation was switched into the short A/O cycle operation (3, 6 and 12 short cycles in Operation III, IV and V). Under the 3～12 short A/O cycles operation, phosphorus was removed completely, although it was not under the 3 short A2 cycles operation. Around day 370 (6 short A/O cycles) the effluent biomass concentration increased from 10 to 50～100 mg-SS/*l* without any specific malfunction (and the SVI [sludge volume index] increased from 30～50 to 50～100 m*l*/g-SS, though it had no influence for removal of the treated effluent). A drastic decrease of the biomass concentration in the SBR occurred (■, ▲ in Fig. 18(b)). This led to incomplete phosphorus removal around day 380. The excess sludge removal was stopped for one week, thereafter complete phosphorus removal occurred under 6 and 12 short A/O cycles.

Long term behaviour of intracellular storage substances: Figure 18(c) and (d) show daily variations of intracellular PHB contents and glycogen concentrations at the beginning and end of the final anoxic/aerobic phase. The intracellular glycogen which is an important factor as a reducing power ($NADH_2$) source on the anaerobic PHB production process, was not limiting.

Under the short A2 cycle operation (II), the PHB content ranged between 15 to 30 (mg-PHB/g-MLSS) in a cycle, while under the ordinary A2 operation (I), it ranged between 15 to 60 (mg-PHB/g-MLSS) in a cycle. The average PHB content in the anoxic phase in the short cycle SBR operation was clearly lower than the average in the ordinary operation. The (average) PHB content under the short A/O cycle operation was clearly lower than the A2 short cycle operation.

Effect of short cycle A2 and A/O operations on the PHB content. The effect of cycle time on phosphorus removal was examined in the short cycle A2 and A/O SBR. Under the short A2 cycles operation, phosphorus removal was unstable, and much phosphorus was detected in effluent. The reason was uncertain, but as above-mentioned, it was difficult to find the optimal nitrate loading rate, and this difficulty in the short A2 cycles operation might lead to unstable phosphorus removal.

Another possible reason for the unstable phosphorus removal might be a lower PHB content in the short A2 cycle operation. The PHB content can be the most important factor in the phosphorus uptake process, because the energy is obtained by the PHB oxidation with nitrate (oxygen) and growth materials for phosphorus removing organisms are obtained from the intracellular PHB. In the short cycle operation, the average PHB content in the anoxic phase was clearly lower than the average in the ordinary operation. The lower PHB content might lead to the unstable nitrate consumption in the short A2 cycle operation. However, phosphorus removal under

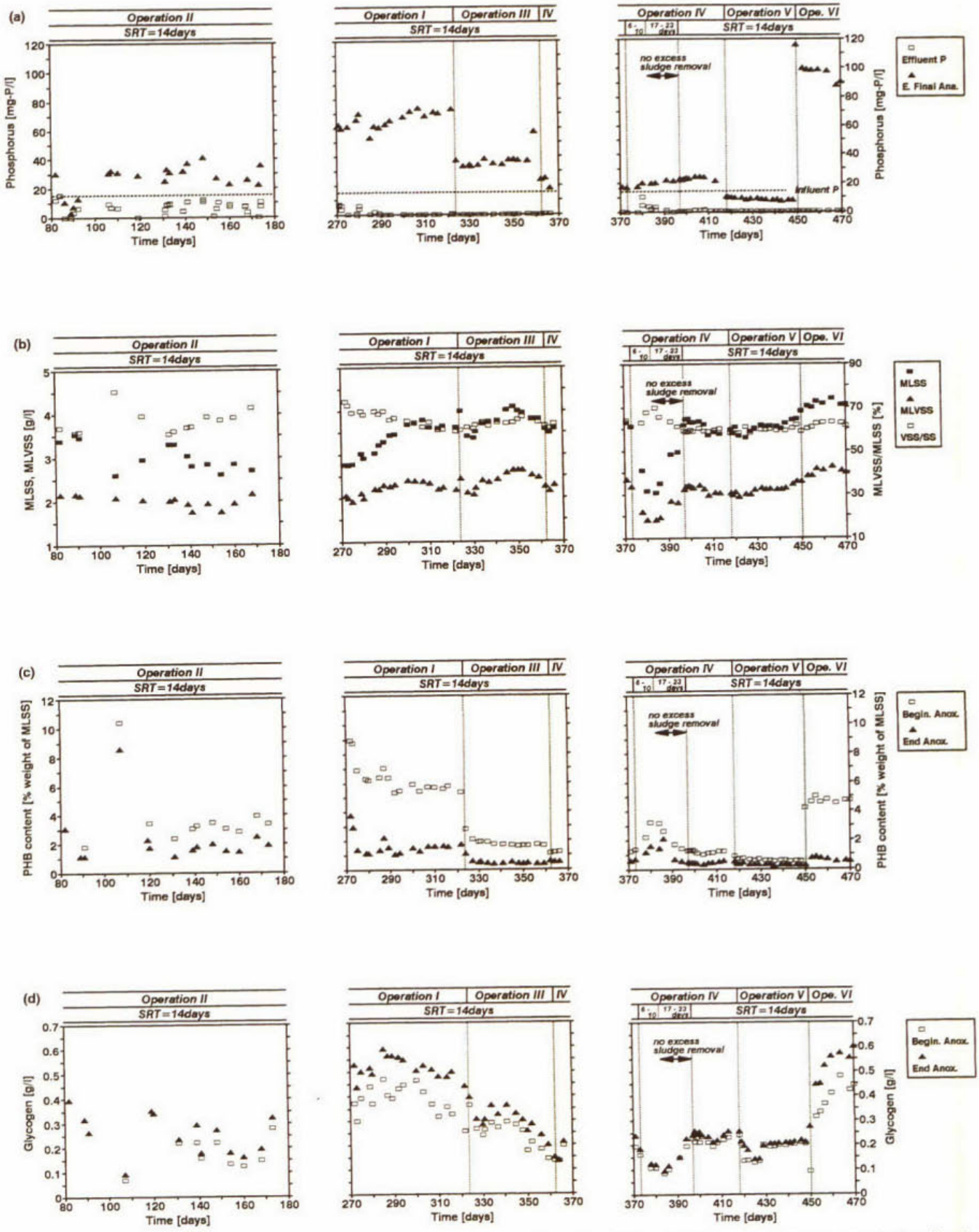

Fig. 18 Daily variation of (a)phosphorus concentrations in effluent (□) and at the end of the final anaerobic phase (▲), (b)MLSS (■) and MLVSS concentrations (▲) and the ratio (□), (c)PHB contents at the beginning (□) and end of the final anoxic/aerobic phase (▲), and (d)intracellular glycogen concentrations at the beginning (□) and end of the final anoxic/aerobic phase (▲).

the short A/O cycle operation was stable, though the PHB content was clearly even lower than the short A2 cycle operation. Since the energy production efficiency in the electron transport phosphorylation with nitrate is quite lower than the efficiency with oxygen (Chapter 3.5), the lower PHB content might have higher influence on the anoxic phosphorus uptake process, rather than in the aerobic process.

From the operation of short cycle A2 and A/O SBR, the following conclusions have been drawn:

(1)Under the short A2 cycle operations, phosphorus removal was unstable and much phosphorus was detected in effluent. After the operation was switched to the ordinary operation, the A2 SBR showed stable and complete phosphorus removal,
(2)It was difficult to find the optimal nitrate loading rate in the short A2 cycles operation. This difficulty on the operation probably leads to the unstable phosphorus removal. Another reason for the unstable phosphorus removal in the short A2 cycles operation might be lower intracellular content of PHB which is a key factor of the phosphorus uptake process,
(3)Short cycle A/O processes proved to be very stable with respect to phosphorus removal.

3.5 Modelling of denitrifying dephosphatation*

Abstract ——— The metabolic model from Smolders for the conventional phosphorus removal process under anaerobic-aerobic (A/O) conditions, was modified for denitrifying dephosphatation under anaerobic-anoxic (A2) conditions. The main difference of the models between the A/O and A2 processes is one metabolic reaction; electron transport phosphorylation with oxygen or nitrate. A value of P/$NADH_2$ ratio in the electron transport phosphorylation with nitrate, δ_N, was determined independently from batch tests with enriched DPB (denitrifying phosphorus removing bacteria) sludge. The measured δ_N in the DPB sludge was approximately 1.0 mol-ATP/mol-$NADH_2$, and the energy production efficiency with nitrate is approximately 40% lower than the efficiency with oxygen. The modified model with the measured δ_N was applied for the simulation of cycle behaviour in an A2 SBR, and the reliability and validity of the modified model were tested. The simulation results showed that the metabolic model derived for aerobic phosphorus removal, can be used also for the denitrifying dephosphatation process, although different model parameters are formed.

Nomenclature

f^{max}:	the maximum intracellular content (mol/mol-X_{DPB}, g/g-COD_{DPB})
K:	saturation constant (mmol/*l*)
m:	maintenance (mol/mol.h)
q:	the maximum specific conversion rate (h^{-1})
r:	conversion rate of substance (mol/mol.h)
S:	concentration of dissolved substance (mmol/*l*)
X:	concentration of particulate substance (mmol/*l*)
Y:	conversion yield coefficient
$\alpha 1$:	required ATP for HAc uptake (mol-ATP/mol-C)
$\alpha 3$:	required ATP for polyphosphate synthesis (mol-ATP/mol-P)
δ:	P/$NADH_2$ ratio in electron transfer phosphorylation (mol-ATP/mol-$NADH_2$)
ϵ:	energy for phosphorus transport (mol-P/mol-$NADH_2$)
κ:	biomass formation and polymerisation constant (mol-ATP/mol-C)

[Subscript]

ana:	anaerobic condition
ano:	anoxic condition
DPB:	active DPB biomass
gly:	glycogen
HAc:	acetic acid
N:	with nitrate
NH3:	ammonium
NO3:	nitrate
P:	phosphorus
PHB:	poly-β-hydroxybutyrate
poly-P:	polyphosphate

3.5.1 Introduction

A metabolic model of the phosphorus removal process under conventional aerobic conditions, has been proposed by Smolders *et al.* (1994a, b, 1995a, b). The model is based on 3 anaerobic and 5 aerobic metabolic reactions. The model can be modified for denitrifying dephosphatation under A2 conditions. The difference between the aerobic and anoxic metabolic reactions, is only the electron transport phosphorylation;

Aerobic: $NADH_2 + 0.5O_2 \rightarrow \delta ATP + H_2O$ · · · (1)

Anoxic: $NADH_2 + 0.4HNO_3 \rightarrow \delta_N ATP + 0.2N_2 + 1.2H_2O$ · · · (2)

Smolders *et al.* (1994b) determined a value of P/$NADH_2$ (P/O) ratio (produced ATP per oxidised $NADH_2$), δ, from the difference of measured oxygen consumption rates in the absence and presence

*: *This subject was studied by Ernst Murnleitner who is an Erasmus-student from Austria (Department of Applied Microbiology, University of Agriculture Wien).*

of phosphorus. In the same way, a value of the P/$NADH_2$ ratio under anoxic conditions, δ_N, can be determined. By using the δ_N, the metabolic model under the conventional aerobic condition is modified for denitrifying dephosphatation under the A2 condition.

In this chapter, the δ_N is determined independently in batch tests with sludge from an A2 SBR. The modified anoxic model based on δ_N is applied to denitrifying dephosphatation by DPB. Cycle behaviour of phosphorus in the A2 SBR is simulated under two different SRT (sludge retention time) conditions, to verify the reliability and validity of the model. Also, the limitations of the present metabolic model will be discussed in this chapter.

3.5.2 Metabolic model under anaerobic and anoxic conditions

Metabolic reactions under anaerobic conditions. The metabolism under anaerobic conditions, is identical between the aerobic organisms and DPB. HAc is taken-up and converted into PHB (poly-β-hydroxybutyrate). The energy (ATP) for this process is obtained from polyphosphate degradation. The reducing power ($NADH_2$) for the PHB formation is assumed to be obtained from glycogen metabolism (Mino *et al.*, 1987; Arun *et al.*, 1988; Smolders *et al.*, 1994a; see Chapter 3.2). The overall process under anaerobic conditions can be described according to the following equation (Smolders *et al.*, 1994a);

$$\underset{[HAc]}{CH_2O} + 0.5\underset{[glycogen]}{CH_{1.67}O_{0.83}} + (0.25+\alpha_1)\underset{[Poly\text{-}P]}{HPO_3}$$

$$\rightarrow 1.33\underset{[PHB]}{CH_{1.5}O_{0.5}} + 0.17CO_2 + (0.25+\alpha_1)\underset{[Pi]}{H_3PO_4} + \{(5/12)-(0.25+\alpha_1)\}H_2O \quad \cdots (3)$$

Metabolic reactions under anoxic conditions. Under aerobic and anoxic conditions, intracellular PHB is oxidised by oxygen or nitrate, and the produced energy is utilized for growth, and synthesis of polyphosphate and glycogen. The difference between aerobic and anoxic conditions is only electron transport phosphorylation with oxygen or nitrate (equation (1), (2)). The P/$NADH_2$ ratio under anoxic conditions, δ_N, will be independently determined from batch tests in section 3.5.3. The P/$NADH_2$ ratio (P/O ratio) under aerobic conditions, δ, was reported to be 1.85 (mol-ATP/mol-$NADH_2$) by Smolders *et al.* (1994b, 1995b).

Anoxic PHB and nitrate conversion rates. From the assumption that no net accumulation of $NADH_2$ and ATP takes place, the overall stoichiometric equation of the PHB conversion rate under anoxic conditions can be expressed as follows, by using three yield coefficients, Y, and one maintenance coefficient, m_{PHB};

$$-r_{PHB} = r_{DPB}/Y_{PHB\text{-}DPB} + r_{poly\text{-}P}/Y_{PHB\text{-}poly\text{-}P} + r_{gly}/Y_{PHB\text{-}gly} + m_{PHB} \cdot X_{DPB} \quad \cdots (4)$$

$$Y_{PHB\text{-}DPB} = (2.25\delta_N+0.5)/(0.635+2.2425\delta_N+\kappa) \quad \cdots (5)$$

$$Y_{PHB\text{-}poly\text{-}P} = (2.25\delta_N+0.5)/(\delta_N/\epsilon_N+\alpha_3) \quad \cdots (6)$$

$$Y_{PHB\text{-}gly} = (2.25\delta_N+0.5)/(2\delta_N+1.5) \quad \cdots (7)$$

$$m_{PHB} = m_{ATP}/(2.25\delta_N+0.5) \quad \cdots (8)$$

The equation (4) describes the amount of required PHB for the biomass production (r_{DPB}), polyphosphate ($r_{poly\text{-}P}$) and glycogen synthesis (r_{gly}), and maintenance (m_{PHB}). X_{DPB} is the active biomass concentration of DPB (see section 3.5.3). The yield and maintenance coefficients are defined by the metabolic model parameters (δ_N, κ, ϵ_N, α_3, m_{ATP}). The formula of these yield coefficients is completely identical between aerobic (Smolders *et al.*, 1994b) and anoxic conditions, when δ_N is replaced by δ.

In the same way, the stoichiometric nitrate conversion rate under anoxic conditions, r_{NO3}, can be expressed by using three yield coefficients ($Y_{NO3\text{-}DPB}$, $Y_{NO3\text{-}poly\text{-}P}$, $Y_{NO3\text{-}gly}$) and one

maintenance coefficient (m_{NO3}).

3.5.3 Measurement of P/$NADH_2$ ratio in electron transport phosphorylation with nitrate

Experimental procedure. In order to determine the P/$NADH_2$ ratio in electron transport phosphorylation with nitrate (δ_N), batch tests were conducted using enriched DPB sludge (SRT=8 days) (see Chapter 2).

In each batch test, 400m*l* DPB sludge was taken from the ordinary A2 SBR at the end of the anaerobic phase. Thus, much PHB was stored inside DPB, and less polyphosphate was stored due to anaerobic phosphorus release with HAc consumption. After centrifugation, the supernatant was replaced by a mineral medium (Chapter 2) without HAc and phosphorus, and the re-suspended sludge was transferred into a 1*l* laboratory fermenter. After addition of proper amounts of phosphorus and nitrate to the pre-treated sludge, a batch test was started.

All batch tests were conducted in the 1*l* laboratory fermenter at 25 °C, using the pre-treated DPB sludge. pH was controlled at 7.0~7.1 by addition of 0.5N HCl or NaOH. N_2 gas was flushing through the head space of fermenter, to prevent from oxygen contamination. In the batch tests, the conversion rate of each substance (phosphorus, NO_3^-, NH_4^+, intracellular glycogen and PHB concentrations) was examined under the anoxic condition using various initial phosphorus concentrations (0, 35, 85, 240 mg-P/*l*).

P/$NADH_2$ ratio under anoxic conditions. The conversion rates of each substance and active biomass concentrations were obtained from 5 batch tests with the enriched DPB sludge. It was assumed that (the measured) MLVSS was the total fraction of PHB, glycogen and the active biomass (X_{DPB}) (Smolders *et al.*, 1995a). The active biomass conversion rate was obtained from the NH_4^+ conversion rate.

If the anoxic P/$NADH_2$ ratio, δ_N, is known, it is possible to calculate all conversion yields, Y, according to the equation (5)~(8), with the model parameters as shown in Table 4. All conversion rates, r, were obtained from the batch tests. Thus, the best estimate of δ_N in each batch test can be determined, as the equation (4) is satisfied.

Table 4 Metabolic model parameters under aerobic (Smolders *et al.*, 1994b) and anoxic conditions.

	Aerobic	Anoxic
P/$NADH_2$ ratio (mol-ATP/mol-$NADH_2$):	$\delta = 1.85$	δ_N = unknown parameter
Energy for transport of phosphorus (mol-P/mol-$NADH_2$):	$\epsilon = 7$	$\epsilon_N = \epsilon \cdot \delta_N/\delta$ *1
Biomass formation and polymerisation constant (mol-ATP/mol-C_{PHB}):	$\kappa = 1.6$*2	
Required ATP for polyphosphate synthesis (mol-ATP/mol-P):	$\alpha 3 = 1.0$*2	
Required ATP for maintenance (mol-ATP/mol-C_{PHB}.h):	$m_{ATP} = 0.019$	$m_{ATP} = 0.010$*3

*1: ϵ_N was estimated from ϵ in proportion to a ratio of δ_N/δ. δ and ϵ are reported values under aerobic conditions (Smolders *et al.*, 1994b).

*2: Reported values under aerobic conditions (Smolders *et al.*, 1994b)

*3: Smolders *et al.* (1994b) reported m_{ATP}=0.019 under aerobic conditions. From the difference of (the anaerobic) secondary phosphorus release rates in the A/O sludge and A2 sludge (Chapter III-1), it was estimated that m_{ATP} under anoxic conditions was 0.01.

The average of obtained δ_N values was approximately 1.0 (mol-ATP/mol-$NADH_2$) under anoxic conditions. Smolders *et al.* (1994b) reported 1.85 (mol-ATP/mol-$NADH_2$) under aerobic conditions. Therefore, the energy (ATP) production in electron transport phosphorylation with nitrate is approximately 40% lower than oxygen. The lower efficiency is probably normal for denitrifying systems, in comparison with aerobic systems.

In section 3.5.4, the metabolic model with the obtained δ_N (=1.0) is applied, and the conversion of each substance is simulated to verify the reliability and validity of the model.

3.5.4 Application of the anaerobic-anoxic model

Anaerobic and anoxic kinetic equations. From the metabolic model parameters (Table 4) including the obtained δ_N (=1.0), all yield coefficients, Y (equation (5)~(8) in the case of the anoxic PHB conversion rate), can be calculated. The anoxic PHB and nitrate conversion rates are the following;

$$-r_{PHB} = 1.628\, r_X + 0.460\, r_{poly\text{-}P} + 1.273\, r_{gly} + 3.64\times10^{-3}\, X_{DPB} \cdots (9)$$

$$-r_{NO3} = 0.568\, r_X + 0.414\, r_{poly\text{-}P} + 0.346\, r_{gly} + 3.27\times10^{-3}\, X_{DPB} \cdots (10)$$

Therefore, by using these yields coefficients, the metabolic reactions under the anaerobic and anoxic condition in Table 5 were obtained.

Table 5 Metabolic reactions under the anaerobic and anoxic condition.

Anaerobic condition	
HAc uptake	$- CH_2O - 0.5\, CH_{1.67}O_{0.83} - 0.36\, HPO_3 + 1.33\, CH_{1.5}O_{0.5} + 0.17\, CO_2 + 0.36\, H_3PO_4 + 0.059\, H_2O = 0$
Maintenance	$- HPO_3 - H_2O + H_3PO_4 = 0$
Anoxic condition	
Biomass synthesis	$- 1.63\, CH_{1.5}O_{0.5} - 0.57\, HNO_3 - 0.2\, NH_3 - 0.015\, H_3PO_4 + CH_{2.09}O_{0.54}N_{0.20}P_{0.015} + 0.28\, N_2 + 0.63\, CO_2 + 0.78\, H_2O = 0$
Phosphorus uptake	$- 0.46\, CH_{1.5}O_{0.5} - 0.41\, HNO_3 - H_3PO_4 + HPO_3 + 0.21\, N_2 + 0.46\, CO_2 + 1.55\, H_2O = 0$
Glycogen synthesis	$- 1.27\, CH_{1.5}O_{0.5} - 0.35\, HNO_3 + CH_{1.67}O_{0.83} + 0.17\, N_2 + 0.27\, CO_2 + 0.29\, H_2O = 0$
Maintenance	$- CH_{1.5}O_{0.5} - 0.9\, HNO_3 + 0.45\, N_2 + CO_2 + 1.2\, H_2O = 0$

Based on these metabolic reactions, the conversion of each substance in the batch tests and the cycle behaviour in the A2 SBR were calculated. Process rate equations and stoichiometry matrix according to "*IAWQ format*" (for instance; Gujer and Henze, 1991), which were used in the simulation, are shown in Table 6 and 7.

Table 6 Process rate equations (Smolders *et al.*, 1995a, b) according to "*IAWQ format*".

Process rate equations		
Uptake of S_{HAc}	$q_{HAc} \cdot S_{HAc} / (S_{HAc} + K_{HAc}) \cdot X_{DPB}$	$\cdot [1 - S_{NO3} / (S_{NO3} + K_{NO3})]$
Anoxic growth	$q_{DPB} \cdot (X_{PHB} / X_{DPB}) \cdot X_{DPB}$	$\cdot S_{NO3} / (S_{NO3} + K_{NO3})$
Storage of $X_{poly\text{-}P}$	$q_{poly\text{-}P} \cdot S_P / (S_P + K_P) \cdot (1 - X_{poly\text{-}P} / X_{DPB} / f_{poly\text{-}P}^{max}) \cdot (X_{PHB} / X_{DPB})^{1/3} \cdot X_{DPB}$	$\cdot S_{NO3} / (S_{NO3} + K_{NO3})$
Storage of X_{gly}	$q_{gly} \cdot (f_{gly}^{max} - X_{gly} / X_{DPB}) \cdot X_{DPB}$	$\cdot S_{NO3} / (S_{NO3} + K_{NO3})$
Anaerobic maintenance	$m_{ana} \cdot X_{DPB}$	$\cdot [1 - S_{NO3} / (S_{NO3} + K_{NO3})]$
Anoxic maintenance	$m_{ano} \cdot X_{DPB}$	$\cdot S_{NO3} / (S_{NO3} + K_{NO3})$

Table 7 Stoichiometry matrix according to "*IAWQ format*" (based on a unit of g-P or g-COD).

Stoichiometry matrix							
Process	S_{NO3}	S_{HAc}	S_P	X_{DPB}	$X_{poly\text{-}P}$	X_{PHB}	X_{gly}
Uptake of S_{HAc}		-1	0.35		-0.35	1.50	-0.5
Anoxic growth	-0.63		0.013	1		-1.63	
Storage of $X_{poly\text{-}P}$	-0.53		-1		1	-0.53	
Storage of X_{gly}	-0.43					-1.43	1
Anaerobic maintenance			1		-1		
Anoxic maintenance	-1					-1	

Simulation of the conversion rates in the batch tests. The following calculation which was a combination of the conversion (the first differential) equations, was performed by simulation software "*AQUASIM*" (ver. 1.0a) (EAWAG: Swiss Federal Institute for Environmental Science and Technology). On "*AQUASIM*", it is easy to define the stoichiometry and kinetic equations in relation to biological conversions, hydraulic effects and reactor configurations. Also parameter estimation and sensitivity analyses can be made. The best estimated kinetic parameters under anoxic conditions in the simulation are shown in Table 8.

Table 8 Kinetic parameters under anoxic conditions in the A2 process.

Parameter	Value	Source
[Anoxic Conditions]		
[*The maximum specific conversion rate*]		
q_{DPB}	0.05 mol-C/mol-C.h	PE
$q_{poly\text{-}P}$	0.1 mmol/mol-C.h	PE
q_{gly}	0.8 mmol-C/mol-C.h	PE
[*Saturation constant*]		
K_P	0.1 mmol/*l*	Smolders *et al.* (1995a, b)
K_{NO3}	0.05 mmol/*l*	Estimation from behaviour of nitrate in the A2 SBR
[*The maximum intracellular content*]		
f_{gly}^{max}	0.3 mol-C/mol-C	PE
$f_{poly\text{-}P}^{max}$	0.3 mol/mol-C	Smolders *et al.* (1995a, b)
[*Maintenance*]		
m_{ano}	3.64×10^{-3} mol-C/mol-C.h	see equation (9)

PE: Parameter estimation from the results of the batch tests, with "*AQUASIM*".

By using the kinetic (Table 6) and stoichiometric equations (Table 5 or 7), and obtained optimal kinetic parameters under anoxic conditions from the parameter estimation on the "*AQUASIM*" (Table 8), it is possible to calculate the conversion rate of each substance in the batch tests (section 3.5.3). Figure 19 shows an example of the measured and calculated P, NO_3^-, NH_4^+, PHA and glycogen concentrations. The simulation results indicated that the model could represent the measured conversion of each substance in various initial concentrations of phosphorus.

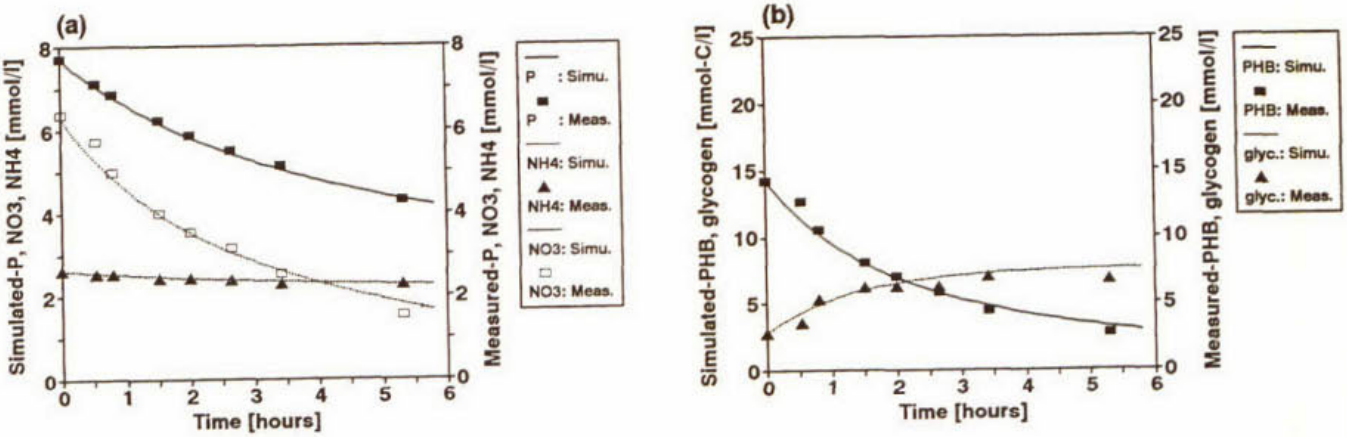

Fig. 19 Calculated and measured conversion of each substance in one of the batch tests (240 mg-P/*l*).

Application of the model to cycle behaviour in the A2 SBR. P, NO_3^-, NH_4^+, HAc, PHA and glycogen concentrations were measured in a steady state cycle, in an A2 SBR (see Chapter 2) under 8 days and 14 days SRT. The cycle behaviour of each substance was simulated by the

above-mentioned model, and the simulation results were compared with the measured data-sets of each substance conversion under 8 days and 14 days SRT. In the anoxic conditions, the calculation was done by using the same kinetic equations, stoichiometric relation and kinetic parameters as above-mentioned. The kinetic parameters under anaerobic conditions are summarized in Table 9. The calculation was done until steady states were obtained (25 A2 cycles = 150 hours).

Table 9 Kinetic parameters under anaerobic conditions in the A2 process.

Parameter	Value	Source
[Anaerobic Conditions]		
[*The maximum specific conversion rate*]		
q_{HAc}	0.2 mol-C/mol-C.h	PE
[*Saturation constant*]		
K_{HAc}	1.0 mmol-C/*l*	Smolders *et al.* (1994b, 1995b)
[*Stoichiometric parameter*]		
$\alpha 1$	0.11 mol-ATP/mol-C	PE
[*Maintenance*]		
m_{ana}	2.5×10^{-3} mol-P/mol-C.h	Secondary phosphorus release rate

PE: Parameter estimation from the results of the batch tests, with "*AQUASIM*".

Now, all kinetic equations, stoichiometric relations and kinetic parameters under anaerobic and anoxic conditions, are specified. The identical kinetic and stoichiometric parameters were used for the simulation under 8 days and 14 days SRT. The measured and simulated cycle behaviour at each SRT are shown in Figure 20(a) and (b).

The model could predict the concentration of all dissolved substances. However a large difference between the measured and simulated PHB concentration was observed. The underestimation of PHB concentration on the model can be due to two possible reasons: (i)deviating influent HAc (or nitrate) concentrations and (ii)sensitivity for the δ_N obtained from independent batch tests.

By using slight higher HAc loading rates, the conversions were recalculated. All other calculation conditions were the same. The recalculations indicated that 2% and 5% differences of the influent HAc concentration brought about approximately 15% and 35% differences on the PHB concentration. 2% ~ 5% higher HAc input can be caused due to an error of making the substrate and inaccurate flow rates of the substrate pump. The influent HAc concentration had large effect on the PHB concentration, but not on the concentration of the other substances. The slight higher influent HAc concentration resulted in very good agreement between simulation and experiment in both SRT conditions. This effect can be understood as follows. According to the kinetic model of the anoxic phase, the rate of growth, poly-P synthesis and glycogen production stop if nitrate concentration is zero (Table 6). This means that the PHB consumption also stops (equation (4) or (9). If there is a slight imbalance, within one cycle, between the PHB-produced in the anaerobic phase and consumed in the anoxic phase, this imbalance will accumulate over many cycles. A small change in the effect on this imbalance of PHB will lead to a significant effect on the ultimate steady state PHB level.

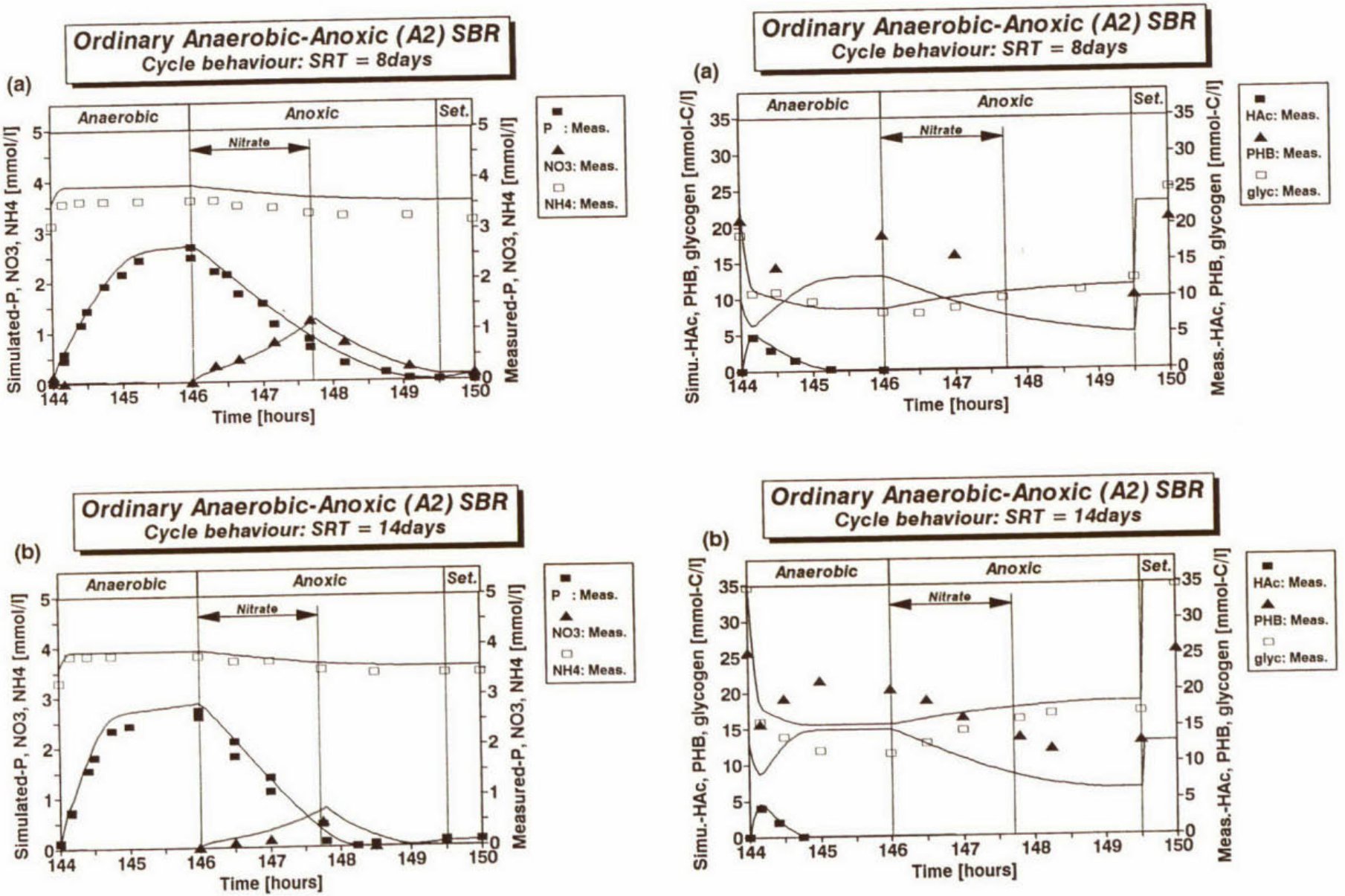

Fig. 20 Calculated and measured conversion of each substance in the A2 SBR, at (a)8 days SRT and (b)14 days SRT.

The second possible reason for the underestimation of PHB concentration can be sensitivity for the δ_N. By using the same kinetic parameters and slight lower δ_N (=0.9, instead of 1.0) the conversions were recalculated in the batch tests and in the cycle behaviour. The model with slight lower δ_N still predicted the measured conversion of each substance in the batch tests, which indicates the δ_N has little influence on the estimation of kinetic parameters (on the *AQUASIM*). The model with slight lower δ_N resulted in very good agreement of the cycle behaviour of PHB between simulation and experiment in both SRT conditions. In this subject, δ_N was independently decided from the batch tests with the enriched DPB sludge, not on calculation. As shown in the recalculation with slight lower δ_N, the δ_N has relatively large effect on the PHB concentration, thus it should be estimated more precisely from many of this type batch test.

The role of PHB in the metabolic model. Two points about intracellular PHB contents on the model calculation, should be emphasized. The first point to notice is the effect of influent HAc loading rates and δ_N on the PHB contents in the long term simulation. As above-mentioned, a small difference of the HAc loading rate and δ_N leads to a large difference of the PHB content in a steady state. Since the PHB content is a key factor for the anoxic/aerobic phosphorus uptake process, these two factors should be evaluated carefully in the model calculation.

The second point to notice is the requirement of a new relationship between the growth rate and PHB contents. The relationship between the PHB content and growth rate is one of the important factors in the modelling of the phosphorus removal process. From the obtained results in Chapter 3.4, the comparison of PHB levels in A2, A/O and standard or short cycle processes all with the same SRT indicates that there is not a unique relationship between SRT and intracellular PHB level. In a metabolic model of the phosphorus removal process proposed by Smolders *et al.* (1995a, b), the growth rate which can be defined by SRT, is related to the PHB content (Table 6). To put it the other way round, the proposed kinetic equation of the growth leads to the same PHB contents in a steady state, at the same SRT conditions (growth rate). However the PHB content in the short cycle operation seemed to be lower than the ordinary operation and also the PHB content of A2 sludge is much higher than A/O sludge, in spite of the same SRT conditions. This might indicate that a new relationship between the growth rate and PHB content is required on the modelling of phosphorus removal process.

Modelling of denitrifying dephosphatation. Only few studies have so far been made at modelling of denitrifying dephosphatation. Phosphorus removing organisms in the Activated Sludge Model No.2 proposed by the IAWQ Task Group, are modelled to be incapable of denitrifying dephosphatation. The first attempt of the introduction of denitrifying dephosphatation into the Activated Sludge Model No.2 was made by Mino *et al.* (1994). They assume that all aerobic phosphorus removing organisms are also active under denitrifying conditions. Contrary, Kerrn-Jespersen and Henze (1993) proposed that phosphorus removing organisms are divided into two groups; one group can utilize not only oxygen but also nitrate, and another can utilize oxygen only. In modelling normal aerobic or denitrifying heterotrophic growth there is no difference. In the modelling of phosphorus removal the results depend however strongly on whether one assumes the existence of one or two groups of organisms. As shown in the next chapter, in principle aerobic and anoxic dephosphatation capacities can be the same. In the Chapter 3.2, it was shown that even in an anaerobic-aerobic SBR without anoxic conditions denitrification activity is present (the anoxic phosphorus uptake activity was approximately 15% of the aerobic activity). Since it is unlikely that under this condition full denitrification enzyme activity is induced, it is unlikely that 15% of phosphorus removing organisms are DPB and another part is "strict" aerobic phosphorus removing bacteria. It seems that oxygen-related cytochrome oxidation enzyme is always present which leads to full aerobic phosphorus uptake activity even after cultivation of the sludge without oxygen, but that the enzyme for denitrification (nitrate reductase) is lost in a part of DPB when the sludge is cultivated without nitrate, and a certain time is required till the full denitrification enzyme activity

is induced.

In this chapter the modelling of denitrifying dephosphatation was discussed. In conclusion:

(1)The measured P/$NADH_2$ ratio in electron transport phosphorylation with nitrate, δ_N, was approximately 1.0 mol-ATP/mol-$NADH_2$. This δ_N value indicates that the ATP-production efficiency with nitrate is approximately 40% lower than with oxygen,

(2)A metabolic model which has been proposed for the conventional aerobic process, can be applied for the denitrifying dephosphatation process. A most important difference between the aerobic and anoxic processes is the electron transport phosphorylation with oxygen (δ) or nitrate (δ_N),

(3)Results of the simulation indicate that the same structure of the model derived for aerobic phosphorus removal, can be used also for the denitrifying dephosphatation process, although different kinetic parameters are formed. Moreover, comparing A/O and A2 systems with standard and short cycles it is clear that the present model structure cannot describe all systems with one set of kinetic parameters.

4 SINGLE-SLUDGE SYSTEMS

4.1 Effect of oxygen exposure in a post-denitrification configuration on the activity of denitrifying phosphorus removing bacteria

Abstract ——— The application of denitrifying phosphorus removing bacteria (DPB) in single-sludge systems with phosphorus removal, denitrification and nitrification requires knowledge of the effect of oxygen on DPB. In this chapter the effect of oxygen on the activity of DPB has been studied. DPB were enriched without oxygen in an anaerobic-anoxic sequencing batch reactor (SBR) over a long time, after which an aerobic phase was introduced into the SBR. The performance on phosphorus and nitrogen removal was examined for the post-denitrification (anaerobic-aerobic-anoxic) and pre-denitrification (anaerobic-anoxic-aerobic) SBR. It could be concluded that oxygen has no detrimental effect on the denitrifying dephosphatation activity. The maximum phosphorus uptake rate by the enriched denitrifying sludge was almost equal for anoxic and aerobic conditions, and full anoxic phosphorus removal activity was kept for at least 5 months after the introduction of the aerobic phase. From the experiments it became evident that full advantage of applying denitrifying phosphorus removing bacteria can only be obtained in a pre-denitrification process like UCT-type processes.

4.1.1 Introduction

Biological phosphorus removal is usually applied with simultaneous nitrogen removal in waste water treatment processes. The last years several publications have been devoted to the study of denitrifying phosphorus removing bacteria (DPB) which are capable of phosphorus removal under anoxic conditions (Vlekke *et al.*, 1988; Pokethitiyook *et al.*, 1990; Wanner *et al.*, 1992; van Loosdrecht *et al.*, 1992; Kerrn-Jespersen and Henze, 1993; Kuba *et al.*, 1993a and 1994e). It was shown in previous research that DPB have the same potential for phosphorus removal as conventional aerobic phosphorus removing bacteria (Kuba *et al.*, 1993a). The main advantage of applying this kind of organisms is the reduced need of COD (and oxygen) for the overall phosphorus and nitrogen removal process. In these processes an aerobic phase is needed for nitrification. This can be achieved in a single- or two-sludge system (Fig. 3). In the single-sludge system which is the subject of this chapter, DPB coexist with nitrifiers, and the mixed sludge goes through all stages such as anaerobic, aerobic and anoxic phases. In a two-sludge system, nitrifiers are separated from the DPB, e.g., in a nitrification biofilm reactor (Bortone *et al.*, 1994) or a nitrification sequencing batch reactor (Kuba *et al.*, 1996a; see Chapter 5.1), and DPB are hardly exposed to oxygen.

In the single-sludge system DPB undergo temporarily aerobic conditions which may disturb their activity. So far the effect of oxygen on DPB has never been discussed. In this chapter, an aerobic phase was introduced into an anaerobic-anoxic sequencing batch reactor (SBR) in which DPB had been enriched over a long period. This can be done in two different ways; post-denitrification, where oxygen is introduced before the anoxic phase (anaerobic-aerobic-anoxic); pre-denitrification, where oxygen is introduced after the anoxic phase (anaerobic-anoxic-aerobic). In this study the post-denitrification was studied in a lab-scale SBR (this chapter), and the pre-denitrification was studied in actual waste water treatment plants (Chapter 4.2, 4.3). The purpose in this chapter is to examine the effect of prolonged cyclic exposure to oxygen on the activity of DPB. Added to this, general requirements and problems in single-sludge phosphorus and nitrogen removal systems incorporating DPB will be discussed.

4.1.2 Experiments

The study was carried out in a lab-scale SBR (3.5～3.6*l* working volume) at room temperature. The SBR had been operated under anaerobic-anoxic (A2) conditions over 2 months before the start of this study (Operation 0 in Fig. 21). Then an aerobic phase (0.25～0.75h) was introduced between anaerobic and anoxic phases (Operation I～V in Fig. 21) to achieve nitrification. In each operation, the cycle time was 6 hours. A 2 hours anaerobic phase was maintained at the beginning of the cycle, and a 0.5 hours settling period was applied at the end of the cycle. 1.75*l* synthetic waste water containing HAc and phosphorus (Chapter 2) was pumped into the SBR during the first 10 minutes of the anaerobic phase. In the appropriate period of the anoxic phase (Fig. 21), nitrate (117 mmol/*l*) was pumped into the reactor with a constant flow rate (1 m*l*/min). Also after the introduction of the aerobic phase nitrate had to be pumped into the SBR in the anoxic phase, because of low nitrification activities in the anaerobic-aerobic-anoxic (AOA) SBR sludge. The quantity of nitrate addition in each operation was reduced in proportion to the length of each aerobic phase. After the settlement, 1.8～1.85*l* supernatant was retrieved from the SBR. The overall hydraulic retention time was 0.5 days, and the overall sludge retention time (SRT) was 20 days. The aerobic SRT was 0.0～7.2 days (Fig. 21).

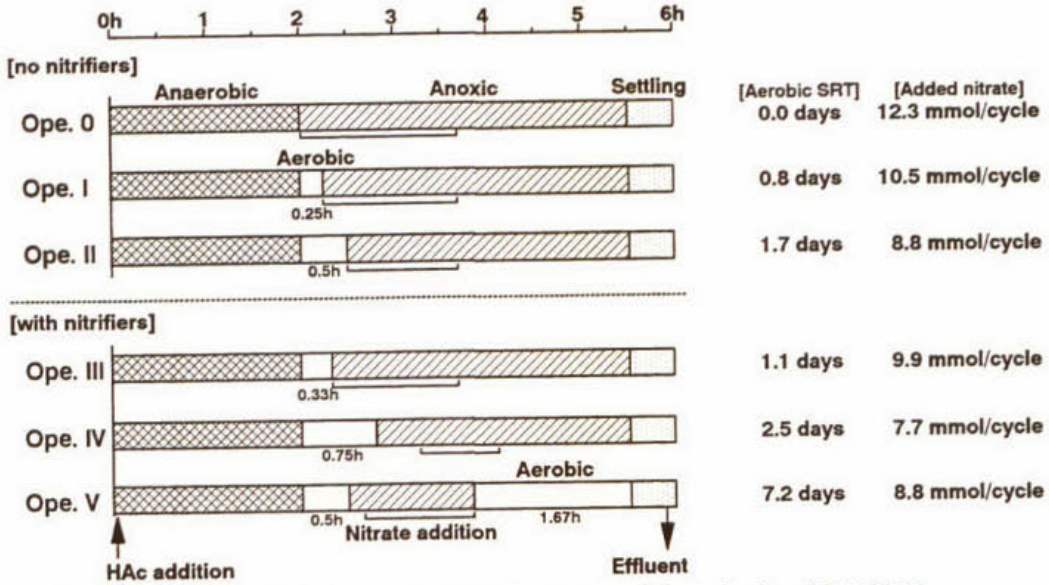

Fig. 21 Schematic diagram of operational conditions in the AOA SBR.

The effect of oxygen on the activity of DPB was investigated over the whole period (5 months) of the SBR operations (I～V). Added to this, in the Operation III～V, nitrification activities in the single-sludge system were investigated. Since the SBR was previously operated under A2 conditions over a long time, nitrifiers were not present in the system. To obtain some nitrification, nitrifying sludge (the nitrification activity was approximately 0.8 mmol-NH_4^+/g-SS.h) which had been enriched with mineral medium containing NH_4^+ in a nitrification SBR, was inoculated into the AOA SBR at day 67 (1.5 g-SS nitrifying sludge) and day 80～103 (6.5 g-SS nitrifying sludge).

To compare phosphorus uptake activities of DPB with nitrate and with oxygen as an electron acceptor, the maximum phosphorus uptake rate was measured in the SBR, at day 45 and 46 (Operation I). In the measurement, the DPB sludge was exposed to full aerobic or anoxic conditions after the anaerobic phase. When the anoxic maximum phosphorus uptake rate was measured, nitrate was pumped into the SBR with twice higher flow rate than the ordinary SBR operation. Nitrate was always present in the measurement which means nitrate limitation for phosphorus uptake didn't occur.

4.1.3 Results and discussion

Effect of oxygen exposure on denitrifying dephosphatation activities of DPB. Before the introduction of the aerobic phase, the SBR had been operated under A2 conditions over 2 months (Operation 0). Before starting the AOA operation (I ~ V), an aerobic phase (20 minutes) was introduced at the end of the anoxic phase for two weeks (pre-denitrification). This introduction of the aerobic phase did not have any effect on phosphorus removal by DPB.

After the enrichment of DPB, an aerobic phase was introduced into the SBR between the anaerobic and anoxic phases, at day 0 (Operation I) (post-denitrification). Immediately after the introduction of the aerobic phase, phosphorus which was released in the anaerobic phase, was aerobically taken-up. This is shown by a difference between solid (■) and open squares (□) in Fig. 22. This observation coincided with previous research (van der Velde, 1992). The aerobic phosphorus uptake didn't increase with time during the Operation I, and 23 ~ 33% phosphorus was removed in the aerobic phase. Until day 40, phosphorus was removed completely during the subsequent anoxic phase (▲ in Fig. 22 and ■ in Fig 23). From day 40 to day 65, secondary phosphorus release (Kuba *et al.*, 1993a; see Chapter 3.11) at the end of the anoxic phase was observed as shown in Fig. 23 (□), and phosphorus was detected in effluent.

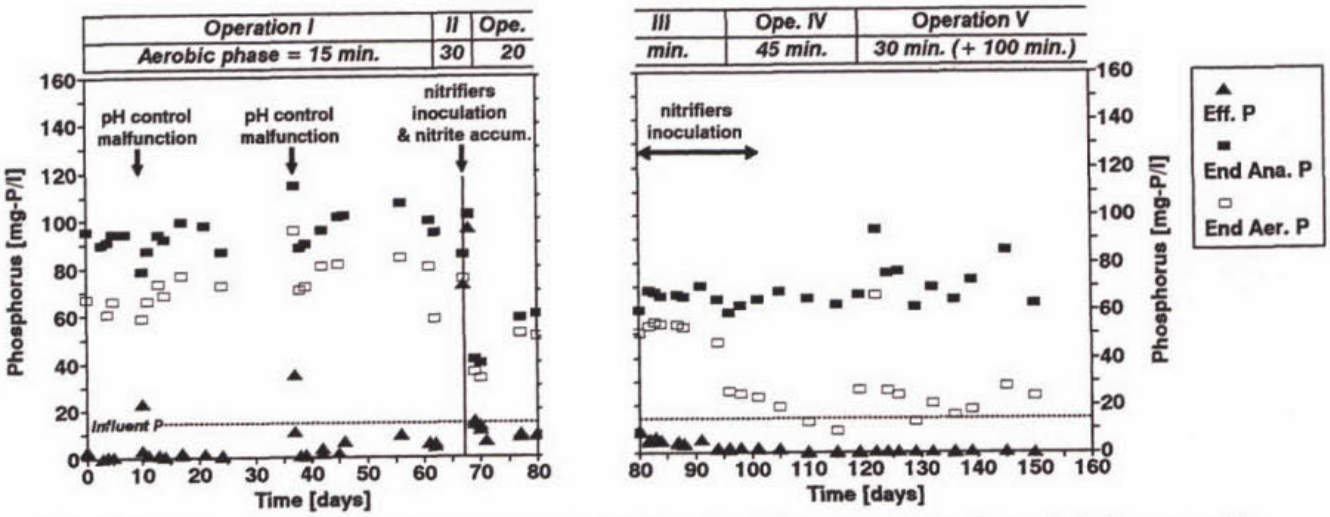

Fig. 22 Daily variation of phosphorus concentrations in effluent (▲) and at the end of the anaerobic (■) and aerobic phase (□).

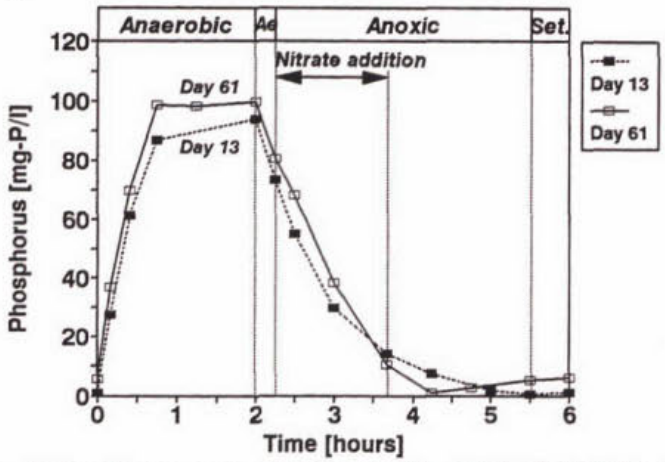

Fig. 23 Cycle behaviour of phosphorus concentrations in the AOA SBR at day 13 and 61 (Operation I)

The amount of aerobic phosphorus uptake increased proportional to the aerobic time applied (Fig. 22). However, in a period of at least 5 months after the introduction of the aerobic phase,

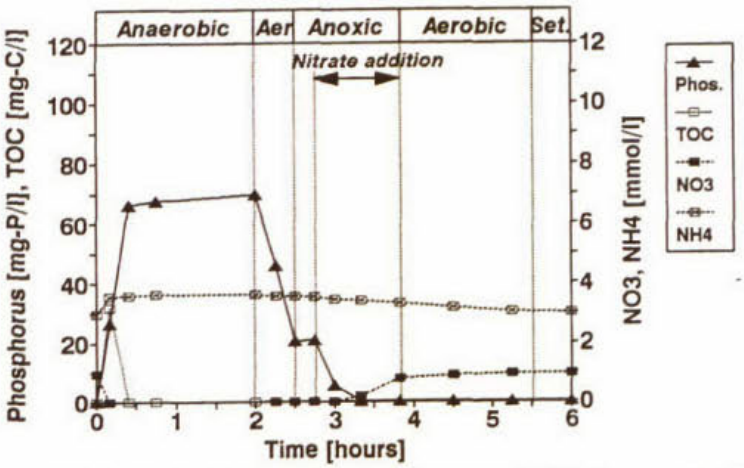

Fig. 24 Cycle behaviour of phosphorus, TOC (HAc), nitrate and NH_4^+ concentrations in the AOA SBR at day 132 (Operation V).

high anoxic phosphorus uptake activities were maintained in the SBR, see Fig. 24. Apparently oxygen has no detrimental effect on the denitrifying dephosphatation activity by DPB.

Phosphorus uptake activities under anoxic or aerobic conditions in denitrifying dephosphatation sludge. As shown in Fig. 23 and 24, the phosphorus uptake rate under aerobic conditions was virtually equal to that under denitrifying conditions. This is also indicated by maximum phosphorus uptake rate measurements, see Fig. 25. There were no differences between the maximum phosphorus uptake rates with oxygen and nitrate as an electron acceptor. Despite the long term enrichment under A2 conditions without oxygen, the DPB sludge showed maximum aerobic phosphorus uptake activity immediately after the introduction of an aerobic phase. It also shows that the phosphorus uptake rates are similar under both redox conditions.

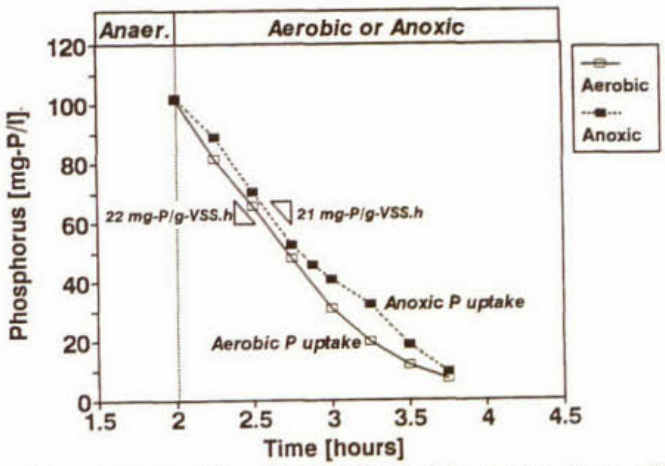

Fig. 25 The maximum phosphorus uptake under full aerobic or anoxic conditions (day 45 and 46).

Cycle behaviour of intracellular PHB content. In the AOA SBR, the short aerobic phase led not only to large quantities of aerobic phosphorus uptake but also to significant aerobic PHB (poly-β-hydroxybutyrate) oxidation (Fig. 26). This aerobic PHB oxidation means that COD is not utilized efficiently for nitrogen removal, because the oxidised PHB (COD) cannot be used anymore for denitrification.

Nitrification activities in the mixed sludge of nitrifiers and DPB. To obtain some nitrification, nitrifiers were inoculated into the SBR at day 67 and continuously from day 80 to 103. Immediately after the inoculation at day 67, large amounts of nitrite (approximately 1 mmol/*l*) accumulated and

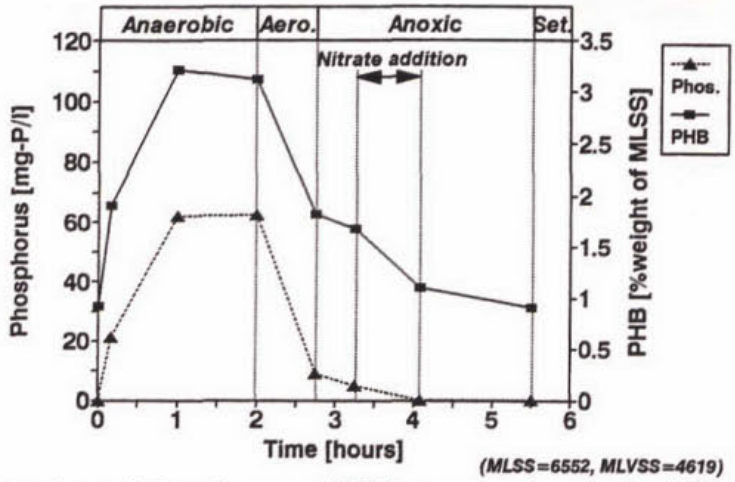

Fig. 26 Cycle behaviour of phosphorus and PHB concentrations in the AOA SBR at day 115 (Operation IV).

a strong inhibition of the phosphorus uptake activities occurred. This points to a strong nitrite inhibition of phosphorus removing bacteria. Whether this can be overcome by adaptation is not known.

Even when the aerobic-SRT was increased to approximately 7 days (Operation V) by the introduction of extra aerobic phase (100 minutes) after the anoxic phase, nitrification activities were still very low, as shown in Fig. 24. Nitrifiers did not accumulate under the 7 days aerobic SRT over a 100 days operation (until day 220 in the experiment). This indicates that a very long aerobic SRT is required for growth of nitrifiers, although it is uncertain whether this is due to an intrinsic problem or due to the experimental conditions.

After the nitrite inhibition accident the anaerobic phosphorus release changed from 95 mg-P/*l* (before day 67) to 65 mg-P/*l* (after day 67). Clearly the phosphorus/HAc-ratio decreased 30%. The reasons for this are presently not known. There could be a sudden accumulation of e.g. "G-bacteria" (Cech and Hartman, 1993) in the sludge. A second possible reason might be carry-over of nitrate into the anaerobic phase. From day 110 nitrate was not completely removed from the liquid (see Fig. 24). This resulted in nitrate introduction into the anaerobic phase. Approximately 25 mg-N nitrate was introduced, and according to "nitrate-driven HAc oxidation" by DPB in previous research (Kuba *et al.*, 1994e; see Chapter 3.2), this can account for 45% less phosphorus release. According to ordinary denitrification with HAc and nitrate, the transferred nitrate can account for 15% less phosphorus release. The observed decrease in phosphorus release (30%) was within this range.

Requirements and problems in single-sludge systems. Ideal conditions for combined phosphorus and nitrogen removal by DPB and nitrifiers in single-sludge activated sludge systems are summarized as follows;

[1]Anaerobic phase -no COD carry-over to the aerobic/anoxic phases
-no nitrate or oxygen input from the anoxic or aerobic phase
[2]Aerobic phase -maximal nitrate production by nitrifiers
-minimal phosphorus uptake, which means minimal PHB oxidation inside DPB
[3]Anoxic phase -complete phosphorus uptake and nitrate utilization
-optimal NH_4^+ utilization by the growth of DPB

These situations can promote maximum selection for the DPB, because the growth of the other bacterial groups such as heterotrophic bacteria and non-polyphosphate accumulating denitrifiers, are prevented.

In single-sludge systems long aerobic retention times are needed in order to allow growth of nitrifiers. Especially in post-denitrification systems (Fig. 27(a)), the long aerobic phase for

nitrifiers conflicts clearly with denitrifying dephosphatation, because PHB (COD) which is present in high concentrations after the anaerobic phase, are aerobically oxidised at a high rate (Fig. 26). This aerobic PHB oxidation means that COD is not utilized efficiently for nitrogen removal by denitrification. In many systems long aerobic periods also lead to a reduction of the denitrification activity due to a suppression of the enzyme (nitrate-reductase) production system. The negative effect of aerobic PHB consumption and the subsequent lower PHB concentrations on the rate of post-denitrification phosphorus removal and denitrification is further enhanced by the observed first order rate kinetics of PHB utilization (Smolders *et al.*, 1995a, b).

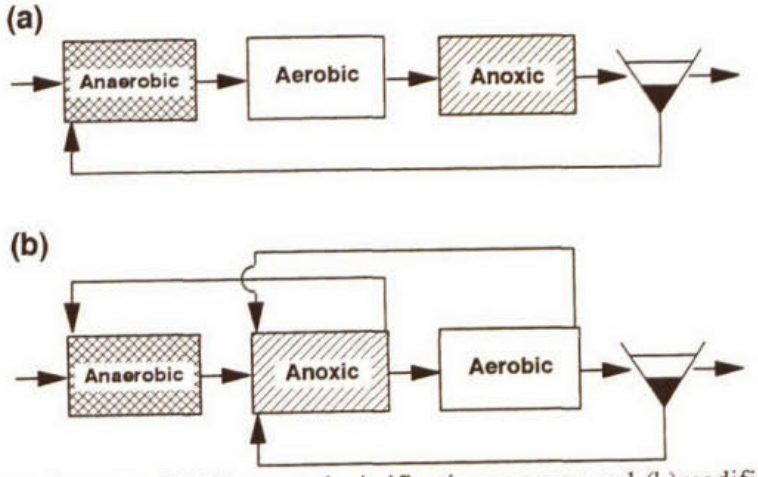

Fig. 27 Schematic diagram of (a)the post-denitrification process and (b)modified UCT-type (pre-denitrification) process.

Pre-denitrification, e.g. UCT-type of process (see Fig. 27(b)), seems to have a better potential for simultaneous phosphorus and nitrogen removal by mixed sludge of DPB and nitrifiers. The sludge entering the aerobic phase has minimal PHB content because most PHB has been used for denitrification and phosphorus accumulation, and the liquid contains minimal phosphorus. This favours the prime use of COD for denitrifying dephosphatation and minimises the growth of competing heterotrophic bacteria and aerobic PHB oxidation.

Observations on a waste water treatment plant in the Netherlands showed indeed that in the modified UCT-type configuration (Fig. 27(b)), significant amounts of DPB can be accumulated. In the waste water treatment plant, 70～80% of phosphorus is removed in the anoxic zone, and the anoxic phosphorus uptake rate is 50～60% of the aerobic rate (see Chapter 4.2). Based on this experience and the results in this chapter it can be stated that for single-sludge systems, the modified UCT-type process operation (pre-denitrification) is advised for making optimal use of DPB.

However, even in the pre-denitrification processes, a long aerobic phase will be required for nitrification. This inevitably leads to aerobic COD usage and possible reduced denitrification activities. It will therefore be more optimal to separate the nitrification from the total sludge, i.e., two-sludge systems. The two-sludge system can result in significant decrease of COD and energy use for phosphorus and nitrogen removal (Kuba *et al.*, 1996a; see Chapter 5.1).

From the research of the effect of cyclic oxygen exposure on the activity of DPB, it can be concluded that:

(1)Oxygen has no direct detrimental effect on denitrifying dephosphatation activities,
(2)Phosphorus uptake activities under anoxic and aerobic conditions can be identical,
(3)Nitrifiers in the mixed sludge system require a long aerobic period, which is unfavourable for denitrifying dephosphatation because of the aerobic PHB (COD) oxidation by phosphorus removing organisms,
(4)For single-sludge systems, modified UCT-type processes (pre-denitrification) are recommended for efficient phosphorus and nitrogen removal using denitrifying dephosphatation.

4.2 Occurrence of denitrifying phosphorus removing bacteria in a pre-denitrification configuration in waste water treatment plants*

Abstract ——— Several batch tests were conducted with activated sludge from the waste water treatment plants in Genemuiden and Holten in order to investigate contribution of denitrifying phosphorus removing bacteria (DPB) for phosphorus removal. Both treatment plants are operated as single-sludge systems and have similar modified UCT-type processes. In both treatment plants significant amounts of phosphorus are removed under denitrifying conditions. Clearly denitrifying dephosphatation occurs in the actual waste water treatment plants. The batch tests showed a clear difference of proportion of DPB between the Genemuiden and Holten sludge. In Genemuiden, DPB are accumulating slowly, but still DPB form a minor part in the sludge. On the other hand, significant amounts of DPB are accumulated in the Holten sludge. The following possible reasons for lower proportion of DPB in the Genemuiden sludge in comparison with the Holten sludge, were suggested; (a)nitrate/oxygen transfer into the anaerobic/anoxic zones due to the internal recirculation inside the treatment plant, and (b)less fatty acids in the influent due to shorter retention time of the waste water in sewer lines.

4.2.1 Introduction

In single-sludge systems, DPB undergo temporarily aerobic conditions (Chapter 4.1). This leads to aerobic intracellular PHB (poly-β-hydroxybutyrate) oxidation by phosphorus removing organisms, especially in post-denitrification processes (Fig. 27(a)). This aerobic PHB (COD) oxidation means that COD is not utilized efficiently for simultaneous phosphorus and nitrogen removal, because the oxidised COD cannot be utilized anymore for denitrification. Therefore, to enhance denitrifying dephosphatation in the single-sludge systems, the aerobic phase should be preceded by the anoxic phase, i.e., pre-denitrification processes (Fig. 27(b)). Waste water treatment plants in Genemuiden and Holten, the Netherlands, have UCT-type configurations (pre-denitrification processes). Observations on the treatment plants have shown that phosphorus uptake occurs under denitrifying conditions.

In this chapter, several types of batch tests were conducted by using the activated sludge from both treatment plants, in order to examine the occurrence of DPB in waste water treatment plants and to investigate the contribution of DPB for the phosphorus removal.

4.2.2 Experiments

Configurations of the waste water treatment plants. In order to examine activities of phosphorus removing organisms in single-sludge systems, several types of batch tests were conducted using the sludge from the waste water treatment plant in Genemuiden and Holten. The batch tests with the Genemuiden sludge were conducted twice; April and July, 1994. The batch tests with the Holten sludge were conducted in July, 1994. In the Holten sludge it was already confirmed in December, 1992, that significant amounts of DPB are accumulated (G.J.F. Smolders *et al.*, TU Delft, unpublished). Both waste water treatment plants have similar modified UCT-type (pre-denitrification) processes (Fig. 27(b)).

The configuration of the waste water treatment plant in Genemuiden is shown in Fig. 28. The treatment plant consists of an anaerobic, an anoxic and an aerobic zone, and (two) settlers. The mixed liquor at the end of the anoxic zone is recirculated to the beginning of the anaerobic zone,

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*: *This research was performed in cooperation with ing. F.A. Brandse from the waterboard Z.W.O.*

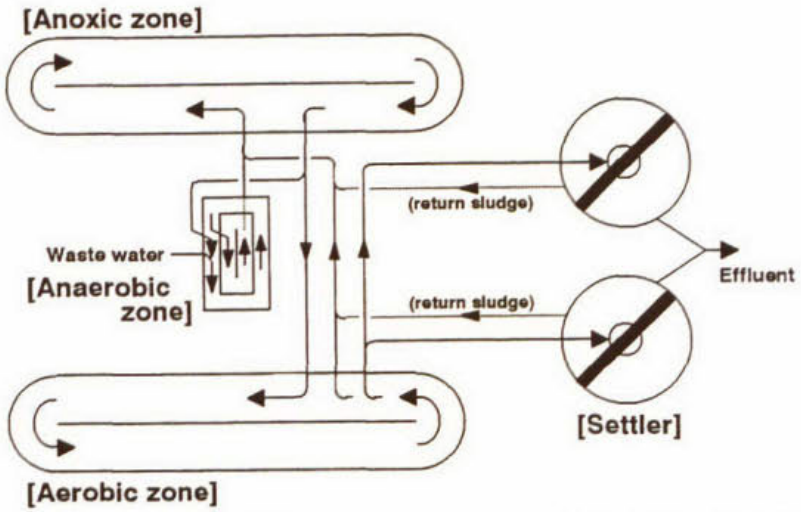

Fig. 28 Configurations of the waste water treatment plant in Genemuiden.

leading to a significant accumulation of DPB in the system. The waste water treatment plant in Holten has similar configurations.

The operations of the waste water treatment plant in Genemuiden were started in December, 1993. The observations on both treatment plants have shown phosphorus removal under denitrifying conditions. In Holten, approximately 70 ~ 80% of phosphorus is removed in the anoxic zone (under dry weather conditions).

Experimental procedure. The return sludge from the settlers in the treatment plants was taken as the original sludge for the batch tests. The original sludge was diluted approximately three times in mineral medium; 0.6 g/*l* $MgSO_4 \cdot 7H_2O$, 0.07 g/*l* $CaCl_2 \cdot 2H_2O$, 0.1 g/*l* NH_4Cl, 0.1 g/*l* KCl, 2 ml/*l* trace mineral solution (see Chapter 2). All batch tests were conducted in a 1*l* or 2*l* laboratory fermenter at 25 °C, using the diluted sludge after the pre-treatment. pH was controlled at 7.0 ~ 7.1 by addition of 0.5N HCl or NaOH.

By using the sludge from the waste water treatment plant in Genemuiden and Holten, the following batch tests were conducted;

(1)*The maximum phosphorus release test*: Excess HAc (acetic acid) was added to the sludge under anaerobic conditions. Phosphorus and HAc concentrations were measured to determine the maximum released phosphorus concentration,

(2)*Aerobic phosphorus uptake rate*: After HAc addition, the sludge was cultivated under anaerobic conditions. The anaerobic sludge was divided into two parts, and one part of the sludge was exposed under aerobic conditions to measure the maximum aerobic phosphorus uptake rates,

(3)*Anoxic phosphorus uptake rate*: Another part of the anaerobic sludge was exposed under anoxic conditions to determine the maximum anoxic phosphorus uptake rates. The phosphorus uptake rates under anoxic conditions were compared with the aerobic conditions, and the proportion of DPB in the sludge was calculated from the comparison (Fig. 12 in Chapter 3.2; see also Chapter 3.5.4 "*Modelling of denitrifying dephosphatation*"),

(4)*Phosphorus release with simultaneous presence of HAc and nitrate*: HAc and nitrate were added to the sludge to examine the effect of nitrate for the phosphorus release (Chapter 3.2). And also ordinary denitrification rates were measured.

4.2.3 Results and discussion

The kinetics and stoichiometry in the batch tests are summarised in Table 10. Table 11

shows kinetics and stoichiometry which have been obtained in a lab-scale anaerobic-aerobic (A/O) and anaerobic-anoxic (A2) SBR in our laboratory (van Loosdrecht *et al.*, 1992; van der Velde, 1992; Kuba *et al.*, 1992, 1993a). These were compared with the results obtained in the batch tests.

The maximum phosphorus release test. Excess HAc was added to the sludge under anaerobic conditions to examine the maximum released phosphorus concentration and the maximum phosphorus release rate.

Table 10 Summary of kinetics and stoichiometry in the batch tests with the activated sludge from waste water treatment plants in Genemuiden (April and July, 1994) and Holten (July, 1994).

		Genemuiden		Holten
		April, 1994	July, 1994	July, 1994
Max. released P concentration [mg-P/g-VSS]		5~7	14~16	30
Max. P release rate [mg-P/g-VSS.h]		7.2	8~9	19
Secondary P release rate [mg-P/g-VSS.h]		0.2	0.0	0.1
P release rate with HAc+NO_3^- [mg-P/g-VSS.h]		3.9	6.8	17
Aerobic P uptake rate [mg-P/g-VSS.h]		5.7	3.8	13
Anoxic P uptake rate [mg-P/g-VSS.h]		1.2	1.6	6
Proportion of P removing organisms [% of MLVSS]		10~20	10~20	40
Proportion of DPB [% of P removing organisms]		20	40	45
Released-P/utilized-HAc ratio [g-P/g-C]	Anaerobic	2.1~2.5	1.2~1.4	1.9~2.3
	Anoxic*	0.2	0.3	0.6
Removed-P/utilized-NO_3^- [mol-P/mol-e^-]		0.04	0.10	0.18
		(0.02)*	(not measure)*	(0.16)*
MLVSS/MLSS ratio [%]		66~69	64~67	72~73

*: P/C ratios during HAc was present or P/N ratios after HAc was gone, in the batch tests with simultaneous presence of HAc and nitrate.

The maximum released phosphorus concentration in the Genemuiden sludge was 5~7 mg-P/g-VSS in the first batch tests and 14~16 mg-P/g-VSS in the second batch tests. The batch test suggested the polyphosphate content was only 1~4% of MLSS. This is not in accordance with low MLVSS/MLSS ratio (31~36% of MLSS was ash content) that are comparable to the enriched sludge in the lab-scale SBRs (Table 11). Although the maximum phosphorus release might be limited by intracellular glycogen contents (Chapter 3.3), this indicates that a lot of inorganic substances except polyphosphate, such as sands or precipitates, might be present in the mixed liquor.

The comparison of the maximum specific phosphorus release rates between the Genemuiden sludge and the enriched SBR sludge showed that the proportion of phosphorus removing organisms was approximately 10~20 % of the total sludge (MLVSS) in April and July.

Table 11 Summary of stoichiometry and kinetics in the lab-scale anaerobic-aerobic (A/O) and anaerobic-anoxic (A2) SBRs.

	A/O SBR	A2 SBR
Max. released P concentration [mg-P/g-VSS]	100	90
Max. P release rate [mg-P/g-VSS.h]	50~70	30~50
Secondary P release rate [mg-P/g-VSS.h]	—	2~4
Aerobic P uptake rate [mg-P/g-VSS.h]	30~50	40~50
Anoxic P uptake rate [mg-P/g-VSS.h]	—	30~50
Released-P/utilized-HAc ratio [g-P/g-C]	1.1~1.3	1.0~1.5
Removed-P/utilized-NO_3^- [mol-P/mol-e^-]	—	0.19
Removed-P/utilized-O_2 [mol-P/mol-e^-]	0.23	—
MLVSS/MLSS ratio [%]	65~70	65~70

In the Holten sludge, The maximum released phosphorus concentration was much higher than the Genemuiden sludge (14~16 mg-P/g-VSS), and this was comparable to another test with the Holten sludge in December, 1992 (G.J.F. Smolders *et al.*, TU Delft, unpublished). The batch tests suggested the polyphosphate content was 7~8% of MLSS. This Holten sludge also showed lower MLVSS/MLSS ratios (27~28% of MLSS was ash content).

The comparison of the maximum specific phosphorus release rates between the Holten sludge and the enriched SBR sludge (Table 11) showed that the proportion of P removing organisms was approximately 40~50% of the total sludge (MLVSS).

Aerobic and anoxic phosphorus uptake test. After the anaerobic cultivation with HAc, the sludge was divided into two parts, then each sludge was exposed under aerobic or anoxic conditions (see Fig. 29(a), (b)).

The comparison of the specific phosphorus uptake rates between the Genemuiden sludge and the enriched SBR sludge (Table 11) showed that approximately 10~20 % of the total sludge (MLVSS) was P removing organisms, which coincides with the above-mentioned proportion of the phosphorus removing organisms estimated from the comparison of the maximum specific phosphorus release rates.

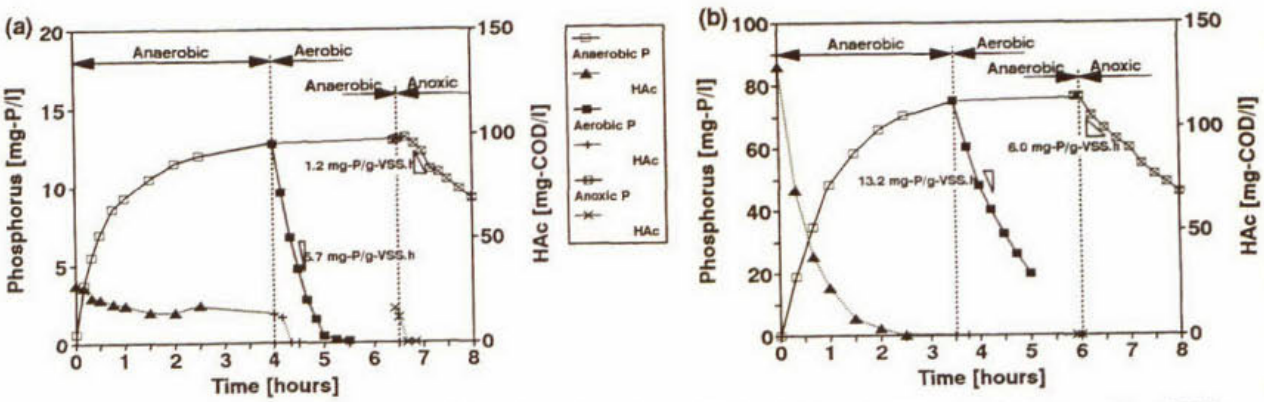

Fig. 29 Phosphorus uptake under A/O and A2 conditions in the (a)Genemuiden (April, 1994) and (b)Holten sludge.

Immediately after the sludge was exposed under the aerobic or anoxic conditions, HAc (5 ~ 10 mg-COD/l, "+" or "×" in Fig. 29(a)) which remained at the end of the anaerobic phase, was oxidised rapidly and disappeared soon. As shown in Fig. 29, clearly denitrifying dephosphatation was observed in the sludge from the actual waste water treatment plants. From the comparison of the phosphorus uptake rates, after HAc was gone, between aerobic and anoxic conditions, the proportion of DPB was calculated to be approximately 20% of the phosphorus removing organisms in April and 40 % in July. This indicates that approximately 2 ~ 8 % of the total sludge (MLVSS) was DPB. It seems that the proportion of DPB in Genemuiden sludge was slightly increased during the start-up of the waste water treatment plant. (The operation of the treatment plant in Genemuiden was started in December, 1993.)

The ratio of removed phosphorus to utilized nitrate (P/N ratio) in the Genemuiden sludge was 0.4 ~ 1.0 g-P/g-N (0.04 ~ 0.1 mol-P/mol-e^-), while it was 2.1 g-P/g-N (0.19 mol-P/mol-e^-, Table 10) in the enriched A2 SBR sludge. The P/N ratio in July was twice higher than the ratio in April, but it was still lower than the ratio in the enriched A2 SBR sludge. This might be due to utilization of nitrate by non-polyphosphate accumulating denitrifiers if they have intracellular carbon storage like PHB (Alleman and Irvine, 1980), or use external (slowly degradable) COD. In this waste water treatment plant, the proportion of non-polyphosphate accumulating denitrifiers might be high, because of nitrate transfer into the anaerobic zone (see below).

In the Holten sludge, the comparison of the specific phosphorus uptake with the enriched SBR sludge showed that approximately 40% of the total sludge (MLVSS) was phosphorus removing organisms, which coincides with the above-mentioned proportion estimated from the comparison of the maximum specific phosphorus release rates.

HAc (▲ in Fig. 29(b)) was completely removed under anaerobic conditions. From the comparison of the phosphorus uptake rates between aerobic and anoxic conditions, the proportion of DPB was estimated to be approximately 40 ~ 50% of the phosphorus removing organisms. This indicates that approximately 20% of the total sludge (MLVSS) was DPB. The proportion was comparable to another test in December, 1992. The proportion of DPB in the Holten sludge was much higher than the Genemuiden sludge.

The ratio of removed phosphorus to utilized nitrate (P/N ratio) in the Holten sludge was 2.0 g-P/g-N (0.18 mol-P/mol-e^-), which was similar to the ratio in the enriched A2 SBR sludge (0.19 mol-P/mol-e^-, Table 11). This indicates that non-polyphosphate denitrifiers form only a marginal fraction of the sludge.

Phosphorus release test with HAc and NO_3^-. In the batch tests, phosphorus release rate was measured in the simultaneous presence of HAc and nitrate.

By addition of nitrate, the HAc consumption was accelerated and the phosphorus release was blocked due to HAc utilization for ordinary denitrification by non-polyphosphate accumulating denitrifiers and/or "nitrate-driven HAc oxidation" by DPB (Kuba *et al.*, 1994e; Chapter 3.2). This leads to lower P/C ratio than the anaerobic conditions (Table 10). It seems that the P/C ratio in the Genemuiden sludge was strongly influenced by nitrate addition, and this might suggest the presence of large quantity of non-polyphosphate accumulating denitrifiers in the sludge.

After HAc was gone, denitrifying phosphorus uptake was observed in the first batch test. The anoxic P-uptake rate was lower than the rate in the "anoxic phosphorus uptake rate" test, because HAc was directly oxidised by non-polyphosphate accumulating denitrifiers and/or DPB, which leads to less PHB production inside the DPB. Also the P/N ratio was lower than the "anoxic phosphorus uptake rate" test. This might be due to that non-polyphosphate accumulating denitrifiers produce PHB in the presence of both HAc and nitrate, and after HAc is gone they utilize nitrate in spite of lack of the extracellular carbon source.

Also in the Holten sludge, by addition of nitrate, the HAc consumption was accelerated and the phosphorus release was slightly blocked due to HAc utilization for "nitrate-driven HAc

oxidation" by DPB. This leads to lower P/C ratio than the anaerobic conditions (Table 10).

After HAc was gone but nitrate was still present, denitrifying phosphorus uptake activities were observed in the batch test. The anoxic phosphorus uptake rate was slight lower than the rate in the "anoxic phosphorus uptake rate" test, because less HAc was converted to PHB inside the DPB. If non-polyphosphate accumulating denitrifiers produce PHB in the presence of both HAc and nitrate, and after HAc is gone they utilize nitrate in spite of lack of the extracellular carbon source, the P/N ratio should be lower than the "anoxic phosphorus uptake rate" test. In fact, if non-polyphosphate accumulating denitrifiers would be expected to be present in significant amounts in the Genemuiden sludge, the P/N ratio would be lower than in the "anoxic phosphorus uptake rate" test. However, the P/N ratio was almost similar to the "anoxic phosphorus uptake rate" test with the Holten sludge, which indicates also that non-polyphosphate accumulating denitrifiers are not present in high amounts in the Holten sludge.

Phosphorus uptake activities under denitrifying conditions in single-sludge systems. Several types of batch test were conducted using the activated sludge from the waste water treatment plants in Genemuiden and Holten, which are single-sludge systems and which have modified UCT-type (pre-denitrification) configurations. The results of the batch tests with the Genemuiden sludge indicated that DPB are accumulating slowly, but still DPB form a minor part in the sludge. On the other hand, the batch tests with the Holten sludge showed that significant amounts of DPB are accumulated in the sludge. Both waste water treatment plants have the similar modified UCT-type (pre-denitrification) configurations, but the proportion and activities of phosphorus removing organisms are obviously different.

Lower enrichment of DPB in the Genemuiden sludge in comparison with the Holten sludge, might result from (ii)nitrate/oxygen transfer into the anaerobic/anoxic zones due to the internal recirculation inside the treatment plant, and (i)shorter retention time of waste water in sewer lines.

(i)*Nitrate/oxygen transfer*: In the waste water treatment plant in Holten, the control is optimised on the prevention of nitrate introduction to the anaerobic zone, and the anoxic zone is never aerated. Also the treatment plant is operated as a plug-flow type process. The waste water treatment plant in Genemuiden is controlled on nitrification primarily. Also the treatment is operated as a completely mixed-type process. These lead to higher oxygen inputs in the anoxic zone and higher nitrate inputs in the anaerobic zone.

In Holten, the introduction of nitrate into the anaerobic zone is controlled strictly by redox sensors. The result of the batch tests with the Genemuiden sludge, i.e., the comparison of P/N ratios between anoxic phosphorus uptake after HAc consumption without nitrate ("anoxic phosphorus uptake rate" test) and with nitrate ("phosphorus release with simultaneous of HAc and nitrate" test), suggests that non-polyphosphate accumulating denitrifiers are present in high concentrations. While the P/N ratios are almost similar in the Holten sludge, and this suggests that non-polyphosphate accumulating denitrifiers are not present in high amounts in the Holten sludge, see Table 12. The difference in control strategy clearly has an effect on the sludge composition. Therefore control procedures and process design should be evaluated both when optimal enrichment for DPB is desired.

Table 12 Rough estimation of biomass proportion in the Genemuiden and Holten sludge.

	Obligate aerobic phosphorus removing bacteria	DPB	non-polyphosphate accumulating denitrifiers
Genemuiden sludge	6~12% of MLVSS	4~8%	4~8%
Holten sludge	14~25% of MLVSS	16~25%	≒1%

(ii)*Retention time of waste water in sewer lines*: The retention time of waste water in the sewer line in Holten is approximately 1 day. Therefore the waste water is digested very well in the sewer line, and significant amounts of fatty acids (especially HAc) which are favourable organic carbon sources for phosphorus removing organisms, are produced before the waste water comes into the treatment plant. The retention time in the sewer line to Genemuiden is approximately 8 hours, and the waste water contains probably a lower concentration of fatty acids.

The following conclusions have been drawn from the activity tests with the sludge from the actual waste water treatment plant as single-sludge systems:

(1)Denitrifying dephosphatation occurs in actual waste water treatment plants,

(2)Although the waste water treatment plants in Genemuiden and Holten have similar UCT-type configurations, the proportion of DPB in the Holten sludge was much higher than in the Genemuiden sludge. A suggested reason for the lower proportion of DPB in the Genemuiden sludge is nitrate/oxygen transfer into the anaerobic/anoxic zones due to the internal recirculation, which leads to competition between DPB and heterotrophic bacteria like non-polyphosphate accumulating denitrifiers. Another suggested reason is short retention time of waste water in the sewer line, which results in lower production of fatty acids as favourable organic carbon sources for phosphorus removing organisms, before the waste water comes into the waste water treatment plant.

4.3 Phosphorus and nitrogen removal with and without presettling of sewage in a waste water treatment plant*

Abstract ——— A modified UCT-type waste water treatment plant where denitrifying phosphorus removing bacteria (DPB) are present, was operated without presettling of sewage for 2 months, in order to evaluate the effect of suspended COD on activities of phosphorus or nitrogen removing organisms. Before and after this alteration of the treatment plant, several kinds of identical batch tests were conducted using the sludge from the treatment plant to quantify the fraction of DPB. After the alteration, the proportion of phosphorus removing organisms in the total sludge decreased to half, but since the production of phosphorus removing organisms (per day) was still the same as before, phosphorus was still removed completely. When the presettling was stopped, the suspended COD in the raw sewage, which might be not available for phosphorus removing organisms in the anaerobic zone, is transferred into the anoxic and aerobic zones, and is available for heterotrophic bacteria in these zones. The batch tests indicated that the sludge production of non-polyphosphate accumulating denitrifiers increased after the introduction of non-presettled sewage.

4.3.1 Introduction

The waste water treatment plant in Holten has a modified UCT-type configuration as a single-sludge system for (denitrifying) dephosphatation and nitrification. Several types of batch tests with sludge from the treatment plant were done in July, 1994, in order to examine the contribution of DPB for the phosphorus removal in the treatment plant (see Chapter 4.2).

A problem in the treatment plant in Holten is insufficient nitrogen removal, probably due to shortage of COD. The previous batch tests using the sludge showed that 50% of phosphorus removing organisms were DPB. In the treatment plant, approximately 70 ~ 80% of phosphorus is removed in the anoxic zone. The modified UCT-type configuration clearly leads to significant accumulation of DPB in the plant, thus, efficient utilization of COD for phosphorus and nitrogen removal is achieved due to denitrifying dephosphatation. However, ammonium- and nitrate-nitrogen are still detected in effluent, probably because of a low COD/N ratio in sewage, even when COD is maximally utilized by denitrifying dephosphatation. To improve nitrogen removal efficiency, the configuration was slightly changed in the treatment plant at the end of October, 1994. Non-presettled sewage was directly introduced into the anaerobic zone, without passing through primary settlers. This led to a lower sludge retention time (SRT), because the biomass concentration was held constant. The SRT was reduced from approximately 45 to 17 days.

Two months after the start of the non-presettled sewage introduction, batch tests using the Holten sludge were conducted in December, 1994. In this chapter, from the comparison of the activities of the sludge in the waste water treatment plant with and without the presettling of sewage, the effect of the suspended COD on phosphorus and nitrogen removal is discussed.

4.3.2 Experiments

Experimental procedure. See Chapter 4.2.

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*: *This research was performed in cooperation with ing. F.A. Brandse from the waterboard Z.W.O.*

4.3.3 Results and discussion

The results of the batch tests in July (Chapter 4.2) and December, 1994, are summarised in Table 12. The kinetics and stoichiometry obtained previously using enriched phosphorus removing organisms (with acetic acid) in the lab-scale SBRs are summarised in Table 11 in Chapter 4.2. The kinetics and stoichiometry are compared with results of the batch tests with the Holten sludge, in the following discussion.

Table 12 Summary of characteristics of the sludge in the waste water treatment plant Holten with and without the presettling of sewage.

	With presettling (July, 1994)*1	Without presettling (December, 1994)
Max. released P concentration [mg-P/g-VSS]	30	14
Max. P release rate [mg-P/g-VSS.h]	19	9
Aerobic P uptake rate [mg-P/g-VSS.h]	13.2	5.4*2
Anoxic P uptake rate [mg-P/g-VSS.h]	5.9	2.3*2
Proportion of P removing organisms	40% of MLVSS	15% of MLVSS
Proportion of DPB	45% of P removing organisms	40% of P removing organisms
Denitrification rate with P uptake*3 [mmol-NO_3^-/g-VSS.h]	0.22~0.24	0.25~0.27*4
Denitrification rate*5 [mmol-NO_3^-/g-VSS.h]	0.44	0.44~0.50
Nitrification rate [mmol-NH_4^+/g-VSS.h]	——	0.2~0.3
MLVSS/MLSS ratio [%]	72~73	73~77

*1: Chapter IV-2.
*2: At the beginning of the batch tests, much HAc (acetic acid) remained.
*3: Endogenous denitrification rate with PHB by DPB.
*4: This is an over-estimated value, because much HAc was consumed with nitrate before denitrifying dephosphatation, which might lead to PHB production from HAc (Alleman and Irvine, 1980), and lead to nitrate consumption by non-polyphosphate accumulating denitrifiers after HAc was gone.
*5: Denitrification rate in the simultaneous presence of HAc and nitrate ("phosphorus release test with HAc and nitrate").

The maximum phosphorus release test. Excess HAc was added to the sludge under anaerobic conditions, to examine the maximum released phosphorus concentration and the maximum phosphorus release rate.

The maximum released phosphorus concentration was 14 mg-P/g-VSS after the introduction of non-presettled sewage into the treatment plant. Before the alteration the same batch tests (in July, 1994) showed that it was 30 mg-P/g-VSS. Clearly the proportion of phosphorus removing organisms in MLVSS decreased after the alteration. The maximum specific phosphorus release rate decreased from 19 to 9 mg-P/g-VSS.h. The rate in the enriched SBR sludge is 40~60 mg-P/g-VSS.h (Table 11 in Chapter 4.2). The comparison between these two rates shows that the proportion of phosphorus removing organisms were approximately 15~20% of the total sludge. The proportion was approximately 40% in July, therefore it decreased to half after the alteration.

Aerobic and anoxic phosphorus uptake rate test. After anaerobic cultivation with HAc, the anaerobic sludge was exposed under aerobic or anoxic conditions, to measure the maximum aerobic and anoxic phosphorus uptake rate. (Much HAc was present at the beginning of the batch tests which might disturb phosphorus uptake under aerobic/anoxic conditions (Chapter 3.2). Thus, the obtained aerobic/anoxic phosphorus uptake rates after HAc was gone, might be lower than real values.)

From the comparison of the phosphorus uptake rates between aerobic and anoxic conditions (after HAc was gone), the proportion of DPB after the introduction of non-presettled sewage was estimated to be approximately 40% of the phosphorus removing organisms (Chapter 3.2; see also Chapter 3.5.4 "*modelling of denitrifying dephosphatation*"). This indicated that approximately 10% of MLVSS was DPB. Before the alteration, the proportion of DPB was 40 ~ 50% of the phosphorus removing organisms and 20 ~ 30% of MLVSS. Although the proportion of phosphorus removing organisms in MLVSS decreased after the alteration, the proportion of DPB in the phosphorus removing organisms was still similar.

Phosphorus release test with HAc and nitrate. There are two purposes in the batch test. One purpose is to see the effect of nitrate on phosphorus release by phosphorus removing organisms, and another is to examine ordinary denitrification rates in the simultaneous presence of HAc and nitrate.

By addition of nitrate, HAc consumption was strongly accelerated, due to denitrification (from 13 to 40 mg-COD/g-VSS.h). As long as HAc was present, phosphorus was released, but the phosphorus release rate under anoxic conditions (1.5 mg-P/g-VSS.h) was much lower than anaerobic conditions (9 mg-P/g-VSS.h). The same inhibition of phosphorus release by nitrate was often observed in the activated sludge (Comeau *et al.*, 1990; Jenkins and Tandoi, 1991; Appeldoorn, 1993), and also in the enriched anaerobic-aerobic or anaerobic-anoxic SBR sludge (Chapter 3.2). The batch test indicates that the "anaerobic" phosphorus release metabolism on phosphorus removing organisms is blocked in the simultaneous presence of electron donors (COD) and acceptors (nitrate or oxygen), which probably leads to disturbances also for the phosphorus uptake metabolism.

The ordinary denitrification rate in the presence of HAc and nitrate was 0.44 ~ 0.50 mmol-NO_3^-/g-VSS.h.

Phosphorus and nitrogen removal in the waste water treatment plant. In the waste water treatment plant without the presettling (at the end of October), still almost all phosphorus was removed from the sewage, and still much phosphorus was removed by DPB in the anoxic zone. As it was expected, nitrate removal efficiency was improved. However, nitrification activities decreased and NH_4^+ was detected in effluent, probably due to low SRT and low temperature. Clearly this shows that a long aerobic retention time is required due to lower nitrification activity in the mixed sludge of the single sludge systems. Therefore, the configurations were returned to the previous conditions and the presettling of sewage was started again at the beginning of December, 1994.

Dephosphatation and denitrification activities of the Holten sludge. The comparison between the aerobic and anoxic phosphorus uptake rates showed that 40% of phosphorus removing organisms were DPB after the alteration. This was still comparable with the proportion before the alteration. Still DPB contributed to large part of phosphorus removal. The maximum specific phosphorus release activities (based on MLVSS) clearly indicated that the proportion of phosphorus removing organisms in MLVSS decreased to half. This is mainly due to a lower SRT. Probably much of the suspended COD (slowly biodegradable COD) is not available for the anaerobic PHB synthesis by the phosphorus removing organisms.

Specific ordinary denitrification rates were measured in the simultaneous presence of HAc and nitrate. As shown in Table 12, the specific denitrification rate (based on MLVSS) after the alteration was almost similar or slightly higher than the rate before the alteration. On the other

hand, the specific (endogenous) denitrification rate with intracellular PHB by DPB after the alteration was similar or lower (see *4 in Table 12) than the rate before the alteration. This indicated that the direct introduction of non-presettled sewage stimulated ordinary denitrification, but not endogenous denitrification by DPB.

Effect of introduction of non-presettled sewage on phosphorus and nitrogen removal: COD input is increased when the presettling of sewage is stopped, but the extra COD input consists of particulate inorganic and slowly biodegradable substances (suspended COD). Especially in the waste water treatment plant in Holten, the sewage is anaerobically well-digested in the sewer line before it comes into the plant (because of 1 day hydraulic residence time in the sewer line). Therefore easily biodegradable COD and probably a part of slowly biodegradable COD are digested, and the sewage contains a lot of fatty acids, especially HAc as preferable substances for phosphorus removing organisms. Almost all easily biodegradable COD is present as fatty acids, and the remainder of the COD might be only slowly biodegradable COD, which is not available for the phosphorus removing organisms under anaerobic conditions.

Since the sludge concentration in the plant was held constant, SRT decreased approximately from 45 to 17 days. With a decrease of SRT to half, the proportion of phosphorus removing organisms decreased to half. This indicated that the sludge production (per day) of the phosphorus removing organisms before and after the alteration, was still the same, and phosphorus was still removed completely in the treatment plant. As above-mentioned, the suspended COD is not available for the phosphorus removing organisms in the anaerobic zone, therefore the production of phosphorus removing organisms after the alteration is still the same as before.

Although COD input was increased, the sludge production of phosphorus removing organisms was the same and the apparent proportion of phosphorus removing organisms in the total sludge decreased. Since the slowly biodegradable substances are not degraded in the anaerobic zone, they are transferred into the anoxic and aerobic zones. These substances might be available for competing heterotrophic bacteria (against DPB), i.e., for non-polyphosphate accumulating denitrifiers in the anoxic zone and for heterotrophic bacteria in the aerobic zone. Therefore, after the alteration the specific ordinary denitrification activities were still similar, or slight higher than before. The alteration led to increase of the proportion of non-polyphosphate accumulating denitrifiers (and probably heterotrophic bacteria) in the total sludge (MLVSS), but not the phosphorus removing organisms.

After the introduction of raw sewage, SRT decreased. This alteration stimulated the ordinary denitrification, and improved nitrate removal efficiency in the treatment plant. However, the lower SRT is a disadvantage for nitrifiers, and the nitrification can become a limiting step for nitrogen removal.

In order to improve the nitrogen removal efficiency, the presettling of sewage was stopped in the waste water treatment plant in Holten. By using the sludge from the treatment plant with and without the presettling, identical batch tests were conducted, to evaluate the effect of suspended COD on phosphorus and nitrogen removal and on the fraction of DPB. The following conclusions from the observation in the treatment plant and the results of the batch tests, have been drawn:

(1)The proportion of phosphorus removing organisms in MLVSS decreased to half, because the extra COD input consists of particulate inorganic substances and particulate COD which are not available for the organisms. However phosphorus removal was complete, since the sludge production of the phosphorus removing organisms was still the same. The proportion of DPB in the phosphorus removing organisms was identical before and after the alteration,

(2)The particulate COD was transferred into the anoxic and aerobic zones, and it might be available for non-polyphosphate accumulating denitrifiers and heterotrophic bacteria. The batch tests indicated that the specific ordinary denitrification activity (based on MLVSS) was still similar or slight higher than before.

5 TWO-SLUDGE SYSTEMS

5.1 Phosphorus and nitrogen removal in an anaerobic-anoxic SBR with a separated nitrification SBR

Abstract ——— A two-sludge system has been studied, in which DPB (denitrifying phosphorus removing bacteria) and nitrifiers were completely separated in two sequencing batch reactors (SBRs). The technical feasibility for simultaneous phosphorus and nitrogen removal in the proposed two-sludge system was evaluated. Also operational strategies for the proposed two-sludge system and benefits of the systems in comparison with single-sludge systems are discussed. The proposed simple two-sludge system showed very stable phosphorus and nitrogen removal. The average removal efficiency was 98% and 89%, respectively. The two-sludge system was suggested to have higher flexibilities on the operations for simultaneous phosphorus and nitrogen removal than the single-sludge system.

5.1.1 Introduction

In two-sludge systems (see Fig. 3 in Chapter 1, and Fig. 30), nitrifiers are separated from DPB as much as possible, e.g., in a nitrification biofilm reactor (Bortone *et al.*, 1994). In this chapter, a simple two-sludge system was proposed, which consists of two SBRs and one "nitrate exchange vessel". One SBR was operated under anaerobic-anoxic (A2) conditions for denitrifying dephosphatation, and another SBR was operated under aerobic conditions for nitrification. In this way, denitrifying dephosphatation sludge and nitrification sludge were completely separated in the two SBRs, and only supernatant was exchanged between these two SBRs. It should be remarked here that in a practical system the lay-out of the process can be such that there is no need for a separate nitrate exchange vessel.

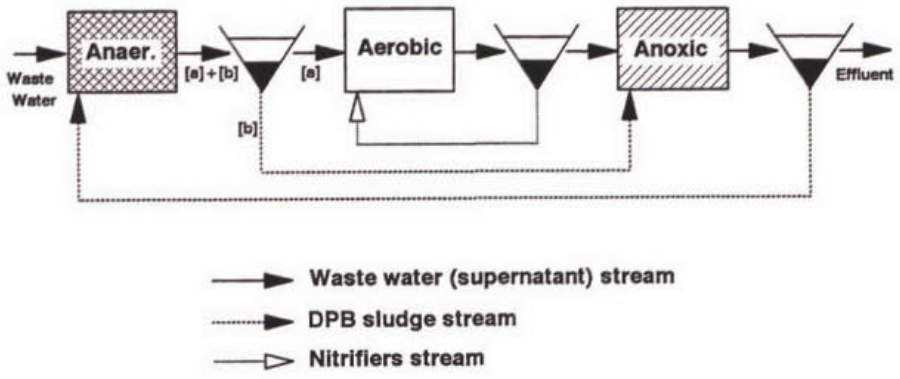

Fig. 30 Schematic diagram of a two-sludge system. [a]: (NH_4^+ and phosphorus rich) Supernatant stream, [b]: DPB sludge stream.

A bottle-neck in two-sludge systems could be NH_4^+ residues in denitrifying dephosphatation sludge streams (stream [b] in Fig. 30). In a settling phase or a settler after anaerobic conditions, the denitrifying dephosphatation sludge is separated from the anaerobic (NH_4^+ and phosphorus rich) supernatant. All NH_4^+ in the supernatant stream is oxidised into nitrate and removed in the nitrification (aerobic) zone. However, NH_4^+ in the DPB sludge stream is transferred into the anoxic zone. NH_4^+ with the DPB sludge stream decreases in the anoxic zone, mainly due to dilution by the supernatant stream and partly due to utilization for growth of DPB. If the NH_4^+ residue in the DPB sludge stream is balanced with NH_4^+ requirement for growth of DPB in the anoxic zone, nitrogen removal efficiency will be 100%. Therefore nitrogen removal in two-sludge systems

depends on a volume exchange ratio (defined by [a]/([a]+[b]) in Fig. 30). With an increasing volume exchange ratio, the nitrogen removal efficiency in two-sludge systems will increase positively. The limit of this ratio is formed by the sludge control of the reactor and SVI of the sludge.

Another possibility for improvement of nitrogen removal is to increase SRT in the denitrifying dephosphatation reactor, which leads to higher NH_4^+ requirements for the growth of DPB in the anoxic zone, because of higher biomass production. In two-sludge systems it can be possible to reduce SRT of the DPB sludge without influencing the nitrification activity. A lower SRT is beneficial for phosphorus removal because of the increase of phosphorus uptake capacity of DPB.

In this chapter, the possibility for simultaneous phosphorus and nitrogen removal was examined in the proposed two-sludge system. Also the effect of SRT in the A2 SBR of the two-sludge system on nitrogen and phosphorus removal was investigated. Especially general operational strategies of two-sludge systems regarding COD/N ratios in influent, will be discussed.

5.1.2 Experiments

Apparatus and methods. The proposed two-sludge system consisted of two SBRs (an A2 SBR and a nitrification SBR) and a "nitrate exchange vessel", as shown in Fig. 31.

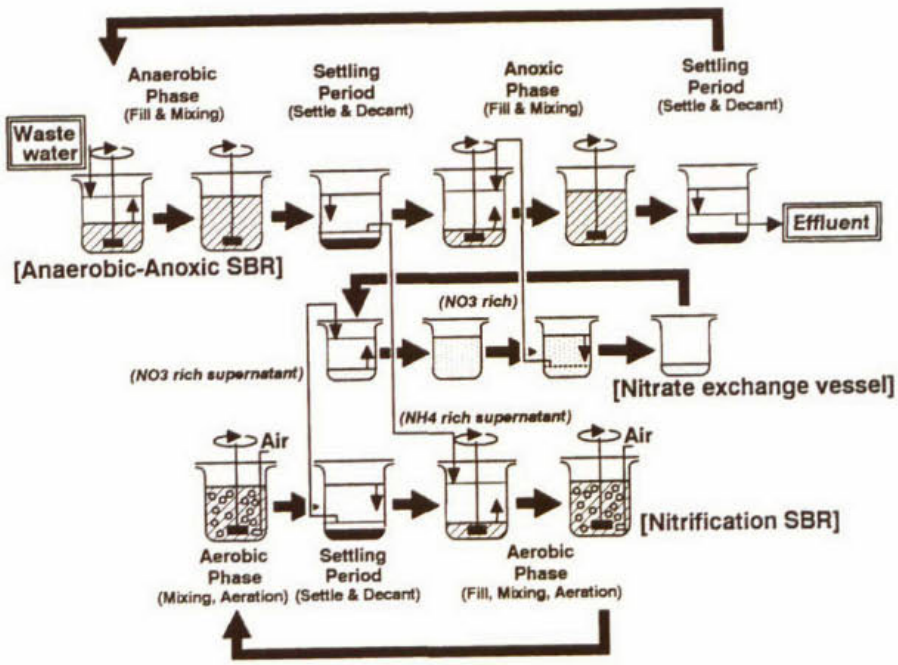

Fig. 31 Schematic diagram of the operation of the proposed two-sludge system.

<u>*Anaerobic-anoxic (A2) SBR*</u>: The working volume of the SBR was 3.5*l*. pH was controlled at 7.0±0.05 by addition of 1N HCl or NaOH. The cycle time in the A2 SBR was 6 hours and the cycle consisted of four phases;

1.5h anaerobic phase: At the beginning of the anaerobic phase (during the first 10 minutes), 1.75*l* synthetic waste water (Table 13) was pumped into the A2 SBR. N_2 gas was flowed through the head space of the SBR. In this phase, COD (HAc) was taken-up and phosphorus was released by DPB. NH_4^+ concentration was almost constant.

0.5h 1st settling period: After the settlement (15 minutes), 2.5*l* anaerobic (NH_4^+ and phosphorus rich) supernatant was directly transferred into the nitrification SBR. Thus, the

volume exchange ratio (defined by [a]/([a]+[b]) in Fig. 30) was 2.5*l*/3.5*l*=71.4%.

3.5h anoxic phase: 2.5*l* nitrate rich supernatant which was transferred from the nitrification SBR and stocked in the nitrate exchange vessel, was continuously pumped into the A2 SBR during the first 1.67 hours (Operation I) or 3.0 hours (Operation II). At the end of the anoxic phase, the excess sludge was removed. In this phase, phosphorus was taken-up by DPB with denitrification.

0.5h 2nd settling period: After the settlement (15 minutes), 1.75*l* supernatant as treated effluent was pumped-out from the A2 SBR.

Nitrification SBR: The working volume of the nitrification SBR was 3.5*l*. pH was controlled at 7.1±0.1 by addition of 1N HCl or $NaHCO_3$. The cycle time in the nitrification SBR was 6 hours and the cycle consisted of two phases;

5.5h aerobic phase: At the beginning of the aerobic phase (during the first 15 minutes), 2.5*l* anaerobic (NH_4^+ and phosphorus rich) supernatant was directly pumped into the nitrification SBR from the A2 SBR. After the transfer of the supernatant, aeration was done, and NH_4^+ was completely oxidised to nitrate by enriched nitrifiers. Phosphorus concentration was almost constant in the nitrification SBR. At the end of this phase, oxygen was removed by flushing N_2 gas into the mixed liquor (15 minutes), to prevent from oxygen contamination into the anoxic phase of the A2 SBR. Also, excess sludge was removed at the end of the aerobic phase. (Aerobic) SRT in the nitrification SBR was kept at approximately 20~40 days.

0.5h settling period: After the settlement (15 minutes), 2.5*l* nitrate rich supernatant was pumped-out into the nitrate exchange vessel.

Experimental procedure. The following parameters have been evaluated; (i)the effect of SRT in the A2 SBR and (ii)the effect of COD/N ratio in synthetic waste water, on phosphorus and nitrogen removal.

(i)*SRT in the A2 SBR*: The A2 SBR was operated under 14 days SRT until day 50, after which the SRT was decreased to 8 days.

(ii)*COD/N ratio in the synthetic waste water*: In the research on the two-sludge system, the synthetic waste water contained a relatively low COD/N ratio (3.4 g-COD/g-NH_4^+-N). NH_4^+ was oxidised into nitrate in the nitrification SBR, and the produced nitrate was transferred to the anoxic phase of the A2 SBR. The amounts of produced nitrate as electron acceptors for DPB, depended on the NH_4^+ concentration in the synthetic waste water. In the first step of this research, the influent NH_4^+ concentration was calculated according to the volume exchange ratio and the previous research about DPB, and it was optimised, on minimising phosphorus (and nitrate) in the effluent, i.e., complete anoxic phosphorus removal. In the second step of the research, the influent NH_4^+ concentration was slightly decreased; 8.5 → 7.5 → 8.0 mmol/*l* (Table 13), in order to examine the effect of the influent COD/N ratio on phosphorus and nitrogen removal efficiency, and to find the optimal ratio as an operational criterium for the two-sludge system.

Table 13 Composition of synthetic waste water for the two-sludge system.

HAc =	400 mg-COD/*l*	
P =	15 mg-P/*l*	
NH_4^+ =	8.5 mmol/*l* (day 0~170, day 193~)	[3.36 g-COD/g-NH_4^+-N]
	7.5 mmol/*l* (day 170~183)	[3.81 g-COD/g-NH_4^+-N]
	8.0 mmol/*l* (day 183~193)	[3.57 g-COD/g-NH_4^+-N]
Trace mineral solution*		

*: See Chapter 2.

5.1.3 Results and discussion

Performance of the two-sludge system. Before the combination, each SBR was operated separately. Phosphorus was completely removed in the anoxic phase of the A2 SBR, and NH_4^+ was completely converted into nitrate in the nitrification SBR.

Cycle behaviour of phosphorus, TOC and nitrogen in each SBR at day 43 (SRT=14 days) after the combination, is shown in Fig. 32. After the first settling period of the A2 SBR, the NH_4^+ rich supernatant was directly transferred into the nitrification SBR. NH_4^+ was completely converted into nitrate, and phosphorus concentration was always constant in the nitrification SBR. After the

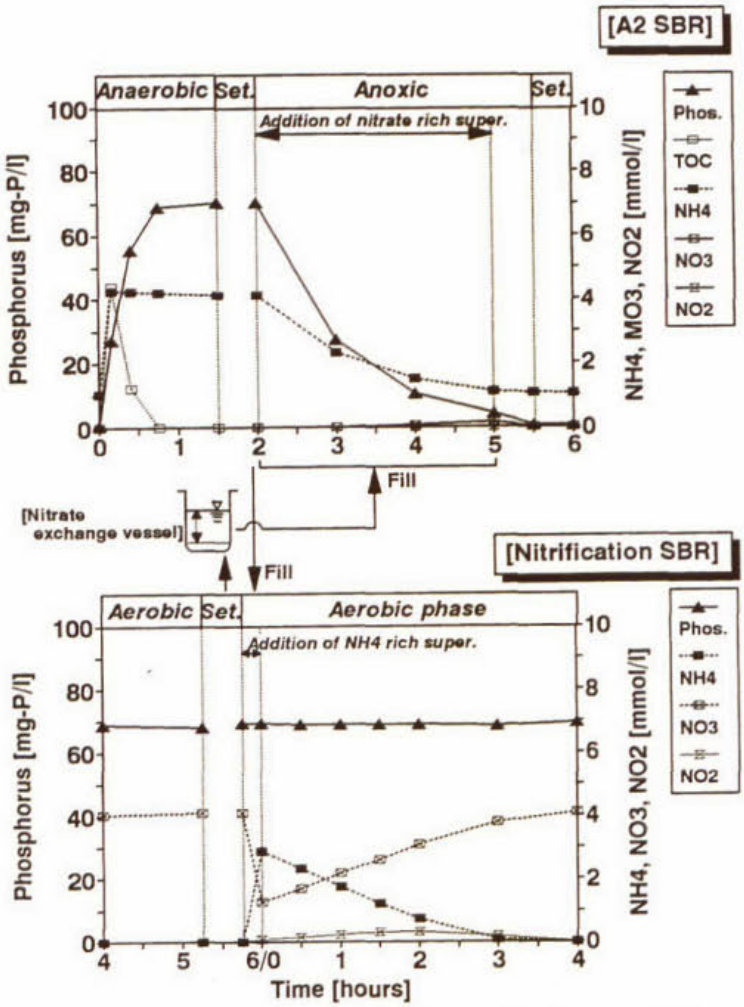

Fig. 32 Cycle behaviour of phosphorus and nitrogen in the two-sludge system at day 43 (Operation II, 14 days SRT).

settling period of the nitrification SBR, the nitrate rich supernatant was stocked in the nitrate exchange vessel. During the first 3.0 hours of the anoxic phase, the nitrate rich supernatant was pumped into the A2 SBR from the exchange vessel. Phosphorus was completely taken up by DPB. Nitrate and nitrite were slightly detected at the end of the anoxic phase, but not detected in effluent, which indicated denitrifying dephosphatation occurred in the A2 SBR. NH_4^+ concentration decreased gradually in the anoxic phase of the A2 SBR, mainly due to dilution by addition of the nitrate rich supernatant (NH_4^+=0.0 mmol/l) from the exchange vessel (the mixed liquor volume increased with time from 1.0*l* to 3.5*l* by the addition of the nitrate rich supernatant), and partly due to utilization of NH_4^+ for growth of DPB. NH_4^+ concentration in effluent was approximately 1 mmol/*l*, which means approximately 90% nitrogen removal efficiency.

Figure 33(a) shows daily variation of phosphorus concentrations at the end of the anaerobic phase and in effluent, and Fig. 33(b) shows nitrogen concentrations in effluent from the A2 SBR. Both SBRs were combined as the two-sludge system at day 0. At day 20, the addition time of the nitrate rich supernatant in the anoxic phase was prolonged from 1.67 (Operation I) to 3.0 hours (Operation II), because of secondary phosphorus release at the end of the anoxic phase (Fig. 8 in Chapter 3.2).

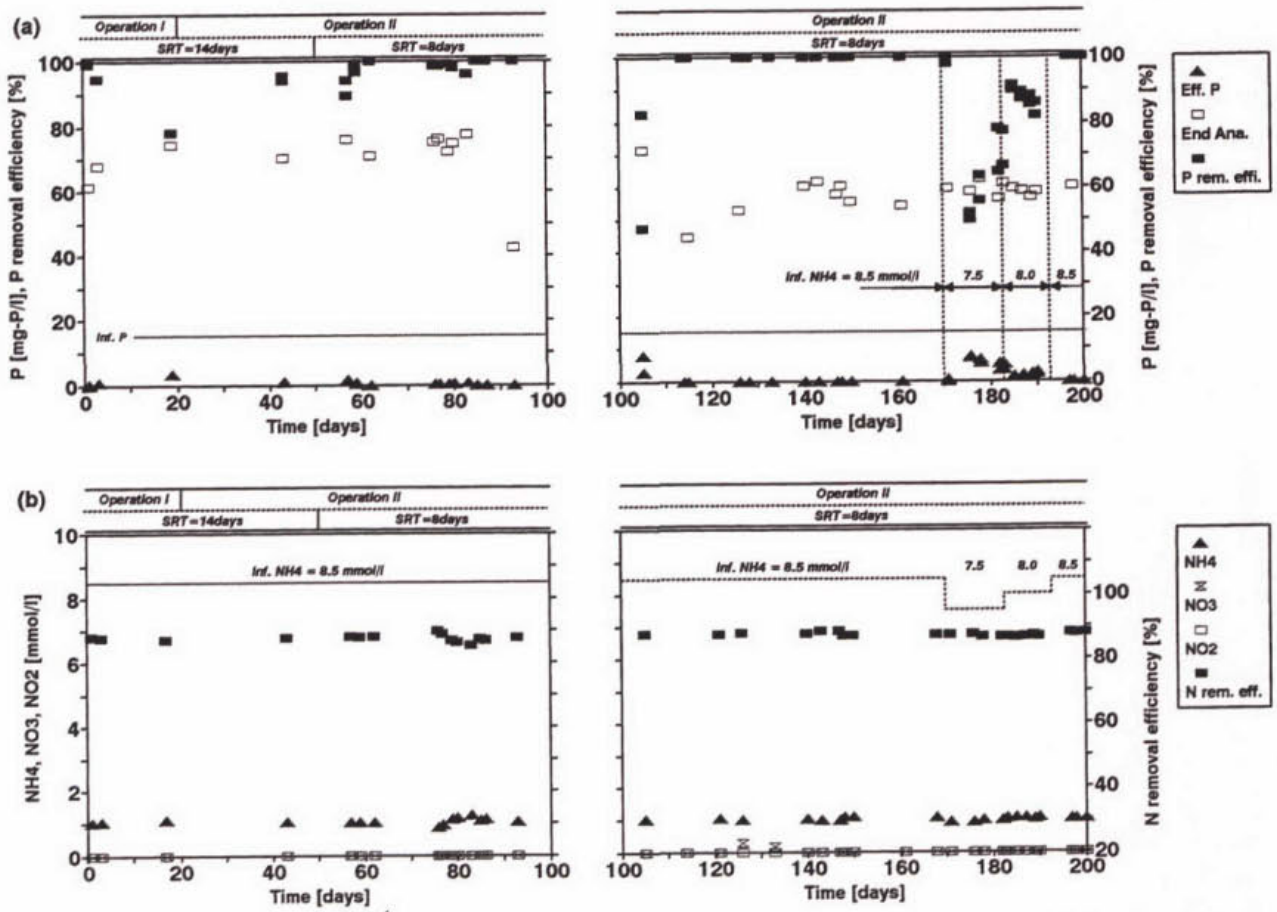

Fig. 33 Daily variation of (a)phosphorus concentrations in effluent and at the end of the anaerobic phase, and (b)NH_4^+ and NO_x^- concentrations in effluent, in the A2 SBR of the two-sludge system.

The phosphorus removal in the two-sludge system was quite stable, and phosphorus removal efficiency was almost 100% (day 0 ~ 170). Nitrogen removal was also stable. Nitrate and nitrite were scarcely detected in effluent, and NH_4^+ removal efficiency was always approximately 90%.

Effect of SRT on nitrogen removal. Although the phosphorus removal efficiency was almost 100%, NH_4^+ was detected in effluent under 14 days SRT of the A2 SBR. In order to improve the nitrogen

removal efficiency, the SRT in the A2 SBR was decreased from 14 to 8 days at day 50. It was expected that the lower SRT led to higher NH_4^+ requirements, because of higher biomass production. The observed growth yield increased slightly, with decrease of the SRT. The observed growth yield was 0.23 ~ 0.24 g-VSS/g-COD at 14 days SRT, and 0.25 ~ 0.28 g-VSS/g-COD at 8 days SRT.

As shown in Fig. 33(a), the phosphorus removal efficiency was always around 100% until day 170, irrespective of the SRT. The lower SRT in the A2 SBR did not bring any negative effect on phosphorus removal. On the other hand, the nitrogen removal efficiency was not improved by the lower SRT, and it was still around 90% at 8 days SRT. The observed growth yield increased with 10 ~ 20% when the SRT was decreased from 14 to 8 days. This means that increment of required ammonium-nitrogen for growth improves only 1 ~ 2% on the nitrogen removal efficiency, if the biomass formula is assumed $CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$ (0.1 g-N/g-VSS). This coincided with the observation on the nitrogen removal efficiency.

It should be concluded that instead of decrease of the SRT of the DPB sludge, the increase of the volume exchange ratio between the A2 and nitrification SBRs probably leads to drastic improvement of the nitrogen removal efficiency. Figure 34 shows a relationship between the volume exchange ratio and nitrogen removal efficiency, on simple calculations. The growth yield of the DPB sludge was assumed to be 0.26 g-VSS/g-COD, and the above-mentioned biomass formula was used on the calculations. Utilized NH_4^+ for growth of nitrifiers in the nitrification SBR was neglected. The two-sludge system has been operated under 71% volume exchange ratio. If the volume exchange ratio is increased from 71% to 76%, the nitrogen removal efficiency will be improved 3 ~ 4% and NH_4^+ concentration in effluent will be less than 10 mg-N/*l*. Since SVI in the A2 and nitrification SBRs is quite low (< 50 m*l*/g-SS), it's easily possible to increase the volume exchange ratio.

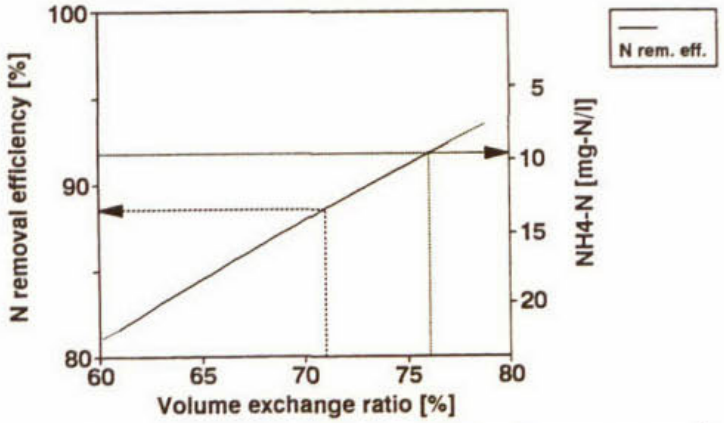

Fig. 34 Relationship between the volume exchange ratio and the nitrogen removal efficiency in the two sludge system.

Phosphorus removal in different influent COD/N ratios. NH_4^+ concentrations in the synthetic waste water were slightly decreased at day 170, to investigate the effect of the influent COD/N ratio on phosphorus and nitrogen removal. The influent NH_4^+ concentration was 7.5 mmol/*l* from day 170 to 183, and it was 8.0 mmol/*l* from day 183 to 193. Influent COD (HAc) concentrations were kept constant (400 mg-COD/*l*).

As shown in Fig. 33, immediately after the influent NH_4^+ concentration was decreased from 8.5 to 7.5 mmol/*l*, the phosphorus removal efficiency dropped to 50 ~ 60%. When the influent NH_4^+ concentration was 8.0 mmol/*l*, the phosphorus removal efficiency was 80 ~ 90%. If the influent COD/N ratio was higher than 3.4 g-COD/g-N (=400 [mg-COD/*l*] ÷ 8.5 [mmol-NH_4^+/*l*] ÷ 14 [g-N/mol-NH_4^+]), clearly phosphorus was not removed completely and detected in effluent, due to shortage of nitrate as an electron acceptor. With increasing the COD/N ratio, the nitrogen removal efficiency decreased slightly, due to lower biomass production because of shortage of nitrate.

Figure 35 shows a schematic diagram of effect of the influent COD/N ratio on the phosphorus and nitrogen removal. In these operations of the two-sludge system (71% volume exchange ratio), the optimal phosphorus and nitrogen removal were obtained at approximately 3.4 g-COD/g-N as the influent COD/N ratio. If the residual NH_4^+-N in effluent is taken into account, approximately 3.8 g-COD/g-N is a critical COD/N ratio for complete phosphorus and nitrogen removal, under ideal operational conditions (an ideal volume exchange ratio, i.e., NH_4^+ residues in the DPB sludge stream are balanced with NH_4^+ requirement for growth of DPB in the anoxic zone).

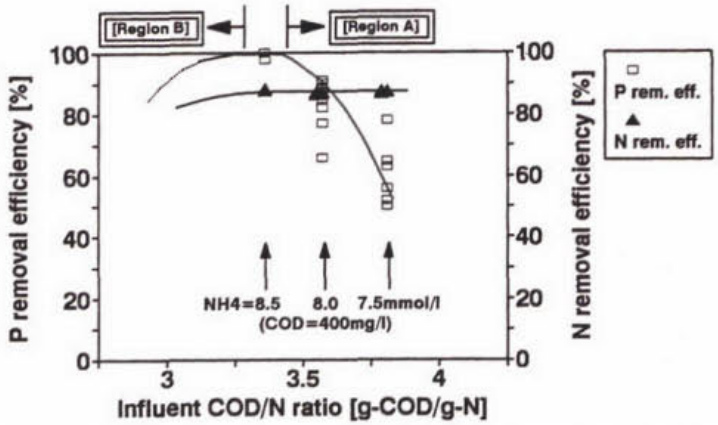

Fig. 35 Schematic diagram of the effect of influent COD/N ratio on the phosphorus and nitrogen removal efficiency.

When the influent COD/N ratio is higher than 3.4 g-COD/g-N ([region A] in Fig. 35), incomplete phosphorus removal occurs due to shortage of nitrate. However, this can be easily resolved by introduction of a short aerobic phase after the anoxic phase in the A2 SBR. The residual phosphorus which is not taken-up by denitrifying dephosphatation, can be removed by DPB with oxygen instead of nitrate (Chapter 4.1).

When the influent COD/N ratio is lower than 3.4 g-COD/g-N ([region B] in Fig. 35), excess nitrate will be supplied from the nitrification SBR and nitrate will be detected in effluent. Probably this disturbs the phosphorus removal, because nitrate will be transferred into the anaerobic phase. In this case, extra COD should be added into the system (preferably into the anaerobic phase of the A2 SBR, not the anoxic phase).

Benefits of two-sludge systems. The proposed two-sludge system showed very stable phosphorus and nitrogen removal. A benefit of two-sludge systems in comparison with single-sludge systems is to be capable to operate under optimal conditions for each step of nitrification and denitrifying dephosphatation. As shown in Chapter 3.1 (or Kuba *et al.*, 1996b), in the single-sludge systems, a long aerobic phase or long aerobic SRT is needed due to low nitrification activities in the mixed sludge of nitrifiers and DPB. However in the two-sludge systems, it is possible to control the SRT separately in the nitrification and denitrifying dephosphatation step. This leads to optimal control of the nitrification step for the two-sludge systems. Moreover, by using a nitrification biofilm reactor, the hydraulic retention time in the nitrification step can be strongly reduced.

Another benefit is a minimized nitrogen concentration in effluent without extensive recycling. As mentioned before (Chapter 4.1), pre-denitrification (like UCT-type) operations (Fig. 27(b)) are recommended for single-sludge systems, because of prevention of aerobic PHB oxidation by DPB. However in the pre-denitrification operations, a large recirculation from the aerobic to anoxic phase is needed to reduce nitrate concentrations in effluent. In this sense, the post-denitrification operations (Fig. 27(a), or Fig. 30) like the proposed two-sludge system are more optimal. In the post-denitrification operations, theoretically it's possible to achieve complete nitrogen removal, because denitrification is achieved after nitrification. This requires a good control of the settling to achieve that a sufficient volume of supernatant and hence a large fraction of the

NH_4^+ goes through the nitrification reactor.

As mentioned above, the two-sludge systems have clearly higher flexibilities on the operations for simultaneous phosphorus and nitrogen removal, rather than the single-sludge systems. The benefits of two-sludge systems for phosphorus and nitrogen removal in comparison with single-sludge systems, are summarized as follows:

(1)Smaller reactor volumes,
(2)Separate optimization for phosphorus and nitrogen removal possible,
(3)Lower energy requirements;
  -utilization of oxygen for nitrification only,
  -minimal utilization of oxygen for phosphorus removal,
  -no large recirculation,
(4)Minimal loss of COD by oxidation.

From the operation of the lab-scale two sludge system, it can be concluded that:

(1)An anaerobic-anoxic SBR with a separated nitrification SBR as a two-sludge system has stable phosphorus and nitrogen removal,
(2)A bottle-neck for nitrogen removal in the proposed two sludge system is the NH_4^+ residue in the DPB sludge and supernatant after the settling period followed the anaerobic phase of the A2 SBR. This will be improved by increment of the volume exchange ratio between the A2 and nitrification SBRs, which can be possible because of high sludge settleability in both SBRs.
(3)The reduction of the SRT from 14 to 8 days in the A2 SBR of the two-sludge system, did not bring about improvement of the nitrogen removal efficiency, because the growth yield of the DPB sludge increased with 10～20% and this corresponds to 1～2% improvement of the nitrogen removal efficiency. The reduction of the SRT had no influence on phosphorus removal by DPB within the range of 8～14 days SRT,
(4)Under the operational conditions in this chapter, complete phosphorus removal and optimal nitrogen removal occurs at approximately 3.4 g-COD/g-N as the influent COD/N ratio, and 3.8 g-COD/g-N as the consumed ratio,
(5)When the influent COD/N ratio is lower than the optimal ratio, extra COD should be added into the system to remove residual nitrate. When the influent ratio is higher than the optimal ratio, simply a short aerobic phase should be introduced after the anoxic phase of the A2 SBR, to remove residual phosphorus due to nitrate shortage,
(6)Two-sludge systems have higher flexibilities on the operations for simultaneous phosphorus and nitrogen removal, in comparison with single-sludge systems.

6 CONCLUSIONS

In this project "*Anaërobe/Denitrificerende Biologische Defosfatering*" (*Biological Dephosphatation under Anaerobic/Denitrifying Conditions*), the integration of biological phosphorus and nitrogen removal from waste water, which is based on denitrifying dephosphatation, has been studied. This project has aimed at developing new denitrifying dephosphatation systems incorporating nitrification, and to provide fundamental information of denitrifying dephosphatation for this development.

The subjects in this project have been investigated by using sludge in lab-scale sequencing batch reactors or sludge from actual waste water treatment plants. The subjects were divided into three research lines; (i)kinetics and stoichiometry of denitrifying dephosphatation, (ii)single-sludge systems, and (iii)two-sludge systems.

In this project, the following main conclusions have been drawn:

(i)***Kinetics and stoichiometry of denitrifying dephosphatation***

(1)Denitrifying phosphorus removing bacteria (DPB) have the glycogen metabolism. The anaerobic metabolism of DPB is completely identical to that of conventional phosphorus removing organisms,

(2)A reduction of phosphorus release by nitrate in biological phosphorus removal systems is partly due to the presence of DPB, which utilize COD for denitrification, not for phosphorus release,

(3)Amounts of intracellular glycogen limit the maximum HAc uptake under anaerobic conditions. The capacity of PHB synthesis or the achievement of complete polyphosphate degradation seems not limiting,

(4)Under short cycle operations (completely mixed-type processes), phosphorus removal was unstable and much phosphorus was detected in effluent. After the operation was switched to ordinary operations (plug flow-type or SBR-type processes), phosphorus was removed stable and complete,

(5)A metabolic model proposed for conventional anaerobic-aerobic (A/O) phosphorus removal processes, can be applied for the denitrifying dephosphatation (A2) process. A difference between the aerobic and anoxic metabolism is only electron transport phosphorylation.

(6)The measured P/$NADH_2$ ratio in the electron transport phosphorylation with nitrate was approximately 1.0 mol-ATP/mol-$NADH_2$, which indicates that the energy production efficiency with nitrate is approximately 40% lower than it with oxygen.

(ii)***Single-sludge systems***

(1)Oxygen has no direct detrimental effect on denitrifying dephosphatation activities. DPB can utilize oxygen for phosphorus removal even immediately after the long cultivation without oxygen, and the phosphorus uptake activities under anoxic and aerobic conditions are almost identical,

(2)For single-sludge systems, pre-denitrification (modified UCT-type) processes are recommended for efficient phosphorus and nitrogen removal using denitrifying dephosphatation,

(3)Denitrifying dephosphatation occurs in significant amounts in actual waste water treatment plants which have the modified UCT-type configurations,

(4)The particulate COD in non-presetting sewage stimulates nitrogen removal in the waste water treatment plant, but not phosphorus removal because the particulate COD is not available for phosphorus removing organisms and transferred into the anoxic zone.

(iii)***Two-sludge systems***

(1)The proposed lab-scale two-sludge system has stable phosphorus and nitrogen removal,
(2)A bottle-neck for nitrogen removal in two-sludge systems is NH_4^+ residue in the DPB sludge stream. This will be improved by increment of the volume exchange ratio between the anaerobic-anoxic and nitrification reactors, which can be possible because of high sludge settleability in the DPB sludge,
(3)Two-sludge systems have higher flexibilities on the operations for simultaneous phosphorus and nitrogen removal, in comparison with single-sludge systems.

Therefore in the process design of nitrogen and phosphorus removal, one should introduce denitrifying dephosphatation, which leads to the saving of COD and energy (aeration) for overall phosphorus and nitrogen removal process. We believe that the results and information derived in this project, will be useful in the process design, when optimal denitrifying dephosphatation is desired.

ABBREVIATIONS

A/O:	anaerobic-aerobic
AOA:	anaerobic-aerobic-anoxic
ATP:	adenosine triphosphate
A2:	anaerobic-anoxic
A2O:	anaerobic-anoxic-aerobic
C:	carbon
COD:	chemical oxygen demand
DPB:	denitrifying phosphorus removing bacteria
$FADH_2$:	flavin adenine dinucleotide
HAc:	acetic acid
HPLC:	high performance liquid chromatography
N:	nitrogen
$NADH_2$:	nicotinamide adenine dinucleotide
P:	phosphorus
P/C-ratio:	a ratio of released phosphorus to consumed HAc
PHA:	polyhydoxyalkanoate
PHB:	poly-β-hydroxybutyrate
PHV:	poly-β-hydroxyvalerate
poly-P:	polyphosphate
POV:	poly-β-iso-hydroxyvalerate
q_{Pa}:	phosphorus uptake rate under anoxic conditions
q_{Po}:	phosphorus uptake rate under aerobic conditions
SBR:	sequencing batch reactor
SRT:	sludge retention time
(ML)SS:	(mixed liquor) suspended solids
SVI:	sludge volume index
TCA:	tricarboxylic acid
TOC:	total (dissolved) organic carbon
UCT:	University of Capetown
VFA:	volatile fatty acid
(ML)VSS:	(mixed liquor) volatile suspended solids

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