Rgw-08

MODELVORMING EN OPTIMALISATIE VAN BIOLOGISCHE DEFOSFATERING VAN AFVALWATER

Ontwikkeling van een metabool model

Stichting voor de Technische Wetenschappen
Technology Foundation

Postbus 3021, 3502 GA Utrecht

Rijkswaterstaat
Rijksinstituut voor Integraal Zoetwaterbeheer
en Afvalwaterbehandeling

Postbus 17, 8200 AA Lelystad

Stichting Toegepast Onderzoek
Waterbeheer

Postbus 8090, 3503 RB Utrecht

ieneratie rioolwaterzuiveringsinrichtingen RWZI 2000

ecretariaat: postbus 17, 8200 AA Lelystad 03200 - 70411

rwzi 2000

MODELVORMING EN OPTIMALISATIE VAN BIOLOGISCHE DEFOSFATERING VAN AFVALWATER

Ontwikkeling van een metabool model

auteur(s):

TU-Delft,vakgroep
Bioprocestechnologie:

dr.ir. G.J.F. Smolders
dr.ir. M.C.M. van Loosdrecht
prof.dr.ir. J.J. Heijnen

RWZI 2000 94-08

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

INHOUD Blz.

VOORWOORD

Naast het onderzoekprogramma PNs 1992 van de STOWA, waarin ervaring uit de praktijk is verkregen op het gebied van biologische fosfaatverwijdering op rwzi's, is binnen het onderzoekprogramma RWZI 2000 aandacht besteed aan de fundamentele kennisopbouw bij biologische defosfatering.
In dit kader is in onderlinge samenwerking tussen de vakgroep Bioprocestechnologie van de Technische Universiteit te Delft en de vakgroep Microbiologie van de Landbouwuniversiteit te Wageningen een onderzoeksproject uitgevoerd. Doel van dit fundamentele onderzoeksproject was enerzijds de nog bestaande leemtes in de microbiële kennis in te vullen en anderzijds deze microbiologische, bioprocestechnologische en zuiveringstechnologische kennis en ervaring te combineren om te komen tot een onderbouwd ontwerpmodel voor biologische defosfatering.

Het voorliggende rapport beschrijft de ontwikkeling van een biochemisch gestructureerd model voor de biologische fosfaatverwijdering, dat is ontwikkeld door dr.ir. G.J.F. Smolders op de Technische Universiteit Delft onder dagelijkse leiding van dr.ir. M.C.M. van Loosdrecht en prof.dr.ir. J.J. Heijnen.

Het onderzoek is financieel ondersteund vanuit de "Stichting Technische Wetenschappen" (projectnr. 90212) en het RWZI 2000 onderzoekprogramma (projectnr. 3225/4).

Begeleiding van dit onderzoek en afstemming met de meer praktische biologische defosfateringsprojecten heeft plaatsgevonden op zogenaamde "fosfaatdagen", waarbij vertegenwoordigers van waterkwaliteitsbeheerders, ingenieursburo's, universiteiten en de financiërende instanties betrokken waren.

Lelystad, januari 1995

Voor de Stuurgroep RWZI 2000

prof.dr. J. de Jong
(voorzitter)

SAMENVATTING

Het in dit rapport beschreven onderzoek had tot doel om een model voor de biologische defosfatering op te stellen. Daarnaast diende het onderzoek het inzicht in het gedrag van biologische defosfaterende systemen te vergroten. Bij het onderzoek is gebruik gemaakt van in het laboratorium gekweekt actief slib dat vrijwel uitsluitend uit defosfateerders bestaat. Dit is mogelijk door alleen Acetaat-CZV aan het systeem te voeden, en ervoor te zorgen dat alle acetaat onder strict anaerobe condities wordt opgenomen.
Bij het modelleren is getracht om uit te gaan van de biologische basis van het proces. Hiertoe wordt het metabolisme van de betrokken microorganismen gemodelleerd en gekoppeld met en procesmodel. Het aldus verkregen model bleek in staat te zijn om met een set parameters het gedrag van een defosfaterende "sequencing batch" reactor te beschrijven. Concentraties van alle van belang zijnde verbindingen konden juist worden voorspeld, bij een grote variatie aan condities (slibleeftijd 5-20 dagen, wisselende invoer van fosfaat, en tijdens de opstart van het systeem wanner competitie met normale heterotrofe organismen van belang is).
Simultaan is door de IAWQ "task group" modellering actief slib processen een voorstel gemaakt voor een model voor defosfatering. Dit model is niet strict op het microbiele metabolisme geent. Het metabole model blijkt (ondanks de hogere mate van complexiteit) uiteindelijk minder modelparameters te hebben dan het voorgestelde model no.2. Dit wordt veroorzaakt doordat een aantal processen op metabool aan elkaar gekoppeld kunnen worden.
Op basis van het verkregen metabool model is een methodiek voor de evaluatie van zuiveringsprocessen opgesteld. Daarbij wordt met name bepaald of (i) de hoeveelheid Acetaat-CZV in het influent voldoende is, of (ii) hoeveel Acetaat-CZV in een striptank moet worden toegevoegd of (iii) hoeveel fosfaat chemisch moet worden geprecipiteerd voor een gewenste effluent fosfaatconcentratie.

Naast het opstellen van het model zijn enkele punten van belang voor de defosfatering naar boven gekomen.
De fosfaatafgifte-acetaatopname tijdens anaerobe condities wordt sterk beinvloed door de heersende pH (bij pH 6-8). Dit betekend dat het meten van de P-afgifte snelheid alleen zin indien de pH wordt gecontroleerd. Daarnaast dient bij het ontwerp van een striptank in een deelstroomproces de pH als kritische parameter te worden meegenomen.
Het metabolisme van de biologische defosfateerders kon eenduidig worden vastgelegd. Glycogeen speelt hierin (naast membraanprocessen) een cruciale rol. De maximale P-afgifte blijkt door de beschikbare hoeveelheid glycogeen in de cel te worden gelimiteerd. Het lijkt erop dat juist deze lastig te bepalen parameter de meest kritisch factor in het polyfosfaatmetabolisme is. Kennis van het glycogeen gehalte in de cellen is o.a. van belang bij het ontwerp van een striptank in het deelstroomproces.
Het zuurstofverbruik voor de fosfaatopname is vrij hoog. Dit betekend dat in principe middels respirometrie de P-opname snelheid kan worden gemeten.

1 INLEIDING

1.1 Algemeen

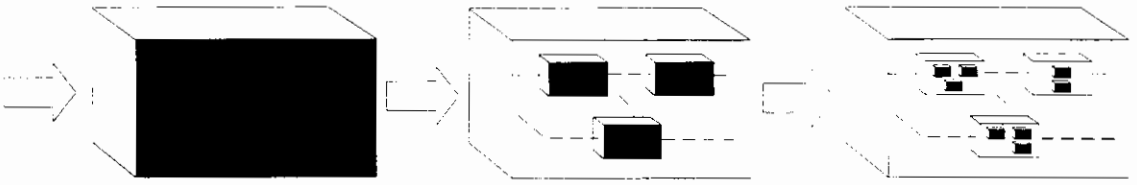
Het ontwerpen en bedrijven van biologische zuiveringsinstallaties voor afvalwater wordt steeds complexer door de toename van het aantal componenten dat verwijderd dient te worden en de steeds hogere eisen die aan het effluent gesteld worden. De aandacht richt zich tegenwoordig, naast de verwijdering van organische stof, vooral op de biologische verwijdering van stikstof en fosfaat. Om al deze processen optimaal te laten verlopen is een gedegen inzicht vereist in de biologische en procestechnologische achtergronden.

In 1990 werd het project modelvorming en optimalisering van de biologische fosfaat-verwijdering uit afvalwater gestart. Dit fundamenteel toepassingsgerichte onderzoeksproject had tot doel om enerzijds de nog bestaande leemte in de microbiële kennis in te vullen en anderzijds de microbiologische, bioprocestechnologische en zuiveringstechnologische kennis en ervaring te combineren om te komen tot een onderbouwd ontwerpmodel voor het biologische fosfaatverwijderings proces. Dit project was een samenwerkingsproject tussen de vakgroep bioprocestechnologie van de TU Delft en de vakgroep microbiologie van de LU Wageningen. De resultaten van het onderzoek verricht in Wageningen staan vermeld in het rapport RWZI 2000 93-03. Het doel van het onderzoek aan de TU Delft was de ontwikkeling van een biochemisch gestructureerd model voor de biologische fosfaatverwijdering.

Gekozen werd voor het maken van een wiskundig model omdat dit een goede manier is om de complexe microbiologische informatie hanteerbaar te maken en zodoende beter inzicht te krijgen in het complexe gedrag van biologische zuiveringsprocessen. Het gebruik van deze modellen is tweeledig: het proces wordt kwantitatief beschouwd waardoor er gediscrimineerd kan worden tussen verschillende theorieën aangaande het proces; en er kan vooraf gesimuleerd worden wat het effect van bepaalde veranderingen op het proces zal zijn. Het model kan dan gebruikt worden om inzicht te krijgen bij het ontwerp of om het proces te optimaliseren. Voorwaarde hiervoor is dat het model in voldoende mate experimenteel gevalideerd is.

1.2 Metabole modellering

De wiskundige beschrijving van microbiële reacties in biologische afvalwaterzuiverings-processen is vaak problematisch door de aanwezigheid van een groot aantal componenten die van belang zijn in het systeem (bijvoorbeeld een afvalwaterzuiveringsproces). Een methode om dergelijke systemen te beschrijven is de macroscopische systeembeschrijving. Hierbij wordt een proces beschouwd in termen van concentraties van chemische componenten, energie, temperatuur of druk. Twee vormen van macroscopische systeem beschrijvingen kunnen onderscheiden worden: het 'black box' model en het 'grey box' model. In het 'black box' model wordt het proces beschouwd als een zwarte doos die enkel componenten uitwisselt met de omgeving. Met andere woorden er wordt uitsluitend naar influent- en effluentwaarden gekeken en op basis daarvan wordt getracht een beschrijving van het proces op te stellen. Een 'black box' model laat zien hoe een systeem zich gedraagt onder bepaalde condities. Wat er zich echter precies afspeelt in het systeem blijft onbekend.



Figuur 1 *schematische weergave van 'black box' (links) en 'grey box' (midden en rechts) modellen. Ook 'grey box' modellen zijn weer opgebouwd uit 'black box' deelmodellen.*

In een 'grey box' model wordt bestaande kennis m.b.t. deelprocessen in het totale proces meegenomen. In tegenstelling tot een 'black box' model waarbij het proces als één geheel wordt beschouwd en er alleen naar de in- en uitgaande stromen van een proces wordt gekeken, wordt in een 'grey box' model het proces onderverdeeld in deelsystemen waarbij kennis aangaande deze deelsystemen kan worden gebruikt. Als voorbeeld kan dienen modellen waar het slib als een organisme wordt beschouwd ('black box') of modellen die de verschillende microbiële populaties, zoals nitrificeerders en heterotrofen, in het slib onderscheiden ('grey box'). Aangezien het vaak onnodig, of onmogelijk, is om tot in alle details elk proces volledig te beschrijven (denk b.v. aan DNA replicatie in de microorganismen of de vele verschillende varianten aan heterotrofe microorganismen in het slib) is een 'grey box' model opgebouwd uit een schakeling van 'black box' modellen (zie figuur 1). In het IAWQ model no. 1 worden de organismen zelf

als een 'black box' beschreven. Bij het modelleren is het de kunst om gegeven de doelen van het uiteindelijk model de juiste mate van complexiteit te vinden. Wanneer in een 'grey box' model expliciet het interne metabolisme van de microorganismen gespecificeerd wordt, wordt het een metabool model genoemd.

Bij het formuleren van een metabool model is de eerste stap een goede definiëring van de onderdelen van het systeem waarop het model betrokken wordt. In een metabool model zijn dat de metabole reacties die zich afspelen in het organisme. Daarnaast is voor de analyse van het systeem de definiëring van de stoichiometrie en kinetiek van deze reacties van groot belang. Met stoichiometrie van een reactie wordt de verhouding tussen de hoeveelheid verbruikt reactant en gevormd produkt bedoeld. De kinetiek van een reactie bepaald de reactiesnelheid en de afhankelijkheid van de componenten die daarop van invloed zijn. Om tot een goede definitie van stoichiometrie en kinetiek in staat te zijn moet er dus eerst bekend zijn welke relevante reacties zich afspelen in het systeem.
Het gebruik van een metabool model heeft een aantal voordelen. Eén van de voordelen is het feit dat de interne deelprocessen waarop het model gebaseerd wordt, minder aan verandering onderhevig zijn dan het systeem als geheel. Hoewel de externe omstandigheden van een microorganisme sterk kunnen veranderen, probeert het micro-organisme doorgaans zijn interne condities zo veel mogelijk constant te houden. Verder zijn de deelsystemen zoals gedefinieerd in een metabool model doorgaans eenvoudiger te beschrijven dan het gehele systeem en vaak al redelijk goed bekend en onderzocht in de microbiologie en biochemie. In een metabool model wordt de fysiologie van het microorganisme beschreven middels een gelimiteerd aantal universele metabole processen (b.v. biomassa vorming of energie produktie). In al deze processen spelen ATP (energie drager) en NADH2 (betrokken bij de reductie/oxydatie reacties) een rol. Doordat een balans over deze stoffen kan worden opgesteld kunnen de diverse metabole processen aan elkaar gekoppeld worden. Extra voordeel is dat deze componenten niet gebruikt of gevormd worden. Hierdoor dient de balans uiteindelijk altijd op nul uit te komen. Vanuit thermodynamisch oogpunt beschouwd zullen de diverse metabole processen een bepaalde minimale energiebehoefte hebben, waarbij micro-organismen door selectie trachten hun efficiency te verhogen om zo dicht mogelijk bij dit minimum te komen. De energie die voor de metabole processen benodigd is kan daardoor min of meer constant verondersteld worden.

Samenvattend, maakt een metabool model maximaal gebruik van kennis omtrent de metabole reacties van het organisme waardoor het model gedefinieerd wordt op basis van een minimaal aantal parameters. De basis van het model is daardoor onafhankelijk van de procescondities of concentraties en kan dus constant veronderstelt worden. Een metabool model biedt in feite een goede methode om zoveel mogelijk biochemische kennis mee te nemen op een relatief eenvoudige manier, hetgeen leidt tot een minimaal aantal parameters.

De reden om een metabool model op te stellen in het geval van de biologische fosfaat-verwijdering is dat het een complex proces is dat grotendeels gebaseerd is op interne opslagprodukten en reacties en een benadering op metabool niveau noodzakelijk maakt.

In hoofdstuk 2 zal uitgelegd worden hoe het model werkt en hoe het gebruikt kan worden. In Hoofdstuk 3 vindt een analyse plaats van de fosfaatverwijdering en acetaatbehoefte van verschillende procesconfiguraties. In de bijlagen is gedetailleerd beschreven hoe de stoichiometry, en de kinetiek zijn gevonden en hoe de modelvalidatie heeft plaatsgevonden.

2 ONTWIKKELING VAN EEN METABOOL MODEL

2.1 Opzet

Relevante componenten

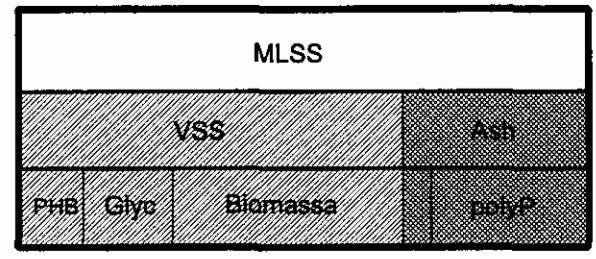
In tabel I zijn de relevante componenten die een rol spelen in de biologische fosfaat-verwijdering weergegeven met hun chemische formule. Alle substraten en polymere verbindingen zoals biomassa, PHB, polyfosfaat en glycogeen worden weergegeven per mol koolstof of fosfaat, wat de berekeningen eenvoudig maakt. Om dezelfde reden worden alle componenten electroneutraal weergegeven.

Tabel 1. *Relevante componenten voor de biologische fosfaatverwijdering.*

Component	Symbool	Elementen compositie	Component	Symbool	Elementen compositie
acetaat	s	CH_2O	glycogeen	gl	$CH_{1.67}O_{0.83}$
fosfaat	p	H_3PO_4	ammonium	n	NH_3
biomassa	x	$CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$	zuurstof	o	O_2
PHB	phb	$CH_{1.5}O_{0.5}$	koolstof	c	CO_2
polyP	pp	HPO_3	water	w	H_2O

Biomassa fracties

De biologische fosfaatverwijdering is een van de meer complexe afvalwaterzuiveringsprocessen, omdat het grootste deel van de omzettingen plaatsvindt op intern opgeslagen substraten en produkten. Het slib bestaat dus niet alleen uit actieve biomassa maar ook uit "inerte" componenten zoals PHB en polyfosfaat. Een accurate modelbeschrijving dient daardoor onderscheidt te maken tussen actieve biomassa en opslagproducten. Eveneens moeten deze opslagproducten zoals polyhydroxybutyraat (PHB), polyfosfaat en glycogeen dus apart worden beschouwd in het model. Een voorbeeld van de verhouding tussen droge stof (MLSS), organische stof (VSS), as, opslagcomponenten en actieve biomassa voor het geval dat vrijwel alle organismen in het slib polyP-organismen zijn, is gegeven in figuur 2. De MLSS en VSS zijn typische afvalwaterparameters die eenvoudig experimenteel te bepalen zijn, maar niet veel informatie geven over de samenstelling van de biomassa. Die is echter juist



Figuur 2 *Biomassa compositie en de relatie met totaal droge stof (MLSS) en organisch stof (VSS)*

in het geval van de biologische P-verwijdering erg essentieel. De direct meetbare droge stof concentratie, is in feite een mengsel van PHB, polyfosfaat, glycogeen en actieve biomassa. Het organisch stof gehalte is het totaal van de fracties PHB, glycogeen en actieve biomassa, zonder de as. Het as-gehalte bestaat uit as van de biomassa (5-10%) en polyfosfaat. De PHB-, polyfosfaat- en glycogeenfracties veranderen sterk tijdens de omzettingen in de anaërobe en aërobe fase. De interne componenten (f_i; PHB, polyP, glycogeen) worden weergegeven als de verhouding van het opslagproduct (C_f) en actieve biomassa (C_x).

$$f_i = \frac{C_i}{C_x} \tag{1}$$

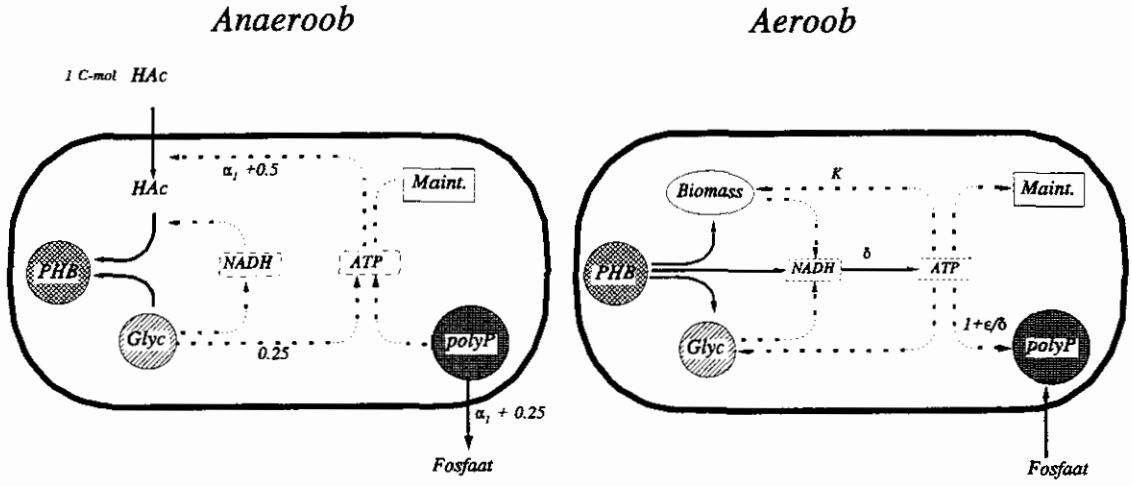
Het waargenomen totale organische stof gehalte in het proces is de som van de concentraties van actieve biomassa, PHB, polyP en glycogeen.

2.2 Stoichiometrie

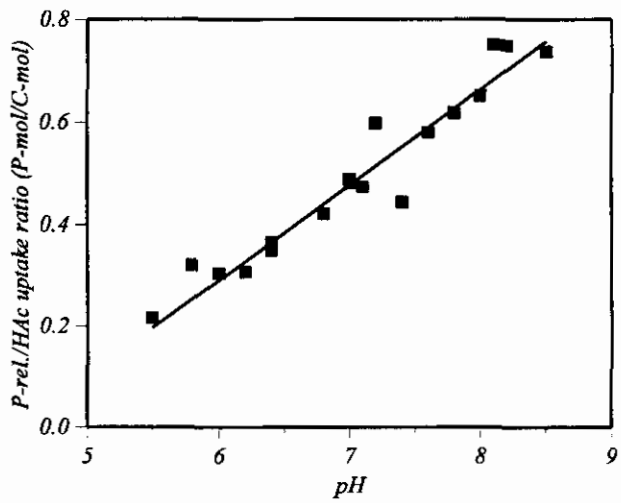
Anaëroob

In de anaërobe fase van de fosfaatverwijdering, wordt acetaat opgenomen en omgezet naar PHB. Hierbij wordt energie (ATP) geproduceerd in de omzetting van polyfosfaat naar ortho-fosfaat. Daarnaast wordt ook energie gegenereerd in de omzetting van glycogeen naar PHB (0.25 mol ATP/C-mol HAc). Deze omzetting (die in de cellen plaats vindt) is noodzakelijk omdat het NADH2 oplevert, hetgeen nodig is voor de omzetting van acetaat naar PHB. De acetaatopname is verdeeld in een transportproces over het celmembraan en een opslagproces, waarbij de energie die nodig is voor het transport van acetaat sterk afhankelijk is van de pH waarde (figuur 3). Bij lage pH is er vrijwel geen energie benodigd voor het transport van het acetaat. Er is bij lage pH (ongeveer 6) alleen energie nodig voor de omzetting van acetaat naar acetylCoA (een tussenstap in de vorming van PHB). Deze energie behoefte komt overeen met 0.5 mol Fosfaat per mol Acetaat. Bij hoge pH (ongeveer 8), is 0.5 mol ATP/C-mol acetaat nodig voor de opname van acetaat in de cel. Het gevolg is dan ook een relatief hogere fosfaat afgifte. Bij tussenliggende pH's neemt de energie behoefte proportioneel toe.

De stoichiometrie van de anaërobe fase kan worden beschreven met slechts één metabole coëfficiënt (α_1; de ATP behoefte voor Acetaat transport over de celmembraan), deze coëfficiënt bepaalt de verhouding tussen de fosfaat afgifte en de acetaat opname onder anaërobe condities (zie voor meer details appendix I). De waargenomen fosfaat/acetaat verhouding laat een variatie zien van 0.25 tot 0.75 P-mol/C-mol (of 0.6 tot 2 mg P/mg HAc) in een pH traject van 5.5 tot 8.5 (figuur 4). De overall stoichiometrie bij pH 7 is gegeven in reactie 1 van tabel 2. Tijdens de anaërobe fase wordt voorzien in de energie benodigd voor onderhouds(maintenance)processen door hydrolyse van polyfosfaat, zoals weergegeven in reactie 2 (Tabel II).



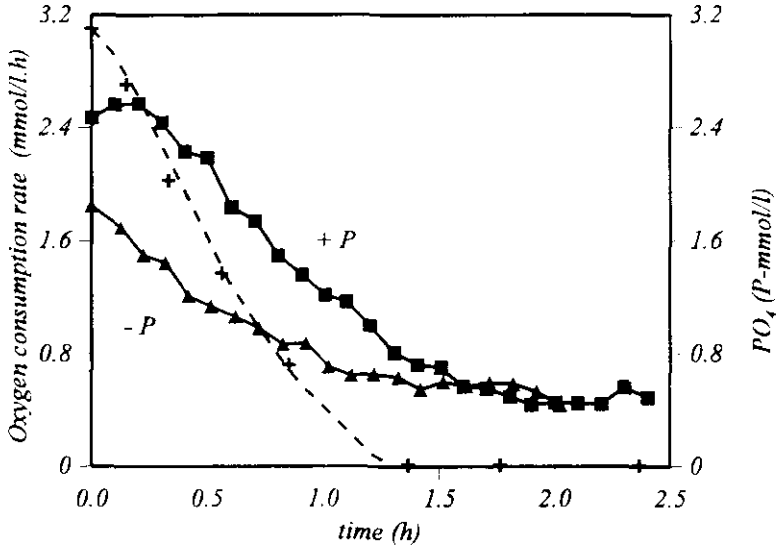
Figuur 3 *Schematische weergave van het metabolisme van polyfosfaat bacteriën tijdens de anaërobe en aërobe fase van een biologisch fosfaatverwijderingsproces.*



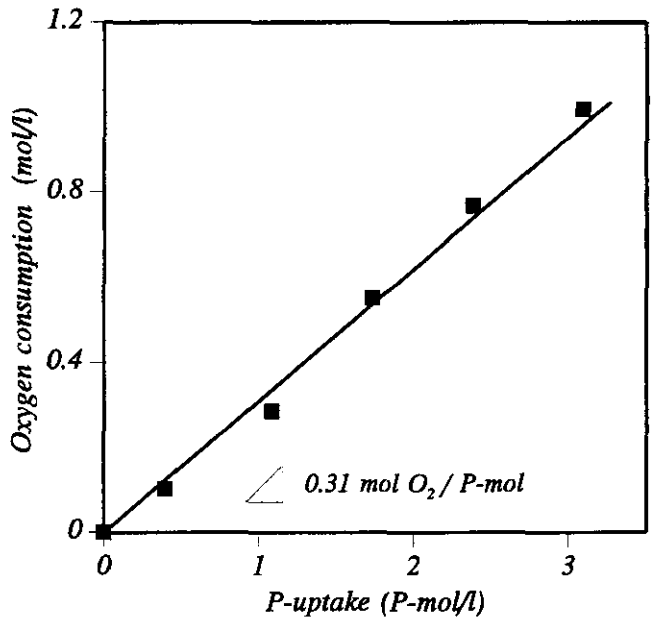
Figuur 4 *De pH afhankelijkheid van de verhouding tussen P-afgifte en Acetaat opname onder anaërobe condities.*

Aëroob

In de aërobe fase (figuur 3) wordt PHB gebruikt voor de productie van biomassa, de opname en opslag van fosfaat, de synthese van glycogeen en onderhouds- of 'maintenance' processen. In de catabole omzetting van PHB en de vorming van biomassa en glycogeen, wordt NADH gevormd dat vervolgens geoxideerd wordt in de oxidatieve fosforylering naar ATP (δ, de P/O ratio, deze ratio geeft weer hoeveel ATP er gevormd wordt per eenheid verbruikt zuurstof). Het gevormde ATP wordt verbruikt in de verschillende metabole processen: biomassa vorming (K), glycogeen produktie, onderhouds- of 'maintenance' processen, *Fosfaatopname en synthese van polyfosfaat.* Voor al deze processen kunnen reactie vergelijkingen worden opgesteld. Deze reacties verlopen allen met een eigen snelheid. Door gebruik te maken van het behoud van elementen, NADH en ATP, (geen van deze stoffen wordt netto geproduceerd of verbruikt in het totale proces) kan aangetoond worden dat er slechts 4 onafhankelijke reacties in het aërobe metabolisme zijn met drie onafhankelijke stoichiometrische parameters (K, ϵ, δ). Door gebruik te maken van experimenteel gevonden waarden (appendix II) worden de reactievergelijkingen gevonden zoals weergegeven in tabel 2.



Figuur 5 *Zuurstofconsumptiesnelheid in aan- (■) en afwezigheid (▲) van fosfaat; (+) fosfaatconcentratie.*



Figuur 6 *Verhouding tussen het specifieke zuurstofverbruik en de opname van fosfaat.*

De energie die benodigd is voor de opname en opslag van fosfaat als polyfosfaat, werd bepaald in een experiment waarbij de zuurstofconsumptiesnelheid van polyP organismen in aan- en afwezigheid van fosfaat werd gemeten (figuur 5). De extra hoeveelheid zuurstof geconsumeerd in het experiment in aanwezigheid van fosfaat is een maat voor de energie (ATP) benodigd voor de polyfosfaatsynthese. Dit experiment laat daarom rechtstreeks de energiebehoefte van het fosfaatmetabolisme zien. De initiële condities zijn gelijk in beide experimenten, en ook de biomassa- en glycogeenproduktie in beide experimenten was vergelijkbaar. In het experiment in aanwezigheid van fosfaat, vindt een verhoogde zuurstofconsumptie plaats zolang als er fosfaatopname is. De extra zuurstofconsumptie was lineair met de fosfaatopname met een verhouding (oftewel yield) van 0.31 mol O_2/P-mol in reactie 4 (figuur 6).

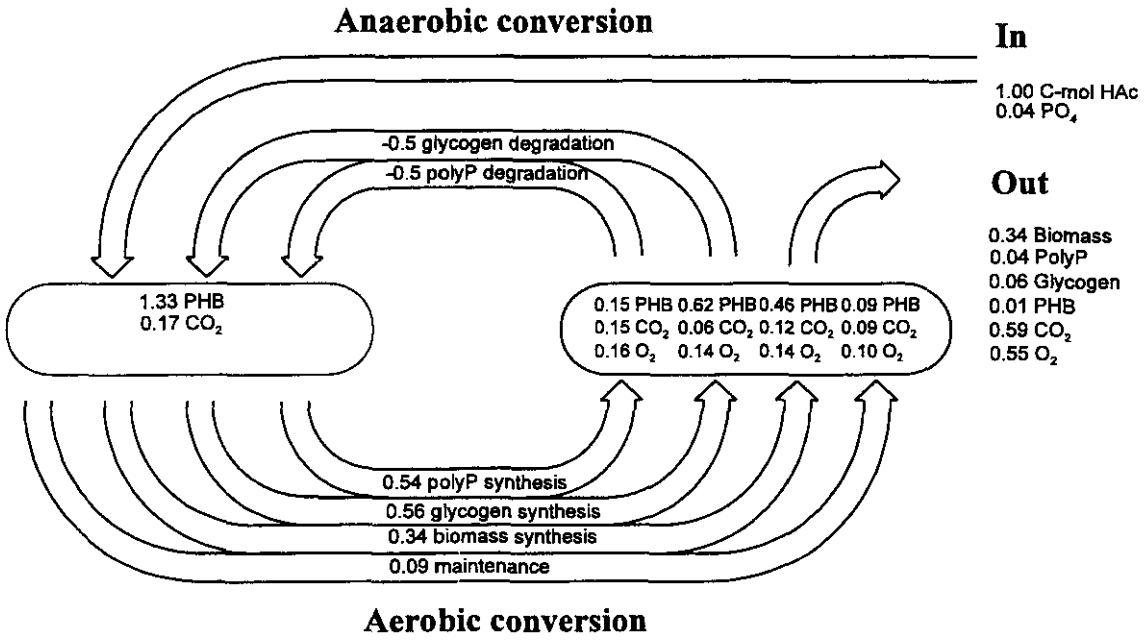
Er kan een relatie tussen de zuurstofconsumptie en ATP produktie afgeleid worden omdat bekend is dat er voor de synthese van 1 mol polyfosfaat 1 mol ATP benodigd is (Kulaev, 1979). In feite kan daarmee δ, de P/O ratio, worden afgeleid. Met deze waarde voor δ, kunnen de maximale yieldwaarden voor groei en glycogeenproductie op PHB worden berekend. De maximale yieldwaarden voor biomassa-, polyfosfaat- en glycogeenproduktie zijn afhankelijk van elkaar omdat ze gekoppeld zijn via de oxydatieve fosforylering. (appendix II).

Tabel 2 *Metabole reacties tijdens de anaërobe en aërobe fase van een biologische fosfaatverwijderingsproces.*

Anaërobe fase		
R_1	Acetaatopname	$-CH_2O - 0.5\ CH_{10/6}O_{5/6} - 0.37\ HPO_3 + 1.33\ CH_{1.5}O_{0.5} + 0.17\ CO_2 + 0.37\ H_3PO_4 + 0.05\ H_2O = 0$
R_2	Onderhoudsenergie	$-HPO_3 - H_2O + H_3PO_4 = 0$
Aërobe fase		
R_3	Biomassavorming	$-1.37\ CH_{1.5}O_{0.5} - 0.20\ NH_3 - 0.015\ H_3PO_4 - 0.42\ O_2 + CH_{2.09}O_{0.54}N_{0.20}P_{0.015} + 0.37\ CO_2 + 0.305\ H_2O = 0$
R_4	Fosfaatopname	$-0.27\ CH_{1.5}O_{0.5} - 0.31\ O_2 - H_3PO_4 + HPO_3 + 0.27\ CO_2 + 1.20\ H_2O = 0$
R_5	Glycogeenvorming	$-1.12\ CH_{1.5}O_{0.5} - 0.26\ O_2 + CH_{10/6}O_{5/6} + 0.12\ CO_2 + 0.007\ H_2O = 0$
R_6	Onderhoudsenergie	$-CH_{1.5}O_{0.5} - 1.125\ O_2 + CO_2 + 0.75\ H_2O = 0$

Het metabolisme van de anaërobe en aërobe fase van de biologische P-verwijdering zijn dus in totaal afhankelijk van 4 fundamentele biochemische coëfficiënten (α_1, δ, K, ϵ) die eerder experimenteel bepaald zijn (Smolders *1994b*). De stoichiometrie van de 4 aërobe reacties werd berekend aan de hand van de gevonden waarden voor de metabole coëfficiënten en is weergegeven in tabel 2.

Met de stoichiometrie van anaërobe en aërobe fase kunnen de conversies van alle componenten tijdens een cyclus van de biologische fosfaatverwijdering berekend worden. Wanneer een systeem in steady state is moet de situatie aan het begin van de cyclus steeds identiek zijn aan de vorige cyclus. Omdat bekend is hoeveel slib afgevoerd gaat worden in elke cyclus (afhankelijk van de slibleeftijd) is bekend hoeveel biomassa glycogeen en polyfosfaat geproduceerd gaat worden. Een voorbeeld van de omzetting van 1 C-mol Acetaat is weergegeven in figuur 7 voor een slibleeftijd van 8 dagen. Opvallend is dat voor de 4 aërobe processen (maintenance, groei, glycogeen en polyfosfaat) ongeveer evenveel zuurstof benodigd is.



Figuur 7 *Conversies (in C-mol) tijdens de anaërobe en aërobe fase van de biologische fosfaatverwijdering, na toediening van 1 C-mol acetaat, SRT 8 dagen.*

2.3 Kinetiek

De conversiesnelheden van alle componenten tijdens de anaërobe en aërobe fase kunnen worden berekend wanneer de snelheden van reactie $R_{1..6}$ van tabel 2 bekend zijn. De stoichiometrie van deze reacties kan worden weergegeven in een stoichiometrie matrix α, waarbij de relevante componenten staan weergegeven in de rijen, en de stoichiometrie van de reacties in de kolommen. We krijgen nu twee stoichiometrische matrices α, één voor de anaërobe fase α^{an} en een voor de aërobe fase $^{aer}\alpha$. De conversiesnelheden van de componenten kunnen weergegeven worden met de vector r, waarbij de subscripts analoog zijn aan de componenten genoemd in tabel 1.

$$r = \begin{pmatrix} r_s \\ r_p \\ r_x \\ r_{phb} \\ r_{pp} \\ r_{gl} \\ r_n \\ r_o \\ r_c \\ r_w \end{pmatrix} \quad \alpha^{an} = \begin{pmatrix} -1 & 0 \\ 0.44 & 1 \\ 0 & 0 \\ 1.33 & 0 \\ -0.44 & -1 \\ -0.5 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0.17 & 0 \\ -0.023 & -1 \end{pmatrix} \quad \alpha^{aer} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ -0.015 & -1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ -1.37 & -0.27 & -1.12 & -1 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ -0.2 & 0 & 0 & 0 \\ -0.42 & -0.31 & -0.26 & -1.125 \\ 0.37 & 0.27 & 0.12 & 1 \\ 0.30 & 1.20 & 0.007 & 0.75 \end{pmatrix}$$

(2)

De specifieke snelheden (op actieve biomassa basis oftewel uitgedrukt in mol per C-mol biomassa per uur) voor reactie $R_{1..6}$ kunnen weergegeven worden met de vector q. Dat levert twee vectors, q^{an} en q^{aer}, voor de anaërobe en aërobe fase op.

Anaëroob

De vector q^{an} beschrijft specifieke snelheden op actieve biomassabasis van reactie R_1 en R_2 gedurende de anaërobe fase. De overall waargenomen snelheid van R_1 is gelijk aan de acetaat opnamesnelheid q_s terwijl de snelheid van reactie R_2 bepaald is door de maintenance coëfficiënt m_{an}.

$$q^{an} = \begin{pmatrix} q_s \\ m_{an} \end{pmatrix} \tag{3}$$

De specifieke acetaatopname snelheid wordt bepaald door de acetaat concentratie volgens de Monod relatie. De anaërobe maintenance coëfficiënt m_{an} is constant veronderstelt en uitgedrukt als mol fosfaat/C-mol biomassa.h.

Aëroob

De vector q^{aer} beschrijft de snelheden van reactie R_3 tot R_6 (eq 4). De reactiesnelheid van R_3, de productiesnelheid van biomassa, wordt bepaald door de groeisnelheid μ, terwijl de synthese van polyfosfaat (R_4) en glycogeen (R_5) respectievelijk bepaald zijn door q_{pp} en q_{gl}. De aërobe maintenance (R_6) is beschreven met m_{aer}.

$$q^{aer} = \begin{pmatrix} \mu \\ q_{pp} \\ q_{gl} \\ m_{aer} \end{pmatrix} \quad (4)$$

Alle conversiesnelheden kunnen nu worden berekend volgens:

$$r = \alpha \cdot q \cdot C_x \quad (5)$$

De concentraties tijdens de anaërobe en aërobe fase van de SBR kunnen worden berekend door integratie van vergelijking (4) over de totale lengte van de anaërobe en aërobe fase.

$$\frac{dC}{dt} = r \quad (6)$$

De conversiesnelheden voor PHB en zuurstof tijdens de aërobe fase worden alsvolgt gegeven:

$$-r_{phb} = 1.37\ \mu\ C_x + 0.27\ q_{pp}\ C_x + 1.12\ q_{gl}\ C_x + m_{aer}\ C_x \quad (7)$$

$$-r_o = 0.42\ \mu\ C_x + 0.31\ q_{pp}\ C_x + 0.26\ q_{gl}\ C_x + 1.125\ m_{aer}\ C_x \quad (8)$$

Slechts vier kinetische relaties zijn dus tijdens de aërobe fase benodigd (groeisnelheid μ, polyfosfaatsynthese snelheid q_{pp}, glycogeen productie snelheid q_{gl}, en de maintenance coefficient m_{aer}) om de conversiesnelheden van alle andere componenten te beschrijven. In Appendix VI is dit model en de bijbehorende parameters opgenomen in IAWQ format, daarbij zijn de conventionele dimensies (mg P, mg CZV) gebruikt.

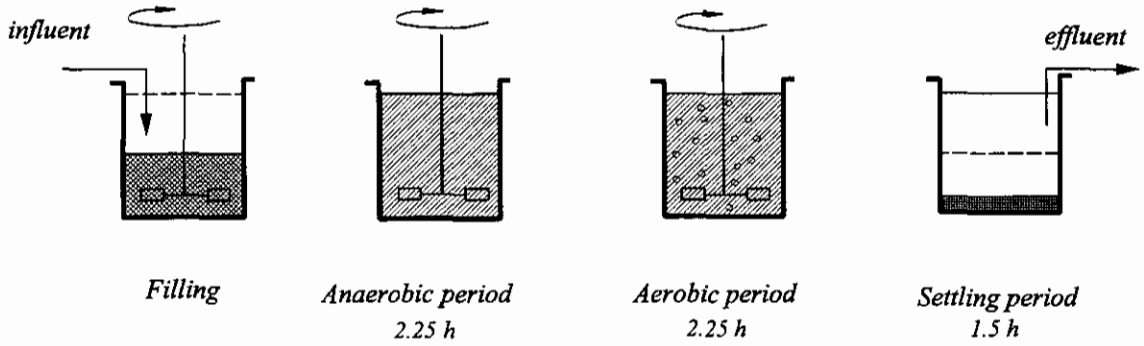
De groei van polyP-organismen wordt bepaald door het PHB-gehalte van de cellen, dat vaak ook het enige beschikbare substraat is tijdens de aërobe fase. De groeisnelheid van de organismen wordt verondersteld afhankelijk te zijn van dit PHB-gehalte en wordt beschreven met eerste orde kinetiek. De groeisnelheid wordt uitgedrukt als C-mol actieve biomassa geproduceerd per C-mol actieve biomassa per uur.

De specifieke synthesesnelheid van polyfosfaat (q_{pp}) is afhankelijk van een drietal factoren: *de externe fosfaatconcentratie* (C_p), *het PHB gehalte* (f_{phb}) *en het* polyfosfaatgehalte (f_{pp}). De polyfosfaat synthesesnelheid hangt af van de fractie polyfosfaat dat reeds in de cellen ligt opgeslagen. De fosfaatopname capaciteit van polyP-organismen is beperkt tot op zekere hoogte. Wanneer het maximale polyfosfaatgehalte (f_{pp}^{max}) van de cellen is bereikt zal de opnamesnelheid van fosfaat nul worden. Het maximale polyfosfaatgehalte van polyP-organismen dat werd waargenomen ligt in de buurt van 0.32-0.38 mgP/mgVSS. Een maximum van 0.3 P-mol/C-mol voor f_{pp}^{max} werd gebruikt in het model.

Glycogeen is een essentiele component van het anaërobe metabolisme. Als er een tekort aan glycogeen is kan de anaërobe opname van acetaat stoppen, door een gebrek aan NADH in de omzetting van acetaat naar PHB. Om een tekort aan glycogeen tijdens de anaërobe fase te voorkomen is het waarschijnlijk dat de produktie van glycogeen zodanig geregeld is dat een bepaald maximum nagestreeft wordt. Bovendien is dit maximum afhankelijk van het PHB gehalte dat in de cellen in de anaërobe fase wordt aangetroffen.

Ter verduidelijking is het goed om hier te stellen dat de kinetiek zoals die in het model wordt gebruikt gekozen is op basis van eenvoud een slechts empirisch bepaald is. Er getracht middels de meest eenvoudige set vergelijkingen een consistente set parameters te verkrijgen waarmee alle experimentele data kunnen worden verwerkt.

2.4 Materialen en Methoden

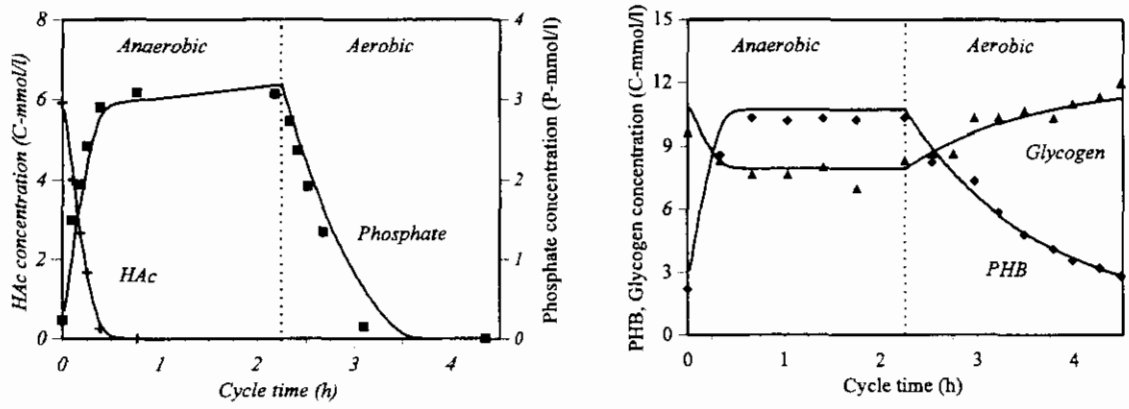


Figuur 8. *Bedrijfsvoering van de sequenced batch reactor (SBR)*

Het onderzoek werd uitgevoerd in een sequencing batch reactor (SBR) met een volume van 2 liter, bij 20 C en pH 7. De reactor werd bedreven met een cyclustijd van 6 uur waarvan 2.25 uur anaëroob, 2.25 uur aëroob en 1.5 uur bezinking (figuur 8). Biologisch P-verwijderend slib werd gebruikt als ent. Gesteriliseerd synthetisch afvalwater werd gebruikt als influent met acetaat als substraat. Eén liter influent met 400 mg COD/l, 28 mg N/l en 15 mg P/l werd bij elke start van een nieuwe cyclus toegevoegd en effluent werd weggepompt aan het eind van een cyclus. Aan het eind van de aërobe fase werd slib weggepompt, afhankelijk van de gewenste slibleeftijd. Omdat al het acetaat in de anaërobe fase volledig werd omgezet, vond er alleen accumulatie plaats van organismen die in staat zijn tot anaërobe acetaatconsumptie. Nitrificatie vond niet plaats, hetgeen gecontroleerd middels metingen van zowel nitriet als nitraat.

2.5 Resultaten

Figuur 9 *Gemeten en berekende concentraties tijdens een cyclus van het*



fosfaatverwijderingsproces.
Links (a): Acetaat en Fosfaat; Rechts (b) PHB en Glycogeen.

De stoichiometrische parameters werden bepaald zoals vermeld in appendix I en II, en zijn weergegeven in tabel 2. De kinetische parameters werden bepaald door meerdere cycli in de SBR te volgen waarbij zowel de anaërobe als aërobe fase intensief bemonsterd werden. Dit is beschreven in appendix III. In tabel 3 staan de waarden van de kinetische parameters vermeld.

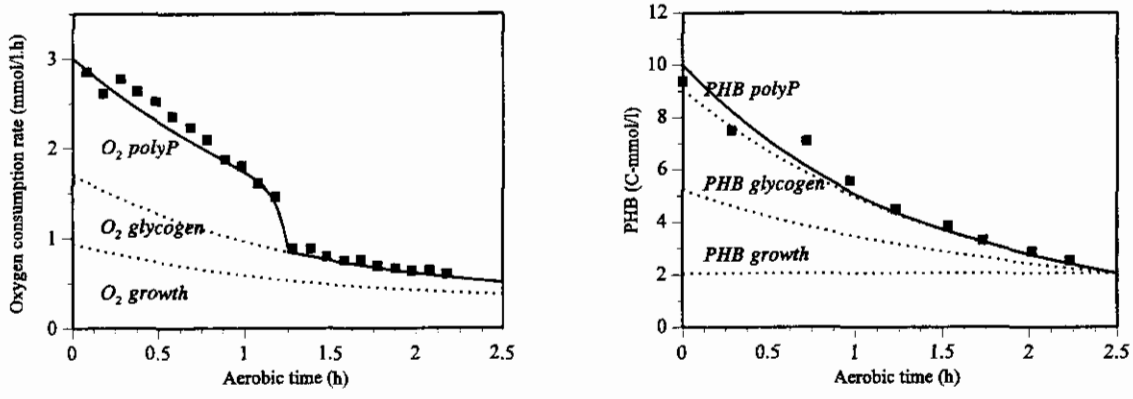
In figuur 9a worden zowel de berekende acetaat- en fosfaatconcentraties tijdens de anaërobe en aërobe fase als de PHB- en glycogeenconcentraties (9b) vergeleken met de metingen tijdens een cyclus. De PHB-consumptie tijdens de aërobe fase is een gevolg van de gelijktijdige biomassa-, polyfosfaat- en glycogeensynthese en maintenance.

De anaërobe maintenance coefficient werd bepaald uit de anaërobe fosfaat afgifte in afwezigheid van een koolstofbron (appendix III). De aërobe maintenance werd bepaald uit de endogene zuurstofconsumptiesnelheid (appendix II).

De zuurstofconsumptiesnelheid tijdens de aërobe fase werd gemeten met een respirometer (appendix II). De zuurstofconsumptiesnelheid is proportioneel met de snelheid van de oxidatieve fosforylering, de productie van ATP, en geeft de snelheid van de interne processen weer. De waargenomen zuurstofconsumptiesnelheid is daarom gelijk aan de som van de zuurstof gebruikt voor groei, polyfosfaat synthese, glycogeen productie en maintenance (zie vergelijking 8). Figuur 10a laat het samengestelde zuurstofverbruikssnelheid tijdens de aërobe fase zien. De

zuurstofconsumptiesnelheid en het cummulatieve zuurstofverbruik werden gebruikt voor het modelmatig vaststellen van de parameters voor groei, polyfosfaat en glycogeen synthese.

De onderste curve in figuur 10a is de berekende bijdrage in de zuurstofconsumptie tengevolge van groei en maintenance, de middelste tengevolge van glycogeensynthese en de bovenste is de totale zuurstofconsumptie snelheid inclusief het verbruik voor de fosfaatopname en polyfosfaatsynthese. Analoog aan de zuurstofconsumptie, kan ook de bijdrage van groei, polyfosfaat en glycogeen synthese in het PHB verbruik berekend worden, wat is weergegeven in figuur 10b. Het is duidelijk dat alleen een klein deel van de PHB gebruikt wordt voor de opname van fosfaat en synthese tot polyfosfaat, terwijl de zuurstofconsumptie sterk beïnvloed wordt door de P-opname.



Figuur 10 *Bijdrage van groei, polyfosfaat- en glycogeensynthese in de totale zuurstofverbruikssnelheid (links, a) en* PHB conversie (rechts, b)

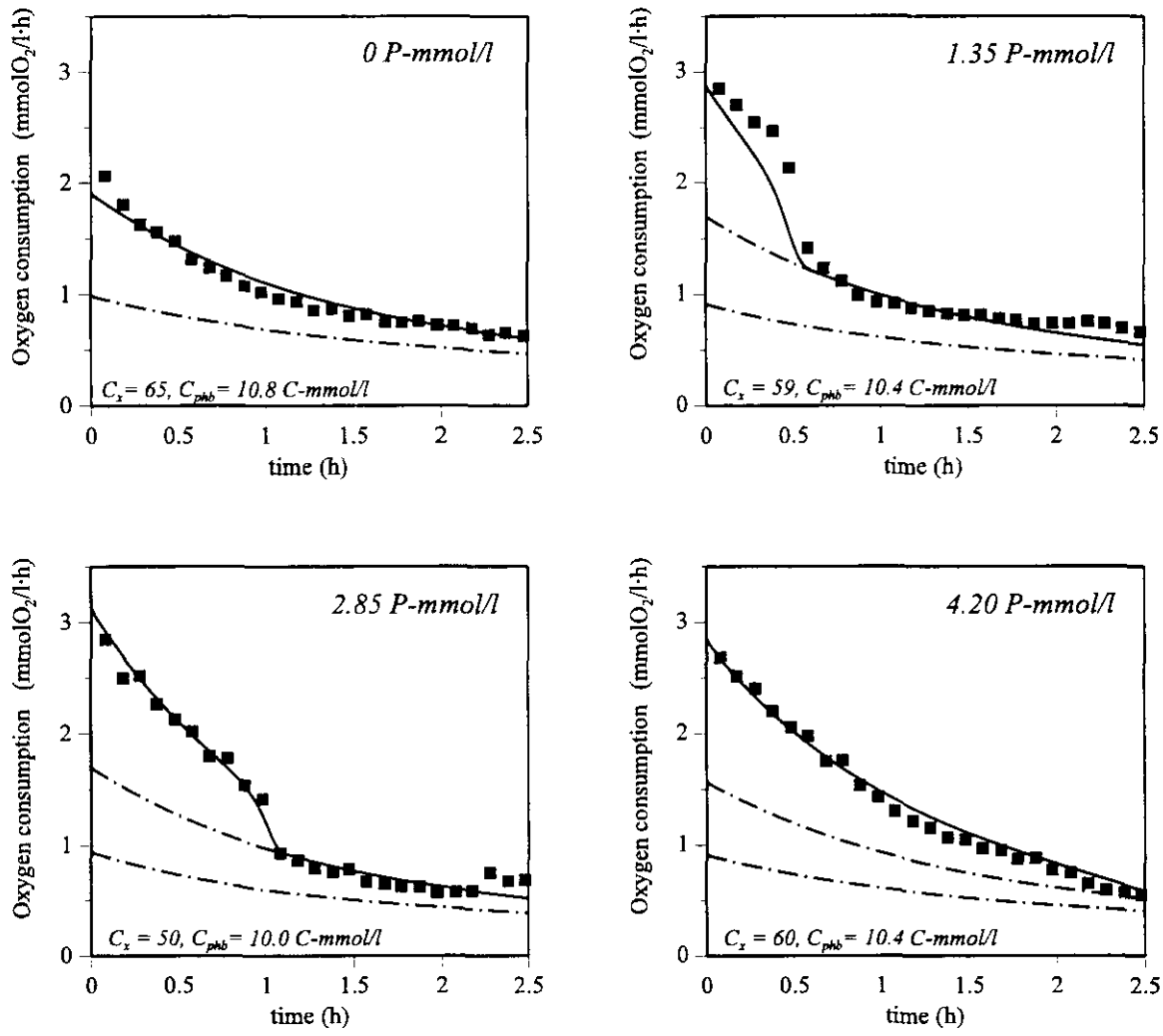
Effect van verschillende fosfaatconcentraties

Om het effect van verschillende fosfaatconcentraties op het zuurstofverbuik en de fosfaatopnamesnelheid te onderzoeken, zijn experimenten uitgevoerd bij verschillende initiële fosfaatconcentraties in een range van 0 tot 6·10-3 P-mol/l. Deze experimenten zijn gebruikt om de voorspellingen, verkregen op basis van het model, te toetsen. Het effect op de zuurstofverbuikssnelheid tijdens deze experimenten is weergegeven in figuur 11.

Validatie van het model onder dynamische condities

Fosfaatverwijderende organismen groeien tijdens aërobe omstandigheden op intern opgeslagen PHB. De vraag is hoe bij deze organismen de groeisnelheid is geregeld. Bij de biologische fosfaatverwijdering in een sequencing batch reactor wordt de

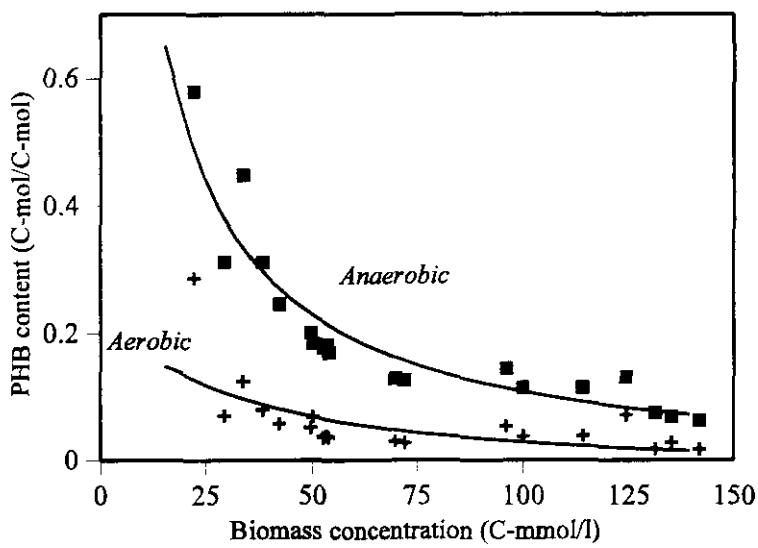
biomassaconcentratie bepaald door de balans tussen de slibafvoersnelheid aan het eind van het proces en de biomassaproduktie tijdens de aërobe fase. De groeisnelheid tijdens de aërobe fase is afhankelijk van het PHB-gehalte van de cellen, dat voornamelijk bepaald wordt door de verhouding tussen acetaattoevoer en biomassa aanwezig in de reactor, de specifieke acetaatbelasting. Een hoge belasting zal leiden tot een hoog PHB-gehalte en daardoor tot een hoge groeisnelheid. De relatie tussen de slibafvoersnelheid en de biomassaconcentratie beïnvloed bovendien de fracties van polyfosfaat en glycogeen in grote mate.



Figuur 10 *Toetsing van het model: gemeten en berekende zuurstofverbuikssnelheid tijdens experimenten met verschillende initiële fosfaatconcentraties.*

Het metabole model werd getoetst door te onderzoeken of het in staat was de conversies te beschrijven van het proces bij een aantal verschillende slibleeftijden. Het bleek dat een enkele set parameters in staat was de conversies van alle reacties als functie van de slibleeftijd te beschrijven. Daarnaast beschreef het model met deze

parameterset de dynamica van alle componenten tijdens de anaërobe/aërobe cyclus. Met het verhogen van de acetaat/biomassa verhouding daalt de biomassaconcentratie en daarmee de hoeveelheid polyP in de reactor. Daardoor wordt op een gegeven moment het polyfosfaat limiterend voor de opname van acetaat en is de maximale gemiddelde groeisnelheid bereikt. Dit punt ligt bij een cyclusverdeling zoals gebruikt in dit onderzoek bij een groeisnelheid van 0.04 h^{-1}. Dit komt overeen met een slibleeftijd van 1 dag.



Figuur 11 *PHB gehalte aan het eind van de anaërobe en aërobe fase als functie van de biomassaconcentratie. De lijnen zijn de modelvoorspellingen voor de anaërobe en aërobe fase.*

Opstart

Het model werd opgesteld onder condities waarbij een stabiele biomassaconcentratie was verkregen. Vervolgens werd aangetoond dat het metabole model in staat is de dynamische omstandigheden die ontstaan tijdens de opstart van het proces in aan- en afwezigheid van heterotrofe organismen te beschrijven met dezelfde set parameters die eerder bepaald was, op de acetaatopnamesnelheid na. In een SBR werden experimenten uitgevoerd waarbij de verrijking van het slib met polyP-organismen werd gevolgd nadat een populatie met heterotrofe organismen beënt werd met een kleine hoeveelheid polyP-organismen. Het effect van verschillende influenttoevoerpatronen voor acetaat en fosfaat werd bestudeerd. De hoogste groeisnelheid van de polyP-organismen die in deze experimenten werd waargenomen was 0.1 h^{-1} terwijl de hoogste gemiddelde groeisnelheid over de gehele aërobe fase 0.045 h^{-1} was. Tijdens de opstart wordt de groeisnelheid van de polyP-organismen bepaald door de aanwezigheid en concentratie van acetaat. De groeisnelheid bleek verder gelimiteerd te worden door de

aanwezigheid van het polyfosfaat, dat op zijn beurt afhankelijk is van de fosfaatopnamesnelheid tijdens aërobe omstandigheden. De aanwezigheid van heterotrofe, geflocculeerde organismen die geen polyfosfaat ophopen verstoort de ingroei van polyP-organismen niet.

3 STEADY-STATE ANALYSE VAN DE FOSFAATVERWIJDERINGS-CAPACITEIT EN ACETAATBEHOEFTE VAN HOOFDSTROOM- EN DEELSTROOMPROCESCONFIGURATIES

3.1 Inleiding

Het ontwerp van biologische P-verwijderende installaties is vaak problematisch door het grote aantal mogelijke procesconfiguraties, de hoge mate van complexiteit van het fosfaatverwijderingsproces en de uitgebreide modellen die een groot aantal parameters gebruiken om het proces te simuleren. In de laatste decennia werden vele verschillende procesconfiguraties ontwikkeld voor de biologische fosfaatverwijdering. De eerste stap in het ontwerp van een P-verwijderende installatie is de keuze van een geschikte procesconfiguratie bij een bepaald afvalwater. Een duidelijk en snel concept om verschillende procesconfiguraties met elkaar te kunnen vergelijken is daarom benodigd in deze fase. De procesconfiguraties voor de P-verwijdering kunnen worden onderverdeeld in hoofdstroom- en deelstroomprocessen, wat aangeeft of de anaërobe fase benodigd voor de P-verwijdering zich bevindt in de hoofd of deelstroom van het proces. Belangrijke punten in de evaluatie van het systeem zijn de verwachte fosfaatverwijderingscapaciteit van de reactor en de CZV behoefte van het systeem om volledige P-verwijdering te krijgen.

Complexe dynamische modellen voor het ontwerp van biologische fosfaatverwijderende systemen zijn ontwikkeld, gebaseerd op de aannamen voor de mechanismen van de P-verwijdering. Recentelijk bracht de IAWQ "Task group modelling activated sludge processes" model no. 2 uit. Dit model is een uitbreiding van het eerder gepubliceerde model no. 1 (dat alleen CZV en N eliminatie beschrijft) met biologische fosfaatverwijdering. Dit model is vooral vanuit een black box benadering opgesteld. Met bekende biochemische mechanismen wordt maar ten dele rekening gehouden. Het model zoals in dit rapport beschreven (zie hoofdstuk 1) is wel gebaseerd op bekende biochemische processen. Al deze dynamische modellen zijn vrij complex en gebruiken een groot aantal stoichiometrische en kinetische parameters. Voor het ontwerp van biologische P-verwijderende installaties zijn in eerste instantie niet al deze complexe modellen nodig. In dat geval is slechts een steady state benadering die alleen op stoichiometrie is gebaseerd nodig.

Voor het dimensioneren van zuiveringsprocessen is de traagste stap in het proces bepalend voor de grootte van de benodigde reactoren. In een biologische fosfaateliminatieproces zijn de defosfaterende organismen vaak niet de snelheidsbeperkende stap. De lengte van de anaërobe fase wordt veelal bepaald door de snelheid waarmee influent-CZV in vetzuren worden omgezet door fermentatieve

bacteriën. De lengte van de aërobe periode wordt bepaald door de nitrificatie snelheid. Dit betekend dat alleen een stoichiometrische berekening vereist is om de fosfaatverwijderingscapaciteit en de acetaat-CZV-behoefte te berekenen (Acetaat vormt het hoofdproduct van een verzuringsproces). Dit betekend dus dat alleen een steady state model nodig is om de P-eliminatie capaciteit te kunnen berekenen als functie van de Acetaat-CZV in het influent (of in een deelstroomproces gedoseerd wordt).

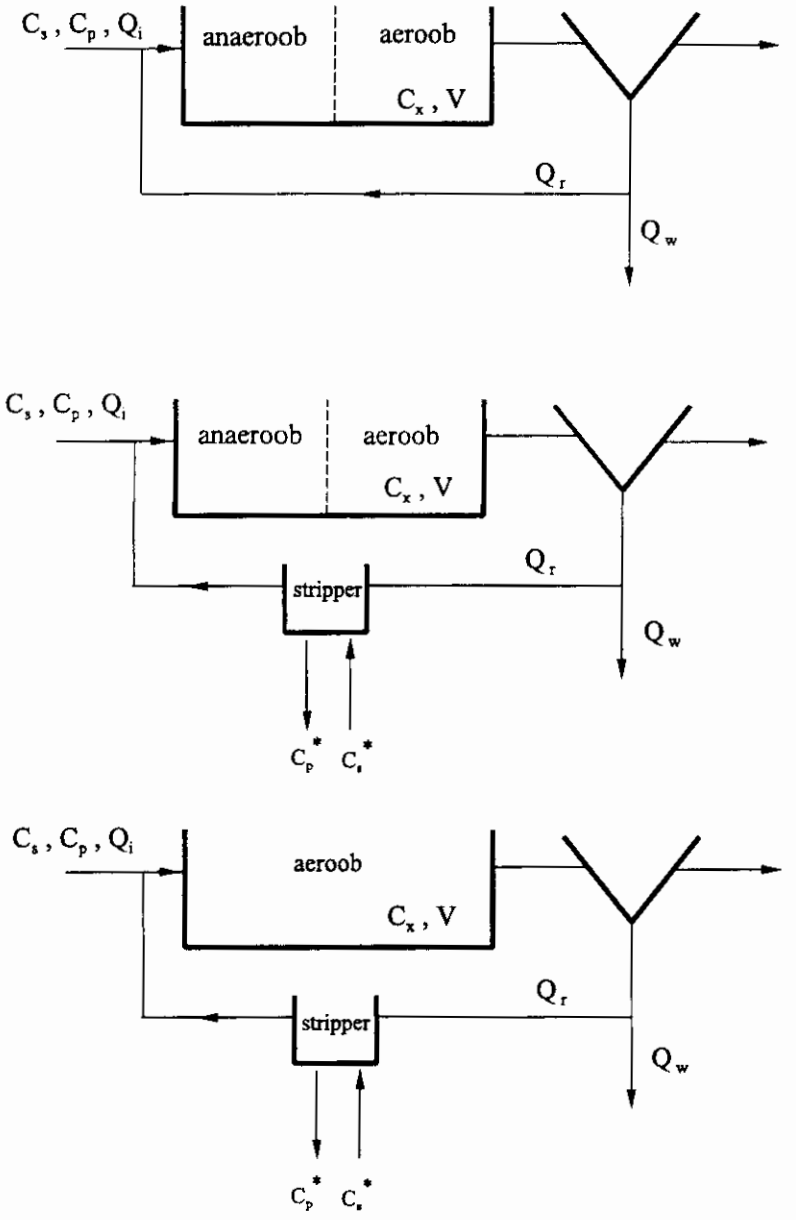
Een steady state model voor de schatting van de effluentfosfaatconcentratie van een hoofdstroom systeem werd voor het eerst ontwikkeld door Wentzel (1990) als een simplificatie van het dynamische model. Maurer en Gujer (1994) ontwikkelden een statisch model voor de berekening van de effluentfosfaatconcentraties van een P-verwijderend proces waarbij het fosfaatgehalte van de cellen constant veronderstelt wordt. Enkel de maximale P-opname capaciteit wordt berekend met dit model. Beide analysemethoden zijn beperkter bruikbaar dan de analyse die in dit hoofdstuk beschreven wordt.

3.2 Doel

Het doel is een methode te ontwikkelen om verschillende procesconfiguraties te kunnen vergelijken met betrekking tot de P-opnamecapaciteit en CZV-behoefte voor de P-verwijdering op basis van de influentkarakteristieken. Drie verschillende P-verwijderende procesconfiguraties zullen daarbij beschouwd worden. Gebaseerd op de fosfaatconcentratie van het influent en de systeemconfiguratie zal de benodigde concentratie polyP-organismen in het systeem berekend worden. Daarna kan de benodigde hoeveelheid acetaat voor de groei van deze organismen worden bepaald. Deze benodigde acetaatconcentratie kan vergeleken worden met het acetaat-CZV in het influent. De werkelijke acetaatconcentratie in het influent kan bepaald worden middels directe meting middels gaschromatografie. Daarnaast kan door een meting van de P-afgifte in een batchtest een schatting worden gemaakt van de hoeveelheid acetaat-CZV die via fermentatie beschikbaar kan worden gemaakt voor de defosfaterende microorganismen. Indien er geen gedetailleerde gegevens beschikbaar zijn kan voor een eerste inschatting de hoeveelheid snel biodegradeerbaar CZV als maat voor de beschikbare Acetaat-CZV worden genomen. Indien het in het influent aanwezige acetaat-CZV niet voldoende is kan berekend worden hoeveel acetaat er aan het proces zou moeten worden gedoseerd voor een optimale P-eliminatie. De term CZV die in de tekst hieronder gebruikt wordt slaat steeds op acetaat-CZV. De waarden van de stoichiometrische parameters die bepaald werden bij de ontwikkeling van het metabole model worden gebruikt als uitgangspunt voor de steady state vergelijking van verschillende procesconfiguraties.

3.3 Procesconfiguraties

Drie basisconfiguraties voor de biologische fosfaatverwijdering zullen beschouwd worden in dit verhaal (figuur 13). Dat zijn het hoofdstroom proces, het hoofdstroom/stripper-proces en het deelstroomproces. Een hoofdstroom- en deelstroomproces zijn genoemd naar de plaats van de anaërobe reactor benodigd voor de P-verwijdering in het systeem: in de (hoofd-) waterstroom of in de (deel-)slibstroom. In een hoofdstroomproces wordt al het fosfaat opgenomen door de polyP-organismen

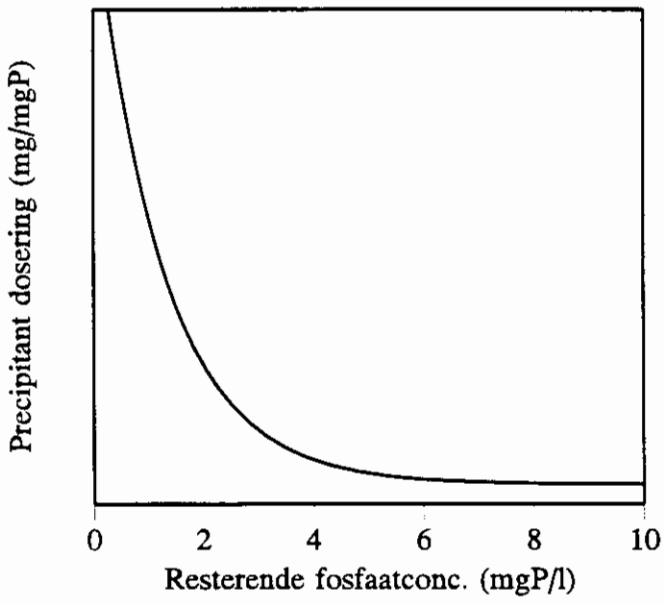


Figuur 13 *Schematische weergave van verschillende biologische fosfaat-verwijderende processen; boven: hoofdstroomproces, midden: hoofd-stroomproces met stripper, en onder: deelstroomproces*

en vindt de verwijdering van fosfaat enkel plaats door de afvoer van deze organismen via het spuislib. Als de fosfaatconcentratie hoger wordt dan de fosfaatopnamecapaciteit van de organismen in het systeem, kan er extra fosfaat verwijderd worden via een strippertank waarin acetaat wordt gedoseerd. De fosfaat die door de acetaatdosering wordt vrijgemaakt uit de organismen kan geprecipiteerd worden en verlaat, in tegenstelling met het hoofdstroomproces, zowel in geprecipiteerde vorm als via het spuislib het systeem. Aangezien de strippertank tevens dienst doet als anaërobe reactor voor de P-verwijdering, kan de anaërobe fase uit de hoofdstroom gelaten worden. Dit wordt het deelstroomproces genoemd. In de praktijk kunnen de chemicaliën voor fosfaatprecipitatie ook aan het einde van een anaërobe zone in het hoofdstroomproces worden gedoseerd. Berekeningstechnisch komt dit overeen met een extern geplaatste striptank.

Een belangrijk verschil tussen deze twee procesalternatieven is dat in het hoofdstroomproces acetaat uit het influent benut wordt voor de fosfaatverwijdering terwijl in het deelstroomproces dit influent-acetaat niet gebruikt wordt en acetaat-CZV gedoseerd moet worden.

In het hoofdstroom/stripper-proces en het deelstroomproces wordt de biologische fosfaat-verwijdering gecombineerd met een chemische precipitatie methode. De reden voor deze combinatie is dat biologische P-verwijdering het fosfaat concentreert in de biomassa, wat dan vervolgens wordt afgegeven in de stripper tank in geconcentreerde vorm. De chemicaliëndosering van de precipitatie methode is afhankelijk van de verlangde fosfaateffluent kwaliteit (figuur 14). Het voordeel van een strippertank is daarom dat de verlangde effluent fosfaatconcentratie niet gehaald hoeft te worden, omdat het effluent van de stripper teruggevoerd wordt naar de aërobe reactor van het proces. Verder kan een lage chemicaliën/fosfaat-verhouding worden gebruikt omdat de precipitatie-efficiency toeneemt bij hogere fosfaatconcentraties.



Figuur 14. Benodigde precipitant dosering als functie van de effluent fosfaat-concentratie.

3.4 Factoren van invloed op de P-verwijdering

De primaire factor voor de selectie van P-verwijderende bacteriën in actief-slibsystemen, is de recirculatie van het slib door anaërobe en aërobe fasen. In de anaërobe zone (geen electronen-acceptor aanwezig) nemen P-verwijderende bacteriën lagere vetzuren op, voornamelijk acetaat en slaan dit op als poly-hydroxy-alkanoaten (PHA, bv: PHB, PHV). De energie voor het transport en opslag van acetaat wordt geleverd door de hydrolyse van intra-cellulair polyfosfaat tot fosfaat, wat afgegeven wordt door de cellen naar de vloeistof. De reduktie-equivalenten en een deel van het ATP wordt voorzien uit de conversie van glycogeen naar PHB. In de aërobe zone wordt het PHB gebruikt om energie te genereren voor groei en synthese van glycogeen en polyfosfaat wat resulteert in de opname van fosfaat uit de vloeistof.

De potentiële fosfaatopnamecapaciteit van een P-verwijderende installatie wordt bepaald door de concentratie polyP-organismen die in het systeem aanwezig zijn. De hoeveelheid polyP-organismen die in het systeem geproduceerd worden is weer afhankelijk van de anaërobe opname van de lagere vetzuren. De concentratie polyP-organismen is over het algemeen slechts een fractie van de totale biomassaconcentratie in het systeem. Het is daarom gewenst de polyP-biomassaconcentratie te onderscheiden van de totale slibconcentratie. Het gemeten P-gehalte van de totale biomassa in het systeem is dus het gemiddelde van de polyP-organismen en de andere niet-polyP-organismen. Hoewel het polyP-gehalte van het totale slib laag mag lijken, kan het dus zijn dat de kleine fractie polyP-organismen aanwezig in dat slib inmiddels al de maximale fosfaatopnamecapaciteit heeft bereikt. In dat geval heeft de installatie dus al de maximale fosfaatopname capaciteit bereikt, hoewel dit niet blijkt uit het P-gehalte van het slib. Om de fosfaatopname capaciteit van het slib vast te stellen, is het belangrijk om het maximale P-gehalte van de P-organismen, f_{PX}^{max}, en de absolute concentratie P-organismen, C_x^{polyP}, in het systeem te kennen.

3.5 Stoichiometrische parameters voor de bepaling van de fosfaat-opnamecapaciteit

Om de fosfaatverwijderingscapaciteit van een actief-slibsysteem te berekenen is het nodig de volgende drie stoichiometrische parameters te beschouwen:

1) Het maximale fosfaatgehalte van de polyP-bacteriën (f_{PX}^{max})
2) De biomassa yield van polyP-bacteriën op acetaat (Y_{SX})
3) De P-afgifte/acetaat opname verhouding, in de anaërobe fase (Y_{SP})

1) Maximaal P-gehalte van de polyP organismen, f_{PX}^{max}

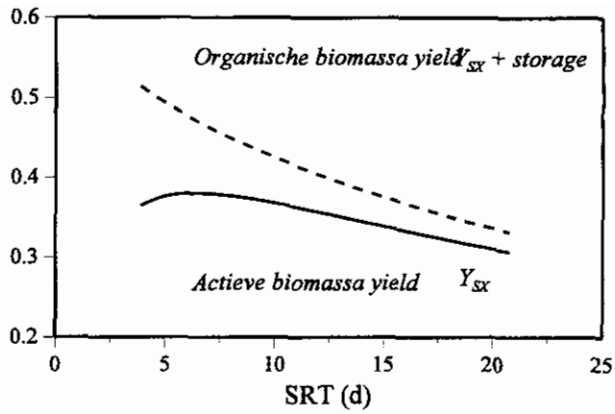
De fosfaatopname door polyP-bacteriën is gebonden aan een maximale waarde. Welke biochemische of energetische limitatie dit maximum veroorzaakt is nog onduidelijk. In de literatuur worden gemiddelde P-gehaltes van polyP-organismen van 140-200 mg P/g VSS (100-145 mg P/g CZV) genoemd voor ophopingscultures die op acetaat ontwikkeld zijn, met een maximum van 380 mg P/g VSS (275 mg P/g CZV; Wentzel et al. 1989). De fosfaatfractie op vaste stof basis (g P/g SS) volgt uit de herberekening van f_{PX}^{max} waarbij een molgewicht van 100 g/mol voor een eenheid polyfosfaat ($K_{1/3}Mg_{1/3}PO_3$) gerekend moet worden. Het fosfaatgehalte van het slib zoals waargenomen in actief-slibsystemen is in het algemeen veel lager, zoals reeds gezegd, door de aanwezigheid van niet-polyP-organismen. Voor het maximale P-gehalte f_{PX}^{max} werd een waarde van 0.15 g P/g biomassa-CZV gebruikt in de berekeningen.

2) Biomassayield van polyP-bacteriën op acetaat, Y_{SX}

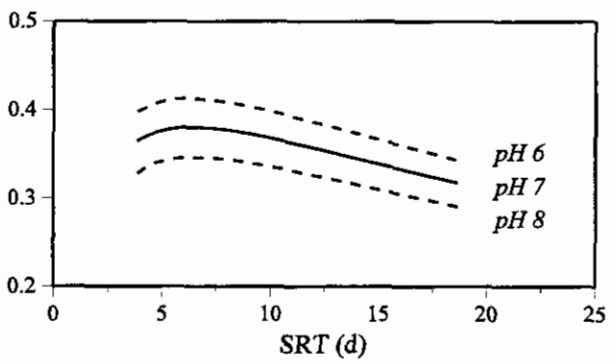
De actieve biomassayield van de polyP-bacteriën is afhankelijk van de groeisnelheid van de organismen welke weer bepaald is door de slibleeftijd (SRT). In figuur 15a is de actieve biomassa (dus zonder opslag produkten) als functie van de slibleeftijd weergegeven. Ook is de organische biomassayield weergegeven als functie van de slibleeftijd. Dit is dus inclusief de opslagproducten PHB en glycogeen. De biomassayield als functie van de SRT is duidelijk niet constant, omdat bij langere SRT er meer energie verloren gaat aan maintenance en de biomassyield dus lager wordt. In figuur 15b is de actieve biomassa weergegeven als functie van de SRT voor drie verschillende pH waarden. De lagere biomassyield bij hogere pH is een gevolg van de toegenomen energiebehoefte voor de opname van acetaat tijdens anaërobe omstandigheden. Figuur 15c laat het effect zien van verschillende fosfaat/acetaat verhoudingen van het influent op de biomassayield bij een pH waarde van 7.0. De waargenomen effecten zijn zeer klein en een gevolg van de toegenomen energie voor de fosfaatopname bij hogere P/CZV verhoudingen.

Uit figuur 15b en 15c kan geconcludeerd worden dat de actieve biomassayield op acetaat zowel niet sterk beïnvloed wordt door de pH als door de P/CZV verhouding van het influent. In theorie zou een lagere SRT gebruikt kunnen worden om de concentratie aan polyP-organismen op te voeren door de hogere yield die dan bereikt wordt en daarmee de fosfaatverwijderingscapaciteit. Echter, het verlagen van de SRT is in het algemeen beperkt door de beperkte groei van nitrificeerders en ook in strijd met het streven naar een lage slibproduktie. Een gemiddelde biomassayield van 0.34 mg CZV/mg HAc-CZV kan gebruikt worden over een range van slibleeftijden van 5 tot 20 dagen bij pH 7. Voor exacte berekeningen dient de actieve biomassayield behorend bij het specifieke geval te worden gebruikt. De berekeningen van figuur 15 zijn gebaseerd op het eerder beschreven metabole model.

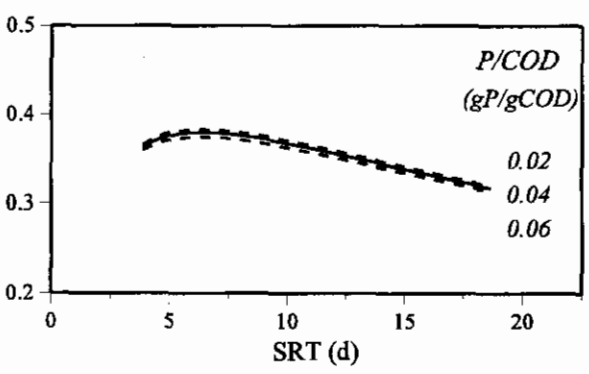
a)



b)



c)

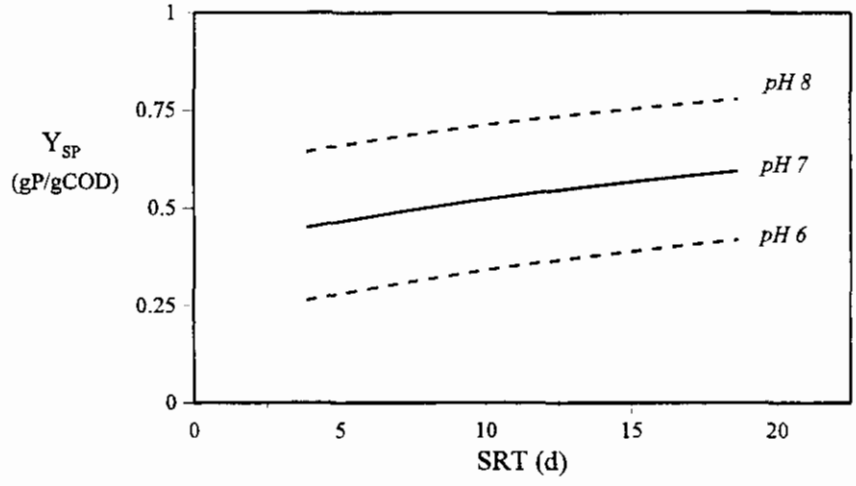


Figuur 15. *Effect van de slibleeftijd: a) op de actieve- en organische biomassayield; b) berekend voor drie slibleeftijden; c) met drie verschillende influent fosfaat/CZV verhoudingen.*

3) fosfaatafgifte/acetaatopname verhouding in de anaërobe fase, Y_{SP}

Wanneer de fosfaatopnamecapaciteit van de polyP-organismen te klein is voor volledige opname van het fosfaat aanwezig in het influent, kan een strippertank gebruikt worden. In een strippertank wordt onder anaërobe condities acetaat-CZV toegevoegd aan de polyP-organismen. De polyP-organismen nemen dit acetaat op waarbij gelijktijdig fosfaat wordt afgegeven, wat dan verwijderd kan worden uit het water door middel van precipitatie. Wanneer een stripper gebruikt wordt in de procesconfiguratie moet de fosfaatafgifte als gevolg van de acetaatdosering bekend zijn om de daling van het polyP-gehalte te kunnen berekenen. De verhouding tussen fosfaatafgifte en acetaat-opname is bovendien niet constant maar sterk beïnvloed door de pH. Wanneer de pH stijgt is meer energie benodigd voor de opname van acetaat en wordt meer fosfaat afgegeven om deze energie te leveren.

In figuur 16 is het pH-effect op de verhouding tussen fosfaatafgifte en acetaatopname weergegeven als functie van de slibleeftijd. De hogere Y_{SP} bij langere slibleeftijden is te danken aan een hogere biomassaconcentratie in de reactor en daardoor ook een hogere bijdrage van anaërobe maintenance-energie. Deze maintenance-energie wordt geleverd door hydrolyse van polyfosfaat. Het effect van verschillende P/CZV verhoudingen in het influent heeft geen significant effect op waargenomen Y_{SP}. In tabel 1 staan de waarden van de in dit hoofdstuk gebruikte parameters.



Figuur 16 *Effect van de slibleeftijd en de pH op de acetaatopname en fosfaatafgifte in de anaerobe fase van een fosfaateliminatieproces.*

3.6 Berekening van de fosfaatopnamecapaciteit

In de analyse wordt aangenomen dat alle reactoren zodanig ontworpen zijn dat het substraat volledig omgezet kan worden en dat de optimale werking van een configuratie berekend wordt. De berekeningen zijn bovendien betrokken op 'steady state'-situaties. Verder is het van belang te weten dat niet al het fosfaat dat in het influent aanwezig is ook daadwerkelijk verwijderd dient te worden door polyP-bacteriën. Een deel van het fosfaat zal gebruikt worden voor de vorming van biomassa van zowel polyP- als niet-polyP-bacteriën. Deze groei is afhankelijk van de slibproduktie en daarmee de slibleeftijd. Verder kan er een fractie zijn die, afhankelijk van de effluenteisen, niet verwijderd hoeft te worden. De hoeveelheid fosfaat die gebruikt wordt voor biomassavorming door niet-polyP-bacteriën is ongeveer 15 mg P/g biomassa CZV. Dit fosfaat is gefixeerd in de biomassa en kan niet vrijgemaakt worden. De hoeveelheid fosfaat dat voor normale celgroei gebruikt wordt kan een aanzienlijk deel zijn van de totale fosfaatbelasting van de installatie en moet dus in rekening worden gebracht. Zie voor een voorbeeld het intermezzo. De overblijvende fosfaatconcentratie is de hoeveelheid die verwijderd moet worden door polyP-organismen. In de berekeningen hieronder, wordt met de influentfosfaatconcentratie alleen deze fractie bedoelt.

INTERMEZZO

Om de fosfaatopnamecapaciteit van een biologisch fosfaatverwijderend proces te berekenen, moet het deel van het influentfosfaat dat daadwerkelijk verwijderd moet worden door bio P-organismen bekend zijn. Niet al het fosfaat hoeft namelijk door de biologische fosfaatverwijdering verwijderd te worden. Een deel van het fosfaat zal gebruikt worden voor celgroei van niet polyP-organismen en een deel zal niet verwijderd hoeven worden afhankelijk van de effluenteis. Enkel het resterende deel dient verwijderd te worden. Voor een influent met 450 mg CZV/l en een effluenteis voor CZV van 50 mg CZV/l, zal 400 mg CZV/l verwijderd moet worden in het systeem. Wanneer deze CZV wordt omgezet met een typische aërobe biomassayield van 0.34 g CZV/g CZV, afhankelijk van de SRT van het systeem, dan zal 136 mg CZV/l biomassa geproduceerd worden. Voor de biomassaproduktie is 15 mg P/g CZV nodig en daarom zal 2 mg P/l van het influentfosfaat daarvoor gebruikt worden. Wanneer de influent P-concentratie 8 mg P/l is, en een effluentfosfaatconcentratie van 1 mg P/l wordt vereist, zal slechts 5 mg P/l verwijderd hoeven te worden middels biologische P-verwijdering.

Criteria voor fosfaatverwijdering in een hoofdstroomproces.

Om vast te stellen of de fosfaatopnamecapaciteit van een hoofdstroomproces voldoende zal zijn, moeten twee factoren bekend zijn. Dat zijn de concentratie polyP-organismen die in het proces geaccumuleerd gaan worden en het fosfaatgehalte van deze organismen. In figuur 13 zijn de verschillende variabelen weergegeven die hieronder gebruikt worden. De volumetrische belastingen van acetaat (r_S , g/l·uur) en fosfaat (r_P , g/l·uur) hangen af van het influentdebiet (Q_i), het totale reactorvolume en de concentratie van substraat in het influent (C_S, C_P) volgens:

$$r_S = \frac{Q_i}{V} \cdot C_S = \frac{C_S}{\tau} \tag{9}$$

$$r_P = \frac{Q_i}{V} \cdot C_P = \frac{C_P}{\tau} \tag{10}$$

waarbij τ de hydraulische verblijftijd is, die gedefinieerd is volgens:

$$\tau = \frac{V}{Q_i} \tag{11}$$

De produktiesnelheid van polyP-biomassa (r_X in g CZV/l·uur) wordt gegeven door de volgende relatie:

$$r_X = Y_{SX} \cdot r_S \tag{12}$$

De biomassayield verminderd van 0.38 - 0.31 mg CZV/mg CZV bij een toename in de slibleeftijd van 5 tot 20 dagen zoals weergegeven in figuur 15. Dit effect van de SRT op de biomassayield heeft slechts een klein effect op de uiteindelijke resultaten. De SRT is gegeven volgens:

$$SRT = \frac{C_X^{polyP}}{r_X} \tag{13}$$

waarin C_X^{polyP} de concentratie polyP-biomassa in g CZV/l reactorvolume is.

Het fosfaatgehalte van het slib, (f_{PX} als g P/g CZV actieve polyP-biomassa) kan nu berekend worden. De fosfaatbelasting van het systeem r_P en de polyP-biomassa-produktiesnelheid bepalen het fosfaatgehalte f_{PX} zoals weergegeven in vergelijking (14). De produktiesnelheid van polyP-bacteriën wordt bepaalt door de substraatbelasting van het systeem en de yield volgens vergelijking (4). Het blijkt dat enkel de biomassyield en de fosfaat/acetaat verhouding in het influent het fosfaatgehalte van de polyP-organismen bepalen:

$$f_{PX} = \frac{r_P}{r_X} = \frac{1}{Y_{SX}} \cdot \frac{C_P}{C_S} \qquad (14)$$

Uit vergelijking (14) volgt direct dat het fosfaatgehalte van de organismen stijgt wanneer de influent fosfaatconcentratie toeneemt. Bij een bepaalde fosfaatconcentratie van het influent wordt het maximale P-gehalte van de organismen bereikt en kan er niet meer fosfaat worden opgenomen. Deze restrictie is de maximale fosfaat/acetaat verhouding van het influent en kan uitgedrukt worden als:

$$\left(\frac{C_P}{C_S}\right)^{max} = Y_{SX} \cdot f_{PX}^{max} \qquad (15)$$

De maximale fosfaat/acetaat verhouding van het influent van een hoofdstroomproces is dus afhankelijk van het maximale P-gehalte van de polyP-organismen. Dit maximale P-gehalte van de organismen kan ook gebruikt worden om de minimaal benodigde hoeveelheid polyP-organismen in het proces te berekenen om al het fosfaat dat aanwezig is in het influent op te nemen. Combinatie van vergelijking (10), (13) en (14) geven deze minimaal benodigde concentratie polyP-organismen voor volledige P-verwijdering:

$$\left(C_X^{PolyP}\right)^{min} = \frac{1}{f_{PX}^{max}} \cdot \frac{C_P}{\tau} \cdot SRT \qquad (16)$$

Combinatie van vergelijkingen (9), (12) en (13) geeft de maximaal bereikbare concentratie polyP-organismen gebaseerd op de hoeveelheid acetaat aanwezig in het influent:

$$\left(C_X^{polyP}\right)^{max} = Y_{SX} \cdot \frac{C_S}{\tau} \cdot SRT \qquad (17)$$

Voor elke situatie dient de minimaal benodigde hoeveelheid polyP-organismen (16) en de maximaal bereikbare hoeveelheid (17) met elkaar vergeleken te worden. Hieruit volgt dat:

$$\frac{\left(C_X^{polyP}\right)^{min}}{\left(C_X^{polyP}\right)^{max}} = \frac{1}{Y_{SX} \cdot f_{PX}^{max}} \cdot \frac{C_P}{C_S} \qquad (18)$$

Wanneer de minimaal benodigde hoeveelheid biomassa lager is dan de maximaal bereikbare, dan is er voldoende acetaat aanwezig in het influent om al het fosfaat te verwijderen (linkerdeel figuur 17). De gehele fosfaataanvoer kan via het spuislib in een hoofdstroomproces worden verwijderd. Wanneer de maximaal bereikbare polyP-biomassa lager is dan de minimaal benodigde voor volledige P-opname, moeten een striptank in het proces worden geïncorporeerd.

Hoofdstroom $$\frac{C_P}{C_S} < Y_{SX} \cdot f_{PX}^{max} \qquad (19)$$

Hoofdstroom/stripper of deelstroom $$\frac{C_P}{C_S} > Y_{SX} \cdot f_{PX}^{max} \qquad (20)$$

Fosfaatstrippen in een hoofdstroomproces

Wanneer de maximaal bereikbare polyP-biomassaconcentratie lager is dan de minimaal benodigde voor volledige P-opname, kan gebruik gemaakt worden van een strippertank, (figuur 13b). In de stripper leidt de dosering van acetaat tot fosfaatafgifte. Het slib wordt na deze tank gescheiden en teruggevoerd naar het proces terwijl het afgegeven fosfaat verwijderd wordt door precipitatie. Om de berekeningen te vereenvoudigen, worden de toegevoegde acetaat (F_A in g/uur) en geprecipiteerde fosfaat (F_P in g/uur) stromen relatief ten opzichte van het influentdebiet, Q_i, gedefinieerd:

$$C_S^* = \frac{F_A}{Q_i} \qquad C_P^* = \frac{P_P}{Q_i} \tag{21}$$

Er wordt verondersteld dat de in- en uitgaande stroom van de stripper (Q_r) gelijk zijn. Deze aanname kan gedaan worden omdat de acetaatvoeding en geprecipiteerde fosfaatstroom verwaarloosbaar zijn op volumetrische basis. Vaak wordt slechts een deel van de retourstroom door de stripper gevoerd, of worden chemicaliën direct aan het eind van de anaërobe fase gedoseerd. In de hier gebruikte benadering zal dat niet tot andere mathematische vergelijkingen leiden. De fosfaatafgifte in de stripper C_P^*, is stoichiometrisch gerelateerd aan de acetaatdosering C_S^*.

$$C_P^* = Y_{SP} \cdot C_S^* \tag{22}$$

In een hoofdstroom/stripper configuratie wordt een deel van het fosfaat verwijderd in de polyP-biomassa via het spuislib, de rest wordt dus via de stripper afgevoerd. De hoeveelheid fosfaat die afgevoerd wordt in de biomassa volgt uit vergelijking (14). Wanneer $f_{PX} = f_{PX}^{max}$ en de acetaatconcentratie gelijk is aan $\hat{C} + C$ door de acetaatdosering C_S^* in de stripper, kan de hoeveelheid fosfaat die afgevoerd wordt door de polyP-biomassa berekend worden middels:

$$Y_{SX} \cdot f_{PX}^{max} \ (C_S + C_S^*) \tag{23}$$

De hoeveelheid fosfaat verwijderd door de stripper is C_P^* en dus:

$$C_P^* = C_P - Y_{SX} \cdot f_{PX}^{max} \ (C_S + C_S^*) \tag{24}$$

Combinatie van vergelijking (22) en (24) en eliminatie van C_P^* levert de benodigde hoeveelheid acetaattoevoeging voor volledige fosfaatverwijdering:

$$C_S^* = \frac{C_P - Y_{SX} \cdot f_{PX}^{max} C_S}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{25}$$

Drie verhoudingen kunnen gedefinieerd worden om de benodigde acetaattoevoeging en de hoeveelheid fosfaat die verwijderd zal worden vast te stellen:

1) De fractie acetaat gedoseerd ten opzichte van de totale hoeveelheid acetaat in het influent:

$$\frac{C_S^*}{C_S} = \frac{C_P/C_S - Y_{SX} \cdot f_{PX}^{max}}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{26}$$

2) De verhouding tussen acetaatdosering en fosfaatverwijdering:

$$\frac{C_S^*}{C_P} = \frac{1 - Y_{SX} \cdot f_{PX}^{max} \cdot C_S/C_P}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{27}$$

3) De verhouding tussen gestript en geprecipiteerd fosfaat, ten opzichte van de totale hoeveelheid fosfaat verwijderd:

$$\frac{C_P^*}{C_P} = \frac{Y_{SP}(1 - C_S/C_P \cdot Y_{SX} \cdot f_{PX}^{max})}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{28}$$

De concentratie polyP-organismen benodigd in het systeem kan berekend worden met vergelijking 17, waarbij de acetaatconcentratie gelijk is aan $C_S+C_S^*$.

$$C_X^{polyP} = \frac{Y_{SX} \cdot (C_S + C_S^*)}{\tau} \cdot SRT \tag{29}$$

Eliminatie van C_S^* met vergelijking 25 geeft:

$$C_X^{polyP} = Y_{SX} \cdot \frac{SRT}{\tau} \cdot C_S \left(\frac{C_P/C_S + Y_{SP}}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \right) \tag{30}$$

De acetaatdosering, fosfaatverwijdering en de benodigde biomassaconcentratie van een hoofdstroom/strippersysteem wordt volledig beschreven door vergelijkingen 26,27,28 en 30.

Fosfaatverwijdering in een deelstroom proces

In een deelstroomproces (figuur 13c), is geen anaërobe zone aanwezig in de waterlijn van het proces. De strippertank heeft in dit systeem de rol van anaërobe selectie zone voor de biologische fosfaatverwijdering overgenomen. Acetaat van het influent komt op deze wijze niet of nauwelijks ten goede aan de polyP-organismen. De hoeveelheid acetaat C_S^* die gedoseerd moet worden in een dergelijk proces, volgt uit vergelijking 25 waarbij C_S in dit geval nul is. De hoeveelheid acetaat om volledige P-verwijdering te bereiken wordt dus:

$$C_S^* = \frac{C_P}{Y_{SX} \cdot f_{PX}^{MAX} + Y_{SP}} \tag{31}$$

De verhoudingen voor de acetaatdosering en de hoeveelheid verwijderd fosfaat voor een deelstroomproces zijn hierdoor anders dan die voor een hoofdstroomproces:

1) De fractie acetaat gedoseerd ten opzichte van de totale hoeveelheid acetaat in het influent:

$$\frac{C_S^*}{C_S} = \frac{C_P / C_S}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{32}$$

2) De verhouding tussen acetaatdosering en fosfaatverwijdering:

$$\frac{C_S^*}{C_P} = \frac{1}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{33}$$

3) De verhouding tussen gestript en geprecipiteerd fosfaat relatief ten opzichte van de totale hoeveelheid fosfaat verwijderd:

$$\frac{C_P^*}{C_P} = \frac{Y_{SP}}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{34}$$

In een deelstroomproces wordt de acetaatdosering enkel bepaald door de fosfaat-concentratie van het influent. Bij vergelijking van vergelijking 26 en 32 wordt het duidelijk dat in een deelstroomproces een hogere acetaatdosering vereist is dan in een hoofdstroomproces. Dit is logisch omdat in een hoofdstroomproces het acetaat van het influent wel benut wordt door de polyP-organismen terwijl dat in het deelstroomproces dat niet het geval is. De concentratie polyP-organismen dat in een deelstroomproces benodigd is om volledige P-verwijdering te bereiken kan berekend worden met vergelijking 17 en 31, waarbij voor C_S dan C_S^* gesubstitueerd moet worden.

$$C_X^{polyP} = Y_{SX} \cdot \frac{SRT}{\tau} \cdot C_S \left(\frac{C_P / C_S}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \right) \qquad (35)$$

In tabel 6 is een overzicht gegeven van de relevante vergelijkingen voor de drie procesconfiguraties.

3.7 Resultaten

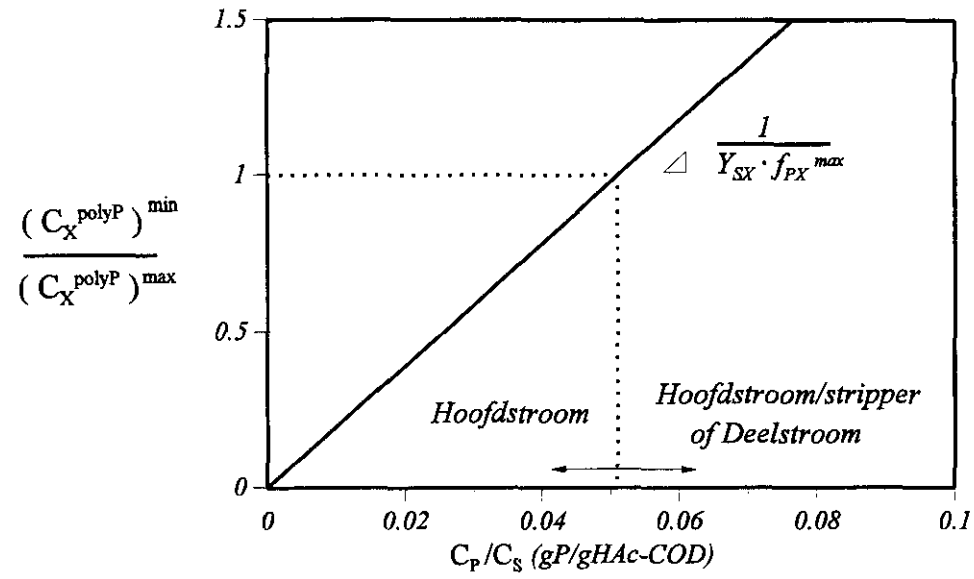
Parameters

In dit hoofdstuk werden parameterwaarden gebruikt zoals weergegeven in tabel 4. Een gemiddelde biomassayield (Y_{SX}) op acetaat van 0.34 g CZV/g HAc-CZV is gehanteerd (figuur 15). Voor het maximale P-gehalte van de biomassa (f_{PX}^{max}) is een relatief lage waarde van 0.15 g P/g biomassa-CZV gebruikt. De fosfaat/acetaat yield parameter (Y_{SP}) is sterk afhankelijk van de pH. Indien niet anders vermeld, wordt de waarde bij pH 7 gebruikt (0.47 g P/g HAc-CZV).

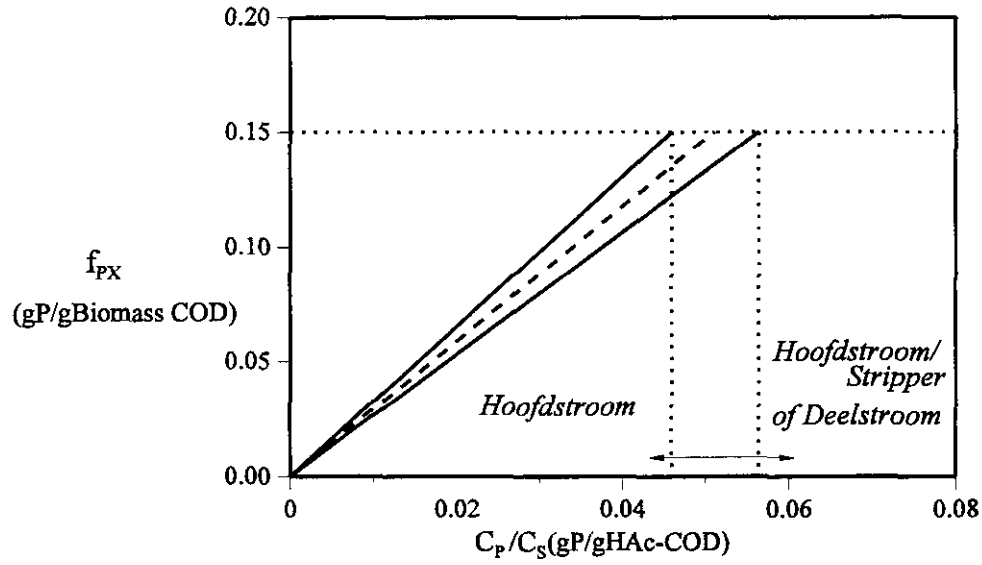
Tabel 4 *Parameters gebruikt voor de berekeningen.*

Y_{SX}	0.34	g VSS/g HAc-CZV	SRT	8	d
Y_{SP}	0.47	g P/g HAc-CZV	HRT,T	12	h
f_{PX}^{max}	0.15,0.25	g P/g Biomassa-CZV	pH	7	

a)



b)



Figuur 17. *a) Minimaal benodigde polyP-biomassaconcentratie voor de verwijdering van fosfaat, en de maximaal bereikbare polyP-biomassaconcentratie op basis van de acetaat-CZV in het influent.*
b) P-gehalte van de polyP-organismen als functie van de fosfaat/acetaat-verhouding van het influent. Bij een verhouding > 1 hebben de polyP-organismen hun maximale P-gehalte bereikt.

Fosfaatverwijdering in een hoofdstroomproces

In figuur 17a is het effect van de fosfaat/acetaat verhouding in het influent op de minimaal benodigde en maximaal bereikbare polyP-biomassaconcentratie weergegeven. Wanneer de minimaal benodigde biomassaconcentratie hoger wordt dan de bereikbare biomassaconcentratie op het acetaat van het influent, dus $C_X^{min}/C_X^{max} > 1$, zal een hoofdstroom/stripper configuratie of deelstroomproces benodigd zijn om volledige P-verwijdering te krijgen. Wanneer het maximale P-gehalte van de polyP-organismen 0.15 g P/g biomassa-CZV is, zal de maximaal toegestane fosfaat/acetaat verhouding van het influent 0.051 g P/g HAc-CZV zijn. De helling van de minimale en maximale biomassa verhouding is afhankelijk van de biomassayield van de polyP-organismen en het maximale P-gehalte dat bereikt kan worden.

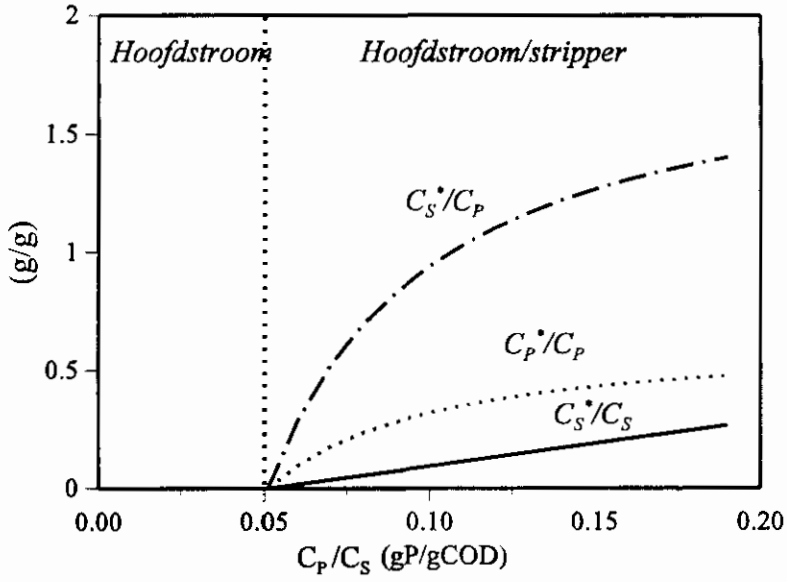
In figuur 17 is het P-gehalte van de polyP-organismen als functie van de influentsamenstelling weergegeven. Het P-gehalte zal toenemen totdat bij een kritische fosfaat/acetaat verhouding van 0.051 g P/g HAc-CZV, de maximale opnamecapaciteit van de organismen bereikt is.

Verlaging van de SRT (van 20 naar 5 dagen) om de biomassayield te verhogen heeft slechts een gering effect op de verbetering van de opnamecapaciteit. De kritische fosfaat/acetaat-verhouding is sterk afhankelijk van de f_{PX}^{max}. Toepassing van het hoogste maximale P-gehalte in het ontwerp van biologische fosfaatverwijderende installaties is niet aan te bevelen, omdat in het geval dat in het systeem geen extra fosfaatopnamecapciteit beschikbaar is om eventuele piekbelastingen op te vangen. Toepassing van deze berekening is mogelijk bij het bepalen van 1) te bepalen of een strippertank nodig is (in het voorbeeld wanneer $C_P/C_S > 0.051$ g P/g HAc-CZV) en 2) de hoeveelheid CZV die overblijft voor andere processen zoals denitrificatie.

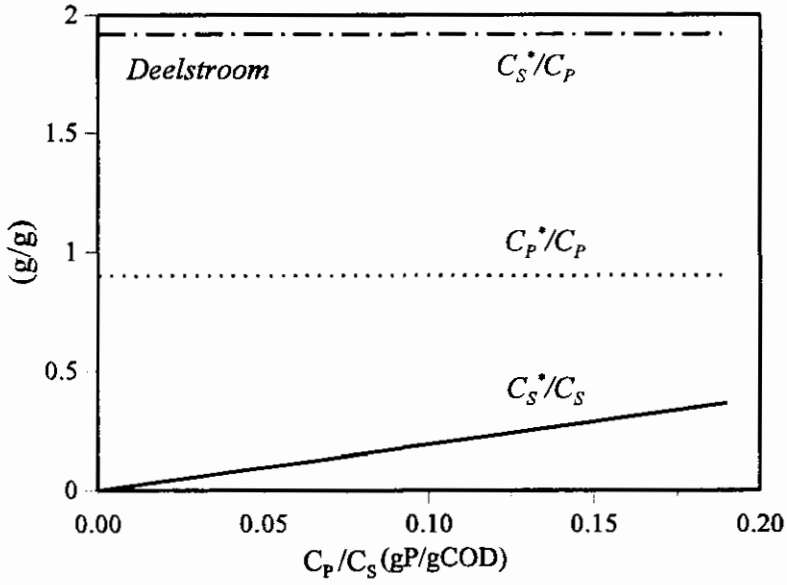
Fosfaatverwijdering in een deelstroomproces

In het deelstroomproces wordt geen gebruik gemaakt van het acetaat aanwezig in het influent. Het is daarom alleen een goed alternatief als er geen of weinig direct beschikbaar CZV aanwezig is. In figuur 18 wordt de hoeveelheid acetaat die toegevoegd moet worden aan een strippertank van een hoofdstroom- (18a) en deelstroomproces (18b) als functie van de influent acetaat- en fosfaatconcentratie (C_S^*/C_S) en (C_S^*/C_P) gegeven. Ook is de relatieve hoeveelheid geprecipiteerd fosfaat (C_P^*/C_P) gegeven.

a)



b)



Figuur 18. *Benodigde acetaatdosering als functie van de fosfaat/acetaat-verhouding van het influent aan een stripper in een a) hoofdstroomproces en b) deelstroomproces.*

Het belangrijkste verschil tussen een hoofdstroom- en deelstroomproces is dat in een hoofdstroom/stripper-proces alleen acetaat gedoseerd hoeft te worden boven een bepaalde fosfaat/acetaat verhouding in het influent, terwijl in een deelstroomproces altijd acetaatdosering nodig is.

De acetaatdosering CS*/CP in een deelstroomproces is ongeveer 2 mg HAc-CZV/mg P van het influent terwijl in een hoofdstroomproces substantieel lagere dosering vereist. Zonder acetaat-CZV in het influent (CS=0) is de acetaatdosering aan de strippertank gelijk voor beide gevallen (tabel 6). De hoeveelheid fosfaat verwijderd in een strippertank door precipitatie (C_P^*/CP) is in het deelstroomproces 0.9 mg P/mg P van het influent. De andere 10% van het influentfosfaat wordt verwijderd in de biomassa van de polyP-organismen.

Tabel 5. *Vereiste acetaatdosering aan een strippertank en de concentratie polyP-organismen benodigd voor volledige P-verwijdering.*
Condities: SRT 8 d, HRT 12 h, pH 7.

Configuratie	Influent			Stripper		Biomassa
	C_P	C_S	P/CZV	C_P^*	C_S^*	$C_{,P}^{poly}$
	mg P/l	mg CZV/l	mg P/g CZV	mg P/l	mg CZV/l	g CZV
(1) Mainstream	15	500	30	-	-	2.72
(2) Mainstream	15	300	50	-	-	1.63
(3) Main/stripper	15	200	75	4.3	9.2	1.14
(4) Side-stream	15	<<1		13.5	28.8	0.16

Tabel 6. *Berekening van de acetaatdosering en fosfaatverwijdering in verschillende P-verwijderende systeemconfiguraties.*

C_P, C_S *fosfaat- en acetaatconcentratie in het influent;*

C_P^*, C_S^* *concentraties gestript fosfaat en gedoseerd acetaat in de stripper per liter influent.*

	Hoofdstroom	Hoofdstroom/stripper	Deelstroom
C_P/C_S	$< Y_{SX} \cdot f_{PX}^{max}$	$> Y_{SX} \cdot f_{PX}^{max}$	$> Y_{SX} \cdot f_{PX}^{max}$
f_{PX}	$\frac{1}{Y_{SX}} \cdot \frac{C_P}{C_S}$	f_{PX}^{max}	f_{PX}^{max}
C_S^*/C_S	0	$\frac{C_P/C_S - Y_{SX}^* \cdot f_{PX}^{max}}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}}$	$\frac{C_P/C_S}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}}$
C_S^*/C_P	0	$\frac{1 - Y_{SX}^* \cdot f_{PX}^{max} \cdot C_S/C_P}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}}$	$\frac{1}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}}$
C_P^*/C_P	0	$\frac{Y_{SP}(1 - C_S/C_P \cdot Y_{SX}^* \cdot f_{PX}^{max})}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}}$	$\frac{Y_{SP}}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}}$
C_x^{polyP}	$Y_{SX}^* \cdot \frac{SRT}{\tau} \cdot C_S$	$Y_{SX}^* \cdot \frac{SRT}{\tau} \cdot C_S \left(\frac{C_P/C_S + Y_{SP}}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}} \right)$	$Y_{SX}^* \cdot \frac{SRT}{\tau} \cdot C_S \left(\frac{C_P/C_S}{Y_{SX}^* \cdot f_{PX}^{max} + Y_{SP}} \right)$

Acetaatdosering en concentratie polyP-organismen

De acetaatdosering aan een strippertank, de fosfaatverwijdering en de vereiste hoeveelheid polyP-biomassa voor een hoofdstroom en deelstroomproces werden berekend voor vier verschillende influent fosfaat/acetaat-verhoudingen zoals weergegeven in tabel 6.

In het eerste voorbeeld van tabel 6 (1) is de influent fosfaat/acetaat verhouding 0.03 mg P/mg CZV. Er zal in dat geval voldoende polyP-biomassa gevormd kunnen worden om al het fosfaat op te nemen; een stripper is derhalve niet nodig voor dit afvalwater. Vergelijking 17 werd gebruikt om de concentratie polyP-organismen uit te rekenen. De uitkomst was 2.72 g CZV/l. Het P-gehalte van de polyP-organismen wordt dan 88 mg P/g biomassa-CZV.

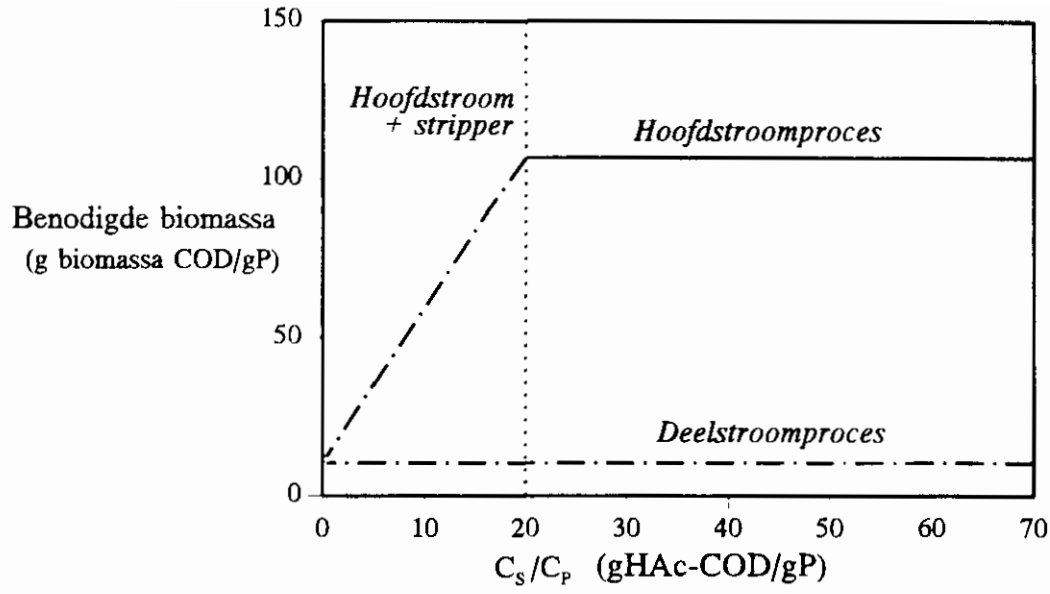
In voorbeeld 2 is de CZV-behoefte uitgerekend voor het geval dat de polyP-organismen hun maximale P-opslagcapaciteit van f_{PX}^{max} = 0.15 g P/g biomassa-CZV gebruiken. Wanneer de fosfaat/acetaat-verhouding boven dit maximum stijgt, zoals in voorbeeld 3, moet een strippertank gebruikt worden om volledige fosfaatverwijdering te krijgen. De vereiste hoeveelheid acetaatdosering en gestript fosfaat is berekend met vergelijking (27) en (28). De polyP-biomassaconcentratie is berekend met vergelijking (30).

Voor een deelstroomproces waarbij het influent CZV niet gebruikt wordt door de polyP-organismen (voorbeeld 4) is de acetaatdosering en fosfaatverwijdering berekend volgens vergelijking (32) en (33). De biomassaconcentratie is berekend met vergelijking (34). Uit tabel 6 blijkt dat de totale CZV-behoefte en de vereiste polyP-biomassaconcentratie voor P-verwijdering verlaagd wordt wanneer een strippertank wordt gebruikt. Dat is logisch omdat in een strippertank het afgegeven fosfaat niet wordt opgenomen in de biomassa maar geprecipiteerd. Daardoor is een lagere biomassaconcentratie toereikend.

In figuur 19 is de vereiste hoeveelheid polyP-biomassa in een systeem weergegeven als functie van de fosfaat/acetaat-verhouding in het influent. Wanneer een stripper gebruikt wordt, vermindert de vereiste hoeveelheid biomassa per gram influent fosfaat.

Effect van de pH op de acetaatdosering in een strippertank

De toevoeging van acetaat in een strippertank is afhankelijk van de biomassa-yield, het maximale P-gehalte en de P-afgifte door de opname van acetaat. Om de acetaatdosering aan een strippertank te optimaliseren, kan het sturen van het proces op deze parameters voordelig zijn. De acetaat/fosfaat yield (Y_{SP}) is de enige stuurbare parameter door het veranderen van de pH in de stripper. Het effect van de pH controle op de fosfaatafgifte kan worden afgeleid uit figuur 16. De fosfaatafgifte per hoeveelheid opgenomen acetaat stijgt bij toenemende pH. Bovendien stijgt de pH ook door het acetaatverbruik.



Figuur 19 *Vereiste poly-P-biomassaconcentratie als functie van de HAc-CZV/P verhouding in het influent*

3.8 Discussie

Hoofdstroom- versus deelstroomproces

Toevoeging van chemicaliën aan afvalwaterzuiveringsprocessen moet in principe geminimaliseerd worden. Bovendien moet de toevoeging van CZV vermeden worden omdat daardoor extra slib geproduceerd zal worden. Een hoofdstroomproces zal op basis hiervan de voorkeur hebben. Alleen wanneer het afvalwater een tekort aan CZV heeft voor volledige P-verwijdering is een strippertank nuttig. Het gebruik van een deelstroomproces, in feite een speciaal geval van een hoofdstroom/stripper configuratie (C_S=0) is pas raadzaam wanneer er nauwelijks acetaat-CZV in het influent beschikbaar is voor de P-verwijdering.

In tabel 6 is de berekeningsmethode voor de acetaatdosering en fosfaatverwijdering voor de verschillende procesconfiguraties samengevat. Uit deze vergelijkingen kan geconcludeerd worden dat de acetaatdosering (C_S^*/C_P) en de gestripte hoeveelheid fosfaat in een hoofdstroom/stripper proces altijd lager zal zijn dan in een deelstroomproces. De benodigde concentratie polyP-organismen daarentegen, zal in een hoofdstroomproces altijd hoger zijn dan in een deelstroomproces. Dat komt omdat in een hoofdstroomproces de polyP-organismen functioneren als opslag van het fosfaat, terwijl in een deelstroomproces de organismen alleen gebruikt worden om het fosfaat

tijdelijk te concentreren, wat dan vervolgens weer afgegeven wordt. De fosfaatbelasting van de aërobe fase in een deelstroomproces is daardoor veel lager dan in een hoofdstroom, waardoor minder biomassa benodigd is voor de P-opname. In een hoofdstroom proces volgt de vereiste hoeveelheid polyP-bacteriën uit de eis dat al het fosfaat gedurende één slibleeftijd kan worden opgeslagen terwijl in een deelstroom-proces de vereiste opslagcapaciteit van het slib equivalent is aan de fosfaatbelasting van één hydraulische verblijftijd.
Het verschil in vereiste polyP-biomassa voor een hoofdstroom- en een deelstroomproces kan worden afgeleid van de vergelijking voor de biomassaconcentratie (30) en een deelstroom (35) en vergelijking (23):

$$\frac{C_x^{polyP}\ Hoofd}{C_x^{polyP}\ Deel} = = 1 + \frac{Y_{sp}}{Y_{sx} \cdot f_{PX}} \qquad (36)$$

Volgens vergelijking (36) met de waarden uit tabel 5 vraagt een hoofdstroomproces 10 keer meer polyP-biomassa dan een deelstroomproces. In een deelstroomproces wordt veel minder acetaat-CZV gebruikt voor de fosfaatverwijdering door de additionele P-verwijdering door precipitatie.

Stripper optimalisatie
Het effect van de pH op de P-afgifte is van belang bij het ontwerpen van de stripper. Optimale instelling van de pH kan de acetaatdosering minimaliseren (figuur 16). De groeisnelheid en het maximale P-gehalte zijn relatief onafhankelijk van een pH-effect, zodat er geen groot effect van de pH op het hoofdstroom proces te verwachten is. Alleen de fosfaatconcentratie aan het eind van de anaërobe fase zal afhankelijk zijn van de pH. Het effect van de pH op de yield Y_{SP} kan worden verklaard uit de toegenomen energie voor de opname van acetaat bij hogere pH's (appendix I). Dit pH effect een verklaring zijn voor het feit dat zoveel verschillende fosfaat/acetaat verhoudingen gevonden zijn in de literatuur. Al deze waarden liggen in de range dat door het pH effect voorspelt wordt. Helaas werd door veel auteurs de pH van de experimenten niet vermeld, waardoor het niet mogelijk is deze relatie aan te tonen met andere data.

Stikstof- en fosfaatverwijdering
Meestal zal bij het ontwerp van een zuiveringsinstallatie zowel stikstof als fosfaat verwijderd moeten worden. Vaak is er slechts een beperkte hoeveelheid CZV beschikbaar in het influent en daarom zal er concurrentie ontstaan tussen stikstof en fosfaatverwijderende organismen. Om in staat te zijn toch volledige N en P-verwijdering te krijgen, is dosering van CZV nodig. Er kan dan gekozen worden om CZV toe te voegen voor de denitrificatie of voor de verwijdering van fosfaat. Voor de verwijdering

van stikstof is 3.5 mg CZV benodigd per mg N. Voor fosfaatverwijdering in een strippertank slechts 2 mg HAc-CZV/mg P gestript en voor P-verwijdering in de hoofdstroom 20 mg HAc/mg P. Het is dus duidelijk dat in het geval van een tekort aan CZV, een strippertank voor de verwijdering van fosfaat de meest efficiënte manier is. Door het gebruik van een strippertank komt er bovendien meer CZV uit het influent beschikbaar voor de stikstofverwijdering.
Een voorbeeld is gegeven in tabel 7, die gebaseerd is op een theoretisch influent met een acetaatconcentratie van 450 mg CZV/l, een stikstofconcentratie van 60 mg N/l en 15 mg P/l. Voor de verwijdering van 15 mg P/l in een hoofdstroomproces zal 450 mg CZV/l nodig zijn wanneer dit CZV ook geheel aan de polyP-organismen ten goede komt. In dat geval zullen de organismen het maximale P-gehalte niet bereiken. Omdat al het CZV geconsumeerd is, is er geen CZV meer voor de denitrificatie. Wanneer de P-organismen wel tot het maximale P-gehalte volgeladen zouden zijn, zou de minimaal noodzakelijke hoeveelheid CZV voor de P-verwijdering bereikt zijn en zou 150 mg CZV/l beschikbaar zijn voor denitrificatie. Voor volledige N-verwijdering is echter 210 mg CZV/l benodigd, wat resulteert in een tekort van 60 mg CZV/l. Wanneer nu een hoofdstroom/stripper configuratie gekozen zou worden met als eis dat volledige stikstofverwijdering plaats moet vinden, wordt slechts 240 mg CZV/l van het influent gebruikt voor de P-verwijdering, en moet de rest van het fosfaat verwijderd worden in de stripper onder dosering van een kleine hoeveelheid CZV. Wanneer een deelstroom-proces gebruikt zou worden en al het fosfaat gestript zou worden, zou slechts 28.8 mg CZV/l nodig zijn voor de P-verwijdering en 421.2 mg CZV/l zou voor de stikstof-verwijdering beschikbaar komen. Vanuit het oogpunt van efficiënt CZV-gebruik in de gecombineerde stikstof/fosfaat verwijdering leid een strippertank duidelijk tot een lagere totale CZV-behoefte. Of dit voordeel ook nog geldt als ook andere aspecten (chemicaliën kosten, extra slibbehandeling en -scheiding) in de evaluatie worden meegenomen valt te bezien.

Tabel 7 *Illustratie van de CZV vraag van verschillende procesconfiguraties voor een influent met 450 mg CZV/l, 60 mg N/l and 15 mg P/l.*
Proces condities: SRT 8 d, HRT 12 h, pH 7.

Configuratie	P-verwijdering door		CZV voor P-verwijdering		CZV voor N-verwijdering		Influent CZV niet voor N/P-verwijdering	Shortage
	Precipitatie	Biomassa	Stripper	Biomassa	Nodig	Beschikbaar		
	(%)	(%)	mg CZV/l	mg CZV/l	mg CZV/l	mg CZV/l	mg CZV/l	mg CZV/l
(1) Hoofdstroom	0	100	0	450	210	0	0	-210
(2) Hoofdstroom, f_{PX}^{max}	0	100	0	300	210	150	0	-60
(3) Hoofd/stripper	20	80	6	240	210	210	0	-6
(4) Deelstroom	90	10	29	0	210	421	240	-29

Resumerend kunnen de volgende conclusies uit het voorgaande worden getrokken:

Met de steady state analyse is het mogelijk de fosfaatverwijderingscapaciteit, de vereiste concentratie aan P-organismen en de acetaatbehoefte van verschillende biologische P-verwijderende procesconfiguraties te berekenen. Op basis van de fosfaatconcentratie van het influent en de systeemconfiguratie kan daarmee rechtstreeks eisen gesteld worden aan de influent P/CZV-verhouding.

De fosfaatopnamecapaciteit van een hoofdstroomproces blijkt vast te liggen met de P/CZV-verhouding van het influent, terwijl de acetaatbehoefte van een deelstroomproces rechtstreeks gekoppeld is aan de P-concentratie van het influent. Fosfaatverwijdering in een strippertank heeft een veel lagere CZV-vraag (2 mg CZV/mg P) dan fosfaatverwijdering in een hoofdstroomproces (20 mg CZV/mg P) en vergt een tien maal lagere polyP-biomassa concentratie dan het hoofdstroomproces.

Wanneer de CZV-concentratie van het influent te laag is om zowel de stikstof als de fosfaatverwijdering te voorzien, kan volledige N- en P-verwijdering verkregen worden door goed gebruik te maken van de lagere CZV-vraag van een strippertank. Verder kan het optimaliseren van de pH bijdragen aan het minimaliseren van de acetaatdosering aan een strippertank.

4 CONCLUSIES EN AANBEVELINGEN

4.1 Conclusies

Het doel van het onderzoeksproject om bestaande leemtes in de microbiele kennis in te vullen en de microbiologische-, procestechnologische- en zuiveringstechnologische kennis te combineren in een model waarmee de fosfaatverwijdering beschreven kan worden is grotendeels bereikt. Het onderzoek heeft geresulteerd in een metabool model voor de biologische fosfaatverwijdering. Dit is een model maakt maximaal gebruik van de microbiële kennis van een proces met een minimaal aantal parameters. Bij een dergelijk metabool model kunnen de stoichiometrische parameters constant worden verondersteld.

In het onderzoek is uitgebreid experimenteel onderzoek verricht naar de reacties in het metabolisme van de fosfaatverwijdering, en hiertoe zijn vrijwel alle relevante componenten die daarbij een rol spelen gemeten. Het metabole model dat in dit onderzoek is opgesteld, is dus grondig experimenteel geverifieerd, een aspect wat tot nog toe veelal ontbrak bij de modellen van de biologische fosfaatverwijdering. Vrijwel alle relevante componenten die een rol spelen bij de verschillende reacties in het metabolisme van de biologische fosfaatverwijdering zijn hierbij bekeken. Het metabole model is getoetst in een sequencing batch reactor onder steady state condities als functie van de groeisnelheid, en onder dynamische condities tijdens de opstart van het proces. In al deze gevallen bleek het model in staat te zijn de conversies van zowel de externe- als interne componenten bevredigend te beschrijven. Tevens bleek het model invariant te zijn voor de procescondities.

Specifieke conclusies die uit het onderzoek naar voren kwamen zijn:

De benodigde reductie-equivalenten benodigd onder anaerobe condities voor de omzetting van acetaat naar PHB komen uit de omzetting van glycogeen naar PHB. Voorgestelde mechanismen waarbij de citroenzuurcyclus betrokken is kunnen op theoretische gronden worden verworpen.

De verhouding tussen opgenomen acetaat en afgegeven fosfaat, in de anaerobe fase van een defosfateringsproces, is sterk afhankelijk van de pH in het traject pH 6-8.

Fosfaatopname leidt tot extra zuurstofverbruik. Meting van dit zuurstofverbruik maakt het mogelijk om de P/O ratio (een parameter in het metabool model) direct experimenteel te bepalen.

Het gehalte aan PHB in de cellen neemt sterk toe met afnemende slibleeftijd.

Fosfaatverwijdering in een deelstroomproces heeft een aanzienlijk lagere HAc-CZV-vraag (2 mg HAc-CZV/mg P) dan voor een hoofdstroomproces (20 mg CZV/mg P). Dit wordt veroorzaakt doordat een veel lager gehalte aan poly-P-bacteriën benodigd is in het deelstroomproces.

Indien de CZV concentratie in het influent te laag is voor volledige P en N eliminatie, heeft een striptank (eventueel met acetaatdosering) de voorkeur. Deze optie heeft een lagere CZV behoefte dan dosering van CZV voor denitrificatie.

pH-regeling zou een bijdrage kunnen leveren aan een verdere optimalisatie van de acetaatdosering aan de striptank.

4.1 Aanbevelingen

De volgende stap zal de toepassing van het model op praktijksituaties moeten zijn, waarbij mogelijk aanpassingen van het model nodig zullen zijn. De volgende aspecten zijn daarbij van belang: a) de ontwikkeling van experimentele methoden om de P-verwijdering op praktijkschaal te kwantificeren, b) de evaluatie van de capaciteit van het model om dynamische influentcondities te voorspellen en c) de relevantie van aspecten die nog niet in het model zijn opgenomen.

ad a)
In het hier uitgevoerde onderzoek werd acetaat als substraat gebruikt. In de praktijk is acetaat deel van het influent-CZV en afkomstig van fermentatie of hydrolyse processen. Voordat de P-verwijdering berekend kan worden moet de beschikbare acetaatfractie voor de P-verwijdering bekend zijn. Verder bestond het slib dat gebruikt werd voor dit onderzoek voornamelijk uit polyP-organismen, terwijl in de praktijk de organismen slechts een deel van het slib uitmaken. Er moeten dus experimentele methoden ontwikkeld worden om de fractie poly-P-organismen in slib te kunnen bepalen.

ad b)
In de praktijksituatie is er altijd een variabel influentdebiet. Deze variatie kan gevolgen hebben voor de P-verwijdering omdat acetaat opgeslagen wordt met behulp van de intern opgeslagen produkten polyfosfaat en glycogeen. Een acetaatpiek in het influent wordt niet altijd ondersteund met voldoende opslagprodukt met als gevolg dat de acetaatopname stopt. Welke gevolgen dit heeft op de totale P-verwijdering en of het een veel voorkomend effect is moet worden bezien.

ad c)
Als laatste zijn in het hier uitgevoerde onderzoek een aantal aspecten niet onderzocht, zoals de maximale P-opnamecapaciteit van de organismen, het effect van de temperatuur, het effect van zuurstof of nitraat op de acetaatopname, en organismen die nitraat als electronenacceptor kunnen gebruiken (denitrificerende defosfateerders).

Samenvattend is de verwachting dat het metabole model zijn waarde zal bewijzen in het ontwerp en de optimalisering van full-scale installaties. Het is onze verwachting dat de genoemde modeluitbreidingen van het metabole model eenvoudig ingepast kunnen worden in de huidige modelstructuur.

5 LITERATUUR

Gujer, W., Henze M., Mino T., Matsuo T., Wentzel M.C., Marais G.v.R, 1994. The Activated Sludge Model No 2: Biological phosphorus removal. IAWQ specialised seminar: Modelling and control of activated sludge processes, Copenhagen.

Kuba, T., Smolders, G.J.F., Van Loosdrecht, M.C.M., Heijnen, J.J., 1993. Biological phosphorus removal from waste water by anaerobic anoxic sequencing batch reactor. *Wat. Sci. Tech. 27,* no.5-6, 241-252.

Mauer, M., Gujer, W., 1994. Prediction of the performance of enhanced biological phosphorus removal plants. IAWQ 17th Biennial International Conference, Budapest.

Niel, E.W.J. van, 1993. Modelvorming en optimalisatie van biologische defosfatering van afvalwater, microbiële aspekten, RWZI 2000 93-03.

Smolders, G.J.F., Van der Meij, J., Van Loosdrecht, M.C.M., Heijnen, J.J., 1994a. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. *Biotech. Bioeng.*, Vol **43**, 461-470.

Smolders, G.J.F., Van der Meij, J., Van Loosdrecht, M.C.M., Heijnen, J.J., 1994b. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process, *Biotech. Bioeng.*, Vol **44**, 837-848.

Smolders, G.J.F., 1995. A metabolic model for de biological phosphate elimination. PhD thesis, *Delft University of Technology*.

Smolders, G.J.F., van der Meij, J., Van Loosdrecht M.C.M., Heijnen, J.J. 1995a. A structured metabolic model for the anaerobic and aerobic stoichiometry and kinetics of the biological phosphorus removal process. Biotech. & Bioeng., submitted.

Smolders, G.J.F., Klop J.M., Van Loosdrecht, M.C.M., Heijnen, J.J. 1995b. A metabolic model for the biological phosphorus removal process; effect of the sludge retention time. Biotech. & Bioeng., submitted.

Toerien D.F., Gerber A., Lötter L.H., Cloete T.E., 1990. Enhanced biological phosphorus removal in activated sludge systems. In: K.C. Marshall, Advances in Microbial Ecology, Vol 11, Vol 11, Plenum, New York.

Wentzel, M.C., G.A. Ekama, R.E. Loewenthal, P.L. Dold and G.v.R. Marais, 1989a. Enhanced polyphosphate organism cultures in activated sludge systems. Part II:Experimental behaviour. *Water SA*, Vol 15, No 2, 71-88.

Wentzel, M.C., Dold, P.L., Ekama, G.A., Marais, G.v.R. 1989b. Enhanced polyphosphate organism cultures in activated sludge systems. Part III: Kinetic model, Water SA Vol. 15, No. 2, 89-102.

Wentzel, M.C., Ekama, G.A., Dold, P.L. and Marais, G.v.R. 1990. Biological excess phosphorus removal -Steady state process design, *Water SA*, **Vol. 16**, No. 11 29-48.

Wentzel, M.C., G.A. Ekama and G.v.R. Marais, 1992. Processes and modelling of nitrification denitrification biological excess phosphorus removal systems - A review *Wat. Sci. Tech. 25*, No 6, 59-82.

Appendix I

Stoichiometry and pH influence of the anaerobic metabolism

In the anaerobic phase of a biological phosphorus removal process, acetate is taken up and converted to PHB utilizing both energy generated in the degradation of polyphosphate to phosphate, which is released, and energy generated in the conversion of glycogen to poly-ß-hydroxy butyrate (PHB). The phosphate/acetate ratio cannot be considered a metabolic constant, because the energy requirement for the uptake of acetate is strongly influenced by the pH value. The observed phosphate/acetate ratio shows a variation of 0.25-0.75 P-mol/C-mol in a pH range of pH 5.5 to 8.5. It is shown that stored glycogen takes part in the metabolism to provide reduction equivalents and energy for the conversion of acetate to PHB. A structured metabolic model based on glycogen as source of the reduction equivalents in the anaerobic phase and the effect of the pH on the energy requirement of the uptake of acetate, is developed. The model explains the experimental results satisfactorily.

Introduction

The primary factor to obtain biological phosphorus removal in an activated sludge system is the recirculation of the sludge through an anaerobic and aerobic zone. A typical example of the change in concentrations during biological phosphorus removal in a sequencing batch process is given in figure 1.

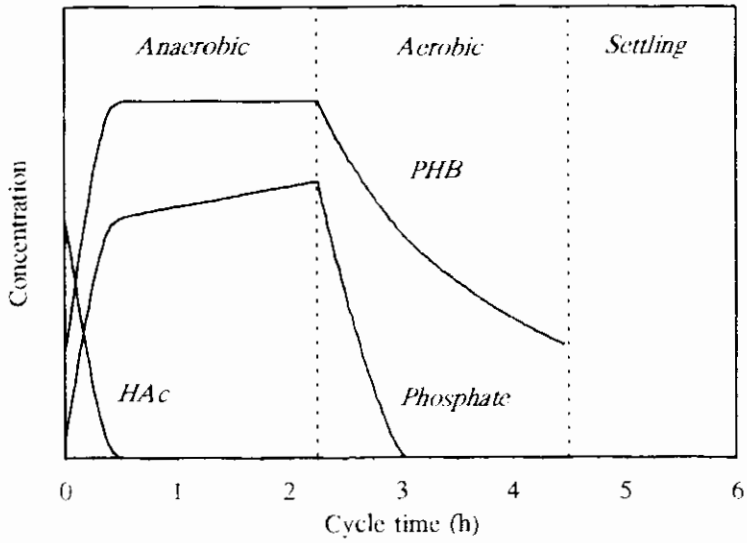


Figure 1 *Change in concentrations during the biological phosphorus removal in a sequencing batch process after addition of the influent.*

In the anaerobic zone (i.e. no electron acceptor is present) P-removing bacteria take up lower fatty acids, mainly acetate (HAc), into the cell and store this as poly-hydroxy-alkanoates (PHA, for instance, PHB). The energy for this transport and storage reaction is thought to be supplied by the hydrolysis of the intracellular polyphosphate (polyP) to phosphate, which is released from the cell to the liquid. In the aerobic zone, PHB is used to generate energy for growth and for polyP synthesis resulting in the uptake of phosphorus. Due to the anaerobic consumption of lower fatty acids, the polyP-organisms accumulate in the sludge and strictly aerobic organisms decrease in number, as they lack substrate in the aerobic phase. In this paper, we will focus on the stoichiometry of the metabolism in the anaerobic zone.

In the anaerobic zone, the uptake and storage of acetate and the degradation of polyP are coupled to each other due to metabolic constraints, and therefore, the ratio between phosphate

release and acetate uptake should be constant. However, data from the literature show that a range of values (0.25-0.75 P-mol/C-mol)) for this ratio has been found;[10] see table I. An explanation for this effect has not been given. Knowledge of the underlying mechanisms of these conversions is crucial, because the anaerobic substrate uptake by the polyP-organisms in the process can be monitored by this ratio.

Two models have been proposed for the anaerobic metabolism of the organisms, with the main point of controversy the origin of the reduction equivalents necessary for the production of PHA from acetate. The first hypothesis [3,16] assumes that reduction equivalents are obtained from $NADH_2$ produced by oxidation of some of the acetate through the tricarboxylic acid (TCA) cycle operating anaerobically. The other hypothesis suggests that degradation of intracellularly stored glycogen in the Embden-Meyerhof (EM) pathway is the source for reducing power needed for $NADH_2$ production.[11] In the degradation of glycogen, ATP is also produced which lowers the required energy contribution of the hydrolysis of polyP, resulting in a decreased ratio for phosphate released per acetate taken up. Afterwards, the latter model was adapted for degradation of glycogen in the Entner-Doudoroff (ED) pathway which yielded different values for the acetate/phosphorus ratio. Since both models predict a constant value for the phosphate/acetate ratio they do not explain the experimentally found variation in this ratio.

Table I *Phosphate/acetate ratios found in literature.*

Author		ref.	ratio (P-mol/C-mol)
Wentzel	1986	16	0.24
Arun	1988	1	0.21-0.39
Mino	1987	11	0.39
Wentzel	1988	17	0.52-0.57
Arvin	1985	2	0.62-0.74
Comeau	1987	4	0.70-0.75

Thermodynamic consideration of the acetate transport

An explanation for the variance in the observed phosphate/acetate ratio is that the energy required for the transport of acetate over the cell membrane is not constant, but dependent on the pH. The pH effect on the energy requirements of the acetate uptake might be related to the influence of the pH on the pH gradient and the change in electric potential difference

($\Delta\psi$) across the cell membrane. Under the assumption that the internal pH and the overall proton motive force (pmf) of a cell is kept constant, the electric potential, $\Delta\psi$, as a function of the pH, can be calculated:[9]

$$\Delta\psi = \Delta p + 2.3RT(pH_{in}-pH_{out}) = \Delta p + 2.3RT\,\Delta pH \quad \text{(kJ/mol)} \quad (1)$$

Where:

Δp	: proton motive force (pmf)	(kJ/mol)
$pH_{in,out}$	: pH inside cell, pH outside cell	(-)
R	: gas constant	(kJ/K· mol)
T	: temperature	(K)

This relation implies that if the external pH is lower than the internal pH, ΔpH is positive. Because the pmf is constant and negative, the contribution of $\Delta\psi$ to the pmf becomes lower. At a higher external pH than the internal pH, the pH gradient is reversed, ΔpH is negative and $\Delta\psi$ must be higher to maintain the constant pmf. The electrical potential difference, $\Delta\psi$, has to increase when the external pH increases. Consequently, for the uptake of a negatively charged compound, more work must be done at a higher pH to overcome $\Delta\psi$. The transport energy, $\Delta G^{\circ\prime\prime}$, for a negatively charged component, like acetate, can be calculated per C-mol according to:

$$\Delta G^{\circ\prime\prime}_{HAc} = n\Delta\psi + 0.5\cdot 2.3RT\log\frac{C_{in}}{C_{out}} \quad \text{(kJ/C-mol)} \quad (2)$$

In equation 2, n is the charge of the transported acetate per C-mol (n=-0.5), C_{in} is the internal concentration and C_{out} the bulk concentration of acetate. The energy for uptake of acetate is described by:

$$\Delta G^{\circ\prime\prime}_{HAc} = n(\Delta p + 2.3RT\Delta pH) + 0.5\cdot 2.3RT\log\frac{C_{in}}{C_{out}} \quad \text{(kJ/C-mol)} \quad (3)$$

The first term represents the work required for transport of the component against the potential difference. The second term is the work done by the system to move a component against a concentration gradient. This work is provided by ATP hydrolysis, in which per P-mol an amount of $\Delta G^{\circ\prime\prime}_{ATP}$ is available ($\Delta G^{\circ\prime\prime}_{ATP}$ = -50 kJ/molATP).[9] If one assumes that this coupling is performed with an efficiency η we can write for α_1, which is the ATP requirement for the acetate transport:

$$\alpha_1 \quad \frac{\Delta G_{HAc}^{'''}}{-\Delta G_{ATP}^{'''} \cdot \eta} \qquad \text{(molATP/C-mol)} \qquad (4)$$

With the assumption that the internal pH and the overall pmf of a cell is kept constant and a value for η = 0.3,[19] a difference of 0.57 molATP/C-mol acetate in the transport energy for acetate appears with a change of the external pH of 3.

Metabolic model of the anaerobic metabolism

Metabolic models of the anaerobic conversions are developed with either the TCA cycle or glycogen as the possible source of reduction equivalents. For both models, the acetate uptake is divided into a transport and a storage process in which the energy necessary for the transport of acetate is highly dependent on the pH, see figure 2.

At low pH no energy is involved in the transport of acetic acid and the observed P-release originates from the ATP used in the conversion of acetate to acetylCoA. The P-release at low pH in the glycogen model is half the amount released in the TCA model, due to the contribution of ATP generated in the conversion of glycogen into PHB. At high pH, α_1 mol ATP/C-mol acetate is involved in the uptake of acetate into the cell (with $\alpha_1 \approx 0.5$) and, consequently, more phosphate is released. The observed phenomena can be described with three basic reactions: the transport and storage of acetate as PHB (reaction 1), polyP degradation (reaction 2) and a reaction which supplies the reduction equivalents needed in reaction 1. This reaction is different for the TCA-model and the glycogen-model (reaction 3a and 3b respectively).

Reaction 1: **Acetate uptake and storage as PHB**

The uptake of 1 C-mol acetic acid (which is equal to 0.5 mol HAc) and conversion to PHB is described in three steps: the uptake of acetate which requires α_1 mol ATP depending on the pH (α_1 = 0 - 0.5), the conversion to acetylCoA which requires 0.5 mol ATP,[7] and the subsequent conversion to PHB which requires 0.25 mol $NADH_2$ per C-mol acetic acid.[5]

$$-\underset{\text{acetic acid}}{CH_2O} - (1/2+\alpha_1)\ ATP - 1/4\ NADH_2 + \underset{\text{PHB}}{CH_{1.5}O_{0.5}} + 1/2\ H_2O = 0 \qquad [1]$$

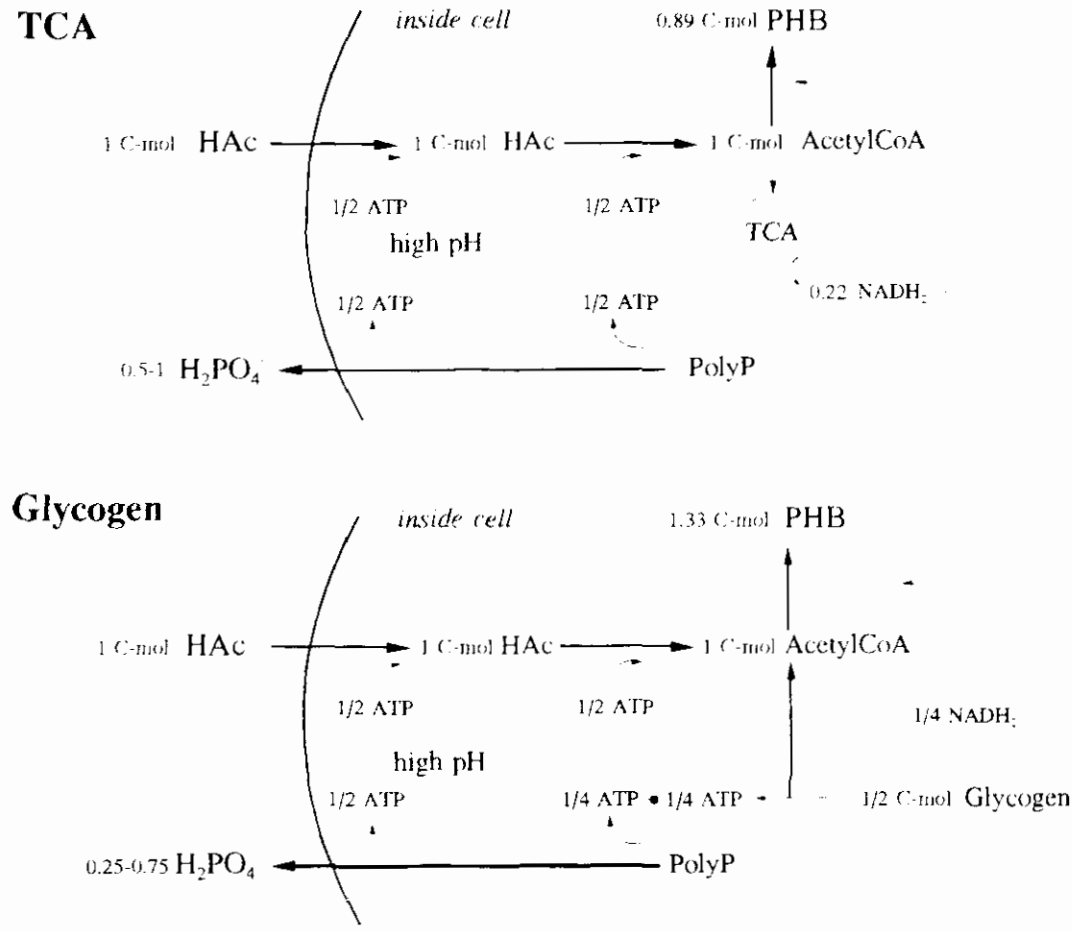


Figure 2 *Schematic representation of the metabolic models: the TCA cycle and glycogen as source of reduction equivalents and the energy requirements of the pH dependent acetate transport.*

Reaction 2: **Polyphosphate degradation for ATP production**

For the uptake and storage of acetate, ATP is produced by the degradation of PolyP. Polyphosphate is represented as HPO_3. The composition of polyphosphate, based on the measured release of phosphorus, magnesium and potassium, was $Mg_{1/3}K_{1/3}PO_3$. Since the elements magnesium and potassium were not considered here the phosphorus group is made electroneutral with a proton. The amount of ATP which is generated from the degradation of polyP is represented by α_2. The hydrolysis of 1 P-mol polyphosphate yields 1 mol ATP and 1 mol phosphate [8] and hence $\alpha_2 = 1$, when presumed that no energy is produced by the export of phosphate.

$$- HPO_3 - H_2O + \alpha_2\ ATP + H_3PO_4 = 0 \qquad [2]$$
polyP

Reaction 3a: **$NADH_2$ production in the TCA cycle**

$NADH_2$ is required in reaction 1 and is produced from the conversion of some of the acetate in the TCA cycle.[16] It is assumed that the amount of ATP produced in the TCA cycle is used for the conversion of $FADH_2$ to $NADH_2$.

$$- CH_2O - (½+\alpha_1)\ ATP - H_2O + 2\ NADH_2 + CO_2 = 0 \qquad [3a]$$

Reaction 3b: **$NADH_2$ production by degradation of glycogen**

In the degradation of glycogen, $NADH_2$ is produced from the conversion of a 0.5 C-mol glycogen via the EM-pathway to acetylCoA which is subsequently converted to PHB, which also yields 0.25 mol ATP.[11]

$$- CH_{10/6}O_{5/6} - 1/6\ H_2O + 2/3\ CH_{1.5}O_{0.5} + 1/3\ CO_2 + 1/2\ NADH_2 + 1/2\ ATP = 0 \qquad [3b]$$
Glycogen PHB

The reactions 1 to 3 are termed the internal reactions and they are based on biochemical knowledge and stoichiometry. These reactions occur in the cell and can not be observed directly. However, the rates of the internal reactions (v_1 to v_3) can be related to the observable conversion rates outside the cell.

TCA cycle as source of $NADH_2$

$$\begin{aligned}
r_s &= -v_1 - v_{3a} \\
r_{phb} &= v_1 \\
r_{pp} &= -v_2 \\
r_p &= v_2 \\
r_c &= 1/3\ v_{3a} \\
r_w &= 1/2\ v_1 - v_2 - v_3 \\
r_{ATP} &= -(0.5+\alpha_1)\ v_1 + \alpha_2\ v_2 - (0.5+\alpha_1)\ v_3 \\
r_{NADH} &= -1/4\ v_1 + 2\ v_3
\end{aligned}$$

Glycogen as source of $NADH_2$

$$\begin{aligned}
r_s &= -v_1 \\
r_{phb} &= v_1 + 2/3\ v_{3b} \\
r_{gl} &= -v_{3b} \\
r_{pp} &= -v_2 \\
r_p &= v_2 \\
r_c &= 1/3\ v_{3b} \\
r_w &= 1/2\ v_1 - v_2 - 1/6\ v_{3b} \\
r_{ATP} &= -(0.5+\alpha_1)\ v_1 + \alpha_2\ v_2 + 0.5\ v_{3b} \\
r_{NADH} &= -1/4\ v_1 + 1/2\ v_{3b}
\end{aligned}$$

The conversion rates can be expressed as a function of the internal reaction rates by a set of linear equations with stoichiometric coefficients composed of the parameters of the internal reactions as shown above. If the assumption is made that no net accumulation of $NADH_2$ and

ATP takes place, $r_{ATP} = 0$ and $r_{NADH} = 0$. The model based on the TCA cycle contains nine unknown rates and eight linear relations. The model based on glycogen degradation contains ten unknown rates and nine linear relations. Thus, both models have one degree of freedom and, therefore, the system can be described with one overall reaction equation for the anaerobic metabolism according to the two alternatives (reaction 4 and 5).

Reaction 4: **Overall reaction TCA cycle as source of $NADH_2$**

$$\underset{\text{acetate}}{-\ CH_2O} - (\tfrac{1}{2}+\alpha_1)/\alpha_2\ \underset{\text{polyP}}{HPO_3} - \{1/3-(\tfrac{1}{2}+\alpha_1)/\alpha_2\}\ H_2O + 0.89\ \underset{\text{PHB}}{CH_{1.5}O_{0.5}} + 0.11\ CO_2 + (\tfrac{1}{2}+\alpha_1)/\alpha_2\ H_3PO_4 = 0 \qquad [4]$$

The overall equations show the stoichiometric coupling of the acetate conversion to the conversions of all other reactants in the anaerobic phase. According to each equation, the PHB/acetate, glycogen/acetate and CO_2/acetate ratios are independent of α_1 and thus pH independent, but different for the two models.

Reaction 5: **Overall reaction glycogen as source of $NADH_2$**

$$\underset{\text{acetate}}{-CH_2O} - 1/2\ \underset{\text{glycogen}}{CH_{10/6}O_{5/6}} - (\tfrac{1}{4}+\alpha_1)/\alpha_2\ \underset{\text{polyP}}{HPO_3} + 1.33\ \underset{\text{PHB}}{CH_{1.5}O_{0.5}} + 0.167\ CO_2 + (\tfrac{1}{4}+\alpha_1)/\alpha_2\ H_3PO_4 + 5/12-(\tfrac{1}{4}+\alpha_1)/\alpha_2\ H_2O = 0 \qquad [5]$$

The phosphate/acetate ratio is depending on the transport of acetic acid (α_1) and on the ATP production in the polyP degradation (α_2). When α_1 varies between 0 and 0.5 mol ATP per C-mol acetate depending on the pH, and α_2 = 1 mol ATP per released phosphate, a ratio between 0.5 and 1 P-mol/C-mol acetate for the TCA model will be found and a ratio between 0.25 and 0.75 P-mol/C-mol for the glycogen model. The production of ATP in the degradation of glycogen causes the main difference in the P-release/acetate ratio between the two models. These ratios were established in a set of experiments to determine the validity of the TCA (reaction 4) or glycogen model (reaction 5).

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

The study was carried out in a laboratory fermenter (Applikon) with a working volume of 2 l, at 20 °C and the pH was maintained at pH 7.0 ± 0.05 using 0.5 M HCl and 1 M NaOH, see table II. The reactor was operated as a sequenced batch (SBR) with a cycle of 6 hours consisting of an anaerobic (2.25 h), aerobic (2.25 h) and settling period (1.5 h). Biological phosphorus removing sludge was used as an inoculum. Because the acetate added in each cycle was completely consumed in the anaerobic zone, only organisms capable of anaerobic acetate consumption were accumulated in the reactor. Methanogens were not present, because part of the time oxygen is present. One litre medium was fed at the start of the anaerobic phase and effluent was removed at the end of the settling period, thus the hydraulic retention time was 12 h. At the end of the aerobic phase 58 ml excess sludge was removed, resulting in a biomass retention of 8.6 days. A stirrer speed of 500 rpm was maintained, except for the settling period. During anaerobic conditions nitrogen gas was bubbled through the reactor at a flow rate of 30 l/h; aeration was provided with an airflow of 60 l/h. The offgas was analysed for CO_2 production. For the batch experiments sludge from the SBR was used.

Table II Operational conditions of the sequenced batch reactor (SBR).

HRT [a]	12	h	Volume	2.0	l
SRT [b]	9	d	Temperature	20	°C
C-load	25	C-mmol/l.d	pH	7.0	
P-load	1.9	P-mmol/l.d	Stirrer speed	500	rpm

[a] HRT : hydraulic retention time
[b] SRT : sludge retention time

Media

Sterilized synthetic medium was used containing per litre: 0.85 g $NaAc \cdot 3H_2O$ (400 mgCOD/l) as carbon source, 107 mg NH_4Cl (28 mgN/l), 75.5 mg $NaH_2PO_4 \cdot 2H_2O$ (15 mgP/l), 90 mg $MgSO_4 \cdot 7H_2O$, 36 mg KCl, 14 mg $CaCl_2 \cdot 2H_2O$, 1 mg yeast extract, 0.3 ml nutrient solution. The nutrient solution contained per litre: 1.5 g $FeCl_3 \cdot 6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4 \cdot 5H_2O$, 0.18 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 $CoCl_2 \cdot 6H_2O$, 10 g EDTA.

Analyses

For dry weight determinations, a 15 ml sample of the sludge was filtered on a Whatman glass microfibre filter. The filter was dried for 24 h at 80 °C and weighed on a microbalance. The ash content was determined by incinerating the dry filters in an oven at 550 °C. The elemental composition of the biomass was measured using washed and freeze dried biomass. Carbon, hydrogen, oxygen and nitrogen were determined using a Perkin Elmer 240B Elemental Analyser. Orthophosphate was determined by the ascorbic acid method. Acetate was determined on a GC with a Hayesep Q 80-100 mesh column at 185 °C and a FID detector.

For PHB determinations, 0.5 to 2 mg benzoic acid in 1-propanol was added to 15 to 20 mg washed and freeze dried biomass in closable tubes of 15 ml. One and one half mililiters of a mixture of 1-propanol and concentrated hydrochloric acid (4:1) and 1.5 ml of dichloromethane were added and the mixture was heated for 2 hours at 100 °C. After cooling, the organic phase was extracted with 3 ml water. One mililiter of the organic phase was dried on Na_2SO_4 and 0.4 µl was injected on a gas chromatograph with a stabilwax (Restek) column at 200 °C with a FID detector at 240 °C.

The gasflows were controlled with massflow controllers (Brooks 5850) for air and nitrogen gas. The offgas was dried over a permapure column and carbon dioxide was measured with a Beckman 870 infrared analyzer. Carbon dioxide contents in the batch experiments were measured with a GC with a molsieve/poropack column at 30 °C and a catharometer as detector. Staining of the cells for glycogen and electron microscopy was done according to Schade[13], 3% glutaraldehyde, 1% osmiumtetraoxide and 1% phosphor-tungstic acid (PTA) were used.

Experimental setup

The measurements to establish the CO_2, PHB and phosphate ratio on acetate were carried out in the SBR as well as in the separate batch experiments using samples of the SBR sludge. For the batch determination of the CO_2 production, sludge from the SBR was washed and resuspended, both with physiological salt-solution; 15-ml tubes with screwcap and septum, were filled with 4 ml sludge. The tubes were flushed with nitrogen gas. Acetate was added in a concentration range of 0 to 10 C-mmol/l. After 160 minutes, the pH was lowered by adding 0.05 ml 4 N HCl to expel the CO_2 from the liquid and the tubes were shaken for 10 minutes. A sample of the gas phase was injected on a GC for detection of CO_2 and N_2 which was used as internal standard. Sludge from the end of the aerobic phase was used for the batch determination of the PHB/acetate ratio. Flasks with 40 ml sludge and 0.8 ml 6 g/l Tris

buffer were flushed with nitrogen gas. The experiment was started by adding acetate in a range of 0 to 7.9 C-mmol/l. After an incubation time of 3.5 hours, all acetate was consumed, and the PHB content of the sludge and released phosphorus were measured. The measurement of the P-release as a function of the pH, was carried out in the SBR. Before the influent was added, the pH was changed to a new setpoint and the phosphate and acetate concentrations were followed during the anaerobic phase. After the measurements the pH was set to its standard value.

Results

Steady state operation

After 50 days of operation, the SBR was in steady-state and after 160 days of trouble-free operation the experiments were performed. The average MLSS (mixed liquor suspended solids) and VSS concentration (volatile suspended solids) was 3.2 g/l and 2.2 g/l. The measured flows in the SBR are shown in table III.

Table III *Average measurements over 16 cycles during 50 days of the SBR in steady state.*

Measured compound	Converted amount	(rel std dev.)	Unit
Acetate consumed	12.07	(0.05)	C-mmol/cycle
Phosphate uptake	0.457	(0.06)	P-mmol/cycle
MLSS increase [a]	176.1	(0.07)	mg/cycle
VSS increase [b]	122.7	(0.06)	mg/cycle
Biomass increase [c]	5.02	(0.07)	C-mmol/cycle
CO_2 produced	6.65	(0.07)	mmol/cycle
H_2CO_3 produced	0.3	(0.2)	mmol/cycle
carbon recovery	99%		-

[a] MLSS : mixed liquid suspended solids concentration
[b] VSS : volatile suspended solids concentration
[c] Biomass : biomass excluding polyphosphate

Due to the short sludge retention time (SRT) nitrification did not occur. Microscopy on cells stained for polyphosphate (Neisser staining)[6] showed that virtually all organisms contained polyphosphate granules. The carbon content of the biomass (MLSS) at the end of the aerobic

phase was 32 % and contained 1.3 % PHB. The carbon recovery over the total cycle was 99%.

CO_2/HAc ratio

The carbon dioxide production in a cycle was measured in the SBR and figure 3 shows the CO_2 production during the cycle. The total amount of CO_2 produced in the anaerobic period was 1.34 C-mmol/l or 0.22 C-mol/C-mol of the added acetate.

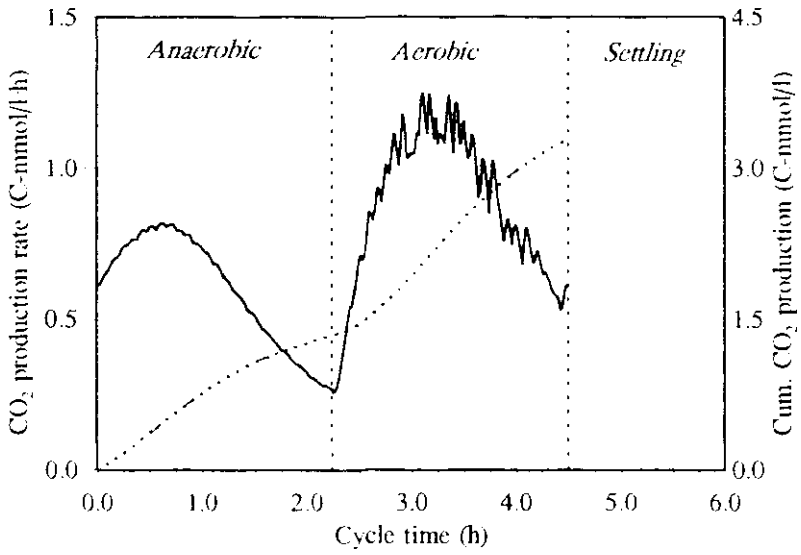


Figure 3 *CO_2 production during a cycle in the SBR, — CO_2 production, ····· cumulative CO_2 production. 6 C-mmol/l acetate was added.*

In batch experiments, (fig. 4), the CO_2 production was measured as a function of the consumed acetate. The observed measurements are generally in accordance with the theoretical value of 0.17 C-mol/C-mol acetate for the glycogen metabolism. From figure 4 it also appears that, in the absence of acetate, a certain CO_2 production still takes place. If this small amount of extra anaerobic CO_2 production also takes place in the reactor the real amount of CO_2 produced coupled to acetate uptake decreases from 0.22 to 0.18 C-mol CO_2/C-mol acetate consumed.

PHB/HAc ratio

Acetate, PHA and phosphorus profiles were measured during a cycle in the SBR (figure 5). From the PHA's, PHB was the component most produced.

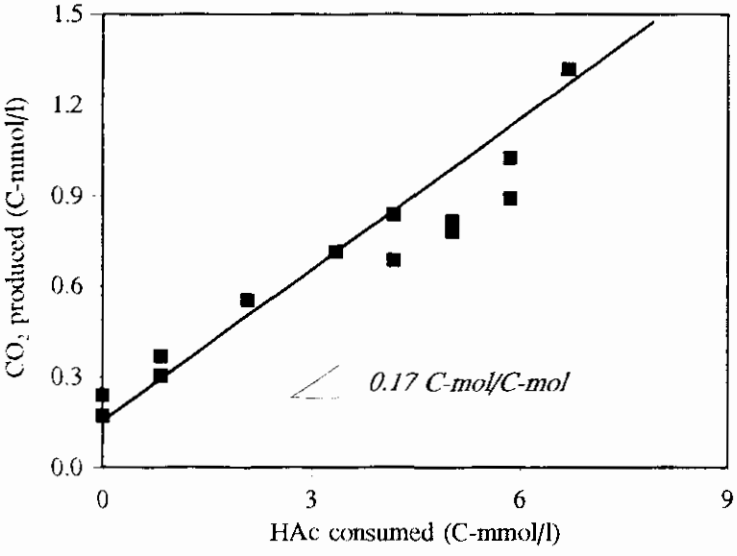


Figure 4 *CO_2 production per C-mol acetate consumped as measured in batch experiments with increasing acetate concentrations.*

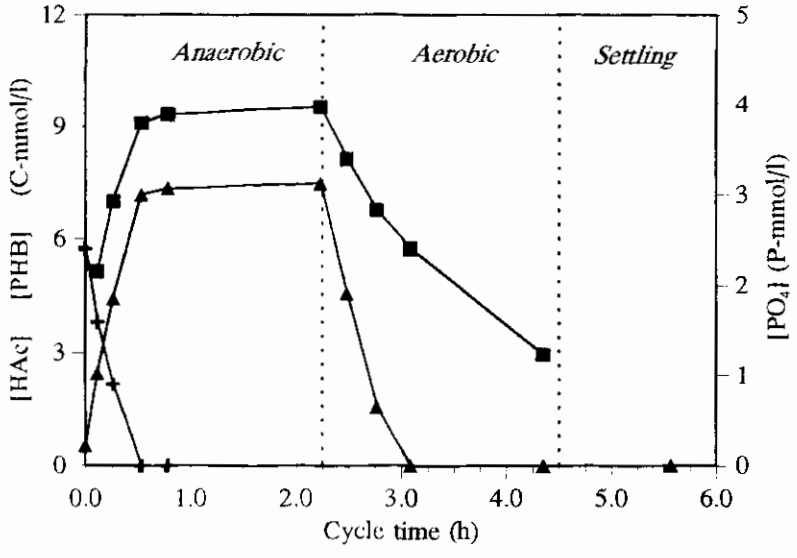


Figure 5 *PO_4 (▲), HAc (+) and PHB (■) profiles during a cycle. Initial acetate concentration 5.75 C-mmol/l, phosphate 0.24 P-mmol/l.*

The amount of PHV (poly-ß-hydroxy valerate) formed was only 10 % of the formed PHB. The carbon in the PHB and PHV conversions were added and further referred to as PHB. PHB was measured as a percentage of the biomass. Since the biomass concentration in a

cycle can not be considered to be constant, due to P-release, PHB formation and glycogen consumption, the MLSS concentration was corrected for these changes. The addition of 5.75 C-mmol/l HAc resulted in a PHB production of 9.6 C-mmol/l at the end of the anaerobic phase. The initial PHB concentration (at the end of the aerobic phase) was 2.6 C-mmol/l, so the amount of PHB produced is 7.0 C-mmol/l, which gives a PHB/acetate ratio of 1.2 C-mol/C-mol. This strongly indicates that an internal carbon source participates in the metabolism. Electron-microscopy on cells, specific stained for glycogen, showed that glycogen was present in the cells (figure 6).

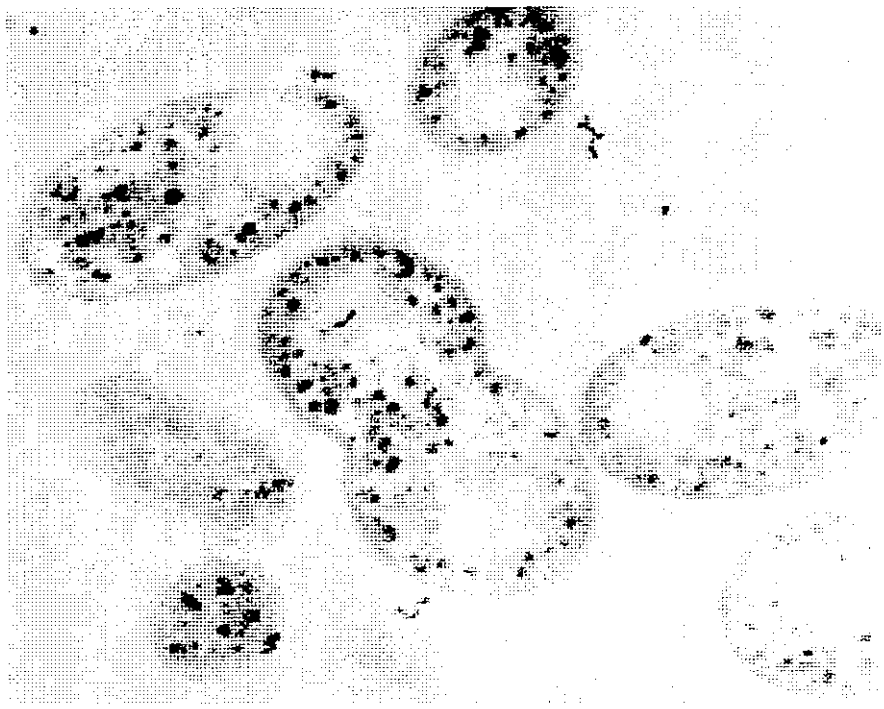


Figure 6 *Electron microscopic detection of glycogen in the sludge at the end of the aerobic phase. The dark spots are stained glycogen granules. Polyphosphate is not present anymore since it disappeared from the cells during the fixation and staining procedure. Magnification 48.000x.*

The PHB measurements in the batch experiments as a function of the acetate consumption are shown in figure 7. The observed ratio was 1.3 C-mol PHB produced per C-mol acetate consumed. The pH in this experiment was 7.4 and did not change.

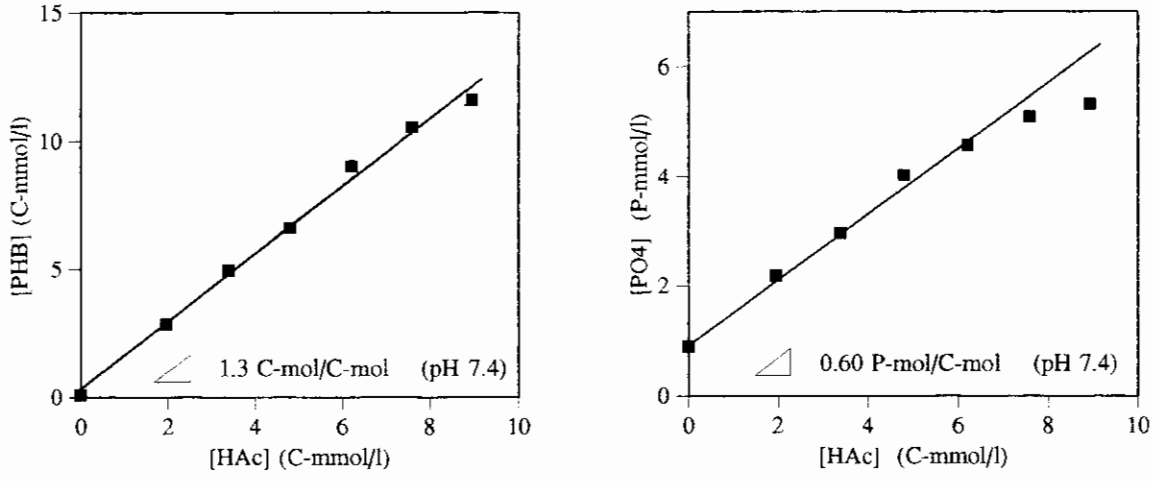


Figure 7 *PHB production (left) and phosphate release (right) per C-mol acetate consumed as measured in batch experiments with increasing acetate concentrations; pH 7.4*

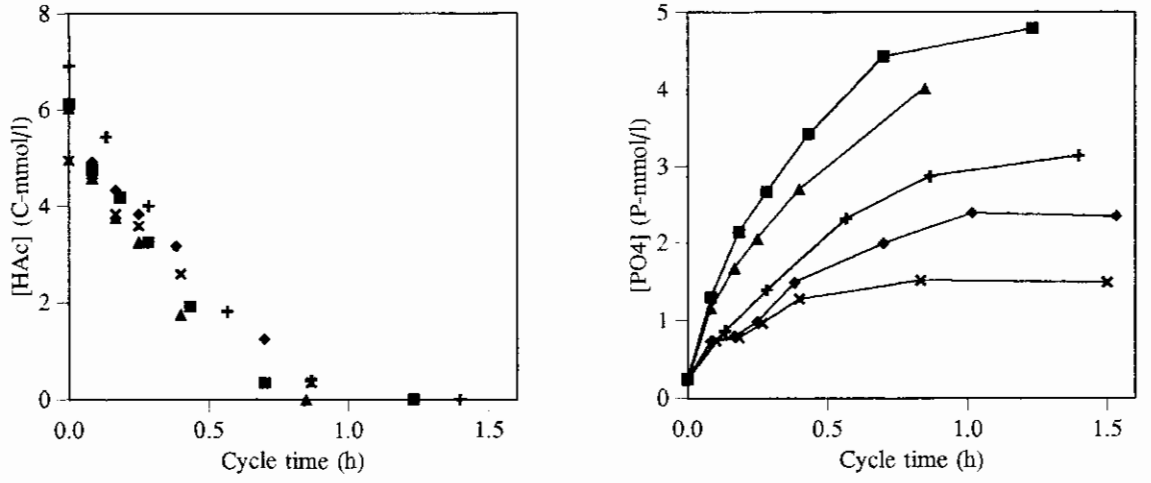


Figure 8 *Acetate uptake (left) and P-release (right) at different pH-values. Average initial acetate concentration 6 C-mmol/l, MLSS 3.2 g/l, pH 5.8 (×), pH 6.4 (♦), pH 7.0 (+), pH 7.8 (▲), pH 8.2 (■).*

Phosphate/HAc Ratio and the pH effect

The amount of phosphate released during the anaerobic part of the cycle at pH 7.0 (figure 5) was 2.88 P-mmol/l, which gives a phosphate/acetate ratio of 0.50 P-mol/C-mol. The phosphate/acetate ratio measured in the PHB experiments at pH 7.4 was 0.60 P-mol released per C-mol acetate consumed (figure 7, right). P-release/acetate uptake experiments were carried out at different pH values in the SBR. The initial acetate concentration was kept constant. During the experiment the pH was controlled. The acetate uptake rate had an average value

of 7.5 C-mmol/l.h and showed no relation with the pH value (figure 8, left). The P-release rate increased with increasing pH (fig. 8, right) and was stoichiometricaly related to the acetate uptake; It varied from 1.45 P-mmol/l at low pH to 4.68 P-mmol/l at high pH. The phosphate/acetate ratio as a function of the pH is shown in figure 9. The ratio for P-release/acetate-uptake varied from 0.25 to 0.75 P-mol/C-mol.

Discussion

Carbon balance

In table IV the theoretical and measured ratios for the anaerobic metabolism are summarized. The measured yield for PHB on acetate of 1.3 shows that an internal carbon source takes part in the anaerobic metabolism and that the ratio obtained is in accordance with the glycogen model. It is striking, that these organisms possess a complete glucose degradation and synthesis route, without addition of glucose in the influent.

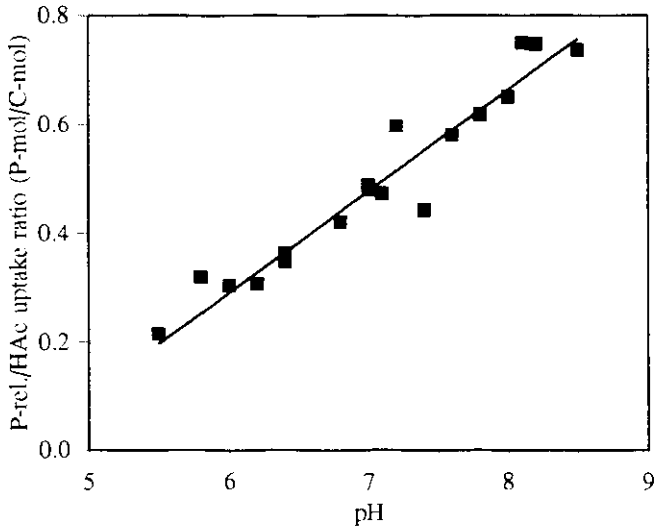


Figure 9 *P-release as a function of the pH. The plotted line was the calculated line based on the increase in $\Delta\psi$ across the cell membrane with increasing pH, according to equation (8).*

Apparently, the presence of glucose is not essential for the metabolism and glycogen is synthesised from PHB aerobically. The yield for carbon dioxide production on acetate of 0.17 C-mol/C-mol also corroborates the glycogen model. The participation of glycogen in the metabolism has consequences for the polyphosphate degradation and P-release since also ATP is produced from the glycogen degradation.

Table IV *Theoretical conversion ratios for the models with the TCA cycle and glycogen as source of reduction equivalents and the measured ratios of the anaerobic metabolism in batch and in the SBR.*

Ratio	Theoretical (mol/mol)		Measured (mol/mol)	
	TCA	Glycogen	Batch	SBR
CO_2/HAc	0.11	0.17	0.17	0.18-0.22
PHB/HAc	0.89	1.33	1.3	1.2
PO_4/HAc	$(0.5+\alpha_1)/\alpha_2$	$(0.25+\alpha_1)/\alpha_2$		0.26-0.76

ATP balance

At low pH (pH 5.5) there is no transport energy required for the uptake of acetate, therefore only energy is used for the conversion of 6 C-mmol/l (Table III) acetate to acetylCoA. This conversion requires 0.5 mol ATP/C-mol thus 3 mmol ATP/l has to be generated. The observed P-release of 1.45 P-mmol/l (figure 8b) is clearly not enough to cover this amount of ATP. Therefore a second energy source takes part in the metabolism (table V). The participation of a 0.5 C-mol glycogen per C-mol acetate in the metabolism, generates 1.5 mmolATP/l which covers, together with the ATP generated in the poly phosphate degradation, the ATP requirements for the acetic acid transport and conversion. This is an additional argument in favour of the glycogen model. Since the acetate consumption rate is constant and the P-release rate is stoichiometricaly related to the acetate uptake rate, also the glycogen degradation kinetics should be pH independent.

Table V *ATP balance over the anaerobic phase. The ATP requirement for the acetate uptake at low and high pH is compared with the ATP generated in the polyP hydrolysis and glycogen degradation. Concentrations in mmol/l.*

pH	HAc uptake	ATP required (mmol/l)			ATP produced (mmol/l)		
		AcetylCoA	transport	Total	polyP	Glycogen	Total
6	6.04	3.02	-	3.02	1.45	1.51	2.96
8	6.04	3.02	3.02	6.04	4.68	1.51	6.19

At high pH the transport of acetate requires 0.5 mol ATP/C-mol acetate, the overall ATP requirement becomes 6 mmol ATP/l. The observed P-release of 4.7 P-mmol/l and participation of glycogen provides the required amount of 6 mmol ATP/l (table V). It can therefore be concluded that the observations strongly support the glycogen model.

Thermodynamic considerations

According to the overall reaction for the glycogen metabolism (r_5) and α_2 = 1 molATP/P-mol, the ratio for the P/acetate ratio for the anaerobic uptake of acetic acid is:

$$Y_{P/HAc} = (¼ + \alpha_1) / \alpha_2 = ¼ + \alpha_1 \qquad \text{(molATP/C-mol)} \qquad (5)$$

Combination with equation 4 gives:

$$Y_{P/HAc} = 0.25 + \frac{n(\Delta p + 2.3RT\Delta pH) + 0.5 \cdot 2.3RT \log \frac{C_{in}}{C_{out}}}{-\Delta G_{ATP}''' \cdot \eta} \qquad \text{(P-mol/C-mol)} \qquad (6)$$

$$Y_{P/HAc} = \frac{2.3nRT}{\Delta G_{ATP}'''} pH_{out} + \frac{2.3nRTpH_{in} + n\Delta p + 0.5 \cdot 2.3RT \log \frac{C_{in}}{C_{out}}}{\Delta G_{ATP}''' \cdot \eta} - 0.25 \qquad (7)$$

Equation 8 describes the pH dependence of the P/acetate ratio and was fitted on the measured data in figure 10. With the assumption of the pmf = - 17.4 kJ/mol (resembling -180 mV/mol), an internal pH of 7 and an acetate concentration outside the cells of 6 C-mmol/l, two parameters remain unknown. With a value of η = 0.3 (-) (19), an internal acetate concentration C_{in} = 0.04 C-mmol/l is found.

$$Y_{P/HAc} = 0.19\ pH_{out} - 0.85 \qquad \text{(P-mol/C-mol)} \qquad (8)$$

These calculations show that the pH effect on the uptake of acetate could be related to the increasing electric potential over the cell membrane with the pH. This concept should hold for the uptake of any negatively charged component over a cell membrane.

Consequences

At low pH the uptake of acetate requires less energy than at high pH. Consequently less phosphate is released at low pH than at high pH. The extra consequence might be that in the aerobic phase less energy is spent on the P-uptake at low pH than at high pH since less phosphate has to be taken up. The remaining energy could be used for other purposes, and for instance, an effect of the pH on the biomass yield is not to be excluded.

The phosphate/acetate ratio is a critical parameter in the description of the biological P-removal process. The control of the pH during the measurement of this parameter is essential. The variation in the ratios published in literature might be an effect of the pH. In some activated sludge systems a stripper tank in the side stream is used in the biological P-removal process to remove the phosphate from the sludge.[14] In such an anaerobic stripper tank acetate is dosed to release the phosphate from the biomass and the phosphate is precipitated or crystallized. For the operation of a stripper tank the effect of the pH is that the required amount of acetate can be reduced considerably if the pH is increased. Therefore it would be better to use acetate-salt than acetic acid.

Conclusions

From the carbon dioxide and PHB measurements it appears that predominantly the glycogen metabolism takes place during anaerobic conditions. The production of 1.3 C-mol PHB per C-mol acetate shows that an internal carbon source is used in the P-metabolism and that the ratio is in accordance with the model. Electron microscopy showed that this carbon source was glycogen. The amount of carbon dioxide produced is also in agreement with the metabolism.

The P-release in the anaerobic phase is strongly influenced by the pH, the release shows a variation of 0.25-0.75 P-mol/C-mol in a pH range of pH 5.5-8.5. The energy generated from the observed P-release at low pH is not enough to convert acetate to acetylCoA, which emphasized the ATP contribution of glycogen conversion in the metabolism.

An explanation for the effect of the pH on the transport of acetate is given by the increasing electrical potential ($\Delta\psi$) difference across the membrane of the cell with increasing pH. Consequently more work must be done to take up a negatively charged ion, like acetate, against the negative electric potential of the cells.

The metabolic model based on glycogen as source of reduction equivalents and ATP and a pH effect on the energy requirement for the transport of acetate explains satisfactorily the stoichiometry of the phosphate-, PHB- and carbon dioxide to acetate ratio during the anaerobic phase of the biological P-removal process.

Nomenclature

α_1	ATP required for uptake of acetate	(molATP/C-mol)
α_2	ATP generated in the degradation of polyphosphate	(molATP/P-mol)
$\Delta G^{\circ\prime\prime}$	transport energy	(kJ/mol)
n	charge of the transported acetate per C-mol	(-)
η	efficiency	(-)
Δp	proton motive force (pmf)	(kJ/mol)
$\Delta \psi$	electric potential difference over the cellmembrane	(kJ/mol)
r	reaction rate	(mol/m^3 · h)
v	internal reaction rate	(mol/m^3 · h)
R	gasconstant	(kJ/K · mol)
T	temperature	(K)

subscripts

s	acetate
phb	poly-ß-hydroxy butyrate
pp	polyphosphate
p	phosphate
c	carbondioxide
w	water
ATP	ATP, elemental composition : '-'
$NADH_2$	$NADH_2$, elemental composition : 'H_2'
1-5	internal reactions

References

1. Arun V., Mino T., Matsuo T., 1988. Biological mechanism of acetate uptake mediated by carbohydrate consumption in excess phosphorus removal systems, Wat. Res. Vol. 22, No. 5, 565-570.
2. Arvin E., Kristensen G.H., 1985. Exchange of organics, phosphate and cations between sludge and water in biological phosphorus and nitrogen removal processes, Wat. Sci. Tech., vol 17, Paris, 147-162.
3. Comeau, Y., Hall, K.J., Hancock, R.E.W., Oldham, W.K., 1986. Biochemical model for enhanced biological phosphorus removal, Water Research, 20, No. 12, 1511-1521.
4. Comeau, Y., Oldham, W.K., Hall, K.J. 1987. Dynamics of carbon reserves in biological dephosphatation of waste water, Biological Phosphate removal from Wastewaters (Advances in Water Pollution Control 4, Ed. R. Ramadori, Pergamon Press, Oxford, 27-38.
5. Dawes, E.A., Senior, P.J. 1973. The role and regulation of energy reserve polymers in micro-organisms, Adv. Microbial Physiol., 10, 135-266.
6. Eikelboom, D.H., Buijsen, H.J.J. van. 1983. Microscopic sludge investigation manual, 2^{e} ed., TNO Research Institute for Environmental Hygiene, Delft, The Netherlands.
7. Gottschalk, G. 1979. Bacterial metabolism, 2^{e} ed., Springer series in microbiology, Springer-Verlag, New york, Berlin, Heidelberg,Tokyo.
8. Groenestijn, J.W. van, Deinema, M.H., Zehnder, A.J.B. 1987. ATP production from polyphosphate in *Acinetobacter* strain 210A, Arch. Microbiol., 148, 14-19.
9. Harold F.M. 1986. The vital force: a study of bio-energetics, W.H. Freeman and Company.
10. Jenkins D., Tandoi, V. 1991. The applied microbiology of enhanced biological phospha e removal - accomplishments and needs, Wat. Res., Vol 25, No. 12, 1471-1478.
11. Mino, T., Arun, V., Tsuzuki, Y., Matsuo, T. 1987. Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process. Biological Phosphate removal from Wastewaters (Advances in Water Pollution Control 4, Ed. R. Ramadori, Pergamon Press, Oxford, 27-38.
12. Roels J.A., 1983. Energetics and kinetics in biotechnology, Elsevier Biomedical Press.
13. Schade, H.A.R. 1973. On the staining of glycogen for electron microscopy with polyacids of tungsten and molybdenum, Electron microscopy and Cytochemistry, 263-266.
14. Toerien, D.F., Gerber, A., Lötter, L.H., Cloete, T.E., 1990. Enhanced biological phosphorus removal in activated sludge systems, Advances in microbial ecology, Marshall K.C., Vol. 11, 173-230.
15. Wentzel, M.C., Dold, P.L., Ekama, G.A., Marais G.v.R., 1985. Kinetics of biological phosphorus release, Wat. Sci. Tech. vol. 17, Paris, 57-71.
16. Wentzel, M.C., Lötter, L.H., Loewenthal, R.E, Marais, G.v.R. 1986. Metabolic behaviour of *Acinetobacter spp.* in enhanced biological phosphorus removal - a biochemical model, Water SA Vol. 12, No 4, 209-224.
17. Wentzel, M.C., Loewenthal, R.E., Ekama, G.A., Marais, G.v.R. 1988. Enhanced polyphosphate organism cultures in activated sludge systems - Part 1: Enhanced culture development, Water SA, Vol. 14, No. 2, 81-92.
18. Wentzel, M.C., Lötter, L.H., Ekama, G.A., Loewenthal, R.E., Marais, G.v.R. 1991. Evaluation of biochemical models for biological excess phosphorus removal, Wat. Sci. Tech, 567-576.
19. Westerhoff, H.V., Dam, K. van. 1987. Thermodynamics and control of biological free-energy transduction, Elsevier Science Publishers B.V.

Appendix II

Stoichiometry of the aerobic metabolism

In the aerobic phase of the biological phosphorus removal process poly-ß-hydroxy butyrate, produced during anaerobic conditions, is used for cell growth, phosphate uptake and glycogen formation. A metabolic model of this process has been developed. The yields for growth, polyphosphate and glycogen formation are quantified using the coupling of all these conversions to the oxygen consumption. The uptake of phosphate and storage as polyphosphate is shown to have a direct effect on the observed oxygen consumption in the aerobic phase. The overall energy requirements for the P-metabolism are substantial: 25% of the acetate consumed during anaerobic conditions and 60% of the oxygen consumption is used for the synthesis of polyphosphate and glycogen.

Introduction

Biological phosphorus removal from waste waters is a process in which phosphorus is removed by bacteria which are able to store intracellular phosphate as polyphosphate. The primary factor to achieve a phosphorus removing bacterial population in an activated sludge plant is the recirculation of the sludge through an anaerobic and aerobic phase. Polyphosphate is produced during aerobic conditions and serves as an energy source for the organisms during anaerobic conditions which enables them to take up and store substrate in the absence of an electron acceptor.[3, 7, 10, 17]

In the anaerobic phase P-removing bacteria transport lower fatty acids, mainly acetate, into the cell and store these as poly-hydroxy-alkanoates (PHA, for instance poly-hydroxy-butyrate, PHB). The energy for this transport and storage reaction is supplied by hydrolysis of intracellularly stored polyphosphate (polyP) to ortho-phosphate, which is released from the cell to the liquid. The reduction equivalents required for the conversion of acetate to PHB are supplied by conversion of intracellularly stored glycogen into PHB. The conversions in the anaerobic phase of the process are well understood [10] and previously we have experimentally validated a metabolic model for this phase.[13] The metabolism of the aerobic phase is much less studied although the effective phosphate removal takes place in this phase. In addition, the synthesis of polyphosphate and glycogen in the aerobic phase are essential for the performance in the anaerobic phase. For a stable process operation the anaerobically consumed polyP and glycogen must be replenished in the aerobic phase, whereas the anaerobically produced PHB is consumed in the aerobic phase.

In the aerobic phase of the P-removal process, the anaerobically produced PHB is used for cell growth, polyphosphate synthesis and glycogen formation. The use of an internal substrate as well as the simultaneous production of two internal storage products during growth is a unique feature of the P-removing organisms. A model for this kind of processes describing also the aerobic phase was developed by Wentzel et al.[17] In the experimental validation of this model however, the conversion of PHB and the separate production of biomass and glycogen were not measured. The role of polyphosphate (polyP) as energy source during anaerobic conditions implies that during aerobic conditions energy has to be spent on the synthesis of polyphosphate and on the transport of phosphate into the cells. In addition it is known that growth and glycogen synthesis from PHB requires energy in the form of ATP. Since ATP

results from oxidative phosphorylation all conversions (growth, polyP- and glycogen synthesis) are coupled to oxygen consumption.

Aim of the present study is the development of a structured metabolic model of the aerobic phase in which the yield on PHB for polyP synthesis, growth and glycogen synthesis is quantified. The coupling of all internal conversions to the oxygen consumption makes it possible to quantify these yields by using a method based on oxygen respiration measurements. In a following paper we will use the established stoichiometry of the anaerobic and aerobic phase to describe the kinetics of the biological phosphorus removal process.

Metabolic model

The metabolic reactions in the aerobic phase can be separated in two groups: the energy generating reactions and the energy consuming reactions, see figure 1.

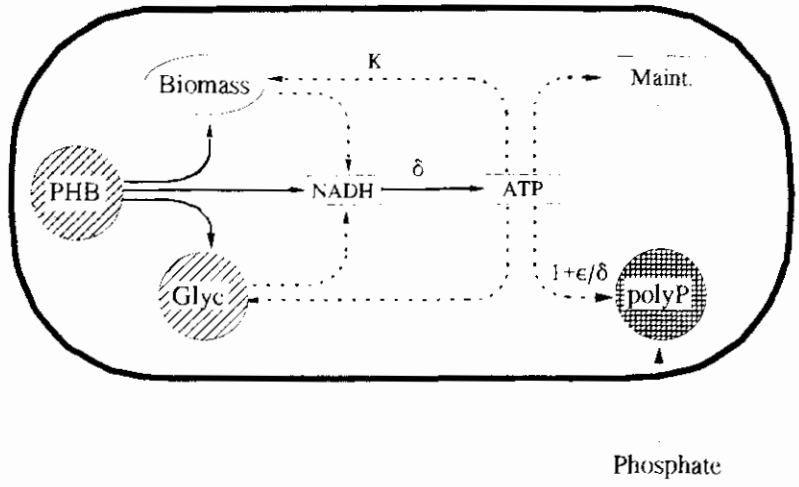


Figure 1 *Aerobic metabolism.*

The energy producing reactions can be described by two equations: PHB catabolism (reaction 1) and oxidative phosphorylation (reaction 2). The energy consuming reactions are described by 3 reactions: production of biomass (reaction 3), polyP synthesis (reaction 4a and 4b) and glycogen synthesis (reaction 5).

Reaction 1: **PHB catabolism**

PHB is degraded to acetylCoA[4] and converted in the tricarboxylic acid cycle (TCA). The $FADH_2$ which is produced is assumed to be equivalent to $NADH_2$.

$$-\underset{\text{PHB}}{CH_{1.5}O_{0.5}} - 1.5\ H_2O + 2.25\ NADH_2 + 0.5\ ATP + CO_2 = 0 \qquad [1]$$

Reaction 2: **Oxidative phosphorylation**

In the oxidative phosphorylation ATP is produced from $NADH_2$. The amount of ATP produced per electron pair is represented by δ, the so called P/O ratio which resembles the efficiency of the oxidative phosphorylation. This process can be represented stoichiometrically by:

$$-NADH_2 - 0.5\ O_2 + H_2O + \delta\ ATP = 0 \qquad [2]$$

Reaction 3: **Biomass synthesis from PHB**

In the synthesis of biomass 0.27 mol CO_2 is produced per C-mol biomass.[5] The amount of ATP needed for the formation of biomass precursors from acetylCoA and polymerisation of these precursors to 1 C-mol biomass is represented by K. According to biochemical analysis[14] the expected value for K = 1.5 mol ATP per C-mol biomass. The term m_{ATP} is the specific ATP consumption due to maintenance processes. The biomass composition shown was corrected for the polyP, PHB and glycogen content and determined by CHON analysis, 1 C-mol active biomass is equal to 26 g. For further symbols see the list of nomenclature.

$$-1.27\ \underset{\text{PHB}}{CH_{1.5}O_{0.5}} - 0.20\ NH_3 - 0.015\ H_3PO_4 - (K+m_{ATP}/\mu)\ ATP - 0.385\ H_2O$$
$$+ CH_{2.09}O_{0.54}N_{0.20}P_{0.015} + 0.615\ NADH_2 + 0.27\ CO_2 = 0 \qquad [3]$$

Reaction 4a: **Phosphate transport**

The transport of phosphate across the cell membrane is a process which requires energy. Phosphate is a negatively charged ion and has to be taken up against the electric potential difference over the cell membrane. Positive ions required for the polyP synthesis (Mg^{2+} and K^+) are taken up without energy costs.[6, 11] The energy used for the transport of phosphate is generated by the import of protons which are subsequently exported over the

cell membrane in the oxidation of $NADH_2$.[6, 11] Therefore a certain amount of phosphate, ϵ, can be transported for each consumed $NADH_2$. The Gibbs energy for oxidation of $NADH_2$ by O_2 per two electrons is -220 kJ/mol.[6] The uptake of 1 mol of negatively charged ions like phosphate ($H_2PO_4^-$) will cost 14.7 kJ/mol.[13] According to Westerhoff and van Dam[19] membrane coupled processes typically have thermodynamic efficiencies of 30 to 60 %. The uptake of phosphate will require at least 14.7/(0.6·220) = 0.11 mol $NADH_2$ per mol phosphate at 60 % thermodynamic efficiency and 0.22 mol $NADH_2$ if the reaction proceeds with a thermodynamic efficiency of 30%. This indicates that $1/\epsilon$ will range between 0.22 and 0.11.

$$- H_3PO_4 \text{ out} - 1/\epsilon \; NADH_2 - 1/2\epsilon \; O_2 + H_3PO_4 \text{ in} + 1/\epsilon \; H_2O = 0 \qquad [4a]$$

Reaction 4b: **Polyphosphate synthesis**

The composition of polyphosphate, based on the measured release of phosphorus, magnesium and potassium, was $Mg_{1/3}K_{1/3}PO_3$. Because the elements magnesium and potassium were not considered in the model, polyphosphate is represented as HPO_3. The amount of ATP which is required for the synthesis of polyP is represented with α_3. For the synthesis of polyphosphate 1 ATP is necessary[8] and hence a typical value of $\alpha_3 = 1$.

$$H_3PO_4 \text{ in} - \alpha_3 \; ATP + \underset{\text{polyP}}{HPO_3} + H_2O = 0 \qquad [4b]$$

Reaction 5: **Glycogen production**

This reaction it based on the production of glycogen from oxaloacetate in glyconeogenesis.[15] Oxaloacetate is produced from PHB through the glyoxylate cycle.[15]

$$- 4/3 \underset{\text{PHB}}{CH_{1.5}O_{0.5}} - 5/6 \; ATP - 5/6 \; H_2O + \underset{\text{Glycogen}}{CH_{10/6}O_{5/6}} + 1/3 \; CO_2 + 1 \; NADH_2 = 0 \qquad [5]$$

The internal reactions, [1..5] are based on biochemical knowledge and stoichiometry. These reactions occur in the cell and can not be observed directly. However, the observed conversion rates outside the cell are a result of the internal reaction rates, which are coupled through $NADH_2$ and ATP.

PHB conversion rate

The conversion rates can be expressed as a function of the internal reaction rates by a set of

linear equations with parameters composed of the stoichiometric coefficients of the internal reactions,[12] as shown above. The rates of reactions [1..5] are $v_1..v_5$, and can be expressed with the transposed reaction rate vector r^T:

$$v^T = (v_1, v_2, v_3, v_{4a}, v_{4b}, v_5) \quad (1)$$

If the transposed conversion rate vector for the 12 compounds is:

$$r^T = (r_{phb}, r_x, r_{gl}, r_{pp}, r_n, r_{p\,out}, r_{p\,in}, r_o, r_c, r_w, r_{ATP}, r_{NADH2}) \quad (2)$$

and α the metabolic reaction matrix:

$$\alpha = \begin{bmatrix} -1 & 0 & -1.27 & 0 & 0 & 4/3 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0.20 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0.015 & 1 & 1 & 0 \\ 0 & 1/2 & 0 & \frac{1}{2\epsilon} & 0 & 0 \\ 1 & 0 & 0.27 & 0 & 0 & 1/3 \\ 1.5 & 1 & 0.385 & \frac{1}{\epsilon} & 1 & 5/6 \\ 1/2 & \delta & (K - \frac{\Pi_{atp}}{\ldots}) & 0 & \alpha_{\ldots} & 5/6 \\ 2.25 & 1 & 0.615 & \frac{1}{\epsilon} & 0 & 1 \end{bmatrix}$$

then the following set of 12 linear relations is obtained, by writing out the compound balances for each of the 12 compounds (PHB..$NADH_2$):

$$r = \alpha * v \quad (3)$$

If the well known[12] assumption is made that no net accumulation of $NADH_2$ and ATP takes place, $r_{ATP} = 0$ and $r_{NADH2} = 0$. Further, the assumption is made that the internal ortho-phosphate concentration is kept constant and therefore $r_{p\,in} = 0$. Now we have a system with 15 unknown rates ($v_1..v_5$ and $r_{phb}..r_w$) and 12 linear equations. With the

measurement of 15-12 = 3 of the unknown rates, all remaining conversion rates in the system can be described. The following unknown rates were chosen: r_x, r_{pp} and r_{gl}. The overall equation for the conversion of PHB in the aerobic phase then becomes:

$$r_{phb} = \frac{1}{Y_{sx}^{max}} r_x + \frac{1}{Y_{spp}^{max}} r_{pp} + \frac{1}{Y_{sgl}^{max}} r_{gl} + m_s C_x \quad (4)$$

The following relations for the defined maximal yields and maintenance, and the metabolic model parameters (δ, K, ϵ, α_3, m_{ATP}) are found:

$$\frac{1}{Y_{sx}^{max}} = \frac{0.635+2.243\delta+K}{2.25\delta+0.5} \quad (5)$$

$$\frac{1}{Y_{spp}^{max}} = \frac{\delta/\epsilon+\alpha_3}{2.25\delta+0.5} \quad (6)$$

$$\frac{1}{Y_{sgl}^{max}} = \frac{2\delta+1.5}{2.25\delta+0.5} \quad (7)$$

$$m_s = \frac{m_{atp}}{2.25\delta+0.5} \quad (8)$$

Equation (4) describes the amount of PHB required for the production of biomass, polyphosphate and glycogen synthesis and maintenance.

Oxygen conversion rate

In an similar way the total amount of oxygen consumed in the aerobic phase as a function of the chosen growth (r_x), polyphosphate (r_{pp}) and glycogen (r_{gl}) rates can be derived from the set of 12 linear relations with 15 unknown rates (eq 3):

$$-r_o = \frac{1}{Y_{ox}^{max}} r_x + \frac{1}{Y_{opp}^{max}} r_{pp} + \frac{1}{Y_{ogl}^{max}} r_{gl} + m_o C_x \quad (9)$$

The following relations hold for the defined maximal oxygen based yields and maintenance coefficient as a function of the metabolic model parameters (δ, K, ϵ, α_3, m_{ATP}):

$$\frac{1}{Y_{ox}^{max}} = \frac{0.714+2.523\delta+1.125K}{2.25\delta+0.5} - 1.121 \tag{10}$$

$$\frac{1}{Y_{opp}^{max}} = \frac{1.125(\delta/\epsilon+\alpha_3)}{2.25\delta+0.5} \tag{11}$$

$$\frac{1}{Y_{ogl}^{max}} = \frac{1.125(2\delta+1.5)}{2.25\delta+0.5} - 1 \tag{12}$$

$$m_o = \frac{1.125m_{atp}}{2.25\delta+0.5} \tag{13}$$

Further, combining (5) to (8) with (10) to (13), it can easily be shown that the following direct relations exist between the substrate and oxygen based maximal yields and maintenance coefficient:

$$\frac{1}{Y_{ox}^{max}} = 1.125\frac{1}{Y_{sx}^{max}} - 1.121 \tag{14}$$

$$\frac{1}{Y_{opp}^{max}} = 1.125\frac{1}{Y_{spp}^{max}} \tag{15}$$

$$\frac{1}{Y_{ogl}^{max}} = 1.125\frac{1}{Y_{sgl}^{max}} - 1 \tag{16}$$

$$m_o = 1.125m_s \tag{17}$$

Determination of the stoichiometric coefficients of the metabolic model

The maximal yield for biomass formation ($1/Y_{ox}^{max}$), polyphosphate synthesis ($1/Y_{opp}^{max}$), and glycogen formation ($1/Y_{ogl}^{max}$) on oxygen are now expressed as a function of the P/O ratio (δ), the coefficient for the transport of phosphate (ϵ), the polymerisation constant (K) and the maintenance energy m_{ATP}. Determination of these coefficients can be achieved by the established relations, eq (4-13), in combination with appropriate measurements, as shown below.

Determination of the P/O ratio, (δ) and transport coefficient, (ϵ)

The P/O ratio is established by measurement of the oxygen/phosphate ratio ($1/Y_{opp}^{max}$) for various amounts of accumulated phosphate. Assuming that cell growth and glycogen production are not influenced by the amount of phosphate accumulation, eq 9 shows that the oxygen/phosphate ratio can be calculated from the difference in oxygen consumption rate (r_o^{+P}-r_o^{-P}) in the absence and presence of phosphate according to the following relation:

$$r_o^{+P} - r_o^{-P} = -\frac{1}{Y_{opp}^{max}} r_{pp} \qquad (18)$$

In a batch situation the cumulative mass of oxygen consumption, M_o and mass of polyphosphate accumulation, M_{pp} are, according to eq 11 and 18, related as:

$$M_o^{+P} - M_o^{-P} = -\frac{1}{Y_{opp}^{max}} M_{pp} = 1.125 \frac{\delta/\epsilon + \alpha_3}{2.25\delta + 0.5} M_{pp} \qquad (19)$$

The coefficient α_3 is known[8] to be equal to 1 and ϵ is expected to have a value between 5 and 9. Figure 2 shows how $1/Y_{opp}^{max}$ depends on the P/O ratio (δ) and the transport coefficient of phosphate, ϵ. The assumed value of ϵ has effect on the calculated P/O ratio from the measured value of Y_{opp}^{max}, however it can be shown that this does not influence the oxygen based yields very much. An average value of ϵ = 7 was taken as an appropriate estimate. Using these values for α_3 and ϵ, an accurate value of the oxygen/phosphate ratio will directly yield the P/O ratio, δ from eq 19.

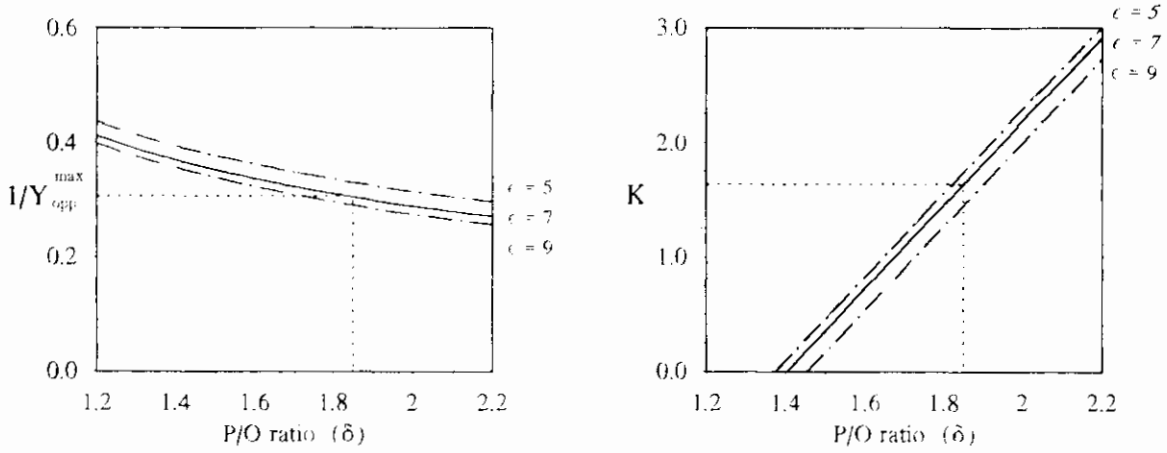


Figure 2 *Dependency of Y_{opp}^{max} (left) and the polymerisation coefficient, K, (right) on the P/O ratio, δ, and the phosphate transport coefficient, ϵ.*

Determination of the maintenance coefficient, m_{ATP}

For the determination of the maintenance coefficient the oxygen consumption rate was followed during 25 h. At the start of the aerobic phase the oxygen consumption is high due to utilisation of PHB for polyP uptake, growth and glycogen formation. The oxygen consumption rate decreases during the aerobic phase due to the decreasing fraction PHB in the biomass. When the oxygen consumption is followed during 25 h, the oxygen consumption decreases further and will finally become constant. This is the oxygen consumption related to maintenance, m_o. Eq 13 leads then to m_{ATP}, using the previously obtained value for δ.

Determination of the polymerisation coefficient, K

With the determination of δ, ϵ and m_{ATP} and the value of α_3=1, the polymerisation constant K can be calculated. The value for K can be derived from the oxygen balance (eq. 9), in combination with eq (10-13):

$$K = \frac{(2.25\delta+0.5)r_o+(0.154+1.125m_{atp}/\mu)r_x+1.125(\delta/\epsilon+\alpha_3)r_{pp}+1.188r_{gl}}{1.125r_x} \qquad (20)$$

For the calculation of K, measurement of the oxygen consumption, (r_o), biomass production, (r_x), polyP accumulation, (r_{pp}) and glycogen production, (r_{gl}) is required.

In figure 2 the relation between the P/O ratio and K is shown for typical experimental values of r_o, r_x, r_{pp}, r_{gl} and C_x in our SBR. The P/O ratio highly influences the value found for the polymerisation coefficient K. An accurate value for the P/O ratio enables the determination of the value for K, while the value for epsilon is of minor importance.

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

The study was carried out in a laboratory fermenter with a working volume of 2 l, at 20 °C. The fermentor was equipped with pH, O_2 and redox electrodes. The pH was maintained at pH 7.0 ± 0.05 using 0.5 N HCl and 1 N NaOH. The reactor was operated as a sequencing batch (SBR) with a cycle of 6 hours consisting of anaerobic (2.25 h), aerobic (2.25 h) and settling (1.5 h) periods. Biological phosphorus removing sludge was used as an inoculum. Since the added acetate was completely consumed in the anaerobic zone, only organisms capable of anaerobic acetate consumption were accumulated in the reactor. Methanogens were not present since part of the time oxygen was present. One litre medium was fed at the start of the anaerobic phase and effluent was removed at the end of the settling period, resulting in a hydraulic retention time of 12 h. At the end of the aerobic phase 63 mL excess sludge was removed, resulting in a biomass retention (SRT) of 8 days, which results in a growth rate (μ) of 0.014 h^{-1} (growth in the aerobic phase only). A stirrer speed of 500 rpm was maintained, except for the settling period. During anaerobic conditions nitrogen gas was bubbled through the reactor with a flow rate of 30 l/h, aeration was provided with an air flowrate of 60 l/h. The dissolved oxygen concentration during the aerobic phase was measured and was always above 50% of the saturation concentration. The offgas was analyzed for carbon dioxide production and oxygen consumption.

Media

Sterilized synthetic medium was used containing per litre: 0.85 g $NaAc.3H_2O$ (400 mgCOD/l) as carbon source, 107 mg NH_4Cl, 75.5 mg $NaH_2PO_4.2H_2O$ (15 mgP/l), 90 mg $MgSO_4.7H_2O$, 36 mg KCl, 14 mg $CaCl_2.2H_2O$, 1 mg yeast extract, 0.3 ml nutrient solution. The nutrient solution contained per litre: 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO_4.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$, 10 g EDTA. The medium for the respiration measurements resembled the medium in the SBR at the end of the anaerobic phase (high concentrations of Mg^{2+}, K^+ and PO_4^{3-}) and contained 0.08

g NH_4Cl, 0.58 g $MgSO_4.7H_2O$, 0.02 g $CaCl_2.2H_2O$, 0.18 g KCl 0.001 g Tris, 0.3 ml nutrient solution and 0.001 g yeast extract. Phosphate was added separately.

Analyses

For dry weight determination a 15 ml sample of the sludge was filtered on a Whatman glass microfibre filter. The filter was dried for 24 h at 80 °C and weighed on a microbalance. The ash content was determined by incinerating the dry filters in an oven at 550 °C. The elemental composition of the biomass was measured with washed and freeze dried biomass. Carbon, hydrogen, oxygen and nitrogen were determined using a Perkin Elmer 240B Elemental Analyser. The elemental composition was corrected for the polyphosphate and stored carbon. Ortho-phosphate was determined by the ascorbic acid method. Acetate was determined on a GC with a Hayesep Q 80-100 mesh. column at 185 °C and FID detector. NH_4^+ was measured with an NH_3 electrode (Metrohm), NO_3^-, NO_2^- were measured by Standard Methods.[1]

For PHB determination, 0.5-2 mg benzoic acid in 1-propanol was added to 15-20 mg washed and freeze dried biomass in closable tubes of 15 ml. 1.5 ml of a mixture of 1-propanol and concentrated hydrochloric acid (4:1) and 1.5 ml di-cloromethane was added and heated for 2 hours at 100 °C. After cooling, the organic phase was extracted with 3 ml water. 1 ml of the organic phase was dried on Na_2SO_4 and 0.4 µl was injected on a GC with a stabilwax (Restek) column at 200 °C with a FID detector at 240 °C.

For glycogen determination, 6 M HCl was directly added to the sample to a final concentration of 0.6 M. The sample was placed in a waterbath at 100 °C for 1 hour. After cooling and centrifugation the glucose concentration of the supernatant was measured on a HPLC.

The gasflows were controlled with massflow controllers (Brooks 5850) for air and nitrogen gas. The offgas was dried over a perma pure column and carbon dioxide was measured with a Beckman 870 infrared analyser. Oxygen was measured with a Servomex 1100 paramagnetic analyser.

Measurement of the respiration rate in the aerobic phase

The oxygen consumption of the biomass in time, exposed to various amounts of phosphate, was followed in an automated respirometer connected to a completely mixed and aerated batch reactor with a volume of 0.5 l. pH was controlled at pH 7 and the temperature was maintained on 20 °C. For measurements in the presence of various amounts of phosphate, biomass was taken at the end of the anaerobic phase of the SBR and washed two times and taken up into medium for the respiration measurements which contained no phosphate.

Phosphate was added separately at the start of the experiment. The biomass was continuously pumped to a small vessel with a volume of 10 ml (also thermostated) which contained the oxygen electrode, see figure 3. Every 3 minutes the flow from the main vessel to the oxygen measurement vessel was switched off. The oxygen consumption rate was measured by following the dissolved oxygen concentration for 3 minutes, after which the pump was switched on again. Data were logged by a computer. The oxygen consumption rate was followed during at least 2.25 h, the length of the aerobic phase in the SBR. During these experiments samples from the main vessel were taken to determine the phosphate and ammonium concentration in the liquid and the PHB and glycogen content of the biomass.

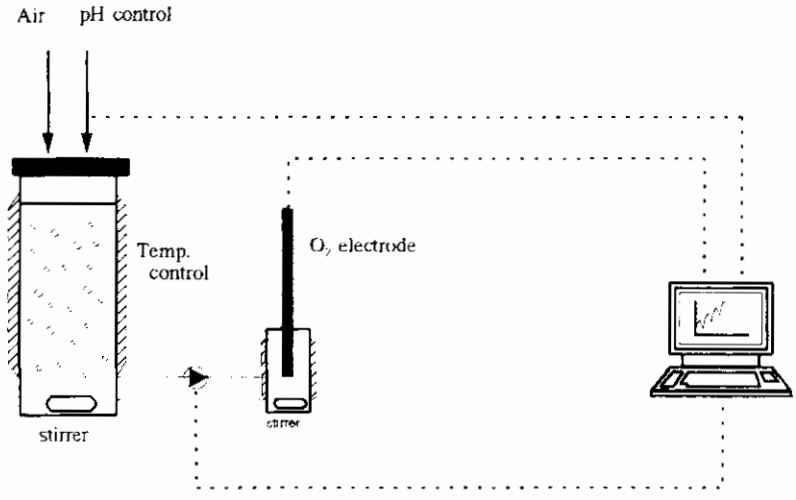


Figure 3 *Schematic representation of the experimental setup for the continuous measurement of the oxygen consumption rate.*

Results

SBR measurements

The SBR was operated for 100 days before the experiments were started and could therefore be considered in steady state. Figure 4a gives an overview of the conversions occurring in the aerobic phase of the biological phosphorus removal process in the SBR. PHB is consumed for the formation of biomass whereas phosphate and glycogen were accumulated in the biomass. The ammonium consumption can be used to calculate the biomass production because nitrification is absent. The nitrite and nitrate concentration were regularly measured

and always zero. The elemental composition of the active biomass, without polyphosphate or carbon storage reserves, was found to be $CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$.

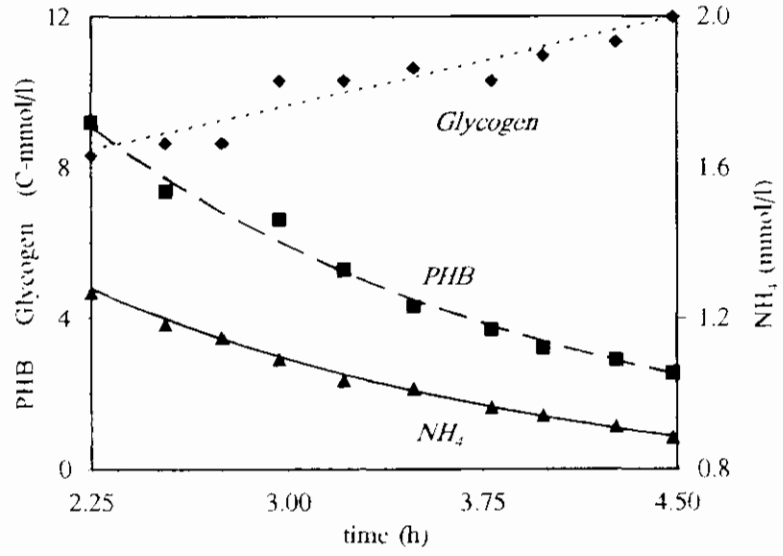


Figure 4a *Conversion of PHB (■), NH4 (▲) and glycogen (♦) during the aerobic phase in the SBR.*

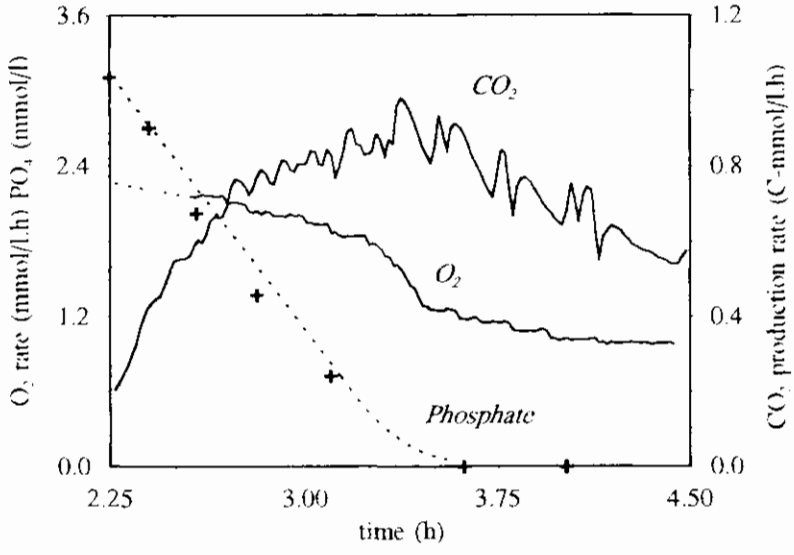


Figure 4b *CO_2 production rate, oxygen consumption rate and phosphate uptake (+) during the aerobic phase of the SBR.*

Figure 4b shows the CO_2 production and O_2 consumption during the aerobic phase in the SBR. The oxygen consumption rate decreases after the phosphate is completely taken up (t=3.4 h). This already indicates that a substantial amount of energy is required for the uptake

of phosphate. The total conversions during the aerobic phase are given in table I. The redox and carbon balance during this phase showed a recovery of 101 and 106 %, which indicates the realibility of the measurements.

Table I Measurements in one cycle of the SBR in steady state.

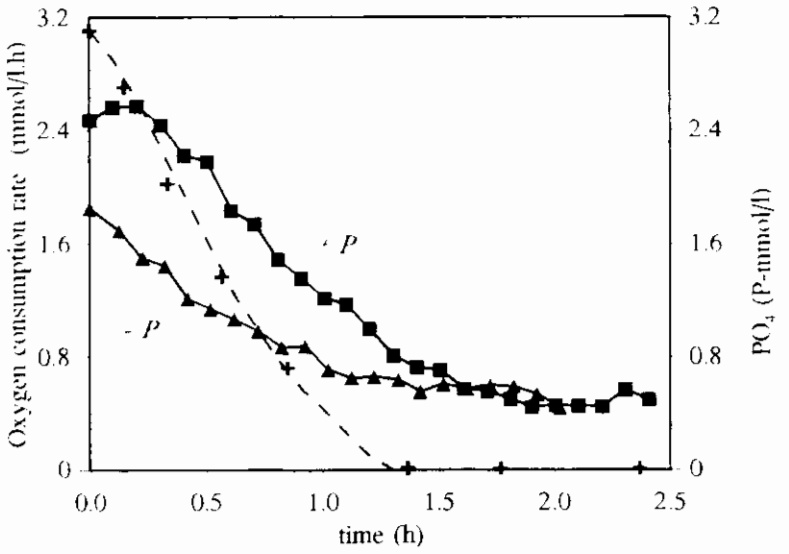
Measured compounts	flow	unit
Conversions over one cycle		
HAc consumed	6.10	C-mmol/l cycle
CO_2 produced	3.37	mmol/l cycle
Conversions in the aerobic phase		
PHB consumed	8.04	C-mmol/l
Ammonium consumed	0.42	mmol/l
Biomass' increase	2.10	C-mmol/l
Phosphate uptake	2.71	P-mmol/l
Glycogen produced	3.62	C-mmol/l
O_2 consumed	2.97	mmol/l

' biomass excluding polyphosphate and carbon reserves

Determination of the P/O ratio from the oxygen/phosphate yield

The effect of the phosphate uptake and synthesis of polyphosphate on the oxygen consumption was studied in a respirometer (Fig 3). Figure 5a gives the result of a measurement in the presence and absence of phosphate in the medium. When phosphate was present, it was completely taken up within 1.5 hour. The oxygen consumption rate during this period was clearly enhanced due to the energy requirement for P-uptake and polymerization to polyphosphate. When phosphate is depleted, the oxygen consumption rate becomes equal to the rate observed in the experiment without phosphate in the medium. Although phosphate is absent in the medium, the organisms can utilize polyphosphate to provide the phosphate required for cell growth [8]. The cumulative oxygen consumption, figure 5b, shows that overall 0.95 mol O_2 is consumed for the total uptake of 3.1 P-mol. The dashed line in figure 5a and 5b representing the phosphate uptake was calculated with the difference in oxygen consumption between the experiments in presence and absence of phosphate and an oxygen/phosphate yield of 0.31 mol/P-mol, see below.

a)



b)

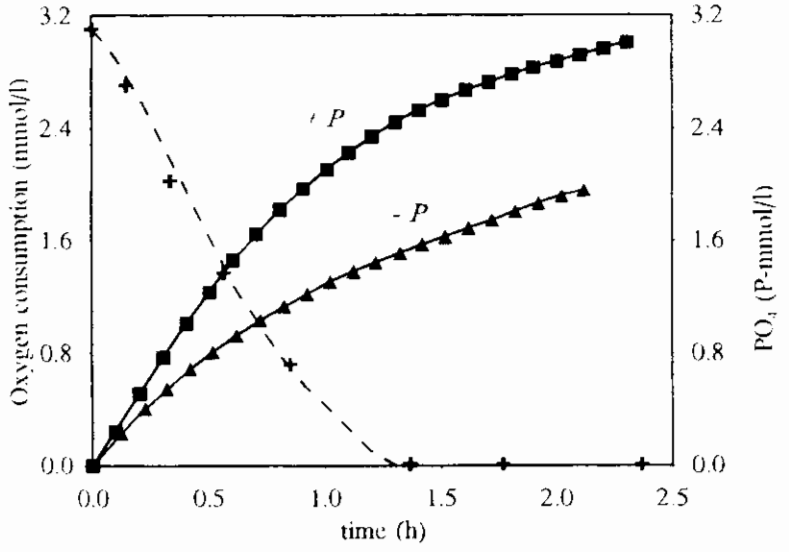


Figure 5 *Oxygen consumption rate (5a) and cummulative oxygen consumption (5b) of the biomass in the presence (■) and absence (▲) of phosphate and the phosphate concentration (+) in the liquid. The line for the phosphate uptake was calculated from the difference in oxygen consumption and a O_2/PO_4 ratio of 0.31 mol/mol.*

The oxygen/phosphate yield has been calculated with equation 19. Comparison of the extra oxygen consumption (M_o^{+P}-M_o^{-P}) with the phosphate uptake M_{pp} (Fig 6) gives a stoichiometric ratio of 0.31 mol O_2/P-mol.

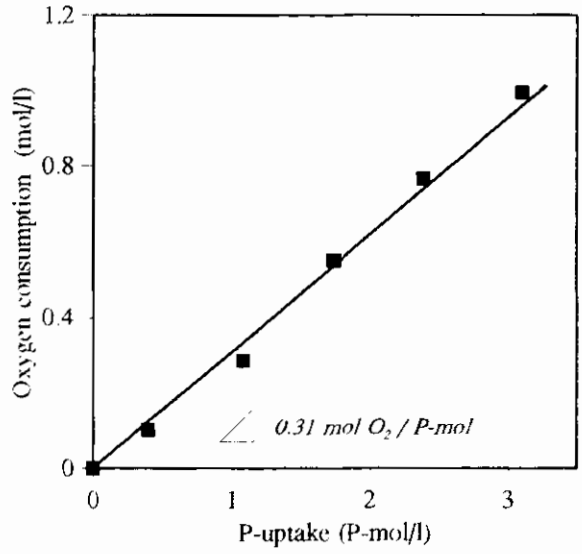


Figure 6 *Stoichiometric relation between the oxygen consumption and phosphate uptake.*

Figure 7 shows the total oxygen consumption M_o of many different experiments whereby the total phosphate uptake differed. Here a yield of 0.30 mol O_2 /P-mol is found. The intercept with the y-axis equals the oxygen consumption for growth and glycogen formation, M_o^{-P}. An oxygen/phosphate ratio of 0.30 mol/P-mol and ϵ = 7 leads to a P/O ratio (δ) of 1.85 (eq. 19).

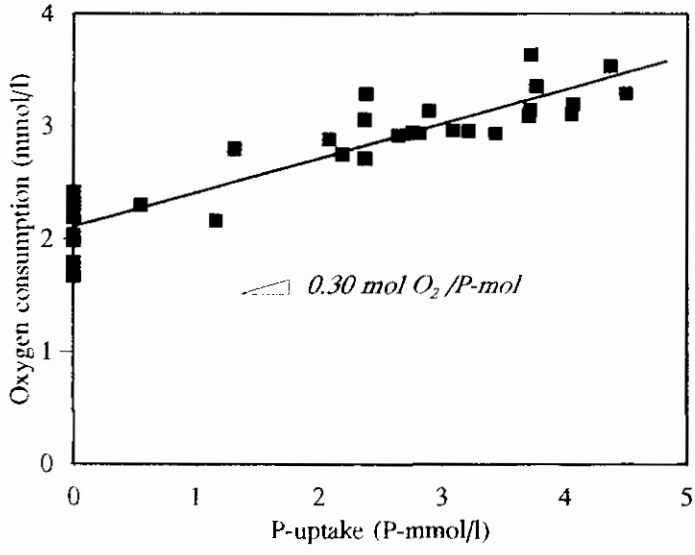


Figure 7 *Effect of various amounts of accumulated polyphosphate on the oxygen consumption of the biomass.*

Alternatively the oxygen/phosphate ratio can be established directly from one measurement of the oxygen consumption rate in the SBR. In figure 8 the oxygen consumption rate of the SBR is shown. After 1 hour (t = 3.2 h) phosphate is consumed and the oxygen consumption rate decreases to the level corresponding with the growth and glycogen synthesis. Extrapolation of the oxygen consumption for cell growth and glycogen production to the start of the aerobic phase is possible since both processes mentioned are not significantly influenced by the phosphate uptake, see below. The amount of oxygen used for phosphate uptake is now given by the difference between the measured curve and the extrapolated line. Calculation of the oxygen/phosphate ratio during this cycle yielded a value of 0.29 mol O_2/P-mol, which is in close agreement with the values of the respirometer. In figure 8, the phosphate uptake was calculated by using the value for the oxygen/phosphate ratio found in the respirometer, and compared with the measured P-uptake.

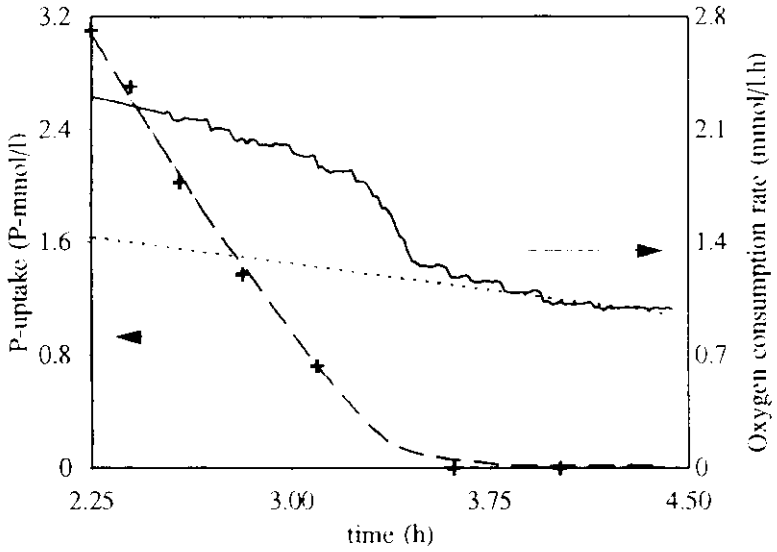


Figure 8 *Total oxygen consumption rate measured in the offgas of the SBR (—) and extrapolated line (····) representing the oxygen consumption for growth and glycogen synthesis. The difference in oxygen consumption between these lines was used to calculate the P-uptake with a value of 0.3 mol/P-mol for the oxygen/phosphate ratio. (+) measured P-uptake.*

Determination of the maintenance coefficient, m_{ATP}

The maintenance coefficient is determined by the oxygen consumption rate which was followed during 25 h in an experiment in the respirometer, see figure 9. At the start of the aerobic phase the oxygen consumption rate is associated with utilisation of PHB for polyP

synthesis, growth and glycogen formation. After 1 hour the drop in oxygen consumption occurs due to depletion of phosphate from the medium. The further drop in oxygen consumption rate is due to the decreasing fraction PHB in the biomass. After 9 h the oxygen consumption finally becomes constant and the observed oxygen consumption rate of 0.25 mmolO_2/l.h is taken as the oxygen consumption for maintenance purposes, m_0 = 4.5 10^{-3}. The maintenance per C-mol biomass, m_s = 4.0 10^{-3} and the m_{ATP} value of 0.019 molATP/C-mol.h is then found, using eq (17) and (13).

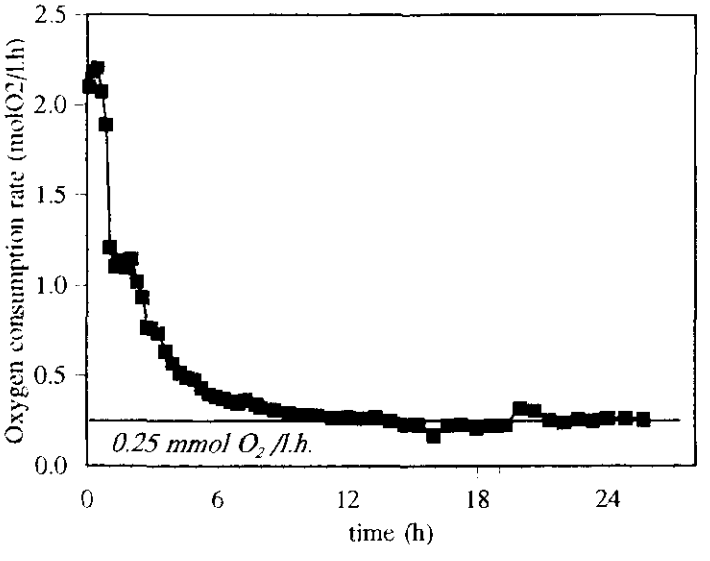


Figure 9 *Determination of the maintenance coefficient from the P-release in absence of substrate during anaerobic conditions and a biomass concentration of 55 C-mmol/l.*

Determination of the polymerisation coefficient, K

To establish the ATP requirement for biomass synthesis several experiments were performed in the respirometer with various phosphate concentrations. PHB, ammonium, phosphate and glycogen concentrations were measured as well as oxygen consumption. Biomass production during the cycle was obtained by taking the ammonium consumption as a measure for cell growth. In figure 10a the biomass production is shown during the aerobic phase, in the absence and presence of phosphate. It can be concluded that there is no difference in biomass production between the two experiments. In both cases the growth rate is gradually decreasing during the cycle. The glycogen production during the aerobic phase is shown in figure 10b. When phosphate is absent in the medium it appears that slightly more glycogen is produced, however the total amount of glycogen produced at the end of the aerobic phase is comparable.

Also the glycogen production rate tends to decrease gradually during the cycle. The PHB consumption during the aerobic phase is shown in figure 10c. Although expected, it can not be concluded from figure 10c that in the absence of phosphate less PHB is used than in its presence. In general it appears however that the effect of phosphate on growth, glycogen and PHB conversions are minor. This validates the above approach to determine the P/O ratio, δ. K was calculated according to equation 20 from 10 different sets of conversion rates measured in 8 different cycles. A value of 1.6 was found with a standard deviation of 0.3.

Calculation of the yield coefficients

With all metabolic stoichiometric coefficients determined, the yields can be calculated according to equation (5) to (8) which are shown in table II. For typical SBR conditions it appears that growth, polyphosphate and glycogen synthesis each consume about equal amounts of oxygen. The accuracy of the obtained yield coefficients is shown in figure 11 where the measured and calculated oxygen (11a) and PHB conversions (11b) are compared. The overall relations for PHB and oxygen consumption in the aerobic phase of the P-removing process are:

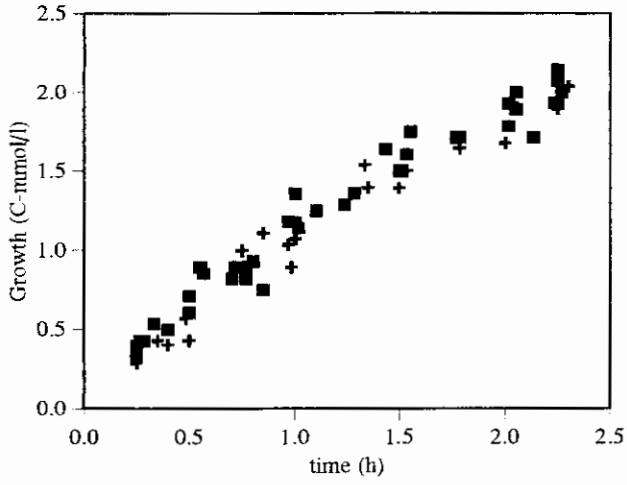
$$-r_{phb} = 1.36\ r_x + 0.27\ r_{pp} + 1.12\ r_{gl} + 4.0\ 10^{-3}\ C_x \qquad (21)$$

$$-r_{o_2} = 0.41\ r_x + 0.31\ r_{pp} + 0.26\ r_{gl} + 4.5\ 10^{-3}\ C_x \qquad (22)$$

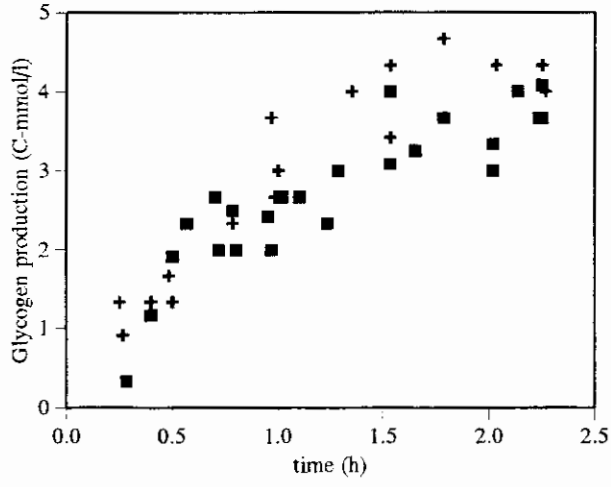
Table II *Maximal yield values found by regression for growth, polyP and glycogen formation on PHB, acetate and oxygen in mol/mol, maintenance: mol/mol.h.*

		PHB conversion		Oxygen conversion
Growth	Y_{sx}^{max}	0.74	Y_{ox}^{max}	2.44
PolyP	Y_{spp}^{max}	3.68	Y_{opp}^{max}	3.27
Glycogen	Y_{sgl}^{max}	0.90	Y_{ogl}^{max}	3.92
Maintenance	m_s	$4.0 \cdot 10^{-3}$	m_o	$4.5 \cdot 10^{-3}$

a)



b)



c)

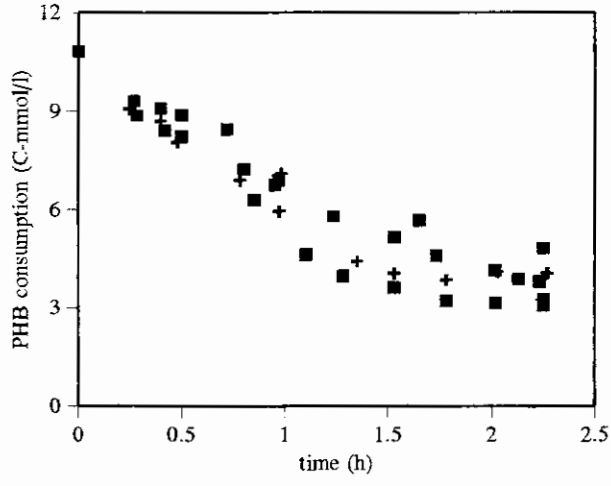
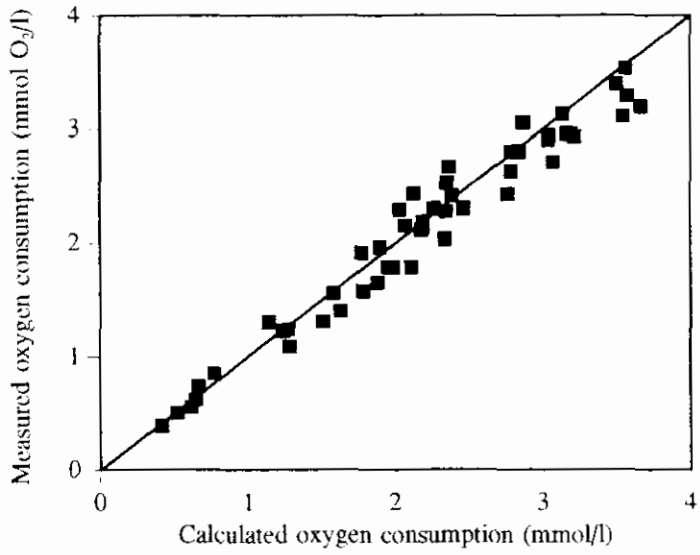


Figure 10 *Effect of phosphate accumulation on the biomass production (a), glycogen formation (b) and PHB consumption (c), in the presence (■) and absence (+) of phosphate. The data points originate from several independent experiments.*

a)



b)

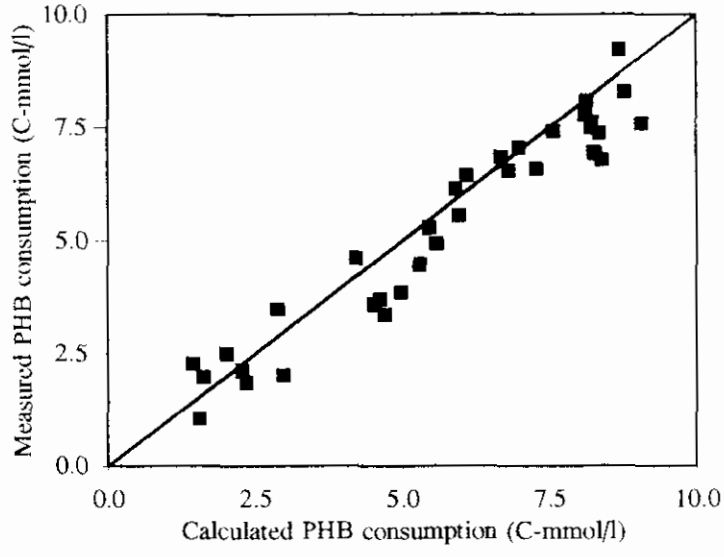


Figure 11 *Comparison of the calculated overall oxygen consumption (a) and PHB consumption (b) composed of the biomass, polyP and glycogen production and the yields of table II, and the overall measured oxygen- and PHB consumption.*

Discussion

In this work the aerobic metabolism of bio-P-bacteria has been studied in a SBR with enriched cultures under non sterile conditions. No attempt for the microbiological characterization of the micro-organisms was made for two reasons: First, the process was

operated in such a way that only organisms of one specific metabolic group were accumulated i.e. organisms which i) accumulate acetate in the cell as PHB under strict anaerobic conditions, using polyphosphate as energy source, ii) in a subsequent aerobic period use the PHB as carbon and energy source for growth and polyphosphate accumulation. Since acetate was never present under aerobic conditions, no normal heterotrophic bacteria were accumulated. Nitrification did not occur in the system thereby excluding nitrifiers and denitrifiers. Under anaerobic conditions HAc can only be converted by polyphosphate bacteria or methanogens. The latter group was not present due to toxicity for oxygen. So due to a strongly selective process we were able to cultivate a homogeneous metabolic group of bacteria and study their physiology. Microscopy showed that virtually all organisms in the sludge contained polyphosphate granules. The second reason was that many researchers have been trying to isolate and identify the bacteria responsible for P removal. Although many bacteria capable of polyP synthesis have been isolated, none of these cultures have shown to exhibit the unique metabolism of the phosphate accumulating sludge.

Coefficients and overall yields

In the presence of oxygen ATP can be derived from the oxidation of $NADH_2$ in the oxidative phosphorylation. This process is generally assumed to be described by the chemiosmotic theory as proposed by Mitchell.[11] The predicted mechanistic stoichiometry for the oxidative phosphorylation according to this theory gives a P/O ratio of 2 or 3 depending on the organism. Roels [12] showed that the treatment of the oxidative phosphorylation in terms of a fixed stoichiometry is not valid. Oxidative phosphorylation is an incompletely coupled process, hence it can have a variable stoichiometric coefficient. The expected operational P/O ratio is roughly 2 for organisms with a mechanistic stoichiometry of 3 and 1.4 for organisms with a mechanistic stoichiometry of 2. In the system considered here with an unknown mechanistic stoichiometry of the organisms, the derived value for the P/O ratio of 1.85 is within the expected range.

The gradual change in growth rate and glycogen production rate during the cycle in presence or absence of phosphate, also justifies the direct determination of the oxygen/phosphate yield from the measurement of the oxygen consumption in the off gas of the SBR by means of extrapolation of the oxygen consumption rate as shown in figure 8.

The maintenance coefficient m_o of $4.5\ 10^{-3}$ found in our experiments can be compared with other values by expressing it as an endogenous mass loss rate (k_d): the amount of biomass lost per g biomass per day. The mass loss rate in our system is 0.06 d^{-1}. A typical value for the k_d in common activated sludge systems is about 0.24 d^{-1}, [9] the observed value for the

biological phosphorus removal is thus lower than the the normally observed value for maintenance. A similar low value for the mass loss rate (k_d = 0.04) for the biological P-removal was found by Wentzel.[15] The derived value for K of 1.6 is close to the theoretical minimal value of 1.5 indicated by Roels.

The effect of external phosphate on oxygen consumption

The high oxygen consumption rate during the P-uptake and drop in oxygen consumption rate after the complete uptake of phosphate was also observed by Wentzel et al.[17] They explained this phenomenon by the kinetic assumption that growth stopped because phosphorus became limiting, although they also stated that the phosphate requirements for normall cell synthesis would generally be supplied from stored polyphosphate. From our measurements (figure 10) it appears however that there is no large change in rate of biomass or glycogen production in the presence or absence of external phosphate. In the present model the well known stoichiometric ATP requirements for the polyP synthesis already explains the observed oxygen consumption satisfactorily. Hence there is no need for other assumptions.

Overall conversions during anaerobic and aerobic phases

Since the anaerobic metabolism[13] and the aerobic metabolism are known, now the overall stoichiometry of the biological phosphorus removal can be formulated. In figure 12 the overall process is shown, which is valid for a SRT of 8 days. With 1 C-mol acetate 1.33 C-mol PHB is synthesised and 0.5 C-mol glycogen is converted anaerobically while 0.5 P-mol phosphate is released. In the aerobic phase 0.15 C-mol PHB is used for the synthesis of polyphosphate, 0.63 C-mol PHB is used for the synthesis of glycogen and 0.54 C-mol is used for biomass synthesis including maintenance. In each cycle 0.34 C-mol active biomass, 0.01 C-mol PHB and 0.06 C-mol glycogen are removed as excess organic matter.

The consumed amount of oxygen for the synthesis of polyphosphate is 0.16 mol O_2, for glycogen 0.14 mol O_2 and for biomass 0.14 mol O_2. The energy requirements for poly-phosphate synthesis represent 10 % of the total PHB consumption and 30% of the total oxygen consumption. Since the production of glycogen is also an essential part of the P-metabolism, in total 25 % of the consumed acetate and 60 % of the oxygen is used for the phosphate metabolism during the aerobic phase.

The overall biomass yield of the polyP-metabolism is low compared to common aerobic growth on acetate. If these polyP organisms were compared to organisms with only aerobic growth on acetate using the same metabolic stoichiometric parameters, the growth yield at μ = 0.014 h^{-1} can be calculated to be 0.47 C-mol biomass per C-mol acetate.

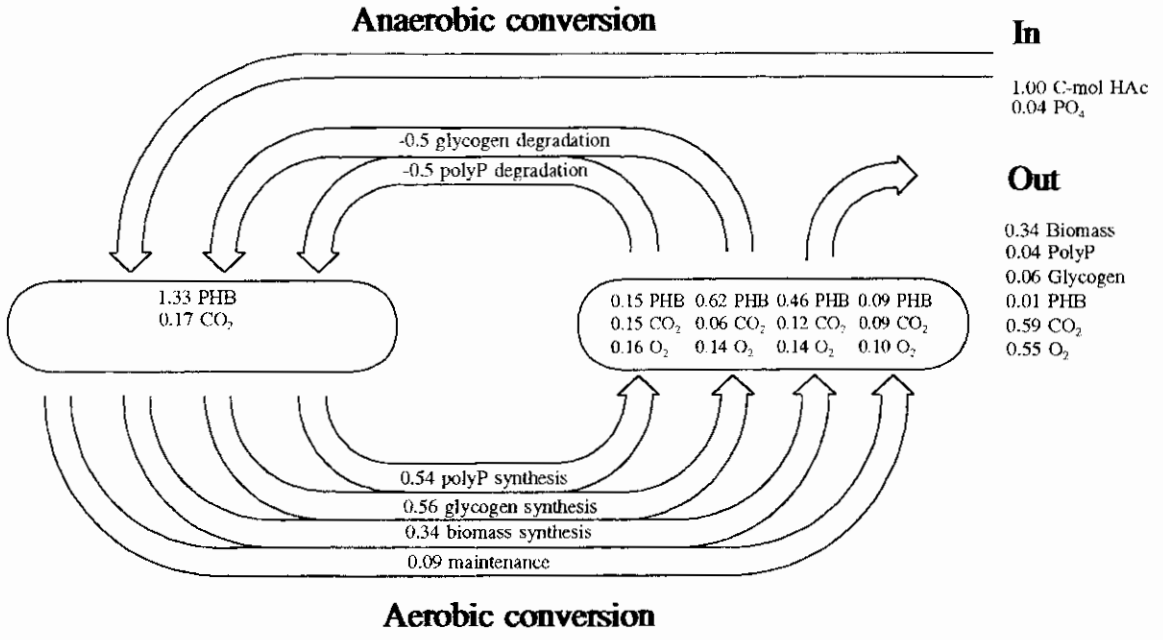


Figure 12 *Conversions, expressed in mol, during the anaerobic and aerobic phase of the biological phosphorus removal process after addition of 1 C-mol acetate, SRT 8 days.*

The oxygen consumption then equals 0.47 mol O_2/C-mol acetate. The total organic biomass yield for the polyP organisms is 0.41 C-mol/C-mol acetate and the consumed amount of oxygen equals 0.55 mol O_2 per C-mol acetate. The metabolic burden of the cyclic storage and consumption of polyphosphate and glycogen can be calculated to be 0.26 molATP/P-mol phosphate and 0.66 molATP/C-mol glycogen. This leads to a 13% decreased organic solids production and 17% increased oxygen production. This observation of energy costs is generally observed in metabolic cycles, as explained by Atkinson.[2] This means that the organic biomass production (VSS) in a waste water treatment system with biological phosphorus removal is decreased. However due to the storage of polyP the total sludge concentration (MLSS) is comparable or higher than a conventional aerobic treatment process (table III). This also would lead to a lower CH_4 production in the excess sludge digester.

Table III *MLSS production for the polyP metabolism compared with normal aerobic growth. Acetate load: 6.1 C-mol per liter per cycle, SRT 8 days.*

	polyP metabolism (mg/l)	aerobic metabolism (mg/l)
Biomass	1612	2295
PolyP	648	
Glycogen	300	
PHB	61	
MLSS	2620	2295

Conclusions

A structured metabolic model based on the biochemical pathways of the process, containing only 3 adjustable parameters (δ, K and m_{ATP}), is very well capable to describe the stoichiometry of the complex conversions of the biological phosphorus removal process. The predicted oxygen and PHB consumption for growth, polyphosphate synthesis and glycogen explains satisfactorily the observed rates. The developed respirometer technique allowed accurate measurement of the stoichiometric coefficients. The special feature of polyP-organisms to take up phosphate enables the direct determination of the P/O ratio of these organisms.

The phosphate and glycogen cycles in biological P-removing organisms have a large energetic effect on the metabolism of these organism: 15% of the added acetate and about 30% of the oxygen consumption is required for the uptake and storage of phosphate. The production of glycogen in the aerobic zone additionally requires 10 % of the added acetate and 25 % of the oxygen consumed.

From the metabolic model it can be calculated that due to the P-metabolism a significant decrease in biomass volatile solids production and an increased oxygen consumption is obtained. However due to stored inorganic phosphate the total (organic and inorganic) biomass production is not changed appreciably.

Nomenclature

α	metabolic reaction matrix	
α_3	ATP required for polyphosphate synthesis	(molATP/C-mol)
δ	P/O ratio	(molATP/mol O)
ϵ	energy for transport of phosphate	(P-mol/mol$NADH_2$)
μ	growth rate	(h^{-1})
k_d	endogenous mass loss rate	(d^{-1})
m	maintenance	(mol/C-mol.h)
r	conversion rate	(mol/m^3.h)
v	reaction rate	(mol/m^3.h)
C_x	bioma concentration, excluding torage product	(C-mol/l)
K	biomass formation and polymerisation constant	(molATP/C-mol)
M	converted amount	(mol/m^3)
Y^{max}	maximal yield	(mol/mol)

subscripts

phb	poly-ß-hydroxy butyrate
x	biomass
gl	glycogen
pp	polyphosphate
n	ammonia
p_{out}	phosphate outside the cell
p_{in}	phosphate inside the cell
o	oxygen
c	carbon dioxide
w	water
s	carbon
ATP	ATP, elemental composition : '-'
$NADH_2$	$NADH_2$, elemental composition : 'H_2'
1-5	internal reactions

References

1) APHA, 1985, Standard Methods for the examination of water and waste wate, 16 th edition. American Public Health Association, Washington, D.C.
2) Atkinson, D.E., 1977, Cellular energy metabolism and its regulation, Academic Press, New York.
3) Comeau, Y., Hall, K.J., Hancock, R.E.W., Oldham, W.K., 1986. Biochemical model for enhanced biological phosphorus removal, Water Research, 20 (12), 1511-1521.
4) Dawes, E.A., Senior, P.J. 1973. The role and regulation of energy reserve polymers in microorganims, Adv. Microbial Physiol., 10, 135-266.
5) Gommers, P.J.F., B.J. Schie, J.P. van Dijken and J.G. Kuenen. 1988. Biochemical limits to microbial growth yields: an analysis of mixed substrate utilization. Biotech. and Bioeng., Vol 32, 86-94.
6) Harold, F.M., 1986. The vital force: A study of bioenergetics, W.H. Freeman and company, New York.
7) Jenkins D., Tandoi, V. 1991. The applied microbiology of enhanced biological phosphate removal - accomplishments and needs, Wat. Res., Vol 25, No. 12, 1471-1478.
8) Kulaev, I.S., 1979. The biochemistry of inorganic polyphosphates, John Wiley and Sons, Chichester, New York, Brisbane, Toronto.
9) Marais, G.v.R., Ekama, G.A., 1976. The activated sludge process: Steady state behaviour. Water SA, Vol. 12, No. 4, 203-208.
10) Mino, T., Arun, V., Tsuzuki, Y., Matsuo, T. 1987. Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process. Biological Phosphate removal from Wastewaters (Advances in Water Pollution Control 4, Ed. R. Ramadori, Pergamon Press, Oxford, 27-38.
11) Mitchell P., Chemiosmotic coupling and energy transduction, 1986. Glynn Research, Bodmin, Cornwall, U.K.
12) Roels J.A., 1983. Energetics and kinetics in biotechnology, Elsevier Biomedical Press.
13) Smolders G.J.F., van der Mey, J., Loosdrecht, van M.C.M, Heijnen, J.J.,. 1994. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. Biotech. and Bioeng, Vol. 43, 461-470.
14) Stouthamer, A.H., 1979. The search for correlation between theoretical and experimental growth yields. Microbial Biochemistry, Vol 21.
15) Stryer, L., 1981. Biochemistry, W.H. Freeman and company, San Francisco.
16) Tijhuis, L., Loosdrecht, van M.C.M., Heijnen, J.J., 1993, A thermodynamically based correlation for maintenance gibbs energy requirements in aerobic and anaerobic chemotrophic growth, Biotech & Bioeng, Vol. 42, 509-519.
17) Wentzel, M.C., Ekama, G.A., Loewenthal, R.E., Dold, P.L., Marais, G.v.R. 1989. Enhanced polyphosphate organism cultures in activated sludge. Part II: Experimental behaviour. Water SA Vol 15., No. 2, 71-88.
18) Wentzel, M.C., Lötter, L.H., Loewenthal, R.E, Marais, G.v.R. 1986. Metabolic behaviour of *Acinetobacter spp.* in enhanced biological phosphorus removal - a biochemical model, Water SA Vol. 12, No 4, 209-224.
19) Westerhoff, H.V., van Dam K. 1987. Thermodynamics and control of biological free-energy transduction, Elsevier, Amsterdam.

Appendix III

Kinetics of the anaerobic and aerobic metabolism

A structured metabolic model is developed that describes the stoichiometry and kinetics of the biological P-removal process. In this approach all relevant metabolic reactions underlying the metabolism, considering also components like ATP and $NADH_2$ are described based on biochemical pathways. As a consequence of the relations between the stoichiometry of the metabolic reactions and the reaction rates of components the required number of kinetic relations to describe the process is reduced. The model describes the dynamics of the storage compounds which are considered separately from the active biomass. The model was validated in experiments at a constant sludge retention time of 8 days, over the anaerobic and aerobic phases in which the external concentrations as well as the internal fractions of the relevant components involved in the P-removal process were monitored. These measurements include dissolved acetate, phosphate and ammonium; oxygen consumption, PHB, glycogen, and active biomass. The model describes the dynamic behaviour of all components during the anaerobic and aerobic phases satisfactorily.

Introduction

The biological phosphorus removal process is one of the more complex waste water treatment processes due to the fact that the main part of the metabolism takes place on internal stored substrates and products. The metabolism is based on the anaerobic consumption of acetate and storage as poly-ß-hydroxybutyrate (PHB) while energy and reduction equivalents are provided in the degradation of internal stored polyphosphate and glycogen. During aerobic conditions the internally stored PHB is oxidized and used for growth, phosphate uptake and production of glycogen. In the overall process three internal storage products (PHB, polyphosphate and glycogen) play an essential role in the metabolism.

Due to its complexity there have been few reports about the mathematical modelling of this process. Most notably is the work of Wentzel et al.[7-10] This model, however, only takes PHB and polyP storage products into account. Recently the involvement of an additional storage polymer, glycogen, has been demonstrated.[2] The model of Wentzel has been validated using only the measured acetate uptake, phosphate release and uptake and oxygen consumption. The dynamics of the storage compounds and the active biomass have not been part of their experimental research. Especially for microbial systems where the biomass composition changes strongly due to the dynamics in the storage polymers it is very relevant to monitor and model such changes. From our previous work the role and dynamics of polyP, glycogen, PHB and active biomass in the anaerobic and aerobic phases have been shown. In this paper a complete structured metabolic model of the stoichiometry and kinetic behaviour of the biological P-process will be presented.

Two process characteristics are essential to the analysis of microbial transformation processes: (1) stoichiometry and (2) kinetics. Stoichiometry of a reaction provides information relating quantities of reactants consumed to quantities of products formed, while kinetics determines the rate of the reaction. The conversion rates of different components are related through stoichiometry of the metabolic reactions and consequently the required number of kinetic relations to describe the process is decreased. The aim of the present study is the development of a structured model for the P-removal process which is based on the metabolic description and stoichiometry with a minimal number of kinetic expressions. In this approach, all relevant metabolic reactions underlying the metabolism can be described, including components like ATP and $NADH_2$. The stoichiometry of the anaerobic and aerobic zone was described in

appendix I and II respectively, here we present the kinetic relations for the conversions in the anaerobic and aerobic zones of the process, obtained for a sludge age of 8 days.

Table I Relevant components in the biological P-removal process.

Compount	symbol	elemental composition
acetate	s	CH_2O
phosphate	p	H_3PO_4
biomass	x	$CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$
PHB	phb	$CH_{1.5}O_{0.5}$
polyP	pp	HPO_3
glycogen	gl	$CH_{1.67}O_{0.83}$
ammonia	n	NH_3
oxygen	o	O_2
carbon dioxide	c	CO_2
water	w	H_2O

Stoichiometry of the P-removal

Relevant components and reactions

In table I the components which play a relevant role in the P-metabolism are shown with their elemental formula. All substrates and polymeric components like biomass, PHB, polyphosphate and glycogen are expressed per mole carbon or phosphorus, which simplifies the calculation. For the same reason all components are electro-neutrally represented. The relevant reactions and their stoichiometry of the anaerobic and aerobic phase are summarized in table II; these reactions are described in appendix I and II.[4,5] The anaerobic metabolism can be described by two reactions: the uptake of acetate (R_1) and the production of energy for maintenance (R_2), (table II). Phosphorus removing organisms take up acetate and store this as poly-hydroxy-butyrate (PHB), see figure 1. The energy for the transport and storage of acetate is supplied by the hydrolysis of intracellular polyphosphate and glycogen. Due to the hydrolysis of polyphosphate, phosphate is released. The energy requirement for the uptake of acetate is dependent on the pH value which is represented with the ATP coefficient α_f. The energy for maintenance purposes is generated by the hydrolysis of polyphosphate.

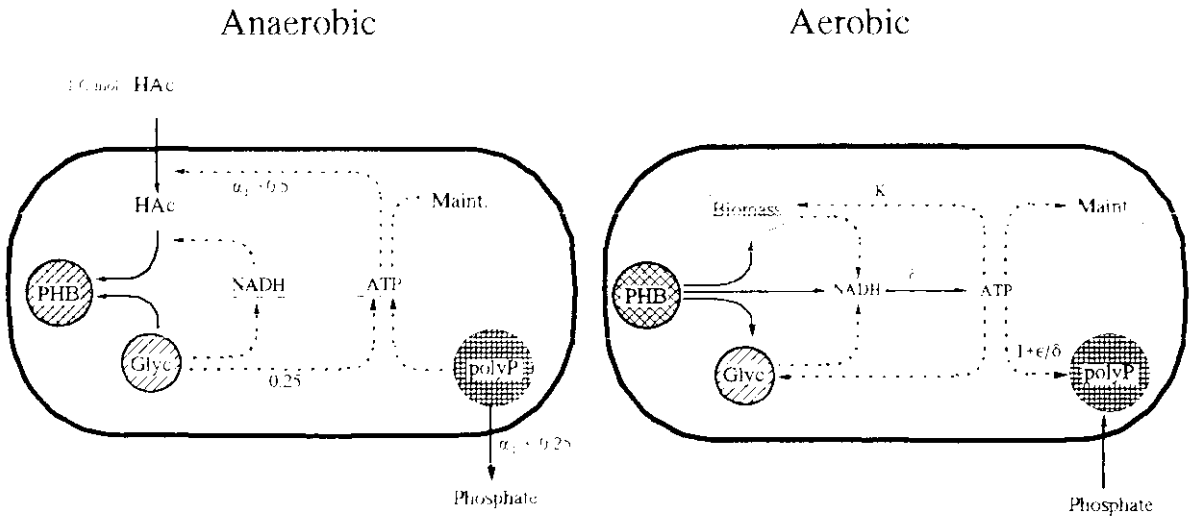


***Figure 1** Metabolism of the anaerobic and aerobic phase.*

The reactions in the aerobic metabolism (figure 1) are described with four reactions all of which use PHB for: the production of biomass (R_3), the synthesis of polyphosphate (R_4) and glycogen (R_5) and the energy production for maintenance (R_6). Three metabolic coefficients determine the stoichiometry of the aerobic metabolism: the P/O ratio, δ, determines the amount of ATP produced in the oxidative phosphorylation, the polymerisation coefficient for biomass synthesis, K, determines the ATP involved in biomass synthesis and the transport coefficient, ϵ, determines the energy involved in the transport of phosphate into the cell, which has only a small contribution in the overall metabolism. The metabolisms of the anaerobic and aerobic phase of the biological P-removal process are only dependent on these four coefficients (α_1, δ, K, ϵ) which were experimentally determined in appendix I and II.[4,5]

Biomass fractions

An accurate mathematical description of the P-removal process has to distinguish between active biomass and organic and inorganic storage products, because the P-removing biomass can be made up for 50 % of internal storage products. Accordingly, in the model internally stored components like PHB, polyphosphate, glycogen and active biomass, are considered separately. A typical example of the relation between MLSS, VSS and active biomass is given in figure 2.

The *MLSS and VSS are typical* waste water parameters which can be easily experimentally determined but do not give much information about the composition of the biomass, which is essential to know in the case of the P-removal process. The overall observed suspended solids (MLSS) is in fact a mixture of PHB, polyphosphate, glycogen and active biomass. The VSS is the total of the fractions PHB, glycogen and active biomass (without the ash). The ash content of the MLSS consist of ash from active biomass (about 5-10% of the active biomass) and polyphosphate.

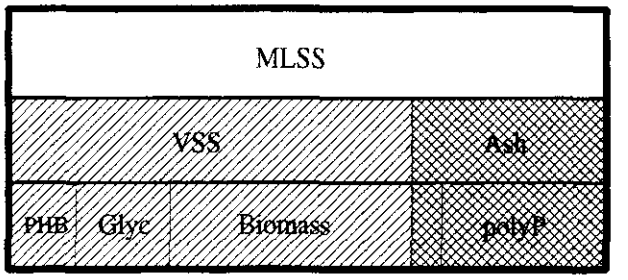


Figure 2 *Relation between MLSS, VSS and ash content and the biomass composition.*

Table II. Metabolic reactions of the anaerobic and aerobic phase of the biological P-removal process.

Anaerobic		
R_1	Acetate uptake	$-CH_2O$ -0.5 $CH_{10/6}O_{5/6}$ -0.44 HPO_3 +1.33 $CH_{1.5}O_{0.5}$ +0.17 CO_2 +0.44 H_3PO_4 -0.023 H_2O = 0
R_2	Maintenance	- HPO_3 - H_2O + H_3PO_4 = 0
Aerobic		
R_3	Biomass synthesis	-1.37 $CH_{1.5}O_{0.5}$ -0.20 NH_3 -0.015 H_3PO_4 -0.42 O_2 +$CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$ +0.37 CO_2 +0.305 H_2O= 0
R_4	Phosphate uptake	- 0.27 $CH_{1.5}O_{0.5}$ - 0.306 O_2 -H_3PO_4 + HPO_3 + 0.27 CO_2 + 1.20 H_2O = 0
R_5	Glycogen formation	-1.12 $CH_{1.5}O_{0.5}$ - 0.26 O_2 +$CH_{10/6}O_{5/6}$ + 0.12 CO_2 + 0.007 H_2O= 0
R_6	Maintenance	- $CH_{1.5}O_{0.5}$ - 1.125 O_2 + CO_2 +0.75 H_2O = 0

The fractions of PHB, glycogen and polyP changes strongly due to the dynamics in the anaerobic and aerobic phase. The fractions of the internal storage components (PHB, polyphosphate and glycogen) are expressed as a ratio with respect to the active biomass (mol/C-mol active biomass):

$$f_i \qquad \frac{C_i}{C_X} \tag{1}$$

The observed biomass concentration in the process is the sum of the concentrations of active biomass, PHB, glycogen and polyphosphate.

Kinetics of the P-removal

Anaerobic metabolism

The conversion rates of all components during the anaerobic phase can be calculated when the rates of reaction R_1 and R_2 are known. The stoichiometry of the reactions R_1 and R_2 (table II) can be represented in a stoichiometry matrix α^{an}, with the rows representing the relevant components and the columns the reaction stoichiometry. If the anaerobic conversion rates are represented by a vector r^{an} and the active biomass specific rates of the reactions with the vector q^{an}, then the conversion rates in the reactor during the anaerobic phase can be calculated according to:

$$r^{an} = \alpha^{an} \cdot q^{an} \cdot C_x \tag{2}$$

$$r \quad \begin{vmatrix} r_s \\ r_p \\ r_x \\ r_{phb} \\ r_{pp} \\ r_{gl} \\ r_n \\ r_o \\ r_c \\ r_w \end{vmatrix} \qquad \alpha^{an} \quad \begin{vmatrix} 1 & 0 \\ 0.44 & 1 \\ 0 & 0 \\ 1.33 & 0 \\ 0.44 & 1 \\ 0.5 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0.17 & 0 \\ 0.023 & 1 \end{vmatrix} \qquad q^{an} \quad \begin{vmatrix} q_s \\ m_{an} \end{vmatrix} \tag{3}$$

The vector q^{an} describes the rates of reactions R_1 and R_2. The overall observed rate of R_1 is equal to the active biomass specific acetate uptake rate q_s and the rate of R_2 is determined by

the active biomass specific anaerobic maintenance coefficient m_{an}. Only the two kinetic relations for the specific acetate uptake rate and the maintenance coefficient have to be found to calculate all conversions in the anaerobic phase, using the two specified reactions, R_1 and R_2.

In the kinetic description of the anaerobic phase the acetate concentration is considered to determine primarily the acetate uptake rate, when all participating components are present in excess. If one of the participating components is not present in excess, the component will finally be exhausted and therefore halting the anaerobic metabolism. In the uptake of acetate, glycogen and polyphosphate are degraded to provide energy and reducing power and might both halt the metabolism if they are exhausted. Previously[4] it was shown that at different pH values the energy requirements for the transport of acetate varied, resulting in an increasing phosphate release rate with an increasing pH. The acetate uptake rate however, remained constant at different pH values, which illustrates that the polyP degradation rate does not affect the rate of the acetate uptake, but that acetate uptake drives the polyP and glycogen degradation. The acetate uptake rate is determined by the acetate concentration and can be described by conventional Monod type of kinetics.

$$q_s = q_s^{max} \cdot \frac{C_s}{C_s + K_s} \tag{4a}$$

Using equation (2) and (3) with α^{an} gives:

$$r_s = -q_s \cdot C_x \tag{4b}$$

The rate of reaction R_2 is determined by the anaerobic maintenance coefficient, m_{an}, which is constant and expressed per C-mol active biomass, as P-mol/C-mol·h. Using equation (2) and (3) and α^{an} this gives for example for the phosphate release, r_p:

$$r_p = 0.44\, q_s C_x + m_{an} C_x \tag{4c}$$

In a similar way all conversion rates are related to q_s and m_{an}.

Aerobic metabolism

The conversion rates of all components during the aerobic phase can be calculated when the rates of reactions R_3 to R_6 are known. The stoichiometry of the reactions of the aerobic phase (table II) are now represented in the aerobic stoichiometry matrix, α^{aer}. If the reactor conversion rates are represented with a vector r^{aer} and the rates of the reactions with the vector q^{aer}, then the conversion rates in the anaerobic phase can be calculated according to:

$$r^{aer} = \alpha^{aer} \cdot q^{aer} \cdot C_x \tag{5}$$

$$r = \begin{vmatrix} r_s \\ r_p \\ r_x \\ r_{phb} \\ r_{pp} \\ r_{gl} \\ r_n \\ r_o \\ r_c \\ r_w \end{vmatrix} \qquad \alpha^{aer} \begin{vmatrix} 0 & 0 & 0 & 0 \\ 0.015 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ -1.37 & 0.27 & 1.12 & -1 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0.2 & 0 & 0 & 0 \\ -0.42 & -0.31 & 0.26 & -1.125 \\ 0.37 & 0.27 & 0.12 & 1 \\ 0.30 & 1.20 & 0.007 & 0.75 \end{vmatrix} \qquad q^{aer} = \begin{vmatrix} \mu \\ q_{pp} \\ q_{gl} \\ m_{aer} \end{vmatrix} \tag{6}$$

The vector q^{aer} describes the active biomass specific rates of reactions R_3 to R_6. The reaction rate of R_3, the production of biomass, is indicated by the growth rate, μ, while the production of polyP, (R_4) and glycogen (R_5) are indicated by q_{pp} and q_{gl}, respectively, and the reaction for maintenance (R_6) is described with m_{aer}. All conversion rates can be calculated according to equation 5 and 6 using α^{aer}. The conversion rates for PHB and oxygen consumption are then as follows:

$$r_{phb} = 1.37\ \mu\ C_x + 0.27\ q_{pp}\ C_x + 1.12\ q_{gl}\ C_x + m_s C_x \tag{7}$$

$$-r_o = 0.42\ \mu\ C_x + 0.31\ q_{pp}\ C_x + 0.26\ q_{gl}\ C_x + 1.125\, m_s C_x \tag{8}$$

The coefficients in these equations represent the inverted maximal yields. For example the value 1.37 in equation (7) indicates that the maximal yield of active biomass on PHB is

1/1.37. These maximal yield values for growth, polyP synthesis and glycogen production are a function of α_1, δ, K, ϵ and dependent on each other since they are related through the production of ATP in the oxidative phosphorylation.[5] Only four kinetic relations are needed (growth rate, μ, polyP synthesis rate, q_{pp}, glycogen production rate, q_{gl}, and a maintenance coefficient, m_{aer}) to be able to describe the conversion rates of all other components. The kinetic relations used in the model are described below.

The growth of the polyP organisms in the aerobic phase is primarily determined by the PHB content of the cells, the only substrate available in the aerobic phase. The growth rate of the biomass is assumed to be dependent on the PHB content of the cells. This is described by first order kinetics in f_{phb}. The growth rate, μ, is expressed as C-mol active biomass produced per C-mol active biomass per hour.

$$\mu = k_x \cdot f_{phb} \tag{9a}$$

$$r_x = k_x \cdot f_{phb} \cdot C_x \tag{9b}$$

The synthesis rate of polyphosphate, q_{pp}, is assumed to be determined by three factors: the external phosphate concentration, C_p, the PHB content of the cells, f_{phb} , and the polyphosphate content, f_{pp} , of the cells. If the external phosphate concentration is exhausted the synthesis rate of polyP will become zero, which is expressed by a conventional Monod type of relation. The synthesis rate depends further on the PHB content of the cells. If the PHB fraction in the cells decreases, the rate to provide energy, required for the synthesis of polyphosphate, decreases also and when the PHB fraction is exhausted, the P-uptake and polyP synthesis will stop. It is assumed that q_{pp} depends linearly on the PHB content of the active biomass. Subsequently, the polyP synthesis rate depends on the fraction polyphosphate stored in the cells. The phosphate uptake capacity of the polyP organisms is limited to a certain extent,[9] and therefore, when the maximal P-content of the cells, (f_{pp}^{max}), is reached the uptake rate of phosphate will become zero. The maximal reported P-content of polyP organisms is in the range of 0.35 mgP/mgVSS.[9] A linear decrease of q_{pp} with f_{pp} is assumed. This leads to the following kinetic expression for q_{pp} and r_{pp}.

$$q_{pp} = k_{pp} \cdot \left(\frac{C_p}{C_p + K_p} \right) \cdot \left(1 - \frac{f_{pp}}{f_{pp}^{max}} \right) \cdot f_{phb} \qquad (10a)$$

$$r_{pp} = q_{pp} \cdot C_x \qquad (10b)$$

Glycogen is an essential component in the anaerobic metabolism.[2, 4] If there is a shortage of glycogen during the anaerobic uptake of acetate, the metabolism will halt, due to a lack of a source of $NADH_2$ to convert acetate to PHB. To avoid a shortage of glycogen during the anaerobic phase, it is not unlikely that the production of glycogen in the aerobic phase is controlled in the metabolism such that a certain maximal value is aimed for. In the kinetic description of the glycogen production this maximal glycogen content is represented with f_{gl}^{max}. The biomass specific glycogen production is assumed to be dependent on the difference between f_{gl}^{max} and the actual value of f_{gl}, according to:

$$q_{gl} = k_{gl}(f_{gl}^{max} - f_{gl}) \qquad (11a)$$

$$r_{gl} = q_{gl} \cdot C_x \qquad (11b)$$

For maintenance it is assumed that there is a constant requirement of PHB. This rate is set equal to m_{aer} C-mol PHB/(C-mol biomass·h). Clearly r_{phb} and r_o are now also found by combining (7), (8) and equation (9-11).

Calculation of the concentrations in the process

The concentrations during the anaerobic or aerobic phase of the SBR can be calculated by integration of equation 12 using eq (2) and (5) over the length of time of the anaerobic or aerobic phase (0-t):

$$\frac{dC}{dt} = r \qquad (12)$$

With the set of kinetic relations (4a, 9 - 11) and the stoichiometry of the reactions (α^{an}, α^{aer}), the development of the concentrations of the components during the anaerobic or aerobic phase can be calculated, using (3) and (6).

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

The study was carried out in a laboratory fermenter with a working volume of 2 l, at 20 °C. The fermenter was equipped with pH, O_2 and redox electrodes. The pH was maintained on pH 7.0 ± 0.05 using 0.5 N HCl and 1 N NaOH. The reactor was operated as a sequencing batch (SBR) with a cycle of 6 hours consisting of an anaerobic (2.25 h), aerobic (2.25 h) and settling period (1.5 h), see figure 3. Biological phosphorus removing sludge was used as an inoculum. Since the added acetate was completely consumed in the anaerobic zone, only organisms capable of anaerobic acetate consumption were accumulated in the reactor. Methanogens were not present since part of the time oxygen was present. Nitrification took not place due to the short biomass retention of 8 days.

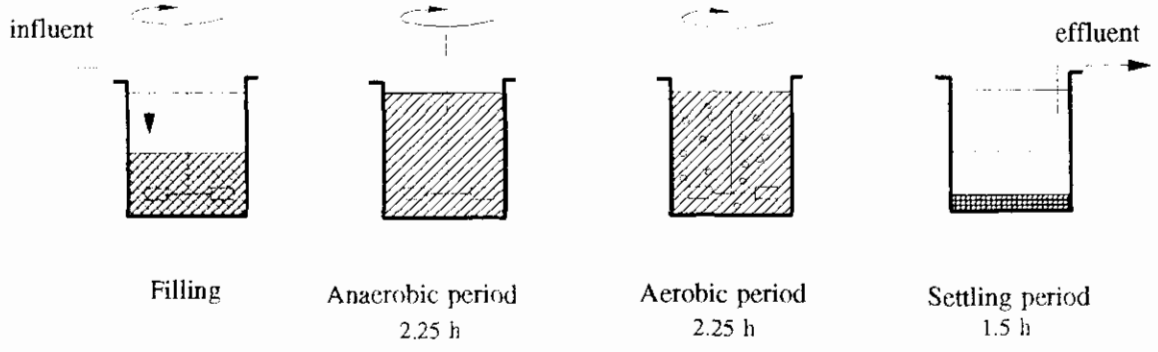


***Figure 3** Operation of the sequencing batch reactor (SBR).*

One litre medium was fed at the start of the anaerobic phase and effluent was removed at the end of the settling period, resulting in a hydraulic retention time of 12 h. At the end of the aerobic phase 63 ml excess sludge was removed, resulting in a biomass retention time (SRT) of 8 days. A stirrer speed of 500 rpm was maintained, except for the settling period. During anaerobic conditions nitrogen gas was bubbled through the reactor with a flow of 30 l/h, In the aerobic period, aeration was provided with an airflow of 60 l/h. The dissolved oxygen concentration during the aerobic phase was measured and was always above 50% of the saturation concentration. The offgas was analyzed for carbon dioxide production and oxygen consumption. The experimental setup is shown in figure 4. The SBR was operated for 100

days before the experiments were started and the microbial population could therefore be considered in steady state.

Media

Sterilized synthetic medium was used containing per litre: 0.85 g $NaAc.3H_2O$ (400 mgCOD/l) as carbon source, 107 mg NH_4Cl, 75.5 mg $NaH_2PO_4.2H_2O$ (15 mgP/l), 90 mg $MgSO_4.7H_2O$, 36 mg KCl, 14 mg $CaCl_2.2H_2O$, 1 mg yeast extract, 0.3 ml nutrient solution, which is described in appendix I and II.

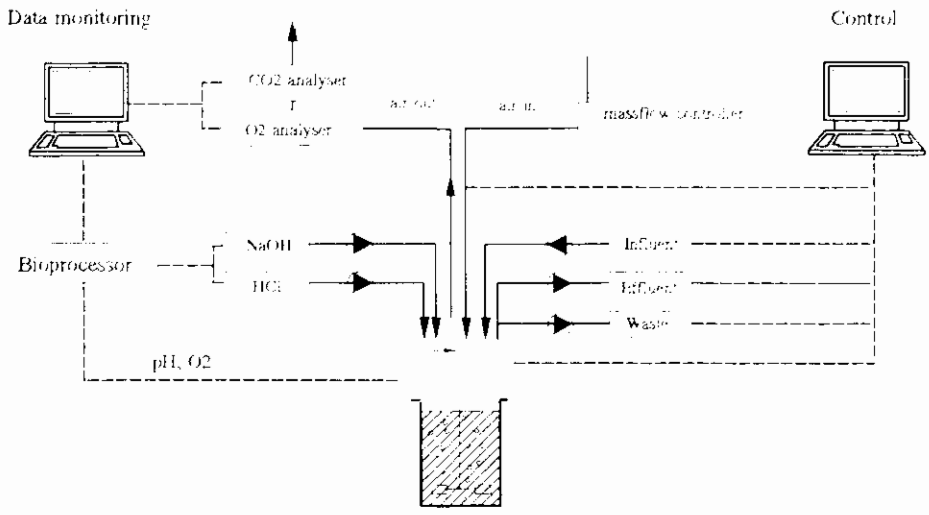


Figure 4 *Experimental set-up used to monitor and control the SBR.*

Measurements and analyses

Data were obtained by monitoring several cycles of the SBR in which the anaerobic and aerobic phase were extensively sampled. The concentrations of acetate, phosphate and ammonium were measured as well as the internal stored fractions of PHB and glycogen. The observed ammonium consumption could be used as a direct measure for growth because nitrification was absent. The nitrite and nitrate concentrations were regularly measured and always zero. Regularly the MLSS and VSS concentrations were measured. During the aerobic phase the SBR was coupled to a respirometer which measured the oxygen consumption rate with intervals of 3 minutes. This equipment is described in appendix II.[4] The oxygen consumption rate and cumulative oxygen consumption were in the presence of highly different P-concentrations both used to fit the kinetic relations for growth, polyP and glycogen

synthesis in combination with the direct measurement of the conversion rates of these components. The percentage oxygen and carbon dioxide were measured in the offgas of the reactor. Analyses were performed as described in appendix I and II. The data sets for evaluation of the kinetic model were derived by measurements over the complete cycle and in batch experiments.

Results and discussion

The stoichiometric parameters in the model were chosen as obtained before (see table II). The kinetic parameters were derived from measurements during several cycles in the SBR in which the anaerobic and aerobic phase were sampled extensively. For the parameter estimation procedure, 6 independent cycles were monitored for the phosphate, acetate and ammonium concentration whereas 4 cycles for the PHB and glycogen content were used. Figure 5a and 5b show examples of these measurements. The data sets derived in this way, were used to fit the kinetic parameters by the criterium of least sum of squares. This was done by calculation of the concentration profiles of all components during the anaerobic and aerobic phase and optimizing the parameters. Not all the measurements were given equal weight during the optimization. This was dependent on the accuracy of the measurement. In table III average values of parameter values are shown.

Table III *Kinetic parameters for the anaerobic and aerobic metabolism*

Anaerobic	parameter	value	Units
Acetate	q_s^{max}	0.43	C-mol/C-mol · h
	K_s	1.6	C-mmol/l
Maintenance	m_{an}	$2.5\ 10^{-3}$	P-mol/C-mol · h
Aerobic			
Growth	k_x	0.16	C-mol/C-mol · h
polyP	k_{pp}	0.55	P-mol/C-mol · h
	K_p	0.1	P-mmol/l
	f_{pp}^{max}	0.3	P-mol/C-mol
Glycogen	k_{gl}	0.8	C-mol/C-mol · h
	f_{gl}^{max}	0.27	C-mol/C-mol
Maintenance	m_{aer}	$4.0\ 10^{-3}$	C-mol/C-mol · h

Anaerobic kinetics

During the anaerobic phase the acetate uptake and phosphate release were measured in the SBR, see figure 5a, as well as the PHB and glycogen conversion, shown in figure 5b. The acetate uptake, phosphate release, PHB and glycogen conversions were calculated with a value for q_s^{max} of 0.43 C-mol/C-mol.h and a constant K_s of $1.6 \cdot 10^{-3}$ C-mol/l, according to eq (2).

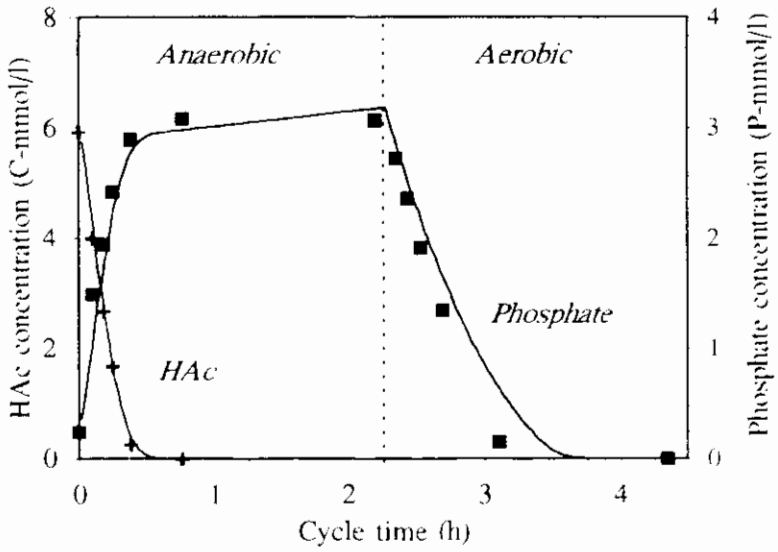


Figure 5a *Acetate (+) and phosphate (■) concentration during the anaerobic and aerobic phase. C_x = 61 C-mmol/l.*

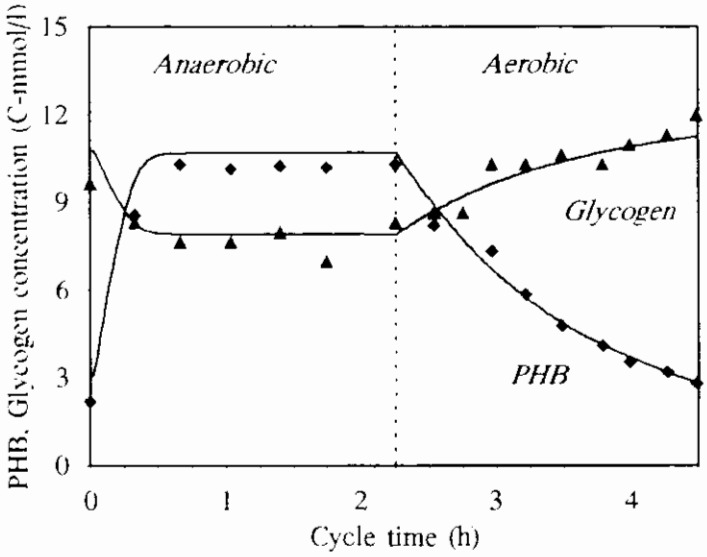


Figure 5b *PHB (♦) and glycogen (▲) concentration during the anaerobic and aerobic phase.*

The anaerobic maintenance coefficient was determined in an experiment in which the sludge was kept anaerobic for several hours in absence of an external carbon source. In figure 6 the phosphate release by the biomass is shown for several experiments. The phosphate release is a result of the degradation of polyphosphate to generate ATP for maintenance purposes. The release rate of phosphate during this period ($2.5 \cdot 10^{-3}$ P-mol/C-mol.h) was taken as the energy requirements for the anaerobic maintenance, m_{an} = $2.5 \cdot 10^{-3}$ molP/C-mol.h. This value (2.4 mgP/gVSS.h) is in the same range as the anaerobic P-release for maintenance purposes found by Wentzel [8] which was 3.7 mgP/gVSS·h.

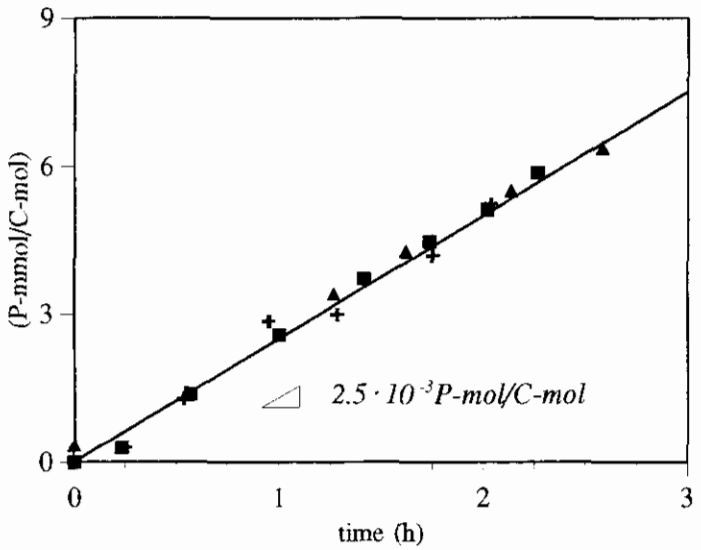


Figure 6 *P-release during the anaerobic phase in absence of an external carbon source in three independent experiments.*

Aerobic kinetics

In figure 5a, 5b and 7 the measured P-uptake, PHB consumption, glycogen production and ammonium consumption in the aerobic phase are compared to the model predictions based on the evaluated parameters values listed in table III. The phosphate uptake during the aerobic phase (see figure 5a) was calculated using equation 10b with the values shown in table III. The line for the measured ammonium consumption in figure 7 was calculated according to equation (9b). The glycogen production during the aerobic phase was calculated according to equation (11b) with a value for the maximal glycogen content f_{gl}^{max} of 0.27 C-mol/C-mol and a production rate k_{gl} of 0.8 C-mol/C-mol·h. The PHB consumption during the aerobic phase is a result of the concerted activity of growth, polyP and glycogen synthesis and maintenance

and was calculated according to equation (7) and shown in figure 5b. The maintenance coefficient m_{aer} was determined as described previously.[5]

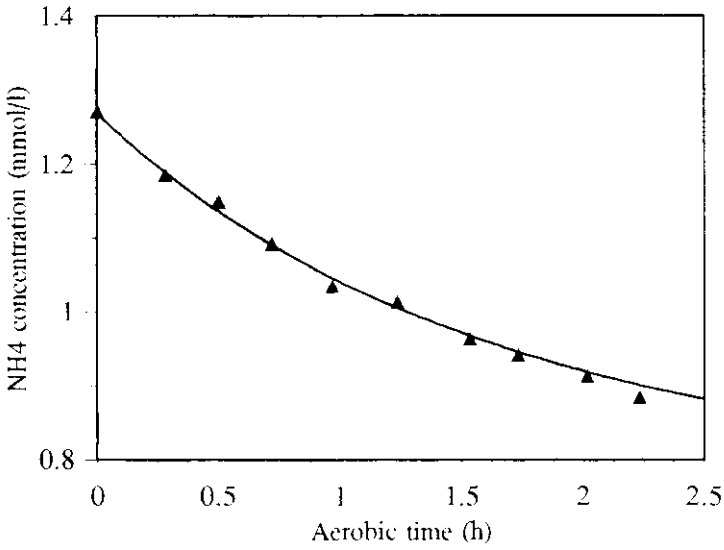
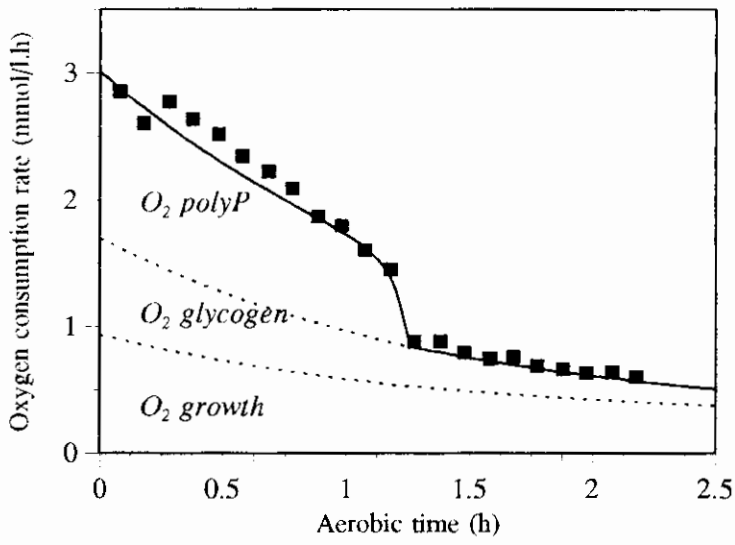


Figure 7 Ammonium consumption during the aerobic phase as a result of growth.

The oxygen consumption rate during the aerobic phase was measured in a respirometer. The oxygen consumption rate is proportional to the rate of the oxidative phosphorylation, the production of ATP. The observed oxygen consumption is therefore equal to the overall sum of the internal rates for growth, polyphosphate and glycogen production and maintenance (see equation 8). Figure 8a shows the oxygen consumption rate during the aerobic phase, 8b shows the cumulative oxygen consumption. The oxygen consumption rate and cumulative consumption were both used to fit the kinetic parameters for growth, polyP and glycogen synthesis. In figure 8a the lowest curve is the calculated contribution in the oxygen consumption rate due to growth and maintenance, the middle curve is the oxygen consumption rate for glycogen (and growth) while the upper curve is the total oxygen consumption rate, including phosphate uptake and storage as polyphosphate.

Analogous to the consumption of oxygen, the contribution of growth (including maintenance), glycogen and polyP synthesis in the consumption of PHB during the aerobic phase can be calculated and is shown in figure 9. It is obvious that only a very small part of the PHB is used in the uptake and storage of phosphate, while the oxygen consumption is strongly influenced by the phosphate uptake.

a)



b)

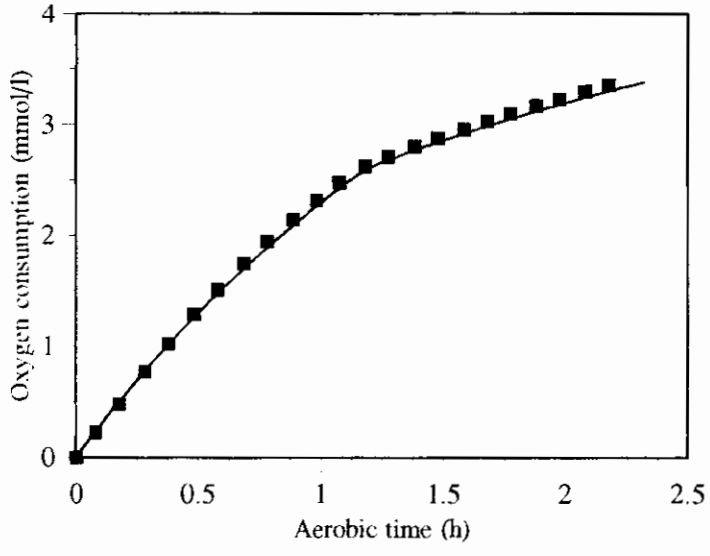


Figure 8 *Oxygen consumption rate (a) and cumulative oxygen consumption (b) during the aerobic phase. Figure 8a shows the contribution of growth, glycogen and polyP synthesis in the total oxygen consumption.*

Effect of the phosphate concentration

To determine the effect of variable concentration of phosphate on the derived kinetic relations several experiments were performed with initial phosphorus concentrations ranging from 0 - $6 \cdot 10^{-3}$ P-mol/l. During these experiments the oxygen consumption rate, ammonium consumption and phosphate concentrations were measured. These experiments are shown in

figure 10. The lines in the figure were calculated using the previously introduced model parameters. The phosphate uptake experiment with an initial concentration of $6 \cdot 10^{-3}$ P-mol/l is used to show that the PHB content as well as the polyP content has an effect on the rate of the polyP production. The polyP content of the biomass increases from 0.05 to 0.15 P-mol/C-mol while the PHB content decreases from 0.19 to 0.05 C-mol/C-mol.

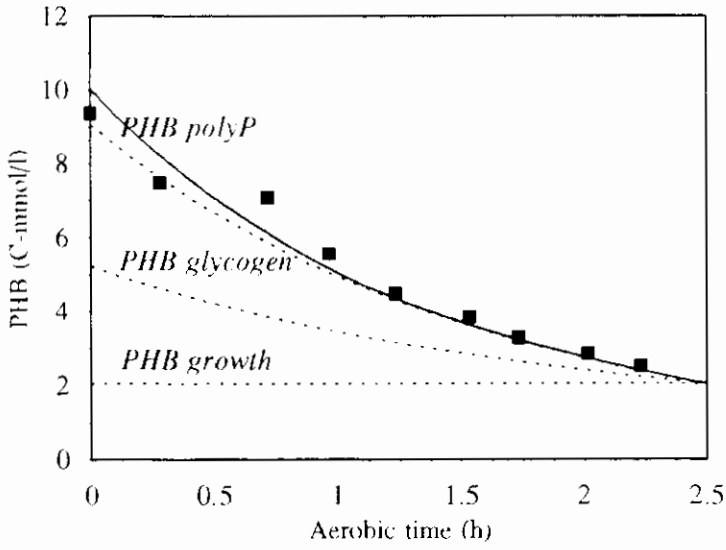


Figure 9 *PHB consumption during the aerobic phase. The contribution of growth, polyP and glycogen synthesis in the PHB consumption are shown.*

Figure 10 (right) shows the best fit for the kinetic relation of the polyP synthesis with the following general relationship:

$$q_{pp} \quad k_{pp} \cdot \frac{C_p}{C_p \cdot K_p} \cdot \left(1 \quad \frac{f_{pp}}{f_{pp}^{\max}}\right)^{m} \cdot f_{phb}^{\,n} \tag{13}$$

Three curves are shown in figure 10b which differ only in the values of m and n: 1) The P-uptake rate is determined by the maximum polyP content as well as the PHB content of the cells, the equation as used before (m=1, n=1). 2) The P-uptake rate is only determined by the PHB content of the cells (m=0, n=1) and 3) The maximal P-content of the cells limits the rate (m=1, n=0). Figure 10 shows that the curves (2) and (3) do not fit the observed data satisfactory, and obviously a combined effect of PHB decrease and polyP increase does limit the uptake rate of phosphate here.

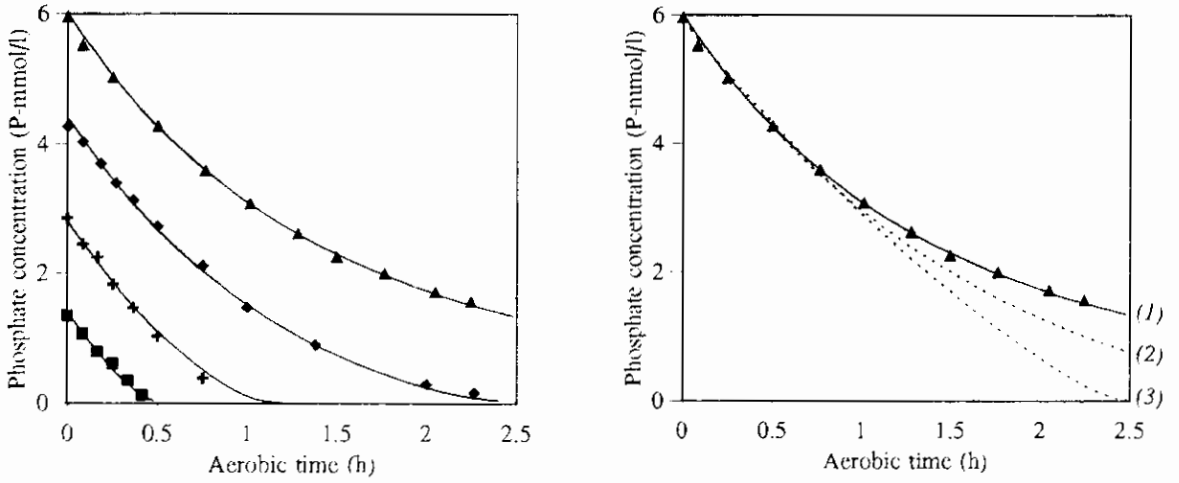


Figure 10 *Phosphate uptake during the aerobic phase in three experiments with phosphate concentrations in the range of 1.4-6 P-mmol/l (left). Comparison of three different kinetic relations to describe the polyP synthesis kinetics (right). The polyP synthesis rate is dependent on the external phosphate concentration and the PHB and polyP content (1), only PHB content (2), or only polyP content (3).*

During the experiments with initial phosphorus concentrations ranging from 0 - $6 \cdot 10^{-3}$ P-mol/l the oxygen consumption rate was measured which is shown in figure 11. With the previously determined kinetic parameters, the oxygen consumption rate profiles were calculated. The model and kinetic parameters describe the oxygen consumption rate satisfactorily. Figure 12 shows the ammonium consumption during these experiments. Also here is good agreement.

The maintenance coefficient based on ATP requirements per C-mol active biomass for the anaerobic phase ($2.5 \cdot 10^{-3}$ molATP/C-mol.h) is about 8 times lower than the aerobic maintenance coefficient ($1.9 \cdot 10^{-2}$ molATP/C-mol.h). The aerobic maintenance value is in the range of the average maintenance value reported by Tijhuis.[6] He also found that maintenance during anaerobic and aerobic conditions was in the same range. Apparently, the anaerobic maintenance coefficient found here, is much lower than the aerobic coefficient. The reasons for this are yet unknown.

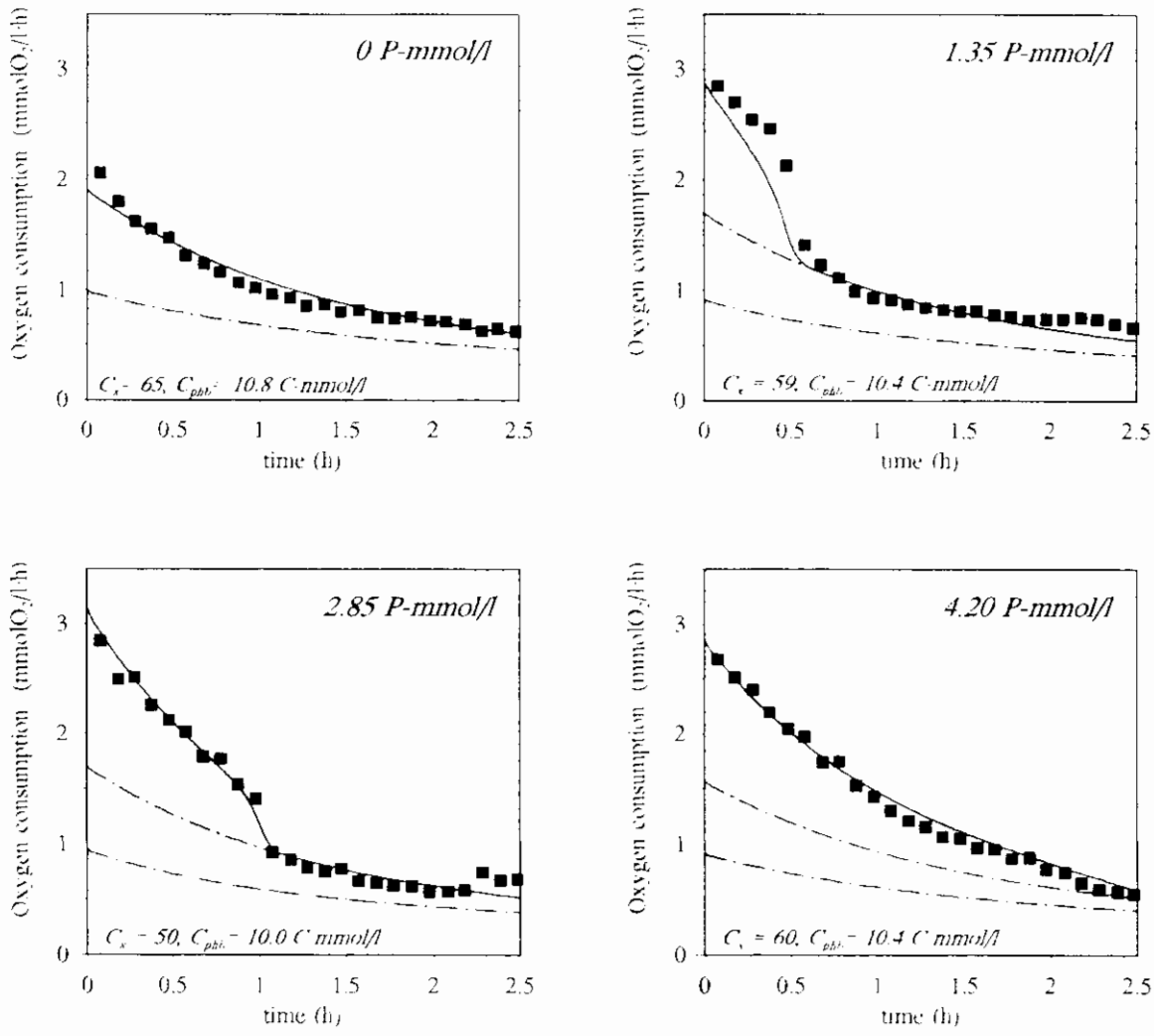


Figure 11 *Oxygen consumption rate in 4 experiments with different initial phosphate concentrations.*

A comparison of the model parameters found here with the results published by Wentzel[9] is not well possible because the model structures are very different. The major differences reside in the following issues:

- In the present model, an energetic evaluation is made of the biological P-removal, in which the metabolic energy involved in the production or consumption of a component was determined. The model is based on the bioenergetics of the metabolism and leads to the stoichiometry of the anaerobic and aerobic phases which is a function of the energetic restrictions expressed by 4 parameters (α_p, δ, ϵ, K). Kinetically, the production of biomass, polyP and glycogen from PHB are not coupled to each other and can all proceed independently with their own kinetics.

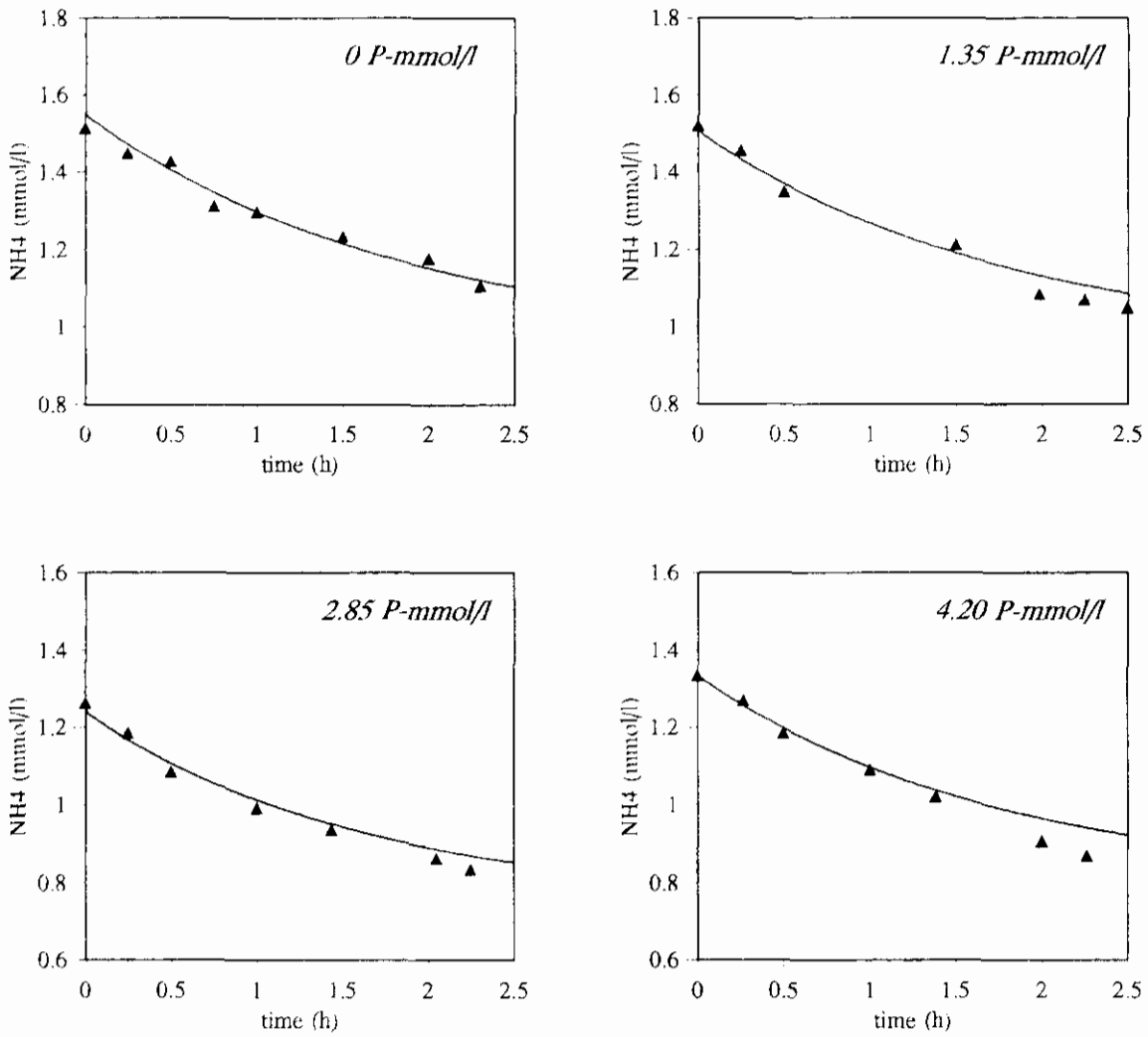


Figure 12 *Ammonium consumption due to growth in 4 experiments with different initial phosphate concentrations.*

The model of Wentzel is different in this respect. Only one yield for biomass production, and one ratio between P-uptake and PHB consumption are defined. Furthermore the description of the aerobic phase contains two separate submodels (P-limiting and P-not limiting) with different kinetic parameters. The present model covers the whole aerobic period.

- In the present model the substrate activity is related to the active biomass only, while in the model of Wentzel biomass activities are based on the total organic mass (active biomass, PHB and glycogen). For activities performed by active biomass it seems more logical to define these activities on the active biomass, with exclusion of the highly dynamic behaviour of non-active storage materials.

- In the present model an additional storage polymer, glycogen which is essential for the P-removal, is introduced. This compound has a substantial place in the aerobic energetics and behaviour of the micro-organisms.
- The use of the maintenance concept in the present model explains the growth rate and biomass yields satisfactory. The model of Wentzel uses the lysis concept.

Conclusions

A structured metabolic model based on the biochemical pathways of the process is very well capable to describe the complex conversions of the biological phosphorus removal process. All conversions of the relevant components participating in the biological phosphorus removal can be described by six independent reactions: two for the anaerobic phase and four for the aerobic phase. The rates of these reactions are described by four kinetic relations and two maintenance terms. It has been shown that with this limited set of parameters it is possible to describe the dynamic behaviour of all components during the anaerobic and aerobic phase very well.

Nomenclature

C_i	concentration of component i	(mol/m^3)
Y	yield	(mol/mol)
r_i	conversion rate of component i	($mol/m^3.h$)
q_i	specific rate of component i	(mol/C-mol.h)
m	maintenance	(mol/C-mol.h)
f_i	fraction of component i	(mol/mol)
μ	growth rate	(h^{-1})
k_i	rate constant of component i	(h^{-1})
K_i	kinetic constant of component i	(mol/l)

subscripts

an	anaerobic
aer	aerobic
s	acetate
p	phosphate
phb	PHB
x	active biomass
pp	polyP
gl	glycogen

References

1) Jenkins D., Tandoi, V. 1991. The applied microbiology of enhanced biological phosphate removal - accomplishments and needs, Wat. Res., Vol 25, No. 12, 1471-1478.
2) Mino, T., Arun, V., Tsuzuki, Y., Matsuo, T. 1987. Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process. Biological Phosphate removal from Waste waters (Advances in Water Pollution Control 4, Ed. R. Ramadori, Pergamon Press, Oxford, 27-38.
3) Roels J.A., 1983. Energetics and kinetics in biotechnology, Elsevier Biomedical Press.
4) Smolders G.J.F., Meij, J. van der, Loosdrecht, van, M.C.M, Heijnen, J.J. 1994. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. Biotech. & Bioeng, 42, 461-470.
5) Smolders G.J.F., M.C.M. van Loosdrecht, J.J. Heijnen. 1994. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotech. & Bioeng, 44, 837-848.
6) Tijhuis L., M.C.M. van Loosdrecht, J.J. Heijnen. 1993. A thermodynamically based correlation for maintenance gibbs energy requirements in aerobic and anaerobic chemotrophic growth. Biotech. & Bioeng., Vol 42, 509-519.
7) Wentzel, M.C., Loewenthal, R.E., Ekama, G.A., Marais, G.v.R. 1988. Enhanced polyphosphate organism cultures in activated sludge. Part I: Enhanced culture development. Water SA Vol 14., No. 2, 81-92.
8) Wentzel, M.C., Ekama, G.A., Loewenthal, R.E., Dold, P.L., Marais, G.v.R. 1989a. Enhanced polyphosphate organism cultures in activated sludge systems. Part II: Experimental behaviour. Water SA Vol 15., No. 2, 71-88.
9) Wentzel, M.C., Dold, P.L., Ekama, G.A., Marais, G.v.R. 1989b. Enhanced polyphosphate organism cultures in activated sludge systems. Part III: Kinetic model, Water SA Vol 15., No. 2, 89-102.
10) Wentzel, M.C., Lötter, L.H., Loewenthal, R.E, Marais, G.v.R. 1986. Metabolic behaviour of *Acinetobacter spp.* in enhanced biological phosphorus removal - a biochemical model, Water SA Vol. 12, No 4, 209-224.

Appendix IV

Validation of the metabolic model: effect of the sludge retention time

The biological phosphorus removal process is a process which depends basically on three internal storage compounds. PHB produced during the anaerobic phase is used as substrate for biomass, polyP and glycogen formation. The reaction rates of the aerobic processes are primarily determined by the PHB content of the cells. This PHB content is highly dynamic due to the conversions during the anaerobic and aerobic phase of the cycle and the ratio between substrate addition and biomass present in the reactor. The amount of biomass present in the reactor is determined by the sludge retention time and growth rate. A metabolic model of the biological phosphorus removal process was developed and verified over a wide range of growth rates. The effect of different growth rates on the internal fractions of stored components was determined and described mathematically. The measured conversions of the relevant components observed in the reactor as a function of the sludge retention time could be described with a single set of kinetic parameters.

Introduction

Biological P-removal in activated sludge systems is characterized by the recirculation of sludge through anaerobic and aerobic phases. During anaerobic conditions, substrate is taken up and stored as intracellular PHB and the required energy is generated in the degradation of polyphosphate and glycogen.[2,5] In the aerobic phase, the internally stored PHB is used for growth, uptake of phosphate and production of glycogen. No external substrate is present in the aerobic phase, and therefore the rate of the conversions during the aerobic phase is determined by the internal fractions of the components. A practical method to study this complex process is the cultivation of biomass in a sequening batch reactor (SBR) in which the biomass is cyclically exposed to anaerobic/aerobic conditions. The biomass is fed at the start of the anaerobic period using an acetate pulse. The waste biomass is withdrawn at the end of the aerobic period.

In previous research a kinetic model has been proposed, [5,6,7] which was validated in an SBR reactor running at a sludge age of 8 days (SRT 8). The PHB content of the biomass is one of the more important control variables in this model because it controls the rate of growth and consequently determines the contents of the other internal compounds, polyP and glycogen. Until now, the model has been validated only over the range of PHB fractions observed during the anaerobic/aerobic cycle at a sludge age of 8 days. However, a more extensive model validation requires a much larger range of PHB fractions in the biomass. In the present research it is shown how this PHB fraction can simply be varied and the proposed model is validated over a wide range of PHB fractions in the biomass.

Biomass concentration and PHB content

The PHB content in the cells is one of the factors that determines primarily the rate of the processes during the aerobic phase.[7] In the biological phosphorus removal, the PHB content of the biomass in the biological phosphorus removal is highly dynamic, and determined by two aspects.

First, the dynamics of the PHB content is determined by the conversions during the cycle. During the anaerobic part of the cycle, the PHB content of the cells is increased by the anaerobic uptake of acetate and in the aerobic part it is decreased by the conversions

associated with biomass, polyP and glycogen synthesis. This dynamic behaviour of components during an anaerobic/aerobic cycle has been described previously.[7]
Secondly, the PHB content depends on the biomass concentration present in the reactor. At a constant acetate feed to the reactor, the PHB content will become high if the acetate is anaerobically taken up into a small amount of biomass, and low if there is a high biomass concentration in the reactor.
The biomass concentration in the SBR reactor can be controlled easily by manipulation of the rate of sludge withdrawal. This withdrawal rate directly influences the biomass solids retention time (SRT) which is closely related to the growth rate (μ). Therefore it can be expected that, at a constant acetate load on the reactor, there will be a relation between SRT and the PHB fraction (f_{phb}) in the biomass. Applications of such different PHB content levels (through SRT variation) then allows more extensive study of the kinetic relations between the PHB content and growth-, polyP- and glycogen kinetics.

A relation between the biomass concentration and the growth rate can easily be derived. The biomass production in a cycle (when all substrate is anaerobically converted to PHB) is given by:

$$\Delta C_x = Y_{sx} \cdot \Delta C_s \qquad (1)$$

with:

ΔC_x	: biomass production per cycle	(C-mmol/l· cycle)
ΔC_s	: substrate addition per cycle	(C-mmol/l· cycle)
Y_{sx}	: biomass yield based on acetate	(C-mol/C-mol)

Growth is determined by the biomass production during the cycle with respect to the biomass concentration and controlled by the removal rate of sludge at the end of the aerobic period. This rate of removal, which is in the steady state equal to ΔC_x, determines the sludge retention time (SRT). The sludge retention time is the reciprocal of the growth rate and is determined according to:

$$SRT \qquad \frac{C_x(0)}{\Delta C_x} \cdot \frac{t_{cyc}}{24} \qquad (2)$$

with:

$C_x(0)$	: biomass at the start of the cycle	(C-mmol/l)
t_{cyc}	: cycle time	(h/cycle)

Combination of equation (1) and (2) shows that the biomass concentration in the reactor depends on SRT, yield and substrate loading rate according to:

$$C_x(0) \quad Y_{sx} \cdot \Delta C_s \cdot \frac{SRT \cdot 24}{t_{cyc}} \tag{3}$$

The appearance of the number 24 in eq. (2) and (3) is due to different time definitions; SRT in days and t_{cyc} in hours. According to this relation, an increasing SRT leads to a higher biomass concentration. An increasing SRT has a decreasing effect on the biomass yield due to the increased contribution of maintenance but this has only a minor effect on the biomass concentration, as will be shown later. The relation is shown for an acetate loading rate of 25 C-mmol/l.d in figure 1.

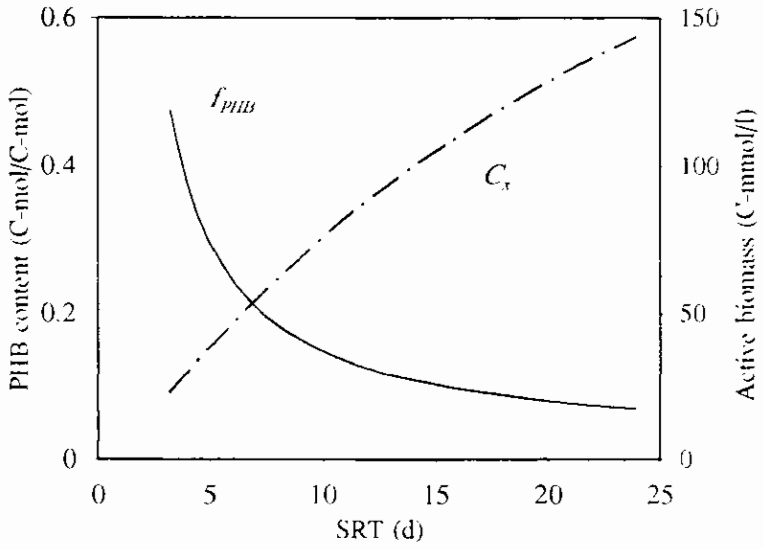


Figure 1. *Effect of the sludge retention time on the PHB content and active biomass concentration in a sequencing batch reactor for an acetate loading rate of 25 C-mmol/l.d.*

The PHB content at the end of the anaerobic phase depends on the biomass concentration and substrate feed to the system according to the following equation where $C_x(0)$ is eliminated using equation (3):

$$f_{phb}(an) \quad \frac{Y_{sphb} \cdot \Delta C_s}{C_x(0)} + f_{phb}(0) \quad - \quad \frac{Y_{sphb}}{Y_{sx} \cdot SRT} + f_{phb}(0) \qquad (4)$$

with

Y_{sphb}	: Yield of PHB based on acetate
$f_{phb}(an)$	: PHB content, end anaerobic phase (fig 2)
$f_{phb}(0)$	: PHB content, start anaerobic phase (fig 2)

From this equation it can be seen that the PHB content is primarily determined by the biomass specific acetate load to the system, $\Delta C_s/C_x(0)$. This load is again determined by the SRT which followes from equation (3) where the biomass concentration was determined through the SRT. Therefore the PHB content is indirectly dependent on the acetate feed to the system or the sludge retention time. In figure 1, the anaerobic PHB content of the cells with a constant volumetric substrate load on the system was calculated according to the model descibed in this paper, as function of the SRT. From this figure it can be seen that a decreasing SRT will increase the PHB content of the cells. The effect of the SRT on the PHB content will directly affect growth kinetics. As a consequence of the change in growth rate, the biomass production will change and therefore the polyP and glycogen content. A different polyP- or glycogen content affects the conversion kinetics of these components and therefore these compounds are indirectly influenced by the PHB content.

Previously, we described and experimentally verified the stoichiometry and kinetics of the polyP organisms in the anaerobic and aerobic phase at a sludge age of 8 days, which represents a biomass concentration of 60-65 C-mmol/l. [7] In this paper, we will determine the effect of different sludge removal rates on the biomass concentrations and internal fractions of stored components, the effect on the kinetics, and try to describe these phenomena mathematically, using the previously mentioned model.

Model development

For the model description of the biological P-removal process in the cyclic SBR process, the mass balance over the full cycle must be made. The accumulation of a component *i* in one

cycle of a sequencing batch system is a result of its conversion in the anaerobic and aerobic phase and the transport Φ_i to or from the system. The accumulation over the total cycle, ΔC_i^{cyc}, if the conversion over a phase is represented by ΔC, becomes:

$$\Delta C_i^{cyc} = \Delta C_i^{an} + \Delta C_i^{ae} + \Phi_i \tag{5}$$

The change in concentration during the anaerobic or aerobic phase, where a constant volume is maintained, is described by:

$$\Delta C_i = \int_0^t r_i \cdot dt \tag{6}$$

The conversion rate, r_i, of component i in the reactor can be calculated according to:

$$r_i = \alpha \cdot q_i \cdot C_x \tag{7}$$

The volumetric concentration of the internal components (PHB, polyP and glycogen) is given by:

$$C_i = f_i \cdot C_x \tag{8}$$

In equation (7), the stoichiometry of the metabolism, see table I, relating quantities of reactants consumed to quantities of products formed, is fixed and represented in the stoichiometry matrix α, which was shown in appendix III. The development of this kind of models was described by Roels.[3] The kinetic relations for the specific rates q of the components are shown in table II. The relations for the anaerobic kinetics are as previously described. In the relations for the aerobic kinetics minor modifications can be found in comparison with previously published work.[7]

In the experiments with different sludge removal rates performed here, different PHB contents were obtained. It appeared that the model did not accurately describe the observed P-uptake, due to the dependency of the P-uptake kinetics on the PHB level. The contribution of the PHB content to the specific uptake rate of phosphate appeared to be less strong. Therefore, the linear function in f_{phb} was changed in a 1/3 power relation. The change of the kinetic relation of phosphate had effect on the previous derived parameter

values. Here, new values are derived for the parameter values in which previous experiments were incorporated. The glycogen content of the cells did increase with the PHB content, as will be shown later. The maximal glycogen fraction which is produced in the aerobic phase is made proportional to the PHB fraction present at the end of the anaerobic phase. This control strategy is very usefull for the organism: if the organism is continuously exposed to an environment where the acetate/biomass load is high, a high PHB content will be reached and a high content of glycogen will be required in the anaerobic phase to garantee full acetate uptake. When only low concentrations of acetate are present, the PHB content will be low and also the glycogen content. A high glycogen content during such circumstances would decrease the yield of the active biomass and be of no advantage to the organism.

Table I *Metabolic reactions of the anaerobic and aerobic phase of the biological P-removal process.*

Anaerobic		
R_1	Acetate uptake	$-CH_2O$ -0.5 $CH_{10/6}O_{5/6}$ -0.37 HPO_3 +1.33 $CH_{1.5}O_{0.5}$ +0.17 CO_2 +0.37 H_3PO_4 +0.05 H_2O = 0
R_2	Maintenance	- HPO_3 - H_2O + H_3PO_4 = 0
Aerobic		
R_3	Biomass synthesis	-1.37 $CH_{1.5}O_{0.5}$ -0.20 NH_3 -0.015 H_3PO_4 -0.42 O_2 +$CH_{2.09}O_{0.54}N_{0.20}P_{0.015}$ +0.37 CO_2 +0.305 H_2O= 0
R_4	Phosphate uptake	- 0.27 $CH_{1.5}O_{0.5}$ - 0.306 O_2 -H_3PO_4 + HPO_3 + 0.27 CO_2 + 1.20 H_2O = 0
R_5	Glycogen formation	-1.12 $CH_{1.5}O_{0.5}$ - 0.26 O_2 +$CH_{10/6}O_{5/6}$ + 0.12 CO_2 + 0.007 H_2O= 0
R_6	Maintenance	- $CH_{1.5}O_{0.5}$ - 1.125 O_2 + CO_2 +0.75 H_2O = 0

The transport term Φ_i is defined as the amount removed or added of component i per litre reactor volume per cycle:

$$\Phi_i = \frac{\Delta V_i}{V} \cdot C_i \qquad (9)$$

The change in volume during a cycle in the SBR is shown in figure 2. After the cycle is

started with the addition of ΔV_i litre influent, the total volume in the SBR becomes V litre. At the end of the aerobic phase the waste sludge is removed with a volume ΔV_w. The effluent, with volume ΔV_e, is withdrawn at the end of the settling phase.

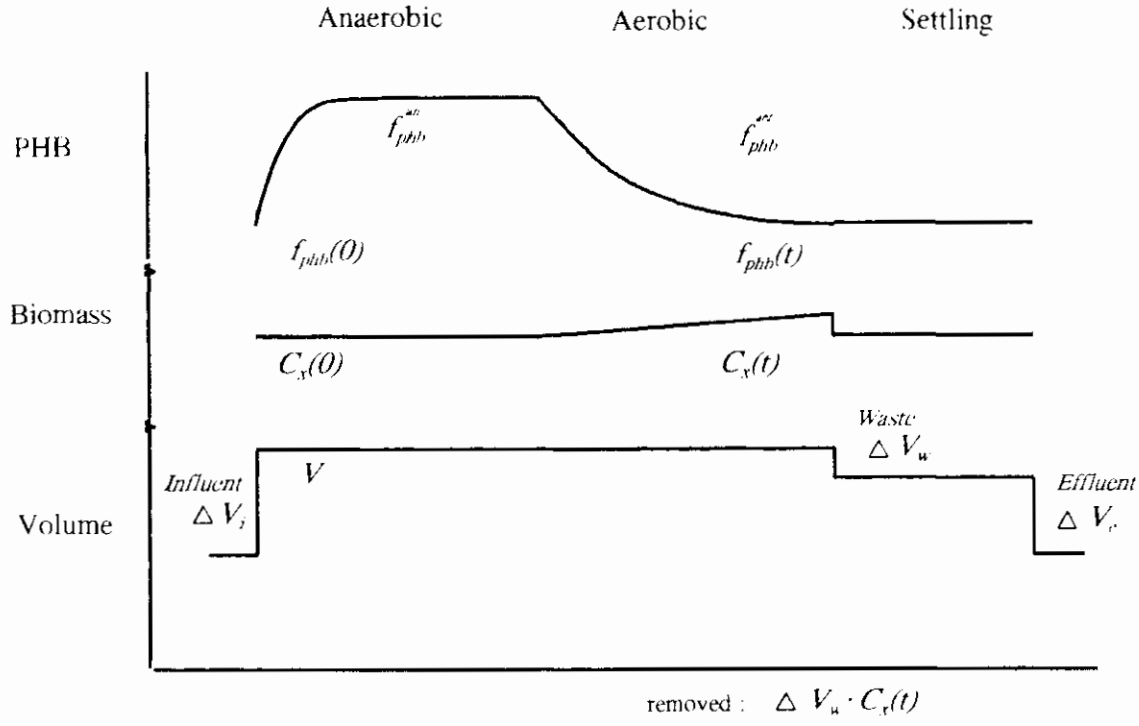


Figure 2 *Volume, biomass concentration and PHB fraction during an anaerobic/aerobic cycle in a SBR due to influent, waste and effluent flows.*

Steady state

In the case of a sequencing batch reactor, a steady state is defined as the situation were no accumulation over a cycle takes place. The concentration of the active biomass and internal components (PHB, polyP and glycogen) at the start of each cycle are therefore identical. For a steady state, equation (5) can be rewritten as:

$$\Delta C_i^{cyc} = \Delta C_i^{an} + \Delta C_i^{aer} + \Phi_i = 0 \qquad (10)$$

The active biomass concentration at the start of the anaerobic phase is $C_x(0)$ and remains constant during this phase, because growth takes place only during aerobic conditions, see figure 2. At the end of the aerobic phase the active biomass concentration is $C_x(t)$,and growth, ΔC_x^{aer} is equal to $C_x(t)$-$C_x(0)$. In a steady state situation, the biomass removal at

the end of the cycle, must equal the growth of biomass during the cycle. This is mathematically expressed as:

$$V \cdot C_x(t) - \Delta V_w \cdot C_x(t) = V \cdot C_x(0) \quad (11)$$

From equation (11) a steady state relation can be found for the active biomass concentration at the end of the aerobic phase, $C_x(t)$, as a function of the biomass concentration at the start of the anaerobic phase $C_x(0)$:

$$C_x(t) \quad \frac{V}{V \cdot \Delta V_w} \cdot C_x(0) \quad (12)$$

In a steady state situation, the amount of active biomass produced during the aerobic phase, ΔC_x^{aer}, equals the amount of biomass removed in the waste. Combination of equation (9-11) and (12) yields the overall biomass balance for a steady state:

$$\Delta C_x^{aer} - \frac{\Delta V_w}{V - \Delta V_w} \cdot C_x(0) = 0 \quad (13)$$

From equation (13) the relation between the SRT and the growth rate ($\bar{\mu}$) can be found when the biomass production during the aerobic phase is expressed as the product of average growth rate and the time period of the aerobic phase:

$$\Delta C_x^{aer} = t_{aer} \cdot \bar{\mu} \cdot C_x(0) \quad (14)$$

The sludge retention time in a SBR is defined as the sludge removal rate over a full cycle. Growth only takes place during aerobic conditions and therefore in the comparison of growth and the SRT a correction for the anaerobic time in a cycle is required. In a steady state, the SRT (in days) is set by the average growth rate, $\bar{\mu}$ (h^{-1}), the aerobic part of a cycle, t_{aer} / t_{cyc}, and the conversion of growth rate from hours to days:

$$SRT = \frac{t_{cyc}}{24 \cdot t_{aer}} \cdot \frac{1}{\bar{\mu}} \quad (15)$$

Therefore, the relation between the SRT and waste flow follows from equation (15),(14) and (13):

$$SRT = \frac{t_{cyc}}{24 \cdot t_{aer}} \cdot \frac{1}{\bar{\mu}} = \frac{t_{cyc}}{24} \cdot \frac{C_X(0)}{\Delta C_X^{aer}} = \frac{t_{cyc}}{24} \cdot \frac{V - \Delta V_w}{\Delta V_w} \qquad (16)$$

For the steady state balance of the internally stored components (polyP, PHB, glycogen) it is assumed that the fraction at the end of the aerobic phase remains constant during the settling phase, see figure 2. The values of f_i at the end and start of the cycle are therefore equal:

$$f_i(t) = f_i(0) \qquad (17)$$

Having defined the total conversions of all components over an anaerobic/aerobic cyle, and the relation of the SRT with the waste flow, the behavior of all components as a function of the waste flow or SRT can be calculated using the complete model which contains the kinetics and transport terms.

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

The study was carried out in a laboratory fermenter with a working volume of 2 l, at 20 °C and pH 7.0. The reactor was operated as a sequencing batch reactor (SBR) with a cycle of 6 hours consisting of an anaerobic (2.25 h), aerobic (2.25 h) and settling period (1.5 h), see figure 2. Biological phosphorus removing sludge was used as an inoculum. Since the added acetate was completely consumed in the anaerobic zone, only organisms capable of anaerobic acetate consumption were accumulated in the reactor. Methanogens were not present since part of the time oxygen was present. At higher SRT's (> 12 days) nitrification took place, which was quantified by the measurement of nitrite and nitrate. One litre medium was fed at the start of the anaerobic phase and effluent was removed at the end of the settling period, resulting in a hydraulic retention time of 12 h. At the end of each aerobic phase excess sludge was removed by a peristaltic pump. For more details about the operation of the SBR, see the preceeding appendices.

Media

Sterilized synthetic medium was used containing per litre: 0.85 g $NaAc.3H_2O$ (400 mgCOD/l) as carbon source, 107 mg NH_4Cl, 75.5 mg $NaH_2PO_4.2H_2O$ (15 mgP/l), 90 mg $MgSO_4.7H_2O$, 36 mg KCl, 14 mg $CaCl_2.2H_2O$, 1 mg yeast extract, 0.3 ml nutrient solution, which was described in appendix I.

Measurements and analyses

The concentrations of acetate, phosphate and ammonium were measured as well as the internally stored fractions of PHB and glycogen. Regularly the MLSS and VSS concentrations were measured by taking samples of 60 ml directly from the reactor of which a volume of 15 ml was used for the measurements, the rest was returned to the reactor. During the aerobic phase the SBR was coupled to a respirometer which measured the oxygen consumption rate with intervals of 3 minutes. This equipment was described in appendix II. Analyses were performed as described in appendix I and II.[5]

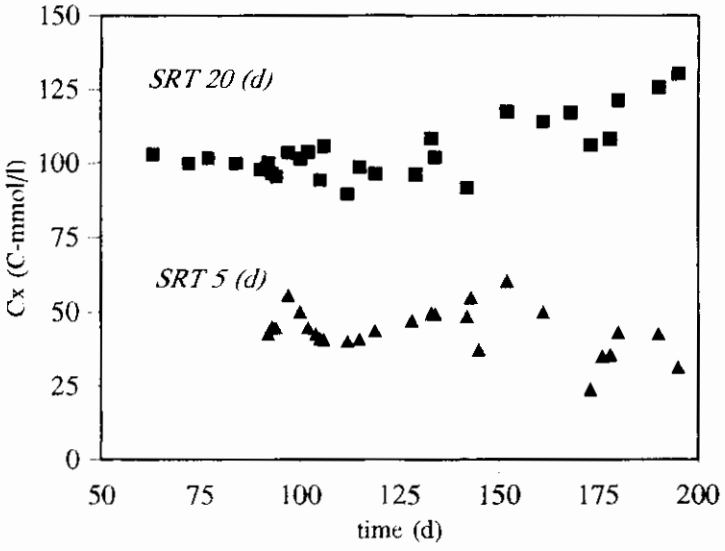


Figure 3 *Active biomass concentrations in the SBR with a sludge retention time of 5 and 20 days at the end of the aerobic phase.*

Experimental setup

Two sequencing batch reactors were operated at a sludge retention time of 5 and 20 days. Therefore from one reactor, 100 ml excess sludge was removed at the end of the aerobic phase in every cycle, to get a SRT of 5 days, while in the other one 25 ml excess sludge was

removed, resulting in a SRT of 20 days. After operation of the SBR's for more than 5 times the SRT the measurements were started. The data were derived by monitoring several cycles of the SBR in which the anaerobic and aerobic phase were extensively sampled.

Results

In figure 3 the active biomass concentration during the experiments for the reactors adapted to a SRT of 5 days and 20 days is shown, with a preeceeding operation time of about 60 days. From this figure it appears that the active biomass does not remain constant over a very long time. Although the waste flow of the SBR's were adjusted to a constant value, this did not lead to a constant biomass concentration over more then 100 days. The SBR adjusted to a SRT of 5 days showed a fluctuation in the active biomass concentration of 35 to 55 C-mmol/l. The active biomass concentration in the SBR with SRT 20 days shows a fluctuation between 90 and 130 C-mmol/l.

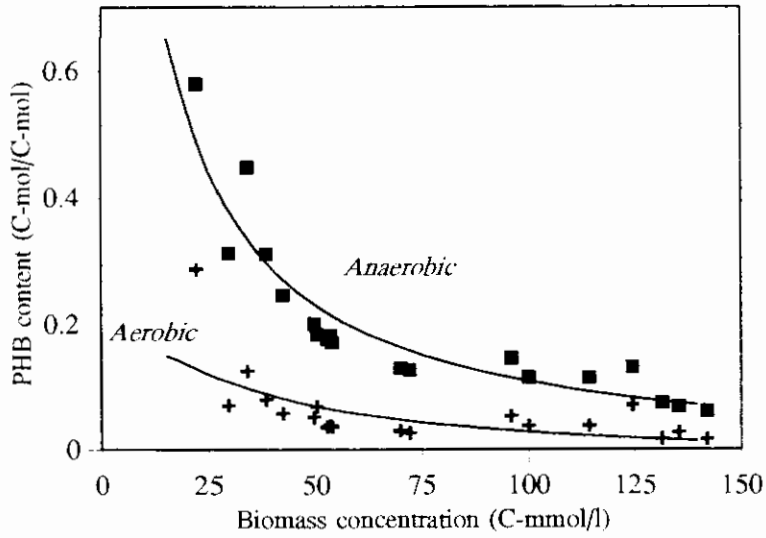
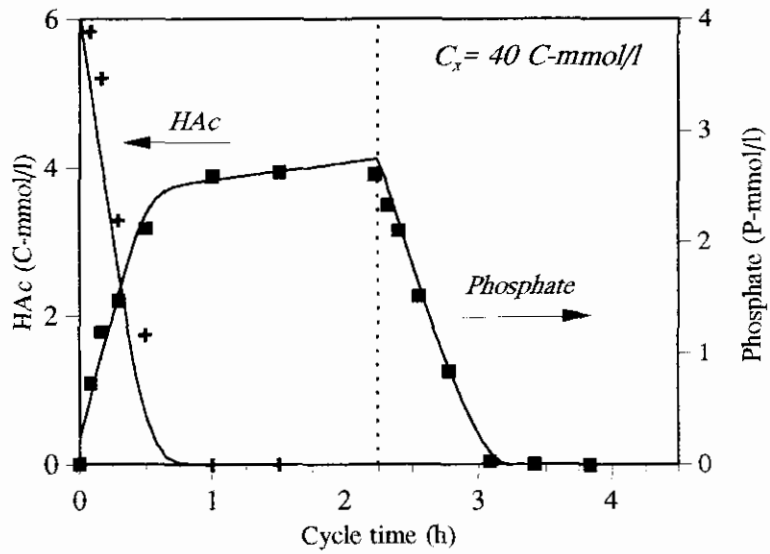


Figure 4 *PHB content at the end of the anaerobic and aerobic phase as function of the biomass concentration. The solid lines are the model predictions for the anaerobic and aerobic phase respectively.*

One of the explanations might be that, for unknown reasons, there is a fluctuation of growth in the aerobic phase. A second, more likely explanation is that the removal of biomass from the reactor fluctuated. This might be due to difficulties in establishing the same biomass

concentration in the waste as in the reactor using the method of removing the suspension with a peristaltic pump. This is supported by the observation that the biomass in the SBR reactor settles very well. Therefore it appears that it is most likely that there have been fluctuations in the actual SRT values. Because the biomass concentration in the reactor has been measured by a method which does not suffer from this sampling problem (high speed suction syringe) the reactor biomass concentration is a much more accurate number than the SRT value.

a)



b)

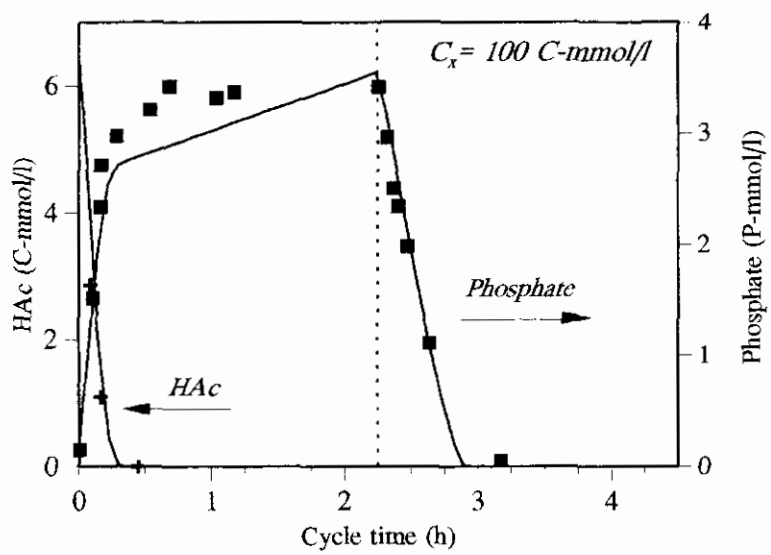


Figure 5 *Acetate and phosphate concentrations during a cycle at two different sludge waste rates resulting in a biomass concentration of a) 40 C-mmol/l and b) 100 C-mmol/l. The lines were calculated using the model.*

Therefore the kinetic analysis will be based on the actual biomass concentration in the reactor. Using this approach, a good correlation was found between the PHB content and biomass concentration. According to the approximate equation (4) the anaerobic PHB concentration is primarily determined by the ratio of acetate added to biomass present in the reactor. In figure 4 the actual measurement of PHB content is compared to the full model prediction. A good relation between the measured data and the model predictions is found both for the anaerobic and aerobic PHB contents.

The results of the measurements during the cycles of both SBR's are shown in figures 5, 7 and 8. The acetate and phosphate concentrations during the cycles for SRT 5 and 20 days are shown in figure 5. The observed phosphate/acetate ratio for the reactor at SRT 5 days was 0.42 molP/molC which was lower than the ratio for the reactor at SRT 20 days, which was 0.55 P-mol/C-mol.

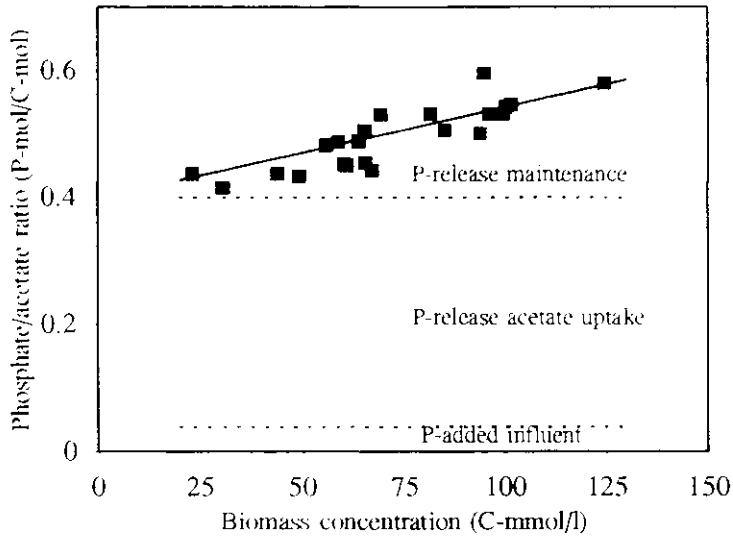
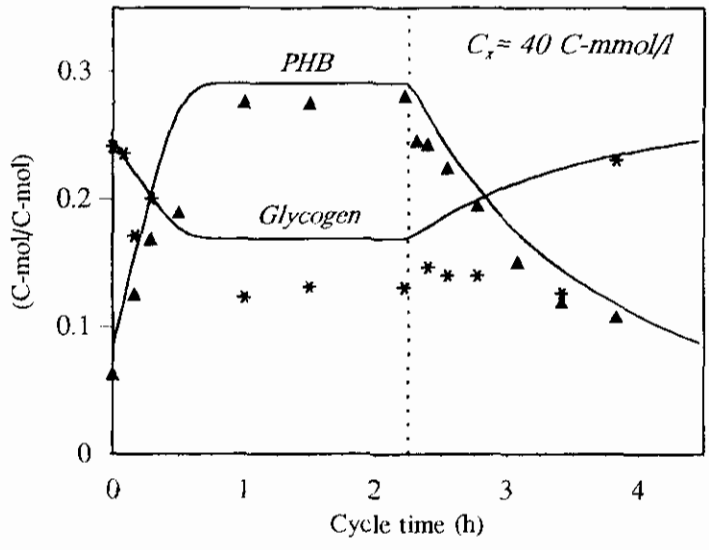


Figure 6 *Effect of the biomass concentration in the reactor on the anaerobic P-release caused by maintenance. Phosphate added with the influent, released in the uptake of acetate, and resulting form maintenance contribute in the total observed phosphate/acetate ratio.*

If the observed phosphate/acetate ratio is plotted as function of the active biomass concentration in the reactor, see figure 6, a distinct trend can be observed. This increase in the observed phosphate/acetate ratio is due to the increased contribution of the maintenance at higher biomass concentrations during anaerobic conditions. The anaerobic maintenance contribution used to plot the line in figure 6 was $4.0 \cdot 10^{-3}$ P-mol/C-mol.h, while the

phosphate/acetate ratio was 0.36 P-mol/C-mol. These values are different from previously found values for a SRT of 8 days, but are considered more reliable and therefore used in the model.

a)



b)

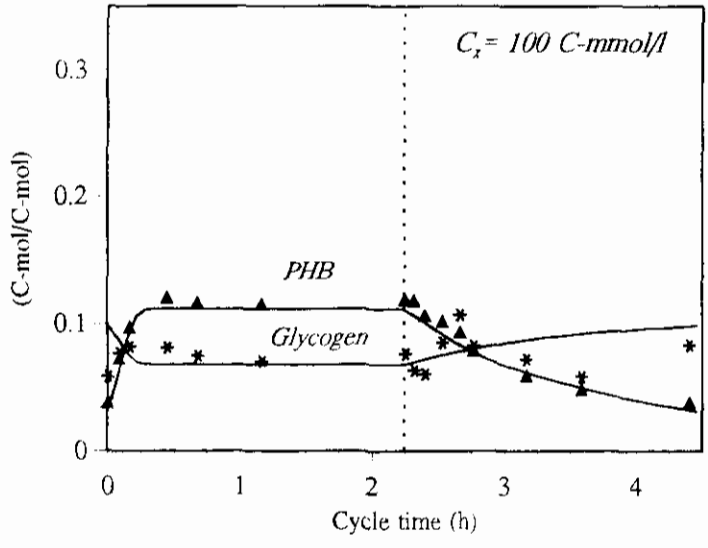


Figure 7 *PHB and glycogen content during a cycle at two different sludge retention times. a) low SRT, C_x = 40 C-mmol/l; b) high SRT, C_x = 100 C-mmol/l. The lines were calculated using the model.*

In figure 7, the PHB and glycogen content during the cycle is shown for the two SRT values. The most important difference between the two figures is the level of the PHB and glycogen content. As expected, at low biomass concentration (SRT ≈ 5 d), the PHB content must be

high as long as all acetate is taken up completely. The glycogen content is in the same range as the PHB content. At a high biomass concentration (C_x =100 C-mmol/l) both the PHB and glycogen content are low. It is clear that the glycogen measurements are not very accurate, however they are at the same level as predicted by the model, which is the most important aspect, as will be explained in the discussion section.

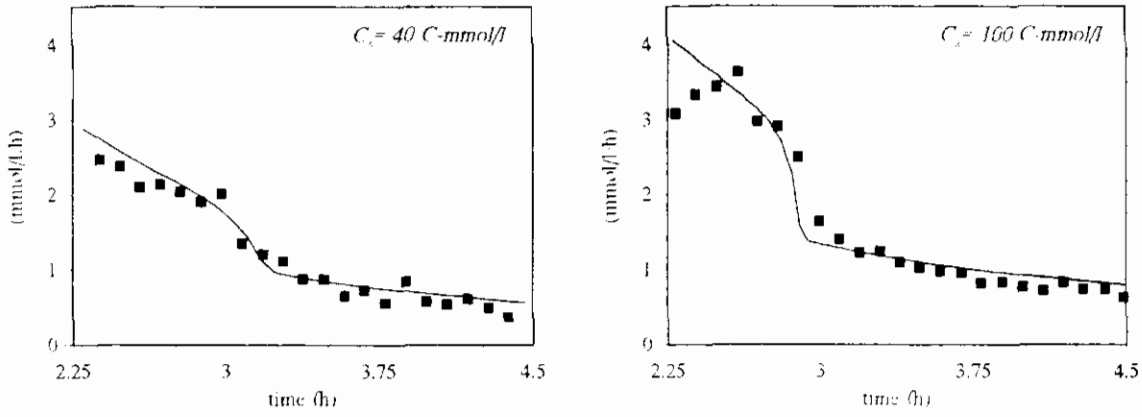


Figure 8a *Oxygen consumption rate during the aerobic phase at a high (left) and low (right) sludge retention time.*

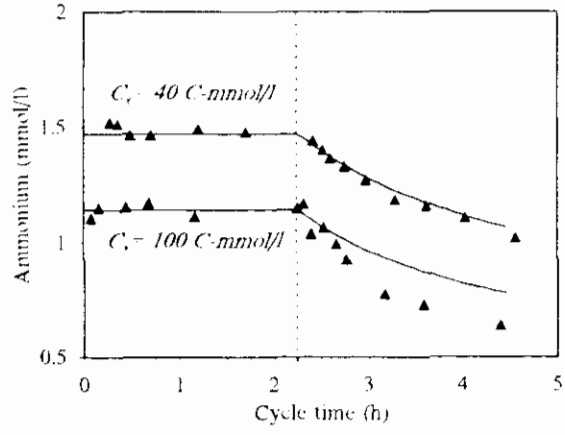


Figure 8b *Ammonium consumption during the cycle at a high (C_x = 40 C-mmol/l) and low (C_x = 100 C-mmol/l) sludge retention time.*

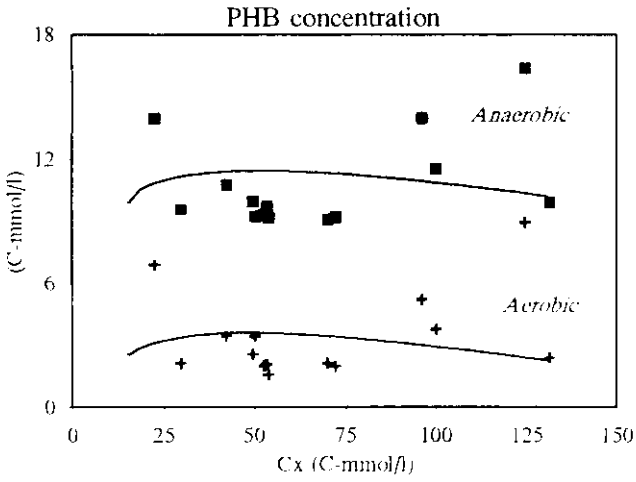
In figure 8 the oxygen consumption rate and ammonium consumption are shown. The oxygen consumption rate at the start of the aerobic phase is enhanced due to the energy requirement for the uptake of phosphate and polymerization to polyphosphate. At a high biomass concentration in the reactor, the increased oxygen consumption rate at the start of the aerobic phase is higher due to the faster uptake of phosphate (see fig. 5). This is an additional

illustration of the coupling between phosphate uptake and oxygen consumption. The ammonium consumption predicted by the model, is not correct for the reactor with the high biomass concentration due to the contribution of nitrification in the ammonium consumption, which takes place at this SRT. The oxygen consumption rate was corrected for the contribution of the nitrification. The difference between measured and predicted ammonium consumption was in accordance with the measured amount of nitrite produced (≈ 0.25 N-mmol/l). The acetate consumption for denitrification of this amount of nitrite after the start of a new cycle would consume only 3% of the influent acetate.
The model predictions for all concentrations, fractions and rates were based on the biomass concentration present in the reactor and not on the adjusted sludge retention time. All conversions were accurately predicted with the same set of kinetic parameters.

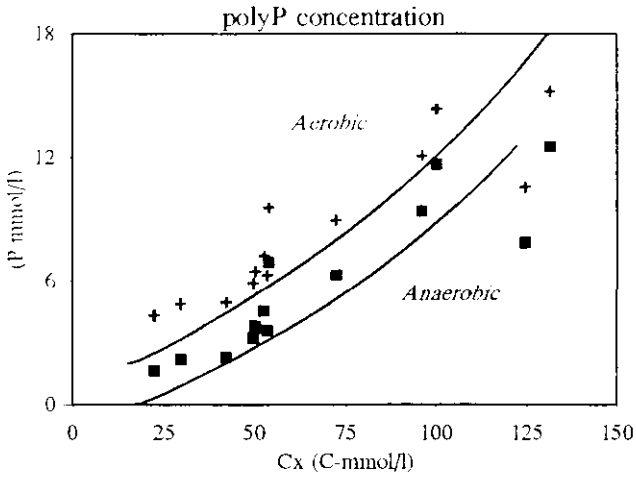
In figure 9 the predicted and measured concentrations of PHB, polyP and glycogen in the reactor at the end of the anaerobic and aerobic phase are shown as a function of the biomass concentration. The measured and calculated PHB fraction as function of the biomass concentration was shown in figure 4, while in figure 10a and 10b the fractions of polyP and glycogen are shown. The anaerobic as well as the aerobic fraction of PHB (fig 4) decreases with an increasing sludge retention time. It is notable that the difference between the anaerobic and aerobic PHB fraction increases at a lower SRT. This is due to the constant acetate load on the system while the biomass concentration in the reactor decreases. Clearly, as long as all acetate can be converted completely, the PHB content of the cells becomes higher at lower SRT.

The anaerobic polyP concentration (fig 9b) as well as the polyP content (fig 10a), decreases with the biomass concentration and thus SRT, while the aerobic polyP fraction decreases initially and then increases. One should realise that the low biomass concentration and the high biomass production rate at a low SRT value results in a lower accuracy of the measurements in comparison to higher SRT values. At a certain minimal SRT, the acetate added in the anaerobic phase can not be taken up completely, due to the shortage of polyP to supply ATP for the uptake. At this minimal SRT not all the acetate is consumed anymore and the maximal loading rate of the cycle is reached. The anaerobic and aerobic glycogen content of the cells (fig. 9c) shows the same trend as the PHB content.

a)



b)



c)

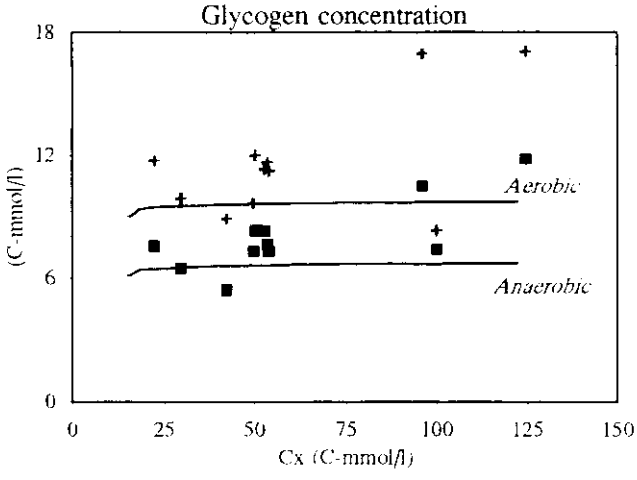
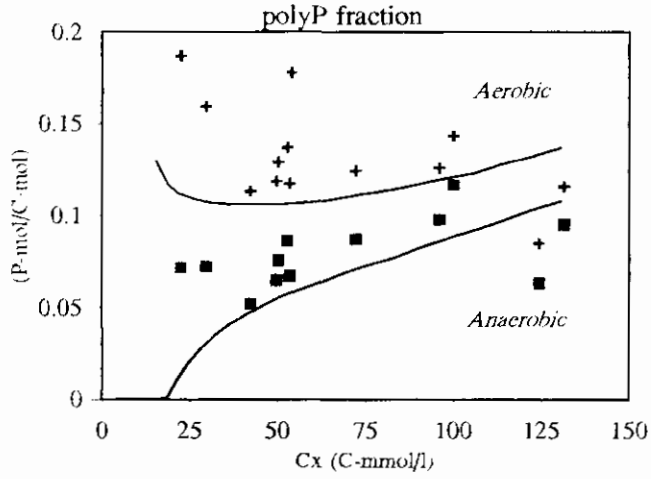


Figure 9 *Concentrations of the internally stored components as a function of the biomass concentration* C_x.

a)



b)

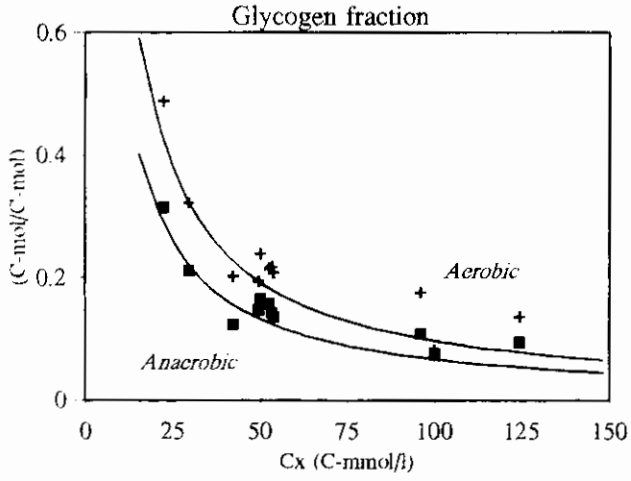


Figure 10 *Fractions of polyP (a) and glycogen (b) as a function of the biomass concentration.*

Discussion

The calculated actual yield of active biomass based on acetate as a function of the sludge retention time is given in figure 11. This figure is only valid for the phosphate/acetate ratio of the influent used in our experiments. A higher phosphate load will decrease the yield due to the increased energy need to run the storage cycle. From the figure it can be seen that there is not a very large change in the biomass yield as a function of the SRT. This is due to the fact that the maintenance value in the biological P-removal is relatively low in comparison with normal heterotrophic growth. Since the biomass yield based on acetate is relatively

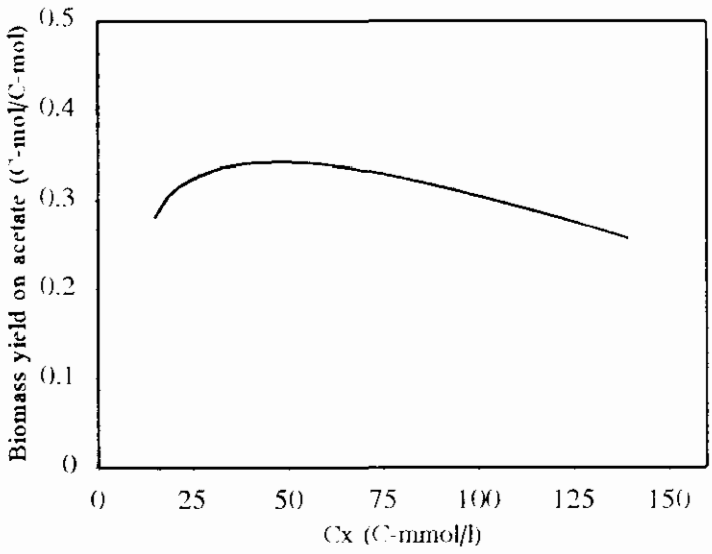


Figure 11. Biomass yield on acetate as a function of the biomass concentration.

From figure 5 to 10 it appeared that the metabolic model is capable to describe the experiments satisfactorily with the set of parameters shown in table II. With this set of parameters, the effect of the sludge retention time on the fractions of PHB, polyP and glycogen during the anaerobic/aerobic cycle can be calculated.

Importance of storage products in the P-removal

The storage compounds in the biological P-removal are highly important since they enable the organism to take up substrate during anaerobic conditions. The sufficient availability of these compounds during anaerobic conditions is therefore essential for the survival of the organism. Exhaustion of one of the components might halt the metabolism, while enough substrate is still available for storage to PHB. Both polyP and glycogen can limit the metabolism of the P-organisms, which is explained below in more detail.

Effect of SRT

The polyP fraction of the cells (fig 10a, 12b) as well as the volumetric polyP concentration (fig 9b) present in the reactor in the anaerobic phase decreases with a decreasing SRT (higher growth rate). At a certain SRT the polyP content present in the cells is not enough for the uptake of acetate during the anaerobic phase (f_{pp} = 0) and consequently acetate is not consumed completely. This means that part of the acetate will reach the aerobic phase. Here it will be possible that there will be interference with the P-uptake.[8] At this SRT the maximal growth rate is reached by limitation of polyP. This occurs at SRT = 3 (d), or using equation (15), at μ = 0.04 h^{-1}. In a continuous bench-scale activated sludge system treating settled domestic waste water supplemented with 50 mg/l acetate, it was observed that the P-removal activity was lost below sludge retention times of 2.9 days.[1] In a full-scale experiment it was observed that the enhanced biological phosphorus removal was eliminated by reducing the SRT from 3 days to 1.5 days.[4] These observations are in accordance with the maximal growth found in our experiments. The glycogen content of the cells is increased at a decreasing SRT and is therefore no limitation in the anacrobic uptake of the normal acetate load at a low SRT.

***Table II.** Kinetics of the anaerobic and aerobic phase of the biological P-removal process.*

			parameter	value	unit
Anaerobic					
q_s	Acetate uptake	$q_s^{max} \cdot \frac{C_s}{C_s + K_s}$	q_s^{max} K_s	0.4 1	C-mol/C-mol.h C-mmol/l
m_{an}	Maintenance		m_{an}	$4\ 10^{-3}$	P-mol/C-mol.h
Aerobic					
μ	Biomass synthesis	$k_x \cdot f_{phb}$	k_x	0.14	C-mol/C-mol.h
q_{pp}	Phosphate uptake	$k_{pp} \cdot \left(\frac{C_p}{C_p + K_p}\right) \cdot \left(1 - \frac{f_{pp}}{f_{pp}^{max}}\right) \cdot f_{phb}^{0.33}$	k_{pp} K_p f_{pp}^{max}	0.2 0.1 0.3	P-mol/C-mol.h P-mmol/l P-mol/C-mol
q_{gl}	Glycogen formation	$k_{gl}(K_{gl} \cdot \Delta f_{phb}^{an} - f_{gl})$	k_{gl} K_{gl}	0.8 1.3	C-mol/C-mol.h C-mol/C-mol
m_{aer}	Maintenance		m_{aer}	$4\ 10^{-3}$	C-mol/C-mol.h

Effect of peak loading

The influent loading of waste water treatment plants is not at all stable, but highly dynamic. The limited uptake of acetate by polyP shortage during a high peak load might be relevant for this reason. When in a system a phosphate stripper is used to release phosphate from the cells by addition of acetate, glycogen might also limit the uptake of acetate and release of phosphate, although it might appear that sufficient polyP is still present in the cells.

The limitation of the acetate uptake by shortage of polyP or glycogen depends on the SRT. At a low SRT the polyP content of the cells is low (explained above) and limits the acetate uptake when a peak load is supplied. With an increasing SRT, the polyP content is increased while the glycogen content is decreased. The glycogen fraction of the cells (fig 10b and 12c) decreases with an increasing SRT, while the volumetric glycogen concentration remains more or less constant. At a higher SRT the limitation of the acetate uptake shifts therefore from polyP limitation to glycogen limitation. This occurs at a SRT higher than 12-15 days. If a peak load of acetate is added to the system at a high SRT the glycogen concentration might limit the uptake of the acetate, as contrasted with polyP which is sufficiently available. Limitation of the acetate uptake by shortage of glycogen occurs when a peak load of acetate enters the system in a concentration 3-4 times higher than the normal steady state concentration.

Parameter estimation

The biological P-removal process is characterized by anaerobic and aerobic phases, in which the reaction rates invert during each phase. For instance, in the anaerobic phase PHB is produced which is consumed during aerobic conditions. The net result at the end of the cycle is the difference between the anaerobic production and aerobic consumption of PHB, (ΔC^{an}-ΔC^{ae}). This small amount (compared to the converted amount per cycle) must be equal to the wasted volumetric PHB concentration according to:

$$\Delta C_{phb}^{an} + \Delta C_{phb}^{aer} = \frac{\Delta V_w}{V-\Delta V_w} \cdot f_{phb}(0) \cdot C_x(0) \qquad (18)$$

In this balance, the net PHB production is very stringently bound to the removed volume ΔV_w, and the PHB content of the cells. From this balance it can be seen that kinetic parameters describing anaerobic and aerobic conversions, derived only from estimates based on measurements over one cycle are not adequate. The reason is that in one cycle it is not necessary that the steady state requirements are satisfied. If e.g. the calculated anaerobic and aerobic PHB conversions are respectively, 8.00 and 7.90 C-mmol/l, at a SRT of 8 days a PHB content of 0.05 C-mol/C-mol is found. If a deviation of 5 % in the fit or in the measurement of the aerobic PHB consumption cycle occurs, due to a small change in the kinetic

parameters, the steady state PHB content, calculated according to equation 18 will increase to 0.27 C-mol/C-mol: a 5 times higher content than measured. In the estimation of the kinetic parameters, it is therefore essential to take the steady state restictions (equation 18) into account. Similar sensitivities and restrictions apply to the glycogen content of the cells as well. These steady state restrictions were used in the parameter estimation procedure, yielding more accurate values than reported previously in appendix III.

Conclusions

In the biological P-removal process in a sequencing batch reactor, the biomass concentration is determined by the balance between the sludge removal rate at the end of an anaerobic/aerobic cycle and the biomass production during the aerobic phase. The biomass production during the aerobic phase is dependent on the PHB content of the cells, which is primarily determined by the ratio between acetate addition and biomass concentration present in the reactor, the specific acetate load. A high load will lead to a high PHB content of the cells and therefore to a high growth rate. The relation between the sludge removal rate and the biomass concentration also affects the fractions of polyP and glycogen to a large extent. The presented metabolic model describes the changes in biomass concentration and internal fractions satisfactory over a wide range of SRT and acetate/biomass loads, with a single set of parameters. Using these parameters the model also describes the dynamic behaviour of the components during the cycle.

At a high acetate/biomass load, the polyP content becomes limiting for the anaerobic acetate uptake. No more acetate can be taken up if the acetate/biomass load is further increased and therefore, at this point the maximal growth rate is reached. The maximal growth rate of the biological P-removing organisms is in the range of 0.04 h^{-1}.

Nomenclature

C	concentration	(mol/l)
r	conversion rate	(mol/l·h)
α	stoichiometry matrix	
q	specific rate	(mol/mol·h)
μ	specific growth rate	(mol/mol·h)
Δ	difference between start and end	
Φ	flow	(mol/l·h)
V	volume	(l)
f	ratio between component and biomass	(mol/mol)
Y	yield	(mol/mol)
t	time of phase, cycle or day	(h)
SRT	sludge retention time	(d)

References

1) Mamais, D., Jenkins, D., 1992. The effect of MCRT and temperature on enhanced biological phosphorus removal. Wat. Sci. Tech., Vol. 26, No. 5-6, 955-965.
2) Mino, T., Arun, V., Tsuzuki, Y., Matsuo, T. 1987. Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process. Biological Phosphate removal from Wastewaters (Advances in Water Pollution Control 4, Ed. R. Ramadori, Pergamon Press, Oxford, 27-38.
3) Roels J.A., 1983. Energetics and kinetics in biotechnology, Elsevier Biomedical Press.
4) Shao, Y.J., Wada, F., Abkian, V., Crosse, J., Horenstein, B. and Jenkins, D., 1992. Effects of MCRT on enhanced biological phosphorus removal. Wat. Sci. Tech. Vol. 26, No 5-6, 967-976.
5) Smolders G.J.F., Meij, J. van der, Loosdrecht, van, M.C.M, Heijnen, J.J. 1994a. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. Biotech. & Bioeng, Vol. 42, 461-470.
6) Smolders G.J.F., Meij, J van der, Loosdrecht, M.C.M. van, Heijnen, J.J. 1994b. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotech. & Bioeng, 44, 837-848.
7) Smolders G.J.F., Loosdrecht, M.C.M. van, Heijnen, J.J. 1994c. A structured metabolic model for the anaerobic and aerobic stoichiometry of the biological phosphorus removal process. Biotech. & Bioeng, accepted.
8) Wentzel, M.C., Ekama, G.A., Loewenthal, R.E., Dold, P.L., Marais, G.v.R. 1989. Enhanced polyphosphate organism cultures in activated sludge. Part II: Experimental behaviour. Water SA Vol 15., No. 2, 71-88.
9) Wentzel, M.C., Lötter, L.H., Loewenthal, R.E, Marais, G.v.R. 1986. Metabolic behaviour of *Acinetobacter spp.* in enhanced biological phosphorus removal - a biochemical model, Water SA Vol. 12, No 4, 209-224.

Appendix V

Validation of the metabolic model: start-up dynamics

A metabolic model of the biological phosphorus removal process has been developed and validated previously for the complex conversions during the process under anaerobic and aerobic conditions at different growth rates in sequencing batch reactors in steady state. For additional validation of the metabolic model, the model was applied to dynamic conditions which occur during the start-up phase of biological P-removal in the presence and absence of non-polyP heterotrophic micro-organisms. In a laboratory scale sequencing batch reactor, experiments were performed to examine the enrichment of the population with polyP organisms during the start-up and the subsequent shift from non-polyP, heterotrophic organisms to polyP organisms in the sludge. The effect of different influent loading patterns for acetate and phosphate was studied. In these experiments the maximal growth rate of the polyP organisms and the behaviour of the internal storage compounds could be determined. The metabolic model was capable to describe the experimental results, with nearly the same set of kinetic and stoichiometric parameters as obtained during steady state conditions.

Introduction

Biological phosphorus removal in waste water treatment systems is accomplished by the introduction of an anaerobic phase in the system ahead of the aerobic phase and recycling of sludge through the anaerobic and aerobic phase. During the anaerobic phase, lower fatty acids are converted to PHB and PHV while energy is supplied by polyphosphate and glycogen conversion.[6] The anaerobic uptake of the lower fatty acids by the polyP organisms accomplishes, that in the aerobic phase no lower fatty acids are left. The polyP organisms use the stored PHB as internal substrate during aerobic conditions for biomass formation, while other aerobic organisms are lacking substrate and consequently do not produce biomass. Due to this competition mechanism, polyP organisms are selectively enriched and a situation is obtained were polyP organisms make up the main part of the population present in the reactor.

When a non P-removing process is transformed to a P-removing process by introduction of an anaerobic phase and inoculum of a limited number of P-organisms in the system, only a small part of the lower fatty acids added with the influent can be taken up during anaerobic conditions. Consequently, the main part of the substrate will become available in the aerobic phase which will provoke growth of aerobic heterotrophic organisms. However, due to growth of the polyP organisms, the amount of acetate removed anaerobically will increase in time and the aerobic available acetate will decrease. This results in a decreased growth of the aerobic heterotrophs which are finally replaced by the polyP organisms, if all substrate is consumed anaerobically. The observed biomass concentration in the reactor during a start-up is therefore a mixture of heterotrophic and polyP organisms. The measurement and interpretation of PHB and glycogen contents of the polyP organisms is for this reason not possible. This phenomenon hinders the proper study of the growth dynamics of polyP organisms during a start-up.

Aim

A metabolic model of the biological phosphorus removal process has been developed previously.[6,7] The model was validated for the complex conversions of the process during anaerobic and aerobic conditions at different growth rates in steady state SBR's.[8,9] For further

validation in the present research, the metabolic model was applied to dynamic conditions during the start-up of the biological P-removal with the same stoichiometric and kinetic parameters that was established previously, except for the specific acetate uptake rate which appeared to be lower. A good indication of the adequateness of a model to describe a system is that the parameters are invariant [3] to the process conditions. If the model is not adequate, different parameter values will be required to compensate for model insufficiencies. The aim was to study the capacity of the model to predict the growth rate of the polyP organisms during a start-up and, consequently, the shift in population from heterotrophic organisms to polyP organisms. The model had to predict the growth rate of the polyP organisms and the behaviour of the internal storage compounds during the start-up. Two situations were studied: (1) the replacement of a heterotrophic population by a polyP population and (2) the effect of different loading patterns of acetate and phosphate on the growth of polyP organisms and heterotrophs.

Experimental design

A sequencing batch reactor (SBR) was used to obtain an enriched biological phosphorus removing population. The reactor was operated with a cycle time of 6 hours consisting of an anaerobic (2.25 h), aerobic (2.25 h) and settling period (1.5 h). One litre medium was fed at the start of the anaerobic phase and effluent was removed at the end of the settling period. Four experiments were performed which differed in the carbon and phosphate loading pattern during the anaerobic phase and the ratio of heterotrophic biomass to polyP biomass present at the start of the experiment (see figure 1 and table II).

I) Controlled acetate feed with aerobic heterotrophic biomass present

The aim of this experiment was to study the growth of the polyP organisms with acetate present during the anaerobic phase only and the interaction with heterotrophic biomass, which did not grow. Hereto, at the start of the experiment a small inoculum of an enriched polyP culture was brought into a sequencing batch reactor in which an aerobic heterotrophic bacterial population was present. From the start, the aerobic heterotrophic organisms could not grow, due to the absence of acetate in the aerobic phase. This was accomplished by feeding acetate with a pH controller, which dosed acetate during the anaerobic phase triggered by the acetate uptake of the polyP organisms. In this way, a high acetate concentration during the anaerobic phase was omitted and it was prevented that acetate became available in the aerobic phase which would lead to growth of the heterotrophic organisms. One litre influent

was added batchwise during each cycle containing the normal phosphate concentration and only a small amount of acetate to trigger the initial uptake by the polyP organisms. The phosphate load per cycle applied to the system was therefore constant in contrast to the carbon/phosphate ratio which increased during the experiment due to increasing acetate feeding, caused by the gradually increased number of polyP bacteria.

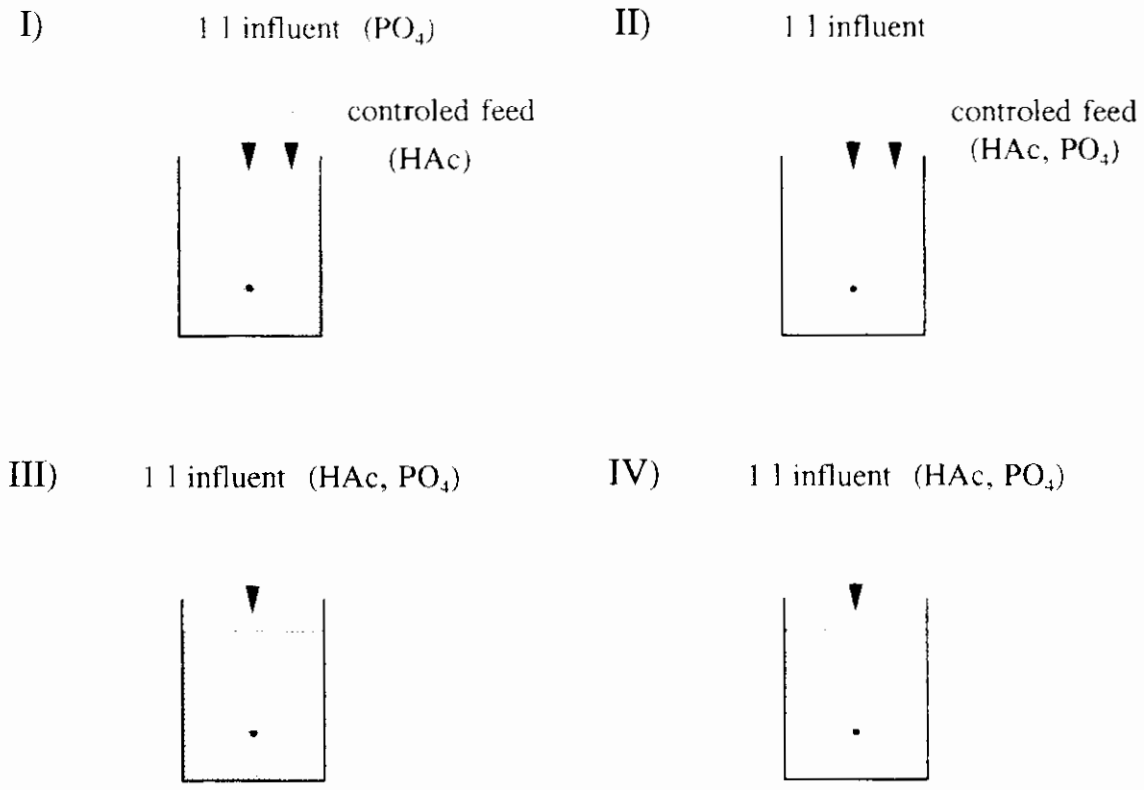


Figure 1 *Schematic presentation of the variation of the acetate and phosphate feed and presence of heterotrophic organisms in the experiments. Influent was batch wise added at the start of the cycle, the controlled feed during the entire anaerobic phase. Four experiments were performed: I) a start-up with heterotrophic organisms present at the start and a controlled HAc-feed, II) no heterotrophic organisms present at the start, controlled HAc- and P-feed, III) heterotrophic organisms present and HAc and P in the influent, IV) No heterotrophic organisms and HAc and P in the influent feed.*

II) Controlled acetate and phosphate feed, no aerobic heterotrophic biomass present

In this experiment (fig. 1) the growth of polyP organisms in the absence of heterotrophs was studied with both carbon and phosphate fed during the anaerobic phase in a constant carbon/-phosphate ratio. A small inoculum of an enriched polyP culture was brought into a SBR in which no other biomass was present at the start. Each cycle, the normal influent volume, containing neither acetate nor phosphate, was added to the SBR. The acetate and phosphate feed were controlled by the pH controller with the acetate/phosphate ratio normally present in the influent. This was done to achieve that phosphate was completely removed during the aerobic phase right from the start of the experiment. The aim was to investigate the effect of low phosphate concentrations during the end of the aerobic phase on the development of the polyP organisms. The specific activity of the phosphate uptake system was reported to be induced at residual phosphate concentrations below the detection limit by *Acinetobacter* strain 210A.[2] It was therefore hypothesized that low phosphate concentrations during aerobic conditions would lead to a higher P-uptake capacity and therewith a faster start-up.

III) Full influent feed with heterotrophic biomass present

In this experiment, the enrichment of polyP organisms was compared with aerobic heterotrophic organisms, while both populations were allowed to grow. This was achieved by adding a small inoculum of polyP organisms to a SBR with aerobic heterotrophic acetate oxidizing biomass present. The full influent volume containing acetate and phosphate was added at the start of the cycle. Because of their limited amount, the polyP organisms could not take up all the acetate during the anaerobic phase leading to large amounts of acetate present in the aerobic phase, allowing growth of aerobic heterotrophic organisms. Due to increased growth of polyP organisms it can be expected that during the start-up the amount of anaerobically consumed acetate increases, which leads to less aerobically consumed acetate.

IV Full in lu nt d with no h t rotrophic biomass pr s nt

A small amount of polyP organisms was present at the start of this experiment. No heterotrophic organisms were present at the start, and the full influent volume containing acetate and phosphate was added to each cycle from the start. The aim of this experiment was to examine the effect of high acetate and phosphate loading rates on the growth of polyP organisms.

Materials and methods

HAc feed controller

Growth of acetate oxidizing heterotrophic organisms in the reactor has been prevented by avoiding the presence of substrate in the aerobic phase. This can be achieved if the acetate feed in the anaerobic phase is adjusted exactly to the acetate uptake and storage capacity into PHB of the polyP organisms during the anaerobic phase. If all the acetate added in the anaerobic phase is taken up, the acetate concentration entering the aerobic phase will be zero. In order to achieve a good balance between acetate addition and uptake the acetate feed was controlled, using the change in pH which occurs during the uptake of acetate by the polyP organisms. At pH 7, most of the acetate is present in dissociated form, while it is consumed by the organisms as acetic acid. The uptake of acetate will therefore lead to an increase of the pH. Using a mixture of acetate/acetic acid as the acid added by a pH controller, the addition of acetate will proceed as long as the polyP organisms take up acetate. When the polyP organisms can no longer take up acetate, the pH will no longer increase and the acetate feed is stopped. In this way, the addition of acetate is elegantly coupled to the demand, and introduction of acetate to the aerobic phase is prevented.

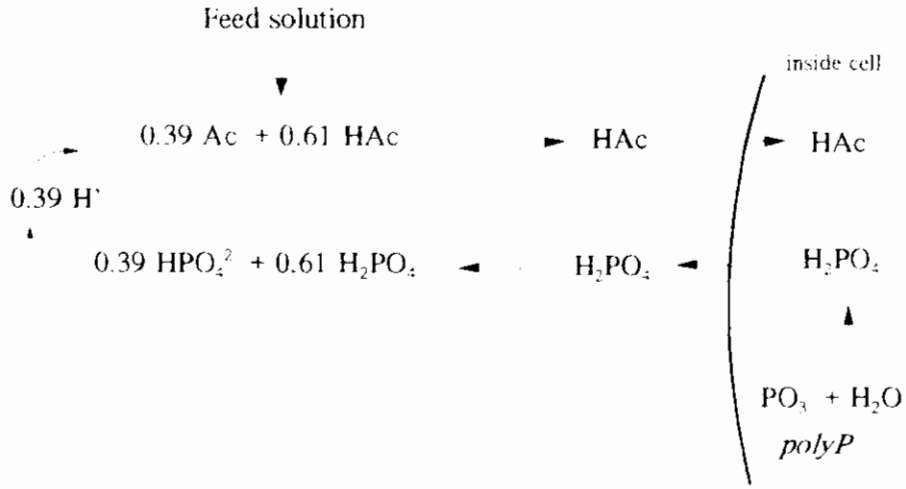


Figure 2 *Required mixture of acetate and acetic acid in the feed of the reactor. During the uptake of 1 molecule acetate (2 carbons, HAc), 1 mol polyP is hydrolysed and consequently 1 mol phosphate ($H_2PO_4^-$) is released. In the subsequent dissociation of phosphate at pH 7.0, 0.39 mol protons are produced. To keep the pH at pH 7, addition of an acetate feed in the ratio 0.39 Ac⁻ to 0.61 HAc is required.*

The required pH in the acetate feed (or the ratio of acetate/acetic acid present) follows from the pH change due to the concomitantly occurring P-release. During the uptake of acetate, phosphate is released from the cells in a ratio of 0.5 P-mol/C-mol acetate at pH 7.0.[6] At this pH, the phosphate will dissociate outside the cells into 0.39 mol HPO_4^{2-} and 0.61 mol $H_2PO_4^-$, see figure 2. Per molecule acetate (2 carbons) taken up, 1 mol phosphate and 0.39 mol H^+ will therefore be released. To keep the pH at 7, the ratio between acetate and acetic acid will have to be 0.39 Ac^- and 0.61 HAc. This is obtained at pH 4.6

SBR

A laboratory fermenter with a volume of 2 l, at a temperature of 20 °C and pH 7.0 was operated as a sequencing batch reactor with a cycle time of 6 hours consisting of an anaerobic (2.25 h), aerobic (2.25 h) and settling period (1.5 h). One litre medium was fed at the start of the anaerobic phase and effluent was removed at the end of the settling period, resulting in a hydraulic retention time of 12 h. At the end of the aerobic phase 62 ml mixed liquor was removed to establish a SRT of 8 days. More details about the SBR were described previously.[7,8] As an inoculum, biological P-removing sludge from the end of the aerobic phase was taken from an acetate fed SBR in steady state at a sludge retention time of 8 days.[7,8] If heterotrophic biomass was required in the reactor at the start, the influent was not supplied at the start of the anaerobic phase but at the start of the aerobic phase allowing growth of heterotrophs. At the start of the polyP startup experiment, when the inoculum was added to the heterotrophic biomass, the influent was again supplied at the start of the anaerobic phase.

Media & analysis

For Experiment III and IV sterilized synthetic medium was used, as described previously,[7,8] containing per litre: 0.85 g $NaAc.3H_2O$ (400 mgCOD/l) as carbon source, 107 mg NH_4Cl (28 mgN/l) and 75.5 mg $NaH_2PO_4.2H_2O$ (15 mgP/l). For Experiment I, the same medium was used without the acetate and in experiment II no acetate and phosphate were present in the medium. The acetate feed for the pH controller contained 0.19 C-mol acetate/l, and the pH was adjusted to pH 4.6. The phosphate acetate ratio was 0.039 P-mol/C-mol acetate in the feed of experiment III. Analysis were performed as described previously.[6,7]

Metabolic model

A metabolic model, which was developed previously,[6,7,8,9] was used to calculate the conversions during the start-up. The metabolic model was based on the participation of glycogen in the metabolism as proposed by Mino.[1,5] For the model calculations, the

stoichiometry was used as described in table I in appendix IV. The same set of kinetic parameters was used for all experiments, except for the specific acetate uptake rate which appeared to be 0.3 instead of 0.4 C-mol/C-mol·h. This set is shown in table I. This set of parameters was validated previously in experiments with steady state sequencing batch reactors, fed with acetate at SRT values between 5 and 20 days.[8,9] The acetate feed patterns during the experiments were measured, fitted and used as influent feed pattern of the model.

***Table I** Kinetics of the anaerobic and aerobic phase of the biological P-removal process*

Anaerobic			parameter	value	unit
q_s	Acetate uptake	$q_s^{max} \cdot \frac{C_s}{C_s + K_s}$	q_s^{max} K_s	0.3 1	C-mol/C-mol.h C-mmol/l
m_{an}	Maintenance		m_{an}	$4 \cdot 10^{-2}$	P-mol/C-mol.h
Aerobic					
μ	Biomass synthesis	$k_x \cdot f_{phb}$	k_x	0.14	C-mol/C-mol.h
q_{pp}	Phosphate uptake	$k_{pp} \cdot \left(\frac{C_p}{C_p + K_p} \right) \cdot \left(1 - \frac{f_{pp}}{f_{pp}^{max}} \right) \cdot f_{phb}^{0.33}$	k_{pp} K_{pp} f_{pp}^{max}	0.2 0.1 0.3	P-mol/C-mol.h P-mmol/l P-mol/C-mol
q_{gly}	Glycogen formation	$k_{gly} \cdot (K_{gly} - \Delta f_{phb} - f_{gly})$	k_{gly} K_{gly}	0.8 1.3	C-mol/C-mol.h C-mol/C-mol
m_{aer}	Maintenance		m_{aer}	$4 \cdot 10^{-2}$	C-mol/C-mol.h

Growth of heterotrophic organisms took only place in experiment III and IV. This was due to the small amount of polyP biomass, not capable of consuming all the acetate provided with the influent, leading to the presence of acetate in the aerobic phase. The growth of the heterotrophic organisms in each cycle was calculated in the model based on the acetate concentration present at the end of the anaerobic phase. It was assumed that all the acetate was completely utilized by the heterotrophic organisms. The growth could therefore be calculated needing an experimentally obtained acetate yield for these organisms of 0.38 C-mol/C-mol acetate only. Although acetate can also be stored as PHB by polyP organisms under aerobic conditions,[10] this was not accounted for in the model.

Results

Heterotrophic growth of non-polyP organisms

During experiment I and III, aerobic acetate oxidizing, heterotrophic, non-polyP biomass was present at the start of the experiments. The heterotrophic biomass was cultivated by addition of the influent volume at the start of the aerobic phase. In figure 3 the development in biomass concentration is shown. During the last 10 days of this experiment the average biomass concentration was ≈ 1.9 g/l VSS (2.1 g/l MLSS), while the SBR was operated at a SRT of 8 days with an acetate load of 6 C-mmol/l·cycle. The heterotrophic biomass yield was calculated to be 0.38 C-mol/C-mol acetate (0.43 gCOD/gCOD-HAc). During the aerobic phase, the average amount of phosphate consumed was 0.08 P-mmol/l and the acetate was consumed within 15 min. Before the polyP organisms were inoculated, the heterotrophic organisms present in the SBR were exposed to one cycle in which the acetate was provided at the start of the anaerobic phase, to measure the anaerobic acetate uptake and P-release capacity of the organisms. Only $15 \cdot 10^{-3}$ P-mmol/l (≈ 0.5 mgP/l) phosphorus was released during this experiment, indicating that no significant number of polyP organisms were present.

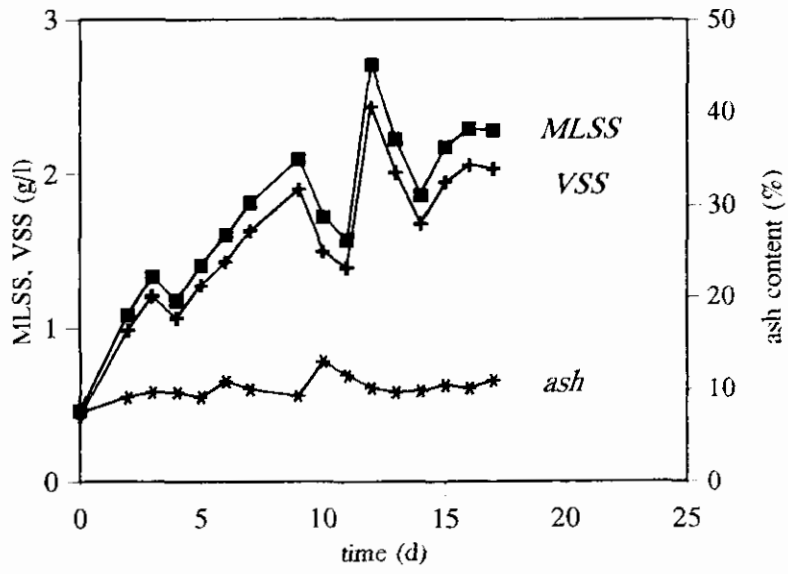


Figure 3 Start-up of a sequencing batch reactor fed with acetate in the aerobic phase.

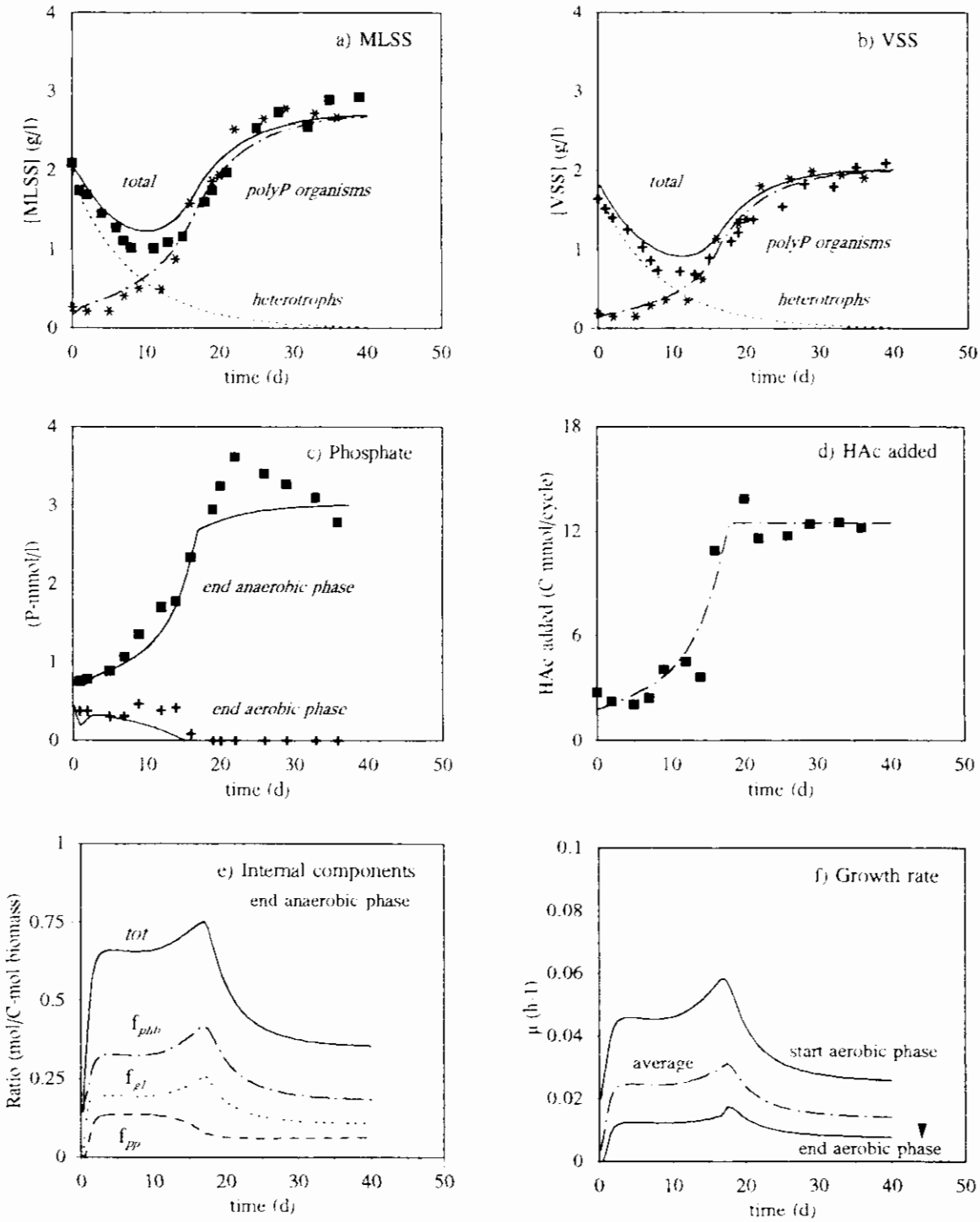


Fig. 4 *Results of experiment I: heterotrophic biomass present and controlled acetate feed. a) Total MLSS (■) and polyP biomass concentration (*); b) Total VSS (□) and polyP biomass concentration (*); c) Phosphate concentration end anaerobic (■) and aerobic phase (□); d) Acetate addition during the start-up; e) Internal compounds to active biomass ratio at the end of the anaerobic phase; f) Growth rate at the start and end of the aerobic phase; and the average biomass production rate during the start-up.*

Experiment I

In Experiment I the acetate flow to the system was controlled by means of the pH controller, while the normal phosphate load at the start of the cycle was provided. The acetate addition during the start-up is shown in figure 4d. The initial feed pattern during the experiment appeared to be linear during the anaerobic phase. 18 days after the start of the experiment the full influent amount of acetate was added. During the first days the pH of the acid mixture of the pH controller was pH 4, because the decrease of the pH due to the P-release was not taken into account and consequently the acetate addition was too small. From day 6 on the pH of the feed was increased to pH 4.6, to obtain the correct acetate/acetic acid mixture. To be able to calculate the conversions during this experiment a continuous acetate addition curve was fitted through the points (fig. 4d). This influent acetate feed profile was used to model the experiments of which the results are shown in figure 4a-4g.

At the start of the experiment, 2.1 g/l MLSS acetate oxidizing heterotrophic biomass was present in the reactor. After the inoculum of 0.19 g/l polyP organisms was added to the reactor, in total 2.3 g/l MLSS (2.0 g/l VSS) biomass was present in the reactor at the start of the experiment. The MLSS and VSS concentrations during the experiment are shown in figure 4a and 4b respectively. From the first day on two processes take place in the system: the wash out of non-growing heterotrophic organisms and the growth of polyP organisms. The wash out of the heterotrophic organisms causes a decrease in MLSS and VSS concentrations until day 12, since the contribution of the growth of polyP organisms is only small during this phase. From day 12 the biomass produced by the polyP organisms is higher than the wash out of organisms from the system and the MLSS and VSS concentrations are increasing. The biomass concentration of the polyP organisms during the start-up was calculated from the ammonium consumption measured during several cycles. The increase in polyP biomass and decrease of heterotrophic organisms was calculated with the model, assuming that no growth of heterotrophic organisms could take place because acetate did not enter the aerobic phase. Therefore, the decrease in biomass concentration of the heterotrophic organisms was calculated considering only the wash out of the biomass. The

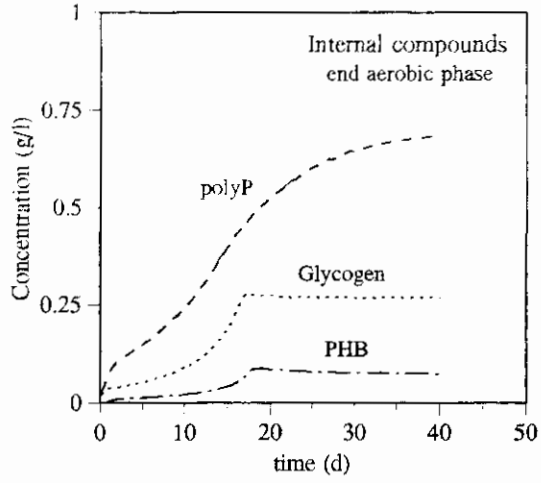


Fig. 4g *Concentrations of the internal components during the start up*

predicted total biomass is the sum of polyP and heterotrophic organisms. The model calculations are in good agreement with the measurements.

Figure 4c shows the phosphate release at the end of the anaerobic phase. After 16 days, all the phosphate in the aerobic phase can be taken up, and after 17 days the maximal release in the anaerobic phase is reached. Again, there is good agreement between the model calculations and the measurements.

The calculated fractions for PHB, glycogen and polyP of the polyP organisms are shown in figure 4e. From this figure, it appears that during the initial phase (0 - 17 days) the ratio between biomass and total storage compounds is 0.75 mol/C-mol active biomass. On a dry weight basis, the polyP biomass is then composed of storage products for 50%. From this figure it can be seen that none of the storage compounds is limiting during the start-up phase. This indicates that the pH controlled influent feed was not properly adjusted to the maximal uptake capacity of the polyP organisms. In other words, the organisms were capable to a higher acetate uptake than provided with the feed.

Figure 4f shows the average growth rate over the aerobic phase as well as the growth rate at the start and end of the aerobic phase during the start-up. The growth rate is coupled to the PHB content and decreases during the aerobic phase due to the consumption of PHB.[9] The highest rate is reached when the PHB content is the highest, after 17 days. The highest average growth rate in the system, defined as the biomass production during the aerobic phase per unit active biomass per length of the aerobic phase is 0.03 h^{-1}. This growth rate is two times lower than the real growth rate (0.06 h^{-1}) of the organisms developed at the start of the aerobic phase. Figure 4g shows the calculated reactor volumetric accumulation of the compounds polyP, PHB and glycogen during the experiment. After the acetate added per cycle has become constant (day 18, figure 4d), the volumetric concentrations of PHB and glycogen stabilize to a constant level, due to the anaerobic stoichiometry. The polyP concentration continues to accumulate, until the amount of phosphate removed with the waste biomass flow equalizes the phosphate added with the influent.

Experiment II

In Experiment II the acetate feed was controlled in the same way as in experiment I, while phosphate was now provided also with the pH controller. Phosphate was present in the acetate feed in a ratio of 26 C-mol/P-mol as used in previous described work.[6,7,8] The realised acetate addition is shown in figure 5d. Because the acetate feed to the system was not fixed, the acetate feed shown in figure 5d was fitted to get a continuous function. This was used to provide the acetate feed profile for the model calculations in this experiment.

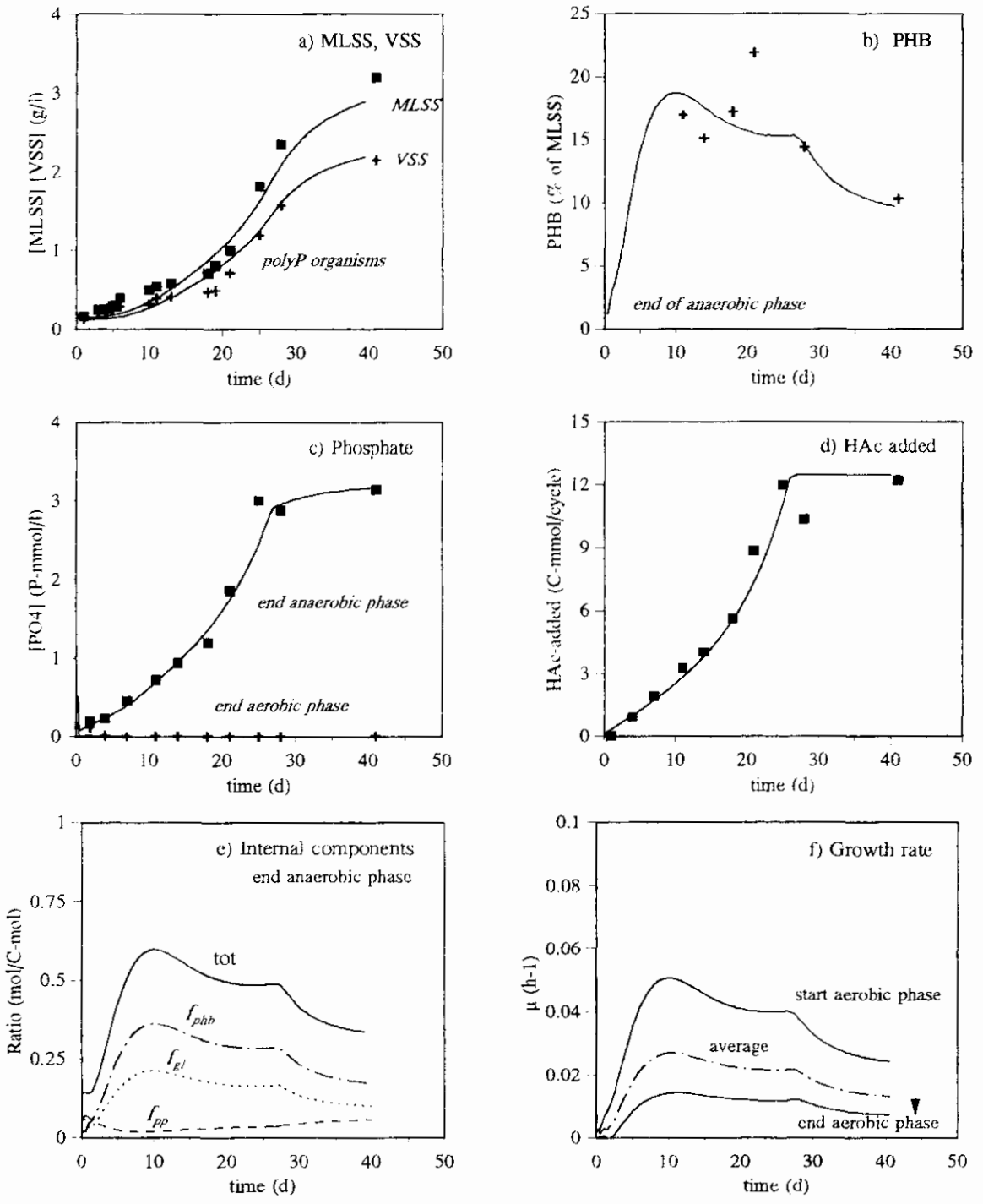


Fig. 5 *Results of experiment II: no biomass present and with a controlled acetate and phosphate feed. a) MLSS (■) and VSS (+) concentrations; b) Percentage PHB of MLSS; c) Phosphate concentration end anaerobic (■) and aerobic phase (+); d) Acetate addition during the start-up; e) Internal compounds to active biomass ratio at the end of the anaerobic phase; f) Growth rate at the start and end of the aerobic phase and the average biomass production rate during the start-up.*

The biomass concentration of the polyP organisms could be measured directly due to the absence of heterotrophic biomass, and is shown in figure 5a. The model and measurements show a good agreement. Figure 5b shows the calculated PHB accumulation as a percentage of the MLSS at the end of the anaerobic phase. Figure 5c shows the phosphate release at the end of the anaerobic phase. After 25 days the maximal release in the anaerobic phase is reached. Clearly a good agreement between the model calculations and the measurements exists. In figure 5e the calculated fractions of PHB, glycogen and polyP in the biomass during the experiment are shown. It is clear that the polyP content is low compared to experiment I, due to the much lower phosphate addition rate. It is difficult to conclude which factor has determined the rate in this experiment. The calculations of the polyP content in figure 5e show that the polyP content is never zero at the end of the anaerobic phase, therefore the acetate provided with the pH controller could have been higher, by an increased pH in the acid feed of the pH controller. The growth rate at the start and end of the aerobic phase and the average growth rate during the experiment are shown in figure 5f and they are slightly lower than in experiment I.

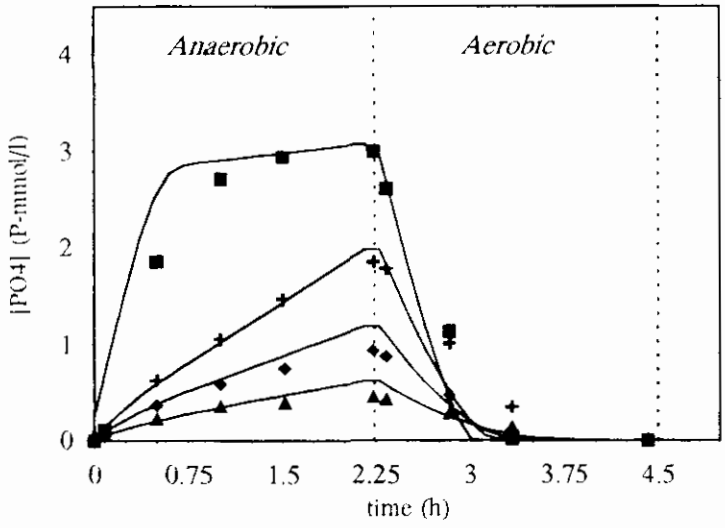


Figure 5g *Phosphate release and uptake during the cycle.*

The development in dynamics in phosphate release and uptake during the start-up in experiment II is shown for each week in figure 5g. The predicted development of the dynamics in phosphate release and uptake is in good agreement with the measurements. From the fact that the P-uptake/release are modelled so well, it appears that the enzymes of the P-removing system are constitutive. There is no effect of high or low P-concentration in last part of the aerobic phase.

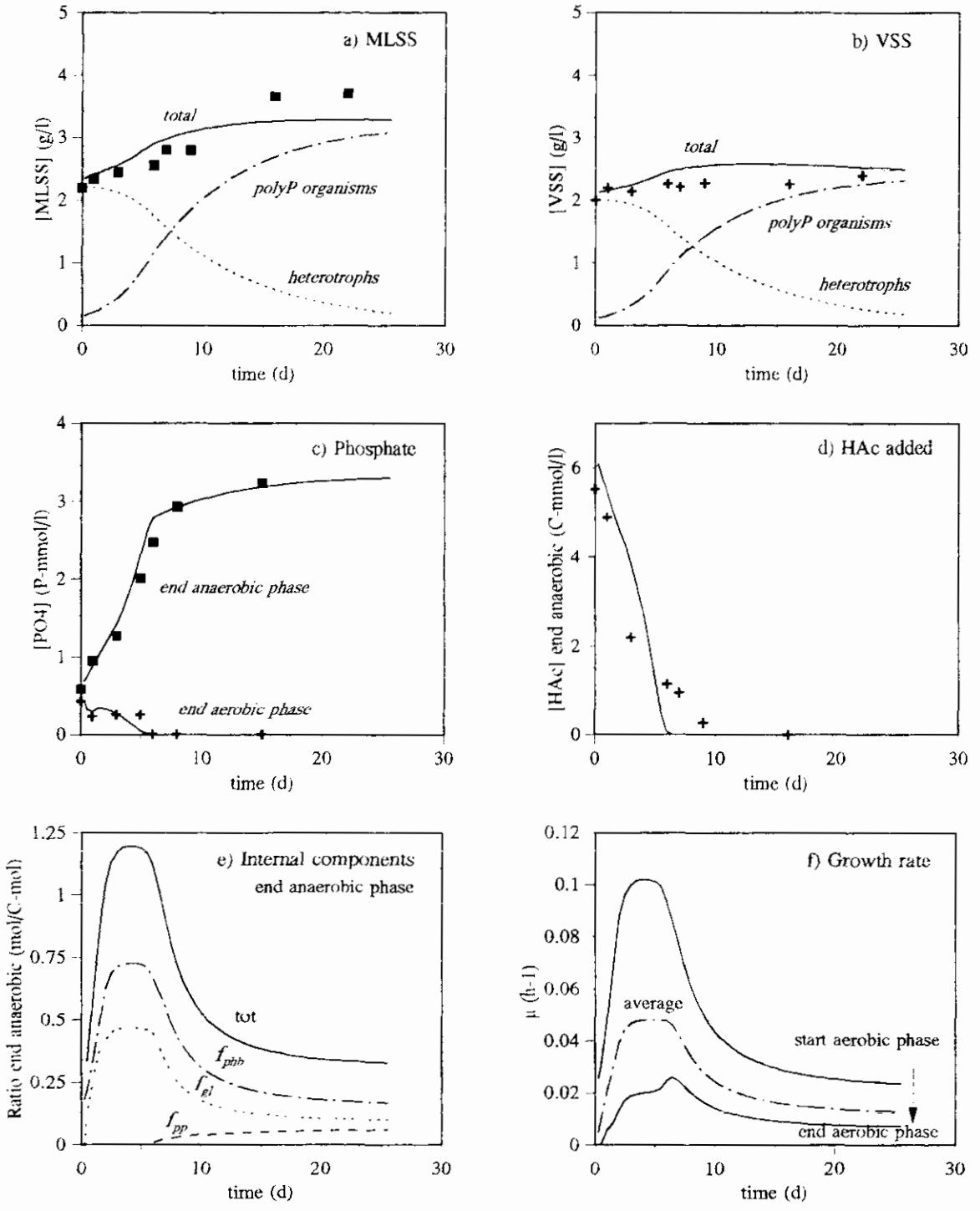


Fig. 6 *Results of experiment III: biomass present at the start of the experiment and with full influent feed. a) Total MLSS concentration (■); b) Total VSS concentration (+); c) Phosphate concentration end anaerobic (■) and aerobic phase (+); d) Acetate concentration at the end of the anaerobic phase; e) Internal compounds to active biomass ratio at the end of the anaerobic phase; f) Growth rate at the start and end of the aerobic phase and the average biomass production rate during the start-up.*

Experiment III

In Experiment III, a small inoculum of 0.19 g/l polyP organisms was added to the reactor in which now 2.0 gVSS/l heterotrophic organisms were present. The full amount of acetate and phosphate was added at the start of each cycle. At the beginning in this experiment, not all the acetate could be consumed by the polyP organisms, leading to the presence of acetate in the aerobic phase. Due to the presence of heterotrophic organisms, the acetate entering the aerobic phase was completely taken up within 15 minutes. Due to the change in population from non-polyP to polyP biomass, the MLSS concentration (fig. 6a) increased from 2.1 g/l to 3.5 g/l, while the VSS concentration during this experiment increased from 2.0 to 2.2 g/l (fig. 6b).

The increase in P-release in the anaerobic phase, figure 6c, is very fast and in 8 days full biological P-removal is obtained. Again good agreement between model and experiment is noted. Figure 6d shows the measured acetate concentration at the end of the anaerobic phase. Initially, the amount of acetate taken up during the anaerobic phase appears to be faster then predicted by the model, while after 7 days the consumption is lower than predicted. A clear effect of the full acetate load on the reactor in this experiment compared to the previous experiment is the higher PHB fraction of the cells, 0.7 C-mol/C-mol active biomass, resulting also in a much higher average growth rate during the cycle, 0.047 h^{-1} (fig. 6f), compared to the previous experiments. The highest growth rate developed at the start of the aerobic phase is in this experiment: 0.1 (h^{-1}). This growth rate shows that polyP organisms are not slow growing organisms but have the capacity to grow rather fast. Due to the kinetic coupling of the growth rate to the PHB content and the fast decrease in PHB content as a result of consumption for growth, a high growth rate will only occur during a short time.

From figure 6f a small increased growth rate at the end of the aerobic phase is observed after day 7. This increase corresponds with the moment were the organisms are capable to consume completely the phosphate present in the aerobic phase, the point were the phosphate concentration at the end of the aerobic phase becomes zero, at day 6 (fig. 6c). An explanation for the higher growth rate is that the PHB which was consumed in the every day increasing P-uptake became constant and after this point is accumulated causing a higher growth rate. The growth rate during the first days of this experiment is limited by the phosphate uptake rate of the organisms. This because during the anaerobic phase the organisms take up acetate till the polyP content becomes zero. However, in the aerobic phase, not all the phosphate is consumed. A higher phosphate uptake would have increased the polyP content of the cells and thus the acetate uptake. The contents of PHB and glycogen increase as long as

polyphosphate is the limiting factor, see figure 6e, and a maximal ratio between total storage materials and biomass of 1.2 mol/C-mol is reached. On dry weight basis, the biomass contains 50 % storage materials.

Experiment IV

In experiment IV, a small inoculum of polyP organisms was added to a SBR without other organism present and the full amount of acetate and phosphate was added in each cycle. The acetate load added with the influent could not be consumed completely by the polyP organisms and came into the aerobic phase were it induced growth of acetate oxidizing, heterotrophic organisms.

Within a few days, the high aerobic concentration of acetate induced growth of fast growing organisms in suspension, the biomass flocs fell apart and the settled biomass concentration decreased during this period. The organisms growing in suspension were removed with the effluent. From the moment the suspended growth disappeared and well flocculated biomass reappeared, the experiment was considered as started (t=0). The problem at this point was that the concentration of polyP organisms in the total biomass present in the reactor was unknown. To be able to model this experiment, the P-release (fig 7c) was used to establish the initial polyP biomass concentration. The initial biomass concentration was chosen in such a way that the fast increase in P-release after day 35, corresponded with the model calculations. A value of $1 \cdot 10^{-3}$ C-mmol/l (= 0.03 mgMLSS/l) resulted. Figure 7a and 7b show the MLSS and VSS concentrations during this experiment.From these figures the change in population in the system can be seen as well as that the MLSS and VSS concentrations are both increased after the change to a polyP population. Comparison of the acetate left at the end of the anaerobic phase during the experiment (figure 7d) shows that there is a deviation between model and measurements between day 17 and 27. Also the predicted anaerobic P-release is much higher. Clearly more acetate is taken up and P is released, than predicted. The reasons remain unclear.

On day 51 a problem with the pH control in the system occurred which affected the organisms: not all the acetate is consumed in the anaerobic phase anymore, and the biomass concentration is decreased.

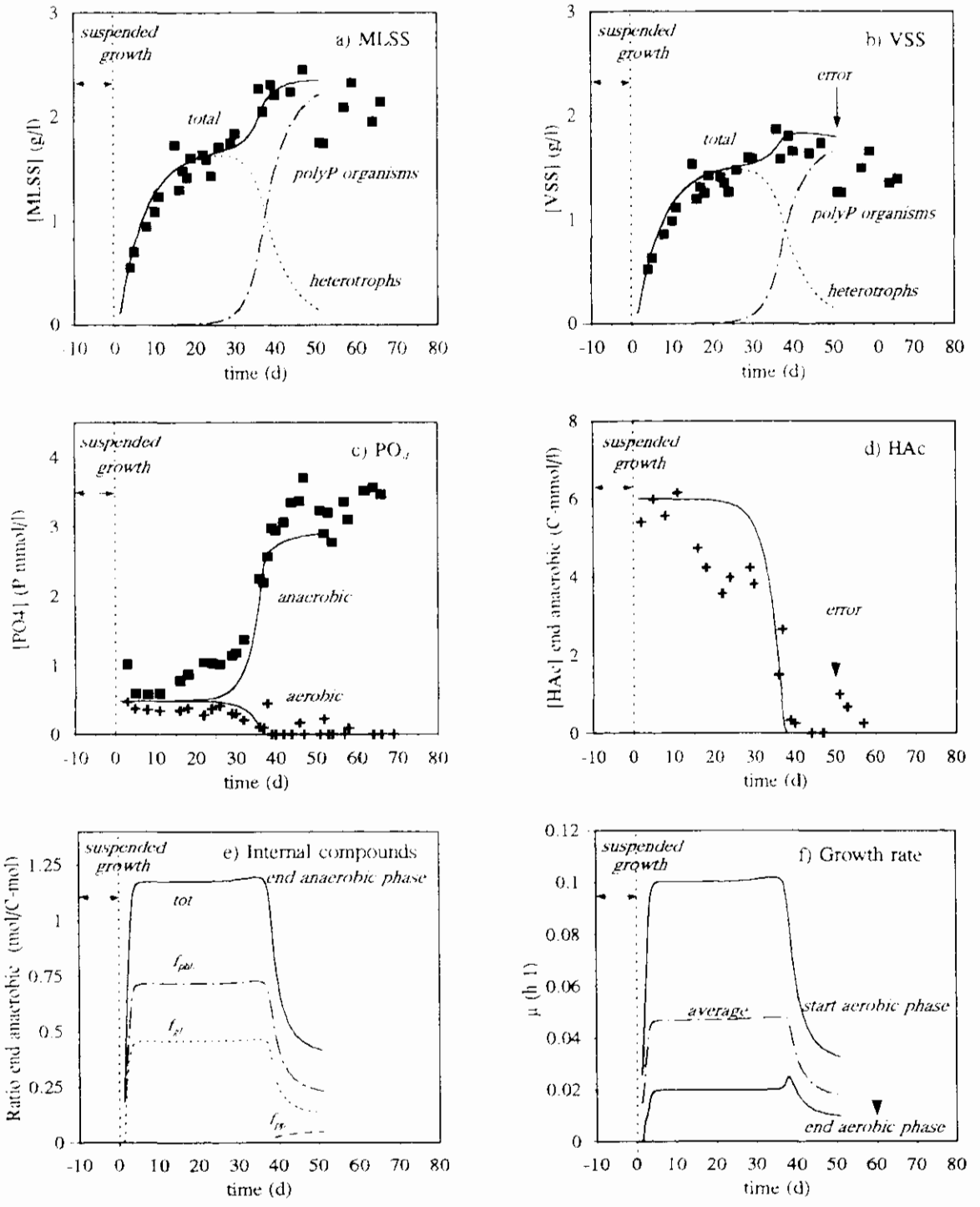


Fig. 7 *Results of experiment IV: a low heterotrophic biomass concentration present at the start of the experiment and with full influent feed. a) Total MLSS concentration (■); b) Total VSS concentration (+); c) Phosphate concentration end anaerobic (■) and aerobic phase (+); d) Acetate concentration at the end of the anaerobic phase; e) Internal compounds to active biomass ratio at the end of the anaerobic phase; f) Growth rate at the start and end of the aerobic phase and the average biomass production rate during the start-up.*

Discussion

Model validation

It has been shown that the metabolic model described the start-up experiments accurately. The MLSS- and VSS biomass concentrations of polyP- and heterotrophic organisms, as well as the phosphate release and -uptake dynamics predicted by the model were in good agreement with the measurements during the experiments. It is emphasized that all experiments described here, could be modelled with a single set of stoichiometric and kinetic parameters. In addition, this set of parameters is the same as the set used in appendix IV, except for the specific acetate uptake rate. The fact that the set of parameters is nearly the same as the set of appendix IV, although established independently under different circumstances, can be seen as an additional indication that the structure of the metabolic model is adequate for the description of the conversions of the biological phosphorus removal process.

Table II *Experimental setup and results*

Exp.	Acetate (mmol/cycle)	Phosphate (mmol/cycle)	Initial polyP biomass (MLSS g/l)	Initial heterotrophic biomass (MLSS g/l)	Maximal average growth rate (h^{-1})	Start-up time (days)
I	fed batch 0 - 6.2	batch 0.24	0.19	2.1	0.031	22
II	fed batch 0 - 6.2	fed batch 0 - 0.24	0.15	0	0.027	26
III	batch 6.2	batch 0.24	0.19	2.0	0.047	7
IV	batch 6.2	batch 0.24	$0.03 \cdot 10^{3}$ *	0	0.047	37

* Estimated from model fit (see text).

Factors effecting the start-up rate

An overview of the experimental results is given in table II. From the experiments described in the results section it appears that there are four factors which can influence the growth rate during a start-up. These are: (1) the anaerobic acetate concentration, (2) the anaerobic polyP content of the cells, (3) the aerobic phosphate uptake rate, (4) presence of other organisms. To increase the rate of a start-up these factors should be optimized:

(1) A higher acetate concentration in the anaerobic phase will increase the uptake rate of acetate, see table I. More acetate will be taken up during each cycle, leading to a higher biomass production. The higher growth rate in experiment III compared to exp. I and II

is probably due to this difference. In experiment I the acetate concentration is always lower than 1.2 C-mmol/l, while in experiment III the initial concentration was ± 6 C-mmol/l acetate.

(2) If the acetate concentration during the anaerobic phase is maximized, the internal storage compounds should be present in stoichiometric amounts. Polyphosphate supplies part of the energy required in the acetate uptake and determines therefore the amount of acetate that can be taken up. During a start-up, when acetate is present in surplus, the acetate uptake will stop when the polyP content of the cells is exhausted. In experiment III and IV the maximal growth rate is restricted by the polyP content of the organisms at the start of the anaerobic phase. The anaerobic polyP content has to be maximized to increase the start-up rate. The polyP content present in the anaerobic phase is a result of the phosphate uptake activity during the aerobic phase.

(3) To maximize the anaerobic polyP content, the phosphate uptake (-rate) in the aerobic phase should be increased. A maximal phosphate uptake during the aerobic phase will supply a maximal amount of polyP for the uptake of acetate in the anaerobic phase. In the operation of the SBR described here, the maximal phosphate uptake rate is reached when at the end of the aerobic phase still phosphate is left in the effluent. This is the case in experiment III and IV: during the first days of the experiment phosphate is still present at the end of the aerobic phase, while the polyP content in the anaerobic phase drops to zero. The uptake rate of acetate could have been higher if there had been more polyP available, which was achieved with a higher phosphate uptake rate. From table I it appears that the phosphate uptake rate is influenced by the phosphate concentration and the PHB content. The growth rate is limited here by the phosphate uptake kinetics. The PHB and glycogen contents increase to values of 0.7 C-mol/C-mol for PHB and 0.5 C-mol/C-mol for glycogen.

(4) The presence of heterotrophic organisms has in general no effect on the growth of polyP organisms. There is however one exception. When there is a high acetate concentration during the anaerobic phase of a start-up, the small number of polyP organisms are not capable of consuming all acetate completely. The remaining acetate will therefore enter the aerobic phase. The presence of non polyP heterotrophic organisms becomes then important. Non-polyP heterotrophs are advantageous because they can consume the acetate quickly. This is important because high acetate concentrations during the aerobic phase induces growth of non-flocculated aerobic heterotrophic organisms growing with a growth rate equal to the HRT. In experiment IV the growth was disturbed by suspended growth for a long time. When the polyP organism finally are growing, the growth rate is

comparable to experiment III.

Maximal growth rate

The growth rate of polyP organisms is determined by their PHB content.[9] The maximal growth rate is therefore not an intrinsic parameter but will depend on the maximal achievable PHB content. PolyP organisms will only develop high growth rates during a short time due to the consumption of PHB as a result of growth. High growth rates will only be obtained during highly dynamic anaerobic/aerobic conditions. The highest initial aerobic growth rate of 0.1 h^{-1} was observed in experiment III. The PHB content that was achieved in this experiment was 0.75 C-mol/C-mol (30% on dry weight basis). A higher PHB content was not achieved in these experiments because the anaerobic production of PHB was limited by the anaerobic polyP content. The polyP content of the organisms is in turn limited by the aerobic phosphate uptake rate. Apparently, for the here operated SBR system the highest obtainable growth rate is 0.1 h^{-1}. The highest average growth rate developed over the aerobic phase is about 0.045 h^{-1}. The growth rates found here are higher then previously found values. In a SBR activated sludge process fed with glucose and polypeptone, the specific growth rate of polyP organisms was estimated to be 0.0014 h^{-1}. [4]

Decreased biomass yield of polyP systems

From experiment III and IV, the biomass yield of heterotrophic and polyP organisms can be compared. The VSS concentration remains more or less constant while the MLSS concentration increases when more polyP organisms grow into the system, which is caused by the accumulation of polyphosphate. This appears to be contradictionary with the statement that the active biomass yield of polyP organisms is decreased due to the P-metabolism.[7] One should realize however that in case of the polyP organisms the carbon storage products are also included in the VSS, and the yield for carbon storage products is much higher then for biomass production. The actual active biomass concentration is not 2.2 gVSS/l but only 1.8 g/l (if 0.2 C-mol/C-mol organic storage products are present). The observed yield for the heterotrophic organisms of 0.38 C-mol/C-mol is higher than the observed active biomass yield on acetate for polyP organisms at a SRT of 8 days: 0.33 C-mol/C-mol.

Conclusions

It has been shown that the enrichment of polyP organisms under different feeding conditions during the start-up of the biological P-removal process is described accurately by the metabolic model, derived originally understeady state conditions, with a single set of parameters. This set of stoichiometric and kinetic parameters was determined previously from steady state SBR's, except for the specific acetate uptake rate which appeared to be slightly lower. The metabolic model is able to describe the dynamics during the anaerobic/aerobic cycle, as a function of the sludge retention time and the start-up dynamics with nearly the same set of parameters.

The highest growth rate of the polyP organisms at the start of the aerobic phase is 0.1 h^{-1}, while the highest average growth rate during the total aerobic phase of a cycle is 0.045 h^{-1}. During the start-up, the growth rate of the polyP organisms is limited by the availability and concentration of acetate. The growth rate is further limited by the polyP content of the cells, which in turn depends on the kinetics of the aerobic phosphate uptake rate. Non-polyP heterotrophic, flocculated organisms present in the system do not disturb the growth of polyP organisms and are advantageous because they consume the surplus of acetate which is not consumed in the anaerobic phase.

References

1) Arun, V., Mino, T., Matsuo, T. 1988. Biological mechanisms of acetate uptake mediated by carbohydrate consumption in excess phosphorus removal systems. Wat. Res. 22, 565-570.
2) Bonting, C.F.C., van Veen, H.W., Taverne, A., Kortstee, G.J.J., Zehnder, A.J.B, 1993. Regulation of polyphosphate metabolism in *Acinetobacter* strain 210A grown in carbon- and phosphate limited continuous cultures. Arch. of Microb., 158, 139-144.
3) Munack, A., 1992. Some improvements in the identification of bioprocesses, In: Modeling and Control of Biotechnical Processes. Eds. Karim M.N. and Stephanopoulos G., Pergamon Press, Oxford. 89-94.
4) Okada, M., Ueno, Y., Lin, C.K. and Murakami, A., 1991. Population dynamics of bacteria for phosphorus removal in sequencing batch reactor activated sludge processes, Journal of JSWE, Vol 14, No. 1, 47-53.
5) Satoh, H., Mino, T., Matsuo, T., 1992. Uptake of organic substrates and accumulation of polyhydroxyalkanoates linked with glycolysis of intracellular carbohydrates under anaerobic conditions in the biological excess phosphate removal processes, Wat. Sci. Tech. Vol 26, No 5-6, 933-942.
6) Smolders, G.J.F., van Loosdrecht, M.C.M., Heijnen, J.J. 1994a. Model of the anaerobic metabolism of the biological phosphorus removal process; stoichiometry and pH influence. Biotech & Bioeng. 43, 461-470.
7) Smolders, G.J.F., van der Meij, J., van Loosdrecht M.C.M., Heijnen, J.J. 1994b. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotech & Bioeng. 44, 837-848.
8) Smolders, G.J.F., van der Meij, J., van Loosdrecht M.C.M., Heijnen, J.J. 1994. A structured metabolic model for the anaerobic and aerobic stoichiometry and kinetics of the biological phosphorus removal process. Biotech. & Bioeng., accepted.
9) Smolders, G.J.F., van der Meij, J., van Loosdrecht M.C.M., Heijnen, J.J. 1994. A metabolic model for the *biological phosphorus removal process; effect of the sludge retention time*. Biotech. & Bioeng., submitted.
10) Wentzel, M.C., Ekama, G.A., Loewenthal, R.E., Dold, P.L. and Marais G.v.R., 1989. Enhanced polyphosphate organim cultures in activated sludge systems. Part II: Experimental behavior, Water SA, Vol 15. No. 2, 71-88.

Appendix VI

IAWQ format for the metabolic model of phosphorus accumulating organisms (PAO):

Stoichiometry Matrix							
Process	S_{O2}	S_A	S_{PO4}	X_{PAO}	X_{PP}	X_{PHA}	X_{GL}
1 Uptake of S_A		-1	0.36		-0.36	1.5	-0.5
2 Aerobic growth	-0.37		-0.013	1		-1.37	
3 Storage of X_{PP}	-0.31		-1		1	-0.31	
4 Storage of X_{GL}	-0.26					-1.26	1
5 Anaerobic maintenance			1		-1		
6 Aerobic maintenance	-1					-1	

Process rate equations	
1 Uptake of S_A	$q_{PHA} \cdot \frac{S_A}{K_A + S_A} \cdot X_{PAO} \cdot \left(\frac{X_{PP}/X_{PAO}}{K_{PP} + X_{PP}/X_{PAO}} \right) \cdot \left(1 - \frac{S_{O2}}{K_{O2} + S_{O2}} \right)$
2 Aerobic growth	$k_X \cdot \left(\frac{X_{PHA}}{X_{PAO}} \right) \cdot X_{PAO} \cdot \left(\frac{S_{O2}}{K_{O2} + S_{O2}} \right)$
3 Storage of X_{PP}	$k_{PP} \cdot \left(\frac{S_p}{K_p + S_p} \right) \cdot \left(1 - \frac{X_{PP}/X_{PAO}}{K_{PP}^{max}} \right) \cdot \left(\frac{X_{PHA}}{X_{PAO}} \right)^{1/3} \cdot X_{PAO} \cdot \left(\frac{S_{O2}}{K_{O2} + S_{O2}} \right)$
4 Storage of X_{GL}	$k_{GL} \cdot \left(K_{GL}^{MAX} \cdot \frac{X_{PHA}^{AN}}{X_{PAO}} \quad \frac{X_{GL}}{X_{PAO}} \right) \cdot X_{PAO} \cdot \left(\frac{S_{O2}}{K_{O2} + S_{O2}} \right)$
5 Anaerobic maintenance	$m_{an} \cdot X_{PAO} \cdot \left(1 - \frac{S_{O2}}{K_{O2} + S_{O2}} \right)$
6 Aerobic maintenance	$m_{aer} \cdot X_{PAO} \cdot \left(\frac{S_{O2}}{K_{O2} + S_{O2}} \right)$

Definition of the soluble and particulate components

Component	definition	units
S_A	Fermentation products, considered to be acetate	gCOD/l
S_{O2}	Dissolved oxygen	g/l
S_{PO4}	Inorganic soluble phosphorus, primarily ortho phosphate	g/l
X_{PAO}	Phosphorus accumulating organisms	gCOD/l
X_{PP}	Polyphosphate	gCOD/l
X_{PHA}	Cell internal stored PHB, PHV	gCOD/l
X_{GL}	Cell internal stored Glycogen	gCOD/l

Definition of values for the kinetic coefficients

Coefficient	value	units
q_{PHA}	0.36	gCOD/gCOD-PAO.h
k_X	0.14	gCOD-PAO/gCOD.h
k_{PP}	0.17	gP/gCOD.h
k_{GL}	0.8	gCOD/gCOD.h
m_{an}	$3.4 \cdot 10^{-2}$	gP/gCOD-PAO.h
m_{aer}	$4.0 \cdot 10^{-2}$	gCOD/gCOD-PAO.h
K_A	32	gCOD/m^3
K_P	3	gP/m^3
K_{PP}^{MAX}	0.34	gPP/gCOD-PAO
K_{GL}^{MAX}	1.2	gCOD/gCOD-PAO
K_{PP} switch	$1 \cdot 10^{-2}$	gPP/gCOD-PAO
K_{O2} switch	$1 \cdot 10^{-3}$	gO$_2$/m^3

Conversions factors

Component		M (g)	COD (g)
Acetate	1 C-mol	30	32
PHB	1 C-mol	21.5	36
Biomass	1 C-mol	26	36
Glycogen	1 C-mol	27	32
Phosphate	1 P-mol	31	0

PUBLIKATIEREEKS "TOEKOMSTIGE GENERATIE RIOOLWATERZUIVERINGSINRICHTINGEN RWZI 2000" [1]

- 'Behandeling van stedelijk afvalwater in de toekomst'
  Een haalbaarheidsonderzoek. I Eindrapport. II Werkrapport
  RIZA, TNO-Maatschappelijke Technologie en Witteveen + Bos Raadgevende ingenieurs B.V.
  Juli 1986

- 'Toekomstige generatie rioolwaterzuiveringsinrichtingen; RWZI 2000'
  Onderzoeksplan
  RIZA, STORA
  Januari 1988

- 'Jaarverslag 1988'
  RIZA, STORA
  Maart 1989

- 'Slibontwatering; een voorstudie'
  TU-Delft, TU-Eindhoven
  RWZI 2000 89-01
  Januari 1989

- 'Knelpunten bij de invoering van defosfatering'
  Witteveen + Bos Raadgevende ingenieurs B.V.
  RWZI 2000 89-02
  April 1989

- 'Selectieve verwijdering van zware metalen uit ruw rioolwater met behulp van een magneetsysteem'
  Smit-Nijmegen, TNO-Maatschappelijke Technologie
  RWZI 2000 89-03
  Oktober 1989

- 'Verwijdering van zware metalen uit zuiveringsslib door electrolyse'
  TNO-Maatschappelijke Technologie
  RWZI 2000 89-04
  Oktober 1989

---

[1] Te bestellen bij:
Hageman Verpakkers B.V., Postbus 281, 2700 AC Zoetermeer
tel. 079-611188 / fax 079-613927

- 'Hydrolyse van zuiveringsslib in combinatie met anaërobe vergisting'
  TNO-Maatschappelijke Technologie
  RWZI 2000 89-05
  Oktober 1989

- 'Het drogen van zuiveringsslib met het Carver-Greenfieldproces'
  TNO-Maatschappelijke Technologie, Witteveen + Bos Raadgevende ingenieurs B.V.
  RWZI 2000 89-06
  December 1989

- 'Natte oxydatie van zuiveringsslib met het Vertech-systeem'
  TNO-Maatschappelijke Technologie, Witteveen + Bos Raadgevende ingenieurs B.V.
  RWZI 2000 89-07
  December 1989

- 'Symposium 'RWZI 2000' d.d. 5 oktober 1989'
  RIZA, STORA
  RWZI 2000 89-08
  December 1989

- 'Jaarverslag 1989'
  RIZA, STORA
  RWZI 2000 90-01
  Maart 1990

- 'AB-systemen; een inventarisatie'
  DHV Raadgevend Ingenieursbureau BV
  RWZI 2000 90-02
  September 1990

- 'Vergisting van aëroob gestabiliseerd slib'
  DHV Raadgevend Ingenieursbureau BV
  RWZI 2000 90-03
  Augustus 1990

- 'Het afleiden van procestechnologische relaties uit bedrijfsgegevens van rwzi's'
  DHV Raadgevend Ingenieursbureau BV
  RWZI 2000 90-04
  December 1990

- 'Automatische regeling van het slibgehalte in beluchtingstanks'
  Adviesbureau BKH
  RWZI 2000 90-05
  September 1990

- 'Verkenning Biodenitro-Biodenipho'
  Witteveen + Bos Raadgevende ingenieurs B.V.
  RWZI 2000 90-06
  Juni 1990

- 'Linpor-sponsjes als dragermateriaal bij de aërobe zuivering van rioolwater'
  TNO-Maatschappelijke Technologie
  RWZI 2000 90-07
  Oktober 1990

- 'Jaarverslag 1990'
  RIZA, STORA
  RWZI 2000 91-01
  Maart 1991

- 'Deep Shaft-systemen; een inventarisatie'
  DHV Raadgevend Ingenieursbureau BV
  RWZI 2000 91-02
  Maart 1991

- 'Perspectives for the utilization of membrane-assisted sludge retention in municipal waste water treatment plants'
  A feasibility study
  RU-Groningen
  RWZI 2000 91-03
  Juni 1991

- 'Jaarverslag 1991'
  RIZA, STOWA
  RWZI 2000 92-01
  Maart 1992

- 'Vergisten van zuiveringsslib; een vergelijking tussen thermofiele en mesofiele slibgisting'
  Haskoning B.V., RIZA, LU-Wageningen, DHV Water B.V.
  RWZI 2000 92-02
  Maart 1992

- 'First Dutch-Japanese workshop on the treatment of municipal waste water'
  8-11 april 1991, Heelsum, The Netherlands. Part I and part II.
  RIZA, STORA, TU-Delft
  RWZI 2000 92-03
  Maart 1992

- 'Biologische fosfaatverwijdering in combinatie met een korrelreactor'
  LU-Wageningen, DHV Water B.V.
  RWZI 2000 92-04
  Augustus 1992

- 'Anaërobe behandeling van stedelijk afvalwater in Nederland'
  Covernota van het uitgevoerde onderzoek 1976 - 1991
  LU-Wageningen, Haskoning B.V.
  RWZI 2000 92-05
  Mei 1992

- 'Vergaande nutriëntenverwijdering op een zeer laagbelaste actief-slibinstallatie'
  Zuiveringsschap Hollandse Eilanden en Waarden, Grontmij N.V.
  RWZI 2000 92-06
  Oktober 1992

- 'Ontwikkeling van een slib-op-drager systeem voor de aërobe zuivering van stedelijk afvalwater'
  Fase II: Onderzoek naar de processtabiliteit en optimalisatie van het zuiveringsrendement.
  TNO-IMW
  RWZI 2000 92-07
  Oktober 1992

- 'Behandeling van stedelijk afvalwater met het schachtreactorsysteem'
  V & P Waste Water Management B.V.
  RWZI 2000 92-08
  Juli 1994

- 'Stikstofverwijdering uit interne stromen op rwzi's'
  DHV Water B.V.
  RWZI 2000 92-09
  December 1992

- 'Jaarverslag 1992'
  RIZA, STOWA
  RWZI 2000 93-01
  April 1993

- 'Onderzoek demonstratie-installaties magnetische defosfatering'
  Envimag B.V.
  RWZI 2000 93-02
  April 1993

- 'Modelvorming en optimalisatie van biologische defosfatering van afvalwater. Microbiële aspekten'
  LU-Wageningen, vakgroep Microbiologie
  RWZI 2000 93-03
  November 1993

- 'Jaarverslag 1993'
  RIZA, STOWA
  RWZI 2000 94-01
  Juli 1994

- 'Fundamentele aspekten van slibontwatering'
  Deel 1: Samenvattend verslag
  Deel 2: Flocculatiemechanismen
  Deel 3: Filtratie-expressie modellering
  Deel 4: Filtratie expressie experimenten
  Deel 5: Slib-water binding
  Deel 6: Karakterisering van slibben
  Deel 7: Ontwikkeling nieuw CST-apparaat
  Deel 8: Congresbijdragen
  TU-Eindhoven, Laboratorium voor Scheidingstechnologie
  RWZI 2000 94-02
  Juli 1994

- 'Fundamenteel onderzoek vermindering slibproduktie'
  VU, werkgroepen Theoretische Biologie en Microbiologie
  RWZI 2000 94-03
  September 1994

- 'Alternatieven voor de slibretentie bij hooggesuspendeerde waterzuiverings-systemen'
  DHV Water BV
  RWZI 2000 94-04
  September 1994

- 'Evaluatie en overzicht van het onderzoekprogramma RWZI 2000'
  RIZA, STOWA
  RWZI 2000 94-05
  December 1994

- 'Aërobe biofilm op gesuspendeerde drager ten behoeve van waterzuivering'
  TU-Delft, vakgroep Bioprocestechnologie
  RWZI 2000 94-06
  December 1994

- 'Het uittesten van de filtratie-expressiecel in de praktijk'
  TU-Eindhoven, Laboratorium voor Scheidingstechnologie
  RWZI 2000 94-07
  December 1994

- 'Modelvorming en optimalisatie van biologische defosfatering van afvalwater: modellering'
  TU-Delft, vakgroep Bioprocestechnologie
  RWZI 2000 94-08
  Januari 1995