STELLINGEN

behorende bij het proefschrift

Modeleren van Biologische Fosfaatverwijdering in Actieve Slib Systemen

Damir Brđanović

9 september 1998, Delft, Nederland

1. Het mag merkwaardig worden genoemd dat de biochemische achtergrond van de biologische defosfatering is uitgezocht door ingenieurs. Waarschijnlijk worden ingenieurs minder gehinderd door traditionele biochemische en microbiële denkwijzen, en was het eenvoudiger nieuwe ecophysiological concepten te formuleren.

Dit proefschrift, hoofdstuk 1.

2. In het kader van afvalwaterzuivering is het onmogelijk, maar ook onnodig, om alle onzekerheden in beschouwing te nemen en een model exact aan de meetdata te fitten. Het doel dient te zijn om de waarnemingen te voorspellen binnen de onbetrouwbaarheid van de metingen zelf.

Dit proefschrift, hoofdstuk 5.

3. Het modelleren van actief slib processen leidt tot een beter begrip van de installatie en kan succesvol worden toegepast om niet alleen de technische processen te verbeteren.
4. Het lijkt erop dat data collectie in praktijk afvalwaterzuiveringen meer wordt bepaald door administratieve dan technische beperkingen.
5. De snelheid van het vernieuwen van afvalwater tekstboeken zou de dynamiek van het nieuws in het veld moeten volgen.
6. Recente ontdekkingen dat bacteriën anders dan *Acinetobacter* verantwoordelijk zijn voor biologische P-eliminatie, en *Nitrobacter* niet verantwoordelijk is voor nitriet oxydatie, betekent meer voor het microbiologisch dan voor het proces t e chOnisch onderzoek.

Blackall L.L., Bond P.L., Burrell P.C., Crocetti G.C. and Keller J. (1988) 'The molecular ecology of sequencing batch reactors', Proceedings: 4th Kollekolle Seminar on Activated Sludge Modelling, Denmark.

7. John Forbes zei vaak "Do not worry...nature balances itself", maar voor hoelang?
8. Het is vreemd hoe weinig we nodig hebben om gelukkig te zijn, het is nog vreemder hoe vaak ook dat weinige ontbreekt we miss.

PROPOSITIONS

belonging to the dissertation

Modeling Biological Phosphorus Removal in Activated Sludge Systems

Damir Brđanović

9th September 1998, Delft, The Netherlands

1. It might be called remarkable that the biochemical models for biological phosphorus removal were developed by engineers. Possibly, the engineers were less hampered by a traditional biochemical and microbial approach and could, therefore, more easily come up with new concepts in microbial ecophysiology.

This dissertation, chapter 1.

2. In the context of wastewater treatment, it is almost impossible, but also unnecessary, to take into account all the uncertainties and to fit the model predictions exactly to the measured data. The aim is to predict the observed data within the inaccuracy of the measurements themselves.

This dissertation, chapter 5.

3. Modeling full-scale wastewater treatment plants brings a better understanding of plant operation and can be successfully applied to improve the control of (more than just) the technological processes.
4. It seems that data gathering in wastewater treatment practice is governed by administrative rather than by technological considerations.
5. The speed of updating wastewater-related textbooks should follow the dynamics of news in the field.
6. Recent discoveries that bacteria other than *Acinetobacter* are responsible for biological phosphorus removal, and that the main nitrite oxidizer in activated sludge is not *Nitrobacter*, but *Nitrospira* (Blackall et al, 1998), will have more impact on the perception of the microbiologist than of the wastewater process engineer.

Blackall L.L., Bond P.L., Burrell P.C., Crocetti G.C. and Keller J. (1988) 'The molecular ecology of sequencing batch reactors', Proceedings: 4th Kolloid Seminar on Activated Sludge Modelling, Denmark.

7. John Forbes used to say "Do not worry....nature balances itself". But, for how long?
8. It is strange how little we need to be happy, and it is even more strange how often just that little we miss.

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MODELING BIOLOGICAL PHOSPHORUS REMOVAL IN ACTIVATED
SLUDGE SYSTEMS

TR3176

Modeling Biological Phosphorus Removal in Activated Sludge Systems

DISSERTATION

Submitted in fulfilment of the requirements of
the Board for Doctorates of Delft University of Technology
and the Academic Board of the International Institute for Infrastructural,
Hydraulic and Environmental Engineering
for the Degree of DOCTOR
to be defended in public on Wednesday, 9th September 1998 at 10:30 h

by

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Cover photograph: Wastewater Treatment Plant Haarlem Waarderpolder, by Damir Brđanović
Cover design: Peter Stroo

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Alice in Wonderland

- *"Would you tell me please, which way I ought to go from here", said Alice.*
- *"That depends a good deal on where you want to get go", said the Cat.*
- *"I don't much care where", said Alice*
- *"Then it doesn't matter which way you go", said the Cat, "if you walk long enough".*

Contents

Nomenclature and Symbols

Summary

Chapter 1	1
Biological Phosphorus Removal Processes	
Chapter 2	25
Temperature Effects on the Physiology of Biological Phosphorus Removal	
Chapter 3	51
Influence of Temperature on Bio-P Removal: Process and Molecular Ecological Studies	
Chapter 4	77
Bioassay for Glycogen Determination in Biological Phosphorus Removal Systems	
Chapter 5	91
Modeling COD, N and P Removal in a Full-Scale WWTP Haarlem Waarderpolder	
Chapter 6	165
Minimal Aerobic Sludge Retention Time in Biological Phosphorus Removal Systems	
Chapter 7	181
Effect of Potassium Limitation on Biological Phosphorus Removal	
Chapter 8	195
Effect of Poly-Phosphate Limitation on the Anaerobic Metabolism of Bio-P Bacteria	
Chapter 9	207
Impact of Excessive Aeration on Biological Phosphorus Removal	
Chapter 10	227
Evaluation and Outlook	

Samenvatting (Summary in Dutch)

Acknowledgments

List of Publications

Curriculum Vitae

Nomenclature and Symbols

The following nomenclature and symbols were used in this dissertation (notation of parameters specifically related to mathematical modeling of activated sludge systems is presented in chapter 5, appendix 2):

Abbreviations

A/O	Anaerobic-Aerobic
A2	Aerobic-Anoxic
ATP	Adenosine Triphosphate
ASM	Activated Sludge Model
BOD	Biological Oxygen Demand
BPR	Biological Phosphorus Removal
COD	Chemical Oxygen Demand
DDGGE	Dry Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
EMP	Emden-Meyerhof Pathway
FID	Flame Ionisation Detector
GAO	Glycogen Accumulating (non poly-P) Organisms (G bacteria)
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
IAWQ	International Association for Water Quality
MLSS	Mixed liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NADH	Nicotinamide Adenine Denucleotide
OUR	Oxygen Utilization Rate
PAO	Phosphorus Accumulating Organisms (bio-P bacteria, P-removing bacteria, poly-P bacteria)
PCR	Polymerase Chain Reaction
PE	Person Equivalent
PHA	Poly-Hydroxy-Alkanoate
PHB	Poly-Hydroxy-Butyrate
PHV	Poly-Hydroxy-Valerate
RBCOD	Readily Biodegradable Chemical Oxygen Demand
RNA	Ribonucleic Acid
SBCOD	Slowly Biodegradable Chemical Oxygen Demand
SBR	Sequencing Batch Reactor
SRT	Sludge Retention Time
TCA	Tricarboxylic Acid
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UCTPHO	University of Cape Town Activeted Sludge Model
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Waste Water Treatment Plant

Symbols

α_i	energy required for acetate uptake	mol ATP/C-mol
Δ	difference between two values	
δ	aerobic metabolic parameter	P mol/mol-C
θ	Arrhenius temperature coefficient	
μ	growth rate of microorganisms	1/day
$\lambda_{SRT,p}$	sensitivity factor	
C	carbon	
C_i	concentration of component i	g/L
$C_{x,to}$	active biomass concentration at the beginning of the aerobic phase	g active biomass/L
ΔC_{HAc}	acetate addition per cycle	g HAc/L
f_i	active biomass specific fraction of component i	g/g active biomass
$f_{pha,to}$	active biomass specific PHA fraction at the start of the aerobic phase	g PHA/g act. biomass
$f_{pha,t}$	active biomass specific PHA fraction at the end of the anaerobic phase	g PHA/g active biomass
Δf_{PHA}^{acr}	active biomass specific PHA conversion under aerobic conditions	g PHA/g active biomass
g_{act}	activation energy	kJ/mol
HAc	acetate	
K	potassium	
k_i	specific kinetic constant for conversion of component i	g/g active biomass
m	maintenance coefficient	P mol/C-mol.h
N	nitrogen	
O ₂	oxygen	
P	phosphorus	
P/O	phosphate/Oxygen ratio	mol ATP/mol O ₂
r	conversion rate	g/L.h
R	gas constant	
q_i	active biomass specific reaction rate of component i	g/g active biomass. h
SRT	sludge age	day
T	temperature	°C
t_o	start aerobic phase	h
t	time (specifically, end aerobic phase)	h
t^{acr}	duration of the aerobic phase	h
Y_{ij}	yield of component i on component j	g/g

Subscripts and superscripts

o	zero
HAc	acetate
x	active biomass
aer	aerobic
anaer	anaerobic
anox	anoxic
max	maximal
min	minimal
pp	poly-phosphate
pha	PHA
phb	PHB
gly	glycogen

Summary

Doctoral thesis

Modeling Biological Phosphorus Removal in Activated Sludge Systems

by

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Biological phosphate removal (BPR) from wastewater has become an established process in wastewater treatment practice. Despite the broad knowledge and practical experience that have been accumulated over the last two decades, there are still certain aspects of BPR which require further research. This dissertation deals with the selected microbiological, biochemical, engineering and modeling aspects; the research was structured in a way to fill the gaps in both operational and fundamental knowledge related to kinetic modeling of BPR.

Presently, it is widely agreed that the models for simulation of activated sludge systems are suitable for application to complex full-scale wastewater treatment plants (WWTPs). The Activated Sludge Model no.1 (ASM1) has been used for more than a decade as a tool for modeling the removal of organic matter and for the nitrification and denitrification processes; considerable experience with this model has been acquired. However, for ASM2 (includes also BPR), the situation is different due to the fact that the model became available only recently, and because its accuracy is being debated. In the same time when ASM2 was proposed, an integrated metabolic model for BPR ("Delft BPR model") was developed. While the information on the practical application of ASM2 is scarce, the "Delft BPR model" was already tested and verified in several studies using BPR cultures enriched under the laboratory conditions and under the temperature of 20°C. The model proved to be well capable of describing the complex conversions of the BPR process. The stoichiometry and the kinetics of BPR processes are considered to be well defined in this model. Presently, there is a strong demand for applications of the mathematical models for simulation of COD, N and P removal at full-scale WWTPs. For this purpose, the "Delft

BPR model" replaced the module for P removal of ASM2 and was combined with the retained equations for COD and N conversion of the ASM2. This combined model was used to simulate the operation of one of the most complex plants of the Netherlands, the WWTP "Haarlem Waarderpolder" with the goal to check how well this model predicts the measured concentrations of the components of both the liquid phase and the biomass. This required proper influent and sludge characterization including an extensive sampling program and a number of biological batch tests to be performed. At the same time, a new bioassay for the determination of the glycogen content of bio-P bacteria needed to be developed, due to the fact that the present methods are often cumbersome and erroneous. Since the BPR processes are also applied in cold and warm seasons and climates, or may experience temperature shock due to industrial discharge, it was decided to study the short- and long-term temperature effects on the stoichiometry and kinetics of the anaerobic and the aerobic phases of BPR. Furthermore, critical but less understood kinetic aspects of BPR related to the impact of extreme conditions on BPR were investigated (notably minimum sludge retention time, potassium deficiency in WWTP influent, performance of BPR under poly-phosphate, poly-hydroxy-alkanoate or glycogen limitation, and excessive aeration in BPR systems). A summary of these research topics is given below.

Chapter 1

Biological Phosphorus Removal Processes

Biological phosphorus removal has become a reliable and well understood process for wastewater treatment. This chapter describes the historical development of the process and the most important microbiological, process engineering and modeling aspects. From a microbiological point of view the role of poly-hydroxy-alkanoate (PHA) as storage material in a dynamic process and the use of poly-phosphate (poly-P) as energy reserve are the most important aspects. From a process engineering point of view the study on BPR has shown that highly complex biological processes can be properly designed and controlled, having a good knowledge of the prevailing microbial processes. As far as the modeling aspect is concerned, there are two different modeling approaches for the simulation of BPR processes: one based on the metabolism of the bacteria (the "Delft model") and the other, based on a black-box approach (ASM2). Presently, BPR models are sufficiently developed to be applied for the description of activated sludge processes. The combination of ASM 2 and the "Delft model" for aerobic and anoxic BPR for simulation of activated sludge systems seems to be very promising.

Chapter 2

Temperature Effects on the Physiology of Biological Phosphorus Removal

P-removing sludge was enriched in an anaerobic-aerobic, acetate fed, sequencing

batch reactor (SBR) at 20°C. Conversion of relevant compounds for biological phosphorus removal was studied at 5, 10, 20 and 30°C in separate batch tests (short-term temperature effects). The stoichiometry of the anaerobic processes was insensitive to temperature changes. Some effect on aerobic stoichiometry was observed. In contrast, temperature had a strong influence on the kinetics of the processes under anaerobic as well as aerobic conditions. The anaerobic P-release (or acetate-uptake) rate showed a maximum at 20°C. However, a continuous increase was observed in the interval 5-30°C for the conversion rates under aerobic conditions. Based on these experiments, temperature coefficients for the different reactions were calculated. An overall anaerobic and aerobic temperature coefficient θ was found to be 1.078 and 1.057 (valid in the ranges $5^{\circ}\text{C} \leq T \leq 20^{\circ}\text{C}$ and $5^{\circ}\text{C} \leq T \leq 30^{\circ}\text{C}$), respectively.

Chapter 3

Influence of Temperature on Bio-P Removal: Process and Molecular Ecological Studies

This chapter describes the impact of long-term (weeks) temperature changes on stoichiometry and kinetics of the anaerobic and aerobic phases of BPR processes. Steady state conversion of relevant compounds for BPR was studied by adapting reactors in subsequent periods to 20, 30, 20, 10 and 5°C. Integrated in the process study, two methods (electron-microscopy and dry denaturing gradient gel electrophoresis) were applied to investigate the composition of the bacterial community of biological phosphorus removing sludge developing at those different temperatures. The temperature coefficient for metabolic conversions obtained from long-term temperature tests was similar to the temperature coefficient observed in short-term (hours) tests ($\theta=1.085$ and $\theta=1.078$, respectively). Temperature had a moderate impact on the aerobic P-uptake process rate ($\theta=1.031$) during long-term tests. However, a strong temperature effect on other metabolic processes of the aerobic phase, such as PHA consumption ($\theta=1.163$), oxygen uptake ($\theta=1.090$) and growth ($\theta>1.110$), was observed. Different temperature coefficients were obtained for the aerobic phase from long-term and short-term tests, probably due to a change in population structure. This change was also visible from molecular ecological studies. The different temperature coefficient found for P-uptake compared to the other metabolic processes of the aerobic phase underlines that in complex processes such as BPR, it is dangerous to draw conclusions from easily observable parameters (like phosphate) only. Such consideration can easily lead to underestimation of the temperature dependency of other metabolic processes of the aerobic phase of BPR.

Chapter 4

Bioassay for Glycogen Determination in Biological Phosphorus Removal Systems

Glycogen plays an important role in BPR from wastewaters. Existing measurement techniques often overestimate the glycogen content of the biomass due to the presence of glucose and/or other carbohydrates than glycogen in the cell material. As an alternative to conventional methods a bioassay for glycogen determination in BPR systems was developed. The bioassay is based on the strict stoichiometric coupling between anaerobic acetate uptake and glycogen consumption. In other words, the glycogen concentration of the sludge was determined indirectly by measuring the maximal total acetate uptake by the activated sludge in anaerobic batch tests. The bioassay was successfully tested for the determination of glycogen content of the sludge taken from the lab-scale, acetate fed, anaerobic-aerobic-settling sequencing batch reactor operating at pH 7.0 ± 0.1 and temperature of 20°C . This determination of glycogen requires that glycogen (not poly-P) is the limiting factor for anaerobic acetate uptake. A method to verify this assumption based on the effect of pH on phosphate/acetate ratio is proposed and used. The bioassay is easy to apply and gives an indirect measure of the glycogen content of bio-P bacteria, but its reliability still needs to be verified at full-scale biological P-removal plants.

Chapter 5

Modeling COD, N and P Removal in a Full-Scale WWTP "Haarlem Waarderpolder"

An integrated activated sludge model was used to describe removal of organic matter, nitrogen and phosphorus in a full-scale wastewater treatment plant Haarlem Waarderpolder in The Netherlands. The plant consists of a conventional anoxic/aerobic activated sludge line, side-stream combined biochemical phosphorus removal line and sludge treatment line. In this integrated model, the "Delft model" for biological phosphorus removal (BPR) replaced the module for P removal of Activated Sludge Model No.2 (ASM2). With the adjustment of only three model parameters (fermentation rate constant, glycogen formation rate constant and the percentage of denitrifying activity of phosphorus accumulating organisms) the model proved well capable of describing the performance of the treatment plant (for both liquid phase and biomass). The model was validated using batch tests with the activated sludge and showed satisfactory results in describing the anaerobic P release and aerobic and anoxic P uptake processes. The model was to a lower extent capable of well predicting nitrification, endogenous P release and especially denitrification processes. Both model simulations and results of the sampling program suggested that the acetate dosage to the system could be reduced up to 30%. It was also shown that pH strongly affects observed phosphate/acetate ratio and possibly fermentation rate in the side-stream BPR process. Simulation of three alternative plant configurations showed that: (1) plant effluent quality would strongly deteriorate if the side-stream process is skipped and the rest of the plant is retained in its original state, (2) good effluent quality

would be achieved in a main-stream process (modified UCT system) where an additional anaerobic tank is added in front of the activated sludge unit, however, the required acetate addition would be sevenfold higher in comparison with a present side-stream configuration.

Chapter 6

Minimal Aerobic Sludge Retention Time in Biological Phosphorus Removal Systems

The methodology for determination of the minimally required aerobic sludge retention time (SRT_{min}^{aer}) in BPR systems is presented in this chapter. Contrary to normal biological conversions, the BPR process is not limited by a SRT_{min} , which results from a maximum growth rate of the organisms. This is because the aerobic SRT should be long enough to oxidise the amount of PHA stored in the anaerobic phase. This makes that the SRT_{min}^{aer} will primarily depend on the PHA conversion kinetics and the maximal PHA content in the cell (storage capacity), and on other operational parameters, such as temperature (the SRT_{min}^{aer} exhibited very high temperature dependency similar to that of nitrifiers) and process configuration. It is expected that in denitrifying BPR systems the minimally required SRT will be around 35% longer in comparison with aerobic BPR. A model equations for determination of the minimal aerobic SRT as a function of above kinetic and process parameters were developed and the model predictions were compared with experimental data used to evaluate several operational aspects of BPR. The model was proved to be capable of describing these data satisfactorily.

Chapter 7

Effect of Potassium Limitation on Biological Phosphorus Removal

A full-scale sewage treatment plant designed for BPR may experience short or long-term shortage of potassium in the influent. In this chapter, using an anaerobic-aerobic SBR system, inoculation sludge from laboratory, pilot and full-scale P-removal plants was exposed to different potassium-phosphorus ratios in the influent. By simulating the conditions which may occur in practice, it was shown that potassium is an essential factor in BPR processes. When the system was exposed to severe shortage of potassium in the influent (a) phosphorus removal was nil, (b) poly-P concentration in the biomass decreased exponentially due to sludge wasting, and (c) the anaerobic P release and the related acetate uptake were only affected after several days of potassium absence, likely due to insufficient content of poly-P in the biomass to allow full acetate uptake under anaerobic conditions. In contrast, the system achieved complete P removal when potassium was present in excess amounts in the feed (in this case 1.25 and 2.5 g K/g P).

Chapter 8

Effect of Poly-Phosphate Limitation on the Anaerobic Metabolism of Bio-P Bacteria

There are two types of microbial populations described in the literature as being capable of anaerobic storage of acetic acid in activated sludge processes: the poly-phosphate accumulating organisms (PAOs) and the glycogen accumulating non-poly-phosphate organisms (GAO). Both groups use the conversion of glycogen to poly-hydroxy-alkanoate (PHA) to produce ATP and NADH; however, the first group can also produce ATP from poly-phosphate (poly-P). No representative pure cultures are available from either group. The question arises: is the observed activity of GAO due to PAO that are depleted in poly-P? In this study, using a laboratory sequencing batch reactor containing an enriched culture, the ability of the enriched PAO to utilize organic substrate (acetate) anaerobically was investigated under conditions of poly-P limitation and surplus glycogen content of the biomass. This study showed clearly that, under these conditions, almost no acetate was taken up. Furthermore, this strongly suggests that PAO can not use glycogen conversion to PHA as the sole energy source under anaerobic conditions, which seems to be restricted to a separate group of GAO. On the basis of the results and literature data, an improved scheme for the anaerobic acetate accumulation is presented.

Chapter 9

Impact of Excessive Aeration on Biological Phosphorus Removal

It has been reported that deterioration of biological phosphorus removal efficiency at some wastewater treatment plants regularly occurred after heavy rainfall or weekends. The deterioration has been attributed to low plant loading that took place during such events. However, it is hypothesized in this study that the cause of such deterioration may have been the excessive aeration that took place due to inadequate control of aeration system during weekends and rainfall periods. In order to test this hypothesis, the influence of excessive aeration (aeration during starvation conditions) on BPR processes was studied using a laboratory anaerobic-aerobic-settling sequencing batch reactor. It was clearly demonstrated that the phosphorus uptake stops due to a gradual depletion of poly-hydroxy-butyrate (PHB) in an over-aerated process. If organic substrate is introduced to the system, phosphorus release is immediately at its maximal rate. However, the released phosphorus cannot be taken-up fully again because the PHB content limits the uptake rate. Consequently, incomplete phosphorus uptake leads to temporarily reduction of BPR efficiency. This causal effect can explain the deterioration of BPR efficiency after heavy rainfall or weekends. Since excessive aeration clearly negatively affects the BPR processes, the aeration should be properly controlled at sewage treatment plants. Some other findings of this study deserve to be

mentioned. It was confirmed that the presence of acetate under aerobic conditions provokes phosphorus release. This may also contribute to deterioration of the BPR efficiency. The aerobic phosphate uptake was found to depend not only on the PHB but also on polyphosphate (poly-P) content of the cells. A maximal poly-P (0.18 g P/g VSS) and minimal PHB content of the cells (2.11 mg COD/g VSS) were observed in the enriched sludge during excessive aeration experiments. It was shown that under aerobic starvation conditions glycogen can not replace PHB for phosphate uptake and is only used for maintenance. During this period no oxygen consumption due to decay processes has been observed

Chapter 10

Evaluation and Outlook

Biological phosphate removal has been discovered in wastewater treatment plants by accident, and has developed from an interesting observation to an established biotechnological process implemented widely at full-scale. Presently, biological phosphorus removal models are developed sufficiently to be successfully applied for the description of activated sludge processes. The combined COD and N module of the activated sludge model no. 2 (ASM2) and the Delft BPR model proved well capable of describing the performance of a full-scale treatment plant in Haarlem. The next step should be the application of this model using also dynamic plant data, since all simulations described in this thesis were performed with static, steady-state, data. Furthermore, the model capability to predict well a start-up conditions should be investigated. This research showed that modeling and simulation of wastewater treatment plants does not only bring more experience from practical application of the model, but also provides better understanding of plant operation and treatment processes and gives the opportunity to find out where the model should be improved. Additionally, it was demonstrated that the activated sludge models can be used for process design as well as for the optimization of performance of wastewater treatment installations. Following the rapid development of the activated sludge models, it can be expected that within few years the ASM4 may appear to describe COD, N and P removal for aerobic and anoxic conditions, possibly based on combination of ASM3 and a modified Delft BPR model. At that stage of model development it may be reasonable to let the model be applied and tested for a certain number of years (as was the case with ASM1) prior to any further serious changes in the model take place.

1 Chapter

Biological Phosphate Removal Processes

Chapter 1 is based on: "Biological Phosphorus Removal Processes: A Mini Review" by Mark C. M. van Loosdrecht, Christine M. Hooijmans, Damir Brdjanovic and Joseph J. Heijnen. Published in *Applied Microbiology and Biotechnology*. No. 48, pp. 289-296, (1997).

Contents

1.1. Introduction	5
1.2. Historical Development	5
1.3. Microbiological and Biochemical Aspects	8
1.4. Process Engineering Aspects	10
1.4.1. Combination of Chemical and Biological Phosphate Removal	13
1.4.2. Combination of Biological Phosphate and Nitrogen Removal	14
1.4.3. Effect of Pre-settling and Sludge Digestion	16
1.5. Modeling Aspects	17
1.6. Scope of the Thesis	18
1.7. Outline of the Thesis	20

Abstract

Biological phosphorus removal has become a reliable and well understood process for wastewater treatment. This chapter describes the historical development of the process and the most important microbiological, process engineering and modeling aspects. From a microbiological point of view the role of poly-hydroxy-alkanoate (PHA) as storage material in a dynamic process and the use of poly-phosphate (poly-P) as energy reserve are the most important aspects. From a process engineering point of view the study on BPR has shown that highly complex biological processes can be properly designed and controlled, having a good knowledge of the prevailing microbial processes. As far as the modeling aspect is concerned, there are two different modeling approaches for the simulation of BPR processes: one based on the metabolism of the bacteria (the "Delft model") and the other, based on a black-box approach (ASM2). Presently, BPR models are sufficiently developed to be applied for the description of activated sludge processes. The combination of ASM 2 and the "Delft model" for aerobic and anoxic BPR for simulation of activated sludge systems seems to be very promising.

1.1. Introduction

Biological phosphate removal (BPR) has become a well established process and is applied in many full-scale wastewater treatment processes. The process as such does not only offer a good opportunity to remove phosphate in an efficient way from wastewater, it is also an interesting study object for microbial ecological research. The organisms involved in BPR have a complex physiology in which formation and consumption of storage polymers (poly-phosphate, glycogen, poly-hydroxy-alkanoates: PHA) play a dominant role.

Biological phosphate removal has been discovered by accident in full scale wastewater treatment plants around 1959; the first designed full scale processes were introduced at the end of the 70's. Initially, most of the research was practically oriented trying to achieve systems with BPR with limited attention for the basic mechanisms underlying the phenomenon. In the 80's the research field became more interdisciplinary, with microbiological and process-engineering research resulting in a better understanding of the basic phenomena. Biological phosphate removal has clearly been a research field where it was virtually impossible to make progress without an interdisciplinary approach. This is for a large part due to the complexity of the organisms involved, which in the research requires a good background in microbial physiology. However, microbial groups applying a strict microbial approach have often ignored important observations from practice leading to research on organisms that do not play a significant role in the process.

In this introductory chapter, the historical developments of the process are given in brief, indicating the mentioned interactions between different research groups. Thereafter, the essential microbiological aspects will be discussed shortly (for a more detailed discussion the reader is referred to Mino et al. 1997) followed by a detailed discussion on the process engineering and modeling aspects.

1.2. Historical Development

The first indication of biological phosphate removal occurring in a wastewater treatment process was described by Srinath et al., (1959) of India. They observed that sludge from a certain treatment plant exhibited excessive (more than needed for cell growth) phosphate uptake when aerated. It was shown that the phosphate uptake was a biological process by demonstrating inhibition by toxic substances and the presence of an oxygen demand. Later, in more (plug flow) wastewater treatment plants this so-called enhanced phosphate removal was confirmed.

Levin and Shapiro (1965) conducted the first structured investigation into the phosphate removal phenomena as observed in several treatment plants. They postulated the hypothesis that the removal was biologically mediated because it only occurred under aerobic conditions. The phosphate could be stored in a form of granules as observed in several bacteria. Levin and Shapiro studied the phosphate removal process on full scale treatment

plants and with batch experiments with sludge retrieved from these plants. Their main observations were that phosphate was released under non-aerated conditions and taken up under aerobic conditions; moreover, the addition of wastewater (substrate) increased the phosphate uptake. Since phosphate was taken up under aerobic conditions, they concluded that the uptake occurred via formation of adenosine-tri-phosphate (ATP) in the oxidative phosphorylation. Uptake via substrate phosphorylation could have taken place anaerobically by the Embden-Meyerhof (EM) pathway. They showed that the process was clearly a biological process since aeration and substrate were necessary, and inhibition of oxidative phosphorylation by 2,4 dichloro-phenoxy-acetic acid led to inhibition of phosphate uptake. By observing that at high pH (9) also no phosphate uptake occurred they suggested that indeed no chemical precipitation was causing the observed phosphate removal. In this and other papers from this period it was assumed that glucose was the main substrate; fermentation processes (in the sewer or treatment plant) were seemingly not recognised.

Later, Shapiro et al., (1967) focussed their research more on the anaerobic stage of the process. They indicated that the phosphate release was not caused by cellular decay but could be enhanced by adding poisons such as KCN. Moreover, the release was directly associated with the amount of sludge present. This pointed again towards a biological basis for the observations. Based on experimental findings they concluded that the redox potential rather than the oxygen tension was triggering the phosphate release. This conclusion influenced many later research projects, even after Randall et al., (1970) who clearly showed that not the redox potential, but conditions that adversely affect cell metabolism (such as lack of oxygen or substrate), caused the phosphate release.

Based on the observed behaviour of systems with excess phosphate removal, Levin (1966) filed a patent for the "Phostrip" process. In this process, the observed phosphate release is used to obtain in a separate tank a high concentration of phosphate which can subsequently be precipitated. This process developed without its proper understanding, is still successfully used in treatment plants nowadays. The concept got, however, only accepted widely in the 80's when the basic background of BPR processes became clear.

In the late 60's and early 70's many researchers tried to find a good explanation for the observed excess phosphate removal in certain full-scale treatment plants. Milbury et al., (1971) defined some basic requirements for phosphate removal by stating that the reactor should be plug flow and the first part of the reactor should not be well aerated. Moreover, they found there was a maximum capacity of the sludge to accumulate phosphate. Until this stage, the research was mainly performed at full-scale systems by civil engineers. This led to large controversy and confusion, basically due to a lack in proper understanding of microbial processes in general. An aspect which was highly important and (when looking back) very obvious, a link between phosphate release and uptake processes, was not really recognised. Full attention for the process design was given to the phosphate uptake process. This phosphate uptake was considered to be dependent on aerobic bacteria, resulting from stress conditions due to the dynamic feeding of activated sludge or the presence of anaerobic conditions in part of the treatment process. The phosphate uptake process was called "overplus" or "luxury" phosphate uptake. These phenomena were described by

Harold (1966) and observed with pure cultures, subjected to stress conditions. Based on this stress-theory, Nicholls and Osborn (1979) came to an advice for process design, which led to well functioning processes although the fundamental assumption of the process later turned out to be wrong.

In the second part of the seventies the research expanded in the field of microbiology and by applying more process engineering principles. Fuhs and Chen (1975) concluded from a range of isolation tests that bacteria of the genus *Acinetobacter* were responsible for the BPR process. These organisms accumulated large amounts of poly-phosphate and could also accumulate poly-hydroxy-butyrate (PHB). They postulated the hypothesis that an anaerobic phase was needed to produce volatile fatty acids (VFAs) which is the substrate to grow phosphate removing organisms. *Acinetobacter* type of organisms could use these substrates under aerobic conditions for growth and excessive phosphate uptake, in line with the prevailing theory among sanitary engineers and the work of Harold (1966). It was therefore not strange that Fuhs and Chen (1975) didn't make a link either between anaerobic phosphate release and the occurrence of polyphosphate accumulating bacteria. It was, therefore, not considered strange either that the isolated bacteria showed anaerobic phosphate release but only at very low rates compared to activated sludge. Since there were no good measurements available from activated sludge systems, it was also not recognised that the responsible organisms take up phosphate under aerobic conditions, whereas *Acinetobacter* sp. only consumes acetate under aerobic conditions.

Later most microbiological studies relied on the isolation procedure of Fuhs and Chen (1975) (e.g. Deinema et al. 1980, Lotter et al. 1986), and it is therefore not surprising that always the same type of organisms were found. These organisms were however not involved in the actual process, as recently also clearly demonstrated by the use of molecular ecology techniques (Bond et al. 1994, Wagner et al. 1994, Mino et al. 1997). Nevertheless, the microbiological research has greatly helped the engineers to derive a basic hypothesis for the metabolism of poly-phosphate accumulating bacteria. The development of this hypothesis was, however, greatly hampered by the absence of a true isolate from the BPR process and developed therefore slowly (Rensink, 1981, Comeau et al. 1986, Wentzel et al. 1986, Arun et al. 1987, Smolders et al. 1994b, Maurer et al. 1997, Mino et al. 1997). It might be called remarkable that the biochemical model was developed by engineers, but all had personal contacts with or knowledge of the microbiological research field. Possibly the engineers were less hampered by a traditional biochemical and microbial approach and could therefore easier come up with new concepts in microbial ecophysiology (such as use of poly-phosphate as energy reserve, role of PHB and glycogen in dynamic bacterial processes) (e.g. Comeau et al. 1986, Wentzel et al. 1986, Arun et al. 1987, Smolders et al. 1994a, Maurer et al. 1997).

Due to the lack of a solid microbiological basis, the development of actual processes depended greatly on good observations on full- and pilot-scale processes. The development of an engineering approach of the BPR process was mainly due to the work of Barnard (1974, 1975) and Nicholls (1975). They recognised that an essential prerequisite for BPR was the existence of a truly anaerobic phase, in which return sludge and wastewater are mixed. The presence of an external electron acceptor in this phase limits the capacity of the

BPR process. Based on this principle, many different process configurations for biological phosphate and nitrogen removal have been proposed and constructed (Johansson, 1994).

1.3. Microbiological and Biochemical Aspects

Rensink (1981) was the first to report that substrate might be sequestered as PHB by strict aerobic organisms under anaerobic conditions at the expense of energy stored as polyphosphate. He was therefore the first to make a direct mechanistic link between phosphate release and uptake in the BPR process. The main function of the anaerobic phase therefore was not to provide a stress factor or only to supply polyphosphate accumulating bacteria with volatile fatty acids, but also to give a competitive advantage for substrate uptake over other heterotrophic bacteria. This basic hypothesis was further developed and put in a more biochemical framework by subsequent researchers (Comeau et al. 1986, Wentzel et al. 1986, Arun et al. 1987, Smolders et al. 1994a, Maurer et al. 1997, Mino et al. 1997). Despite the lack of a pure culture of bacteria involved in the BPR process (Van Loosdrecht et al. 1997a), this biochemical framework has been well underlined by detailed measurements on enrichment cultures by traditional methods (Wentzel et al. 1988, Arun et al. 1987, Smolders et al. 1994a) or NMR techniques (Pereira et al. 1996, Maurer et al. 1997). Figure 1.1 gives a schematic representation of this biochemical model (after Smolders et al. 1994b).

Figure 1.1 Metabolic processes of organisms involved in biological phosphorus removal.

Under anaerobic conditions the bacteria use stored poly-phosphate as energy source for ATP production with the aid of the enzyme Poly-P: AMP-phosphotransferase (Van Groenestijn et al. 1987). ATP is used for the uptake of VFAs and subsequent formation of PHA. The reduction equivalents needed for the reduction VFA to PHA is derived from the conversion of glycogen to PHA (Arun et al. 1987, Smolders et al. 1994a). Since the transport energy for VFA and phosphate over the cell membrane is strongly influenced by the pH, the pH has a strong effect on the ratio between VFA uptake and phosphate release (Smolders et al. 1994a).

When oxygen, nitrate or nitrite are present in the absence of substrate, PHA is used as substrate. Under these conditions the bacteria not only produce new biomass, but also restore the storage pools of poly-phosphate and glycogen. This leads to a net uptake of phosphate in the overall process. If external substrate, as well as electron acceptors, are present, the substrate is predominantly converted into PHA instead of being used for growth (Kuba et al. 1994, Brdjanovic et al. 1997). Formation of storage materials rather than using substrate for growth seems to be a basic characteristics of micro-organisms in systems with feast-famine regimes as occur in wastewater treatment processes (Van Loosdrecht et al. 1996). Unfortunately, this aspect gets only limited attention from microbial researchers who prefer to work in batch or continuous cultures rather than in dynamic cultures.

It is clear that the BPR process has introduced a range of interesting aspects for applied microbial research, which certainly require further elaboration. Firstly, there is the recognition that "strictly" aerobic organisms can be active under conditions without electron acceptors present. Secondly, the role of storage polymers in microbial competition processes has become evident, and finally, it was found that the growth rate of these organisms is not directly related to e.g. substrate availability, as generally assumed. The organisms seem to use the available substrate (PHA) primarily for the formation of poly-phosphate and glycogen and for maintenance processes. Growth results from the difference between PHA consumption rate and PHA use for the aforementioned processes (Murnleitner et al. 1997).

The amount of the polymers (such as PHA, glycogen and poly-P) stored in phosphorus accumulating organisms: PAOs (also called bio-P bacteria, BPR organisms or P-removing bacteria) at various phases of BPR process is highly variable (for example, PHA is high, and glycogen and poly-P are low at the end of the anaerobic zone, while the situation is reversed at the end of the aerobic or anoxic zone). So far, there is a lack of information on the influence of extreme concentrations (close to zero or to the maximal storage capacity) of storage polymers in the biomass on the performance of BPR systems. Therefore, the behaviour of the PAOs in situations when one or more of the storage products is depleted, should be further investigated. The same applies on the reversed, saturation conditions, where the bacteria's full storage capacity is attained.

Furthermore, information on the temperature influence on BPR is relevant from both practical and microbiological aspects. The reported data concerning temperature impact on the metabolism of bio-P bacteria, as well as on the composition of a microbial population of the BPR system as such, are scarce and inconsistent. It is expected that the temperature

will influence process conversion rates and, consequently the performance of BPR system. To investigate this, a detailed study of stoichiometry and kinetics of the anaerobic and the aerobic phase of BPR is required under both short- (hours) and long-term (weeks) temperature changes. If the microorganisms are exposed to change in temperature for a relatively long time, the microbial population may adapt to the new process conditions and a change in population composition may occur. To prove this hypothesis, innovative molecular ecological technique, such as denaturing gel gradient electrophoresis (DGGE) could be applied on the 16S-rDNA which is extracted from the BPR biomass cultivated at different temperatures. Using DGGE, it might be possible to observe changes in microbial structure during the course of long-term experiments and to estimate a number of different types of bacteria present in the BPR sludge used in the experiments. When molecular characterization is performed with BPR sludge, it is strongly recommended that an acetate fed, pH controlled, anaerobic-aerobic batch test is performed with measurement of acetate, phosphate, PHA, glycogen and P content of the sludge. Such a test would help to interpret the data obtained from the application of molecular methods properly, in the context of BPR (Mino et al. accepted).

From the biochemical aspects there are several topics which need to be mentioned. Firstly, the PAOs require various nutrients in the form of cations (such as potassium, magnesium, calcium and iron). Potassium defines cell membrane permeability, plays a major role in the phosphate transport between surrounding environment and cell, is an essential counterion for poly-P in the cell, is in general an important factor in the cell's energy generation, and therefore is necessary for poly-P accumulation in BPR systems. A WWTP may be exposed to variations in cations concentration in the plant influent. It is believed that BPR has deteriorated at full-scale treatment plant in Bandigo, Australia, due to shortage of potassium in the influent. So far, there is no reported data that could explain this assumption. This could be checked by monitoring the performance of a BPR system (an anaerobic-aerobic sequencing batch reactor) fed with the influent containing different potassium/phosphate ratios. Secondly, the energy budget of the PAOs and GAOs (glycogen accumulating non-poly-P organisms), which are the only known microbial populations capable of anaerobic utilization of organic substrate in the BPR process, is not fully understood yet. Specifically, it is not yet clear whether the PAOs are capable of using the glycogen conversion to PHA as the sole energy source without poly-P hydrolysis, as it is characteristics of GAOs. And thirdly, it is also not yet known how the simultaneous presence of both external organic substrate (acetate) and electron acceptors (oxygen and/or nitrate) influences the kinetics of BPR.

1.4. Process Engineering Aspects

The traditional method for phosphate removal from wastewater is addition of precipitating chemicals (iron or aluminium salts) to wastewater. Besides the fact that in waste treatment addition of chemicals should be minimised, there are several negative aspects on this practise. The counterion of the salts (usually chloride) remains in the water, resulting in increased salinity of surface waters. The chemical precipitate accumulates in the sludge

leading to extra costs for the treatment of the excess sludge. Moreover, since the sludge content in a treatment system is limited to a maximum amount, large treatment reactors are required in order to maintain the same amount of biological sludge. Due to the low costs of chemical addition, the easiness and stability, and the lack of affinity towards biological processes among civil engineers, chemical phosphate removal has been for long time the dominant treatment process. In most countries where phosphate removal is required, biological phosphate removal has however become the preferred process, occasionally supplemented by chemical precipitation. In evaluating full-scale treatment plants, it should however be realised, that precipitation might contribute to the overall phosphate removal process. This depends strongly on the cation composition of the influent and the prevailing pH conditions (Maurer, 1996). The biological process can induce the precipitation because in the anaerobic phase phosphate and the cellular counterion magnesium are released in the liquid leading to increased concentrations.

BPR processes are dependent on the accumulation of bacteria capable of storing large amounts of poly-phosphate inside the cells (Marais et al. 1983). This means that the efficiency of the process is directly coupled to the formation of poly-phosphate accumulating bacteria. For BPR processes this means that the incoming wastewater needs to be mixed with the sludge in a true anaerobic zone (i.e. no oxygen or nitrate presence). In this zone VFA present in the wastewater, or formed by fermentation processes, can be accumulated into PHA by poly-phosphate accumulating bacteria. The adequate design of the anaerobic phase is thus essential for a good BPR process and will depend heavily on the wastewater characteristics. Sewage from anaerobic sewers will be partly fermented into VFA, therefore small anaerobic reactors can be applied. When the wastewater does not contain VFA (as e.g. with aerobic sewers), the anaerobic phase has to be designed on the slower fermentation process, resulting in a larger anaerobic reactor. The length of the aerobic phase in the treatment process will usually not be limited by the phosphate uptake process but by the nitrification process, due to the slower growth rate of nitrifying bacteria.

In practice there are many different BPR process configurations. All these process configurations can however be grouped into two basic types of processes: full biological processes and combined biological chemical processes. A basic scheme for a biological phosphate removal process is shown in figure 1.2.

In the anaerobic phase, substrate is taken up by poly-phosphate accumulating organisms (PAOs). This results in phosphate release in the liquid phase. In the cells, the VFAs-substrate is stored as PHB with glycogen conversion to PHA as NADH source and poly-P as energy source. In the aerobic phase the organisms grow and accumulate phosphate in the cells, and regenerate their glycogen and poly-P reserves. Due to a net growth of cells the phosphate is removed. The PAOs have a clear competitive advantage due to the ability to accumulate the VFAs-substrate in the cells without the need of an external electron acceptor. When the sludge gets aerated (or nitrate is available), other heterotrophic organisms have no substrate left while the PAOs can grow at the expense on their stored substrate.

Figure 1.2 Schematic representation of a BPR process. The graph gives a schematic representation of the change in concentrations in a plug-type of process.

From the process design point of view, it is essential to have a sewage properly characterized, as this directly influences the process choice and selection as well as the plant sizing and configuration. A detailed knowledge of the influent to a WWTP will allow a sound design which is a prerequisite to achieve the desired performance and operation of the system. The more detailed the characterization, the more reliable the design will be. Proper sewage characterization is not only of extreme importance for the design of new plants, but also for the optimization, retrofitting and upgrading of the existing plants. Since the prominent role of storage polymers in BPR has become evident, there is in addition a need for reliable sludge characterization techniques concerning primarily the P-removing fraction of the activated sludge. In comparison with poly-P and PHA, the determination of glycogen is the least reliable method. The currently available (bio)chemical methods measure glycogen either as a carbohydrate content of the biomass or as glucose extracted from the biomass. Both methods overestimate the glycogen content of the biomass that is specifically related to the BPR due to presence of glucose of the cell material or carbohydrates other than glycogen in the activated sludge. Therefore, there is a strong need for the development of a new method for determination of the glycogen content of the exclusively bio-P bacteria.

Furthermore, two crucial aspects of process design and operation would benefit from a thorough understanding of their underlying mechanisms. Firstly, the climate and the weather seasons as well as a discharge of industrial effluents into a sewerage causes fluctuation of sewage temperature (recorded value range from as low as 5°C to as high as 35°C). Therefore, the consideration of temperature impact on the process design and

operation of such systems is an absolute must. In comparison with the ordinary heterotrophs, autotrophs and denitrifiers, the temperature dependency of PAOs is still not investigated. Since there are a number of plants employing BPR built in cold as well in warm climates, the effects of temperature on the stoichiometry and kinetics of the BPR processes, preferably under defined laboratory conditions need to be studied. Secondly, it has been reported at some treatment plants in Germany and Switzerland for example, that a deterioration of BPR efficiency regularly occurred after heavy rainfall or weekends. The deterioration has been attributed to low plant loading that takes place as a consequence of such events. However, it can be hypothesized that the cause of such deterioration may rather have been the excessive (over) aeration during weekends and/or heavy rain events which results in a total depletion of PHA in PAOs. Once the organic substrate is introduced in the system, the anaerobic phosphate release will occur, but the aerobic phosphate uptake may be limited by the depleted PHA levels. This hypothesis still needs to be examined.

1.4.1. Combination of Chemical and Biological Phosphate Removal

As already mentioned above, BPR is heavily dependent on the formation of PAOs. These organisms can accumulate approx. 12% phosphorus on a dry weight basis, compared to 1-3 % for normal bacteria. In several cases the chemical oxygen demand to phosphate ratio (COD/P) in the influent is too low to produce enough biomass for poly-P storage. The biomass production is often lowered because a long sludge age (low growth rate and therefore low net biomass yield) is maintained to support growth of nitrifying bacteria. In these cases, it is possible to supplement the BPR process with chemical phosphate precipitation. Removal of phosphate by chemical and biological methods requires a well controlled addition of chemicals. If too much of the chemicals is added, the phosphate will be fixed as precipitate and is not available for the bacteria to form poly-P. If this polymer is lacking, the PAOs cannot accumulate the substrate under anaerobic conditions and thereby lose their competitive advantage over normal heterotrophic bacteria.

Combination of chemical and biological phosphate removal has the advantage that the biological process is highly selective. If low effluent phosphate concentrations have to be reached, a large overdosing of chemicals is required. The bacteria have a very high affinity for phosphate and therefore a phosphate concentration below 0.1 mg P/L can easily be achieved. Recently, Smolders et al., (1996) presented an evaluation of biological or biological/chemical phosphate removal. In the first case approx. 20 g COD/g P-removed was needed. If the biological process is only used to concentrate the phosphate in a certain section of the process where it is efficiently precipitated, the minimal COD requirement drops to 2 g COD/g P-removed.

Chemicals can be added to the main sludge line, but this has as disadvantage that the chemical precipitate accumulates in the sludge; leading to adverse effects on the

nitrification process. As alternative the Phostrip process, as originally designed by Levin (1966), can be used (see the scheme in figure 1.3). A fraction of the return sludge is introduced in a "stripper" tank in which anaerobic conditions are maintained. By the addition of acetic acid or influent, the phosphate release is stimulated. After sludge-water separation an enriched phosphate containing flow is obtained. This can be treated by flocculation or crystallisation (Eggers et al. 1991). In the latter process, it is possible to obtain a reusable form of phosphate. Crystallisation is however hampered by the high bicarbonate content of the water from the "stripper" tank. In order to remove this bicarbonate, large amounts of extra chemicals are needed for acidification and subsequent bringing the pH back to 8.

The phostrip process requires relatively large investments in extra infrastructure. By the Dutch water board 'Groot Salland' an elegant solution has been developed in which baffles at the end of the anaerobic reactor compartment induce a quiescent zone in which the sludge partly settles (Van Loosdrecht et al. 1997b). The phosphate-rich supernatant can be pumped from the activated sludge tank and precipitates in the sludge thickener.

Figure 1.3 Schematic representation of the phostrip process.

1.4.2. Combination of Biological Phosphate and Nitrogen Removal

It has already been mentioned that biological phosphate and nitrogen removal require adverse conditions with respect to sludge age (SRT). Moreover, it is traditionally assumed that denitrification and phosphate removal processes compete for the same substrate and electron donor. This assumption is based on the observation that when nitrate was

introduced in the anaerobic tank, the biological phosphate removal process deteriorated (Hascoet et al. 1985). In order to prevent the presence of nitrate in the anaerobic tank, UCT (University of Cape Town) - type of processes are used (figure 1.4). Hereby, the nitrate containing return sludge is firstly introduced in a denitrification reactor after which the nitrate-free sludge water mixture is partly recycled to the anaerobic tank.

Figure 1.4 Schematic representation of a UCT - type process.

The negative effect of nitrate on phosphate release is caused by a direct substrate competition between heterotrophic denitrifiers and PAOs. This competition is won by the denitrifiers. This observation and the fact that the bacterial group thought to be responsible for BPR, *Acinetobacter*, cannot denitrify, induced a general belief that P and N removal are competing processes. Recently, several authors (Vlekke et al. 1988, Kuba et al. 1993) have shown that also denitrifying bacteria do have BPR properties. Moreover, it was shown that these bacteria can contribute significantly to BPR in UCT - type processes (Van Loosdrecht et al. 1997c, Kuba et al. 1997). In these processes organisms are cycled between anaerobic and denitrifying conditions, which stimulate the growth of these organisms.

The use of denitrifying PAOs has as main advantage that less COD is needed in the nutrient removal process, thereby expanding the operational range of the biological processes. The finding of denitrifying PAOs has opened the opportunity to design new wastewater treatment processes in which the slow growing nitrifiers and fast growing aerobic and denitrifying heterotrophs are separated (Wanner et al. 1992, Kuba et al. 1996, Sorm et al. 1996). After the anaerobic reactor, the sludge-water mixture is separated. The water containing ammonium is nitrified in a separate system, and thereafter, mixed with the sludge again in a denitrification-phosphate uptake reactor.

The SRT is one of the most important process design parameters since it is one of the factors which directly determines the biomass concentration and the composition of the microbial community in the system. From the design aspect, it is of great interest to determine the minimally required SRT for the particular system in order to optimize the units sizing and, therefore, minimize the related investments. In activated sludge systems designed for COD and N removal, the SRT is directly linked to the growth rate of the

microorganisms. For ordinary heterotrophs in which internal storage of polymers does not take place, the minimally required SRT corresponds only to their maximal growth rate ($\mu_{\max}$) which is the property of the organisms. However, in the BPR systems, where storage materials play an important role in bacterial metabolism, the determination of the minimally required SRT depends on PHA conversion kinetics, the maximal PHA content in the cell, and on a number of process and operating conditions. Consequently, a bio-P population may have more than one $\mu_{\max}$. Since the calculation of the anaerobic SRT (the time needed for the uptake of external substrates) is quite straight-forward, there is a need for development of the methodology for determination of minimally required aerobic SRT in BPR systems.

1.4.3. Effect of Pre-settling and Sludge Digestion

Wastewater treatment processes have to fulfill a large number of requirements. These are sometimes seemingly conflicting. On one hand, COD is needed for N and P removal, on the other hand, it would be worthwhile to separate, as much as possible, COD in a primary settling tank to produce methane. The methane can be used in a gas generator to produce energy for the operation of the wastewater treatment plant.

Siebritz et al., (1983), concluded from several experimental observations, that only the "readily biodegradable fraction" of the influent COD (RBCOD) was used by the PAOs. Since the RBCOD fraction is soluble, pre-settling as such should not have a direct effect on BPR efficiency.

In a recent study, the fate of N, P and COD in a full-scale treatment plant with and without pre-settling was followed in detail (Van Loosdrecht et al. 1997b). This study showed, that in this process particulate COD (which was removed during pre-settling) was not used by PAOs for cell growth. Addition of extra, particulate, COD in the absence of pre-settler led to a shorter sludge age (higher growth rate) due to the higher required sludge wasting. The decreased sludge retention time (SRT) led to more biomass formation, since less substrate is used for maintenance. With pre-settling iron had to be used to supplement the BPR; without pre-settling enough poly-P accumulating biomass was produced to accumulate all the phosphate.

The positive effect of adding non-settled sewage (no chemicals needed for BPR) was largely negative balanced by the extra energy consumption (and therefore CO_2 production), since not enough methane was produced in the digester. Moreover, it turned out that the amount of nitrate denitrified was not increased by addition of the particulate COD, and extra nitrogen removal was due to extra assimilation of ammonium in the surplus sludge.

Application of primary settling and sludge digestion of primary and secondary sludge

therefore seems to be a logical process for nutrient removal processes. This can however lead to a large recycle of nutrients from the digester back to the treatment plant. For nitrogen removal, specific treatment technologies have been developed (Hellinga et al. 1997), and for phosphate removal the recycling of phosphate depends heavily on the prevailing conditions. If sufficient magnesium or calcium is present in wastewater, the majority of the phosphate will be precipitated in the sludge digester (Jardin and Popel, 1996). If this is not the case, some iron needs to be dosed to the digester. The latter is often done anyway in order to control sulphide levels in the methane gas.

1.5. Modeling Aspects

Several different mathematical models for the simulation of activated sludge process in wastewater treatment are available. The most recent models allow for dynamic simulation of complex activated sludge systems and include carbon oxidation, nitrification, denitrification, chemical and biological phosphorus removal, and the formation of filamentous organisms (bulking). Besides different microbial conversion reactions, these models may also take into account dynamic feeding regime, influence of temperature, pH, dissolved oxygen, various hydraulic patterns, process control, and effects of aeration rate.

In order to promote the development and facilitate the application of practical models to the design and operation of biological wastewater treatment systems, the IAWPRC (now IAWQ) formed in 1983 the Task Group on Mathematical Modeling for Design and Operation of Biological Wastewater Treatment. Four years later the Task Group proposed a general model for removal of organic matter, nitrification and denitrification, called Activated Sludge Model no. 1 - ASM1 (Henze et al. 1987). Since its appearance, this model has greatly encouraged the use of mathematical models. The increased requirement for nutrient removal during the last decade has created a need to extend ASM1 by inclusion of BPR. In 1989, Wentzel et al. presented a kinetic model for aerobic BPR. This model served as a basis for development of the Activated Sludge Model no. 2 - ASM2 (Gujer et al. 1995). Soon after, Mino et al. (1995) extended ASM2 by including the glycogen metabolism and BPR under anoxic conditions. Simultaneously, Smolders and co-workers (1995) developed a structured metabolic model for BPR (also incorporating the glycogen metabolism) which served as a basis for the metabolic model for denitrifying BPR (Kuba et al. 1996). In their integrated metabolic model for aerobic and anoxic BPR (so-called "Delft Model" for BPR), Murnleitner et al. (1997), successfully described the two known BPR processes with the same kinetic equations and parameters. The metabolic model is based on the bioenergetics and stoichiometry of the bacterial metabolism, and describes all relevant metabolic reactions underlying the metabolism of PAOs by six independent reactions: two for the anaerobic metabolism and four for the aerobic/anoxic metabolism. In this model the ATP/NADH₂ ratio (called δ value) is the only model parameter that is different for aerobic

and anoxic BPR. The process reaction rates are described by four kinetic relations and two maintenance terms. The comparison between the ASM2 and the "Delft BPR model" is discussed elsewhere (Van Loosdrecht, 1996). Very recently, the Task Group proposed the ASM2d (Henze et al. 1998) - a minor extension of ASM2 by inclusion of additional two processes to account for denitrifying BPR. In the same time the Task Group also proposed a structured model for simulation of oxygen consumption, sludge production, nitrification and denitrification: ASM3 (Gujer et al. 1998). ASM 3 is related to ASM1 and includes several improvements compared to ASM1, such as addition of storage of organic substrates as a new process, and replacement of lysis process with endogenous respiration process. Following this fast development of the activated sludge models, it can be expected that within few years the ASM4 may appear, possibly as a combination of ASM3 and a slightly modified "Delft BPR model".

So far, the "Delft BPR model" was verified and tested over a range of SRT values (Smolders et al. 1994c) and oxygen or nitrate as electron acceptor (Murnleitner, 1997), as well as during both start-up and steady state conditions (Smolders et al. 1995) - all in BPR lab-scale SBR systems. Recently, the "Delft model" was successfully applied for the first time on the full-scale installation WWTP "Holten" in The Netherlands (Van Veldhuizen et al. submitted). For this application the "Delft model" for P removal was combined with the equations for COD and N conversions of ASM2 following the structure of ASM2 closely. This should further encourage future application of this combined model on the plants operating in a "steady-state" as well as on highly dynamic situations which occur, for example, during start-up conditions. Once again, the extreme importance of proper wastewater and sludge characterization for good simulation of the system's performance is underlined.

1.6. Scope of the Thesis

Biological phosphate removal (BPR) from wastewater has become an established process in wastewater treatment practice. Despite the broad knowledge and practical experience that have been accumulated over the last two decades, there are still several aspects of BPR which require further research. This dissertation deals with the selected microbiological, biochemical, engineering and modeling aspects; the research was structured in a way to fill the gaps in both operational and fundamental knowledge related to kinetic modeling of BPR. The "rationale" of the thesis is presented below (see also figure 1.5).

The stoichiometry and the kinetics of BPR processes can be considered as presently well defined, however only for a temperature of 20°C. Since the BPR processes are also applied in cold and warm seasons and climates, and since industrial discharges and atmospheric precipitation affect the sewage temperature, it was decided to study the short- and long-term

temperature effects on the stoichiometry and kinetics of the anaerobic and the aerobic phases of BPR.

- Chapter 2 Temperature effects on the physiology of biological phosphorus removal
- Chapter 3 Influence of temperature on biological phosphorus removal: Process and molecular ecological studies
- Chapter 4 Bioassay for glycogen determination in biological phosphorus removal systems
- Chapter 5 Modeling the wastewater treatment plant "Haarlem Waarderpolder"
- Chapter 6 Minimal aerobic sludge retention time in biological phosphorus removal systems
- Chapter 7 Effect of potassium limitation on biological phosphorus removal
- Chapter 8 Effect of poly-phosphate limitation on the anaerobic metabolism of bio-P bacteria
- Chapter 9 Impact of excessive aeration on biological phosphorus removal

Figure 1.5. The scope and structure of this dissertation.

Since modeling of the full-scale activated sludge systems using the metabolic approach was applied recently for the first time, there is a strong demand for more applications in order to test the model. Therefore, an integrated metabolic model for BPR ("Delft model") combined with the equations for COD and N conversion of the Activated Sludge Model no. 2 (ASM2) was used to model one of the most complex plants of the Netherlands, the WWTP "Haarlem Waarderpolder". This required: (a) the process and flow scheme of the plant to be made, (b) proper influent and sludge characterization including an extensive sampling program and a number of biological batch tests to be performed, and (c) the process model of the plant to be created. The SIMBA® 3.2+ simulation program (Matlab®/Simulink®) was used for simulations of the plant operation and performance. At the same time, a new bioassay for the determination of the glycogen content of bio-P bacteria was developed, since the present methods are often cumbersome and erroneous. Furthermore, critical but less understood kinetic aspects of BPR related to the impact of extreme conditions on BPR were investigated (notably minimum SRT, potassium

deficiency in WWTP influent, performance of BPR under poly-phosphate, poly-hydroxy-alkanoate or glycogen limitation, and excessive aeration in BPR systems). Since the primary purpose of this research was to check the performance and the metabolic approach on a normal operating wastewater treatment practice, these extremes were not included in the model. An outline of these research topics is given below.

1.7. Outline of the Thesis

So far, no structured study of the impact of the temperature on both stoichiometry and kinetics of the anaerobic and aerobic BPR processes under defined laboratory conditions is available. This is important for a full-scale WWTP due to likely large differences in sewage temperature on short and on long term scale, e.g. between summer and winter periods. Moreover, with the application of mathematical modeling on activated sludge process, the knowledge of temperature coefficients becomes even more important. However, ASM1, ASM2 and the "Delft model" still have default values for parameters that are mostly determined at 20°C.

The study on the effect of temperature on BPR was performed in two parts. In the first part, described in chapter 2, the P-removing sludge was enriched in an anaerobic-aerobic-settling, acetate fed, SBR at 20°C. Conversion of relevant compounds for BPR was studied at 5, 10, 20 and 30°C in separate batch tests over a period of a couple of hours (therefore also called "short-term" temperature tests). Based on these experiments, the temperature coefficient of the Arrhenius equation for the different reactions was calculated.

In the second part, described in chapter 3, the strategy was changed. The impact of "long-term" temperature changes (couple of weeks) was investigated by studying the steady-state conversion of relevant compounds for BPR at different temperatures, namely 5, 10, 20 and 30°C. It can be assumed that during short-term temperature experiments the BPR population ecology will not change. However, if microorganisms are exposed for a relatively long time to a new temperature, the microbial community may be expected to adapt to the new process conditions and a change in population structure may occur. Such change may lead to different temperature coefficients obtained from "short-term" and "long-term" temperature experiments. Two methods (electron microscopy and dry denaturing gradient gel electrophoresis - DDGGE) were applied in order to investigate the bacterial population composition at different temperatures. Second aim of this study was to evaluate whether or not the "short-term" temperature coefficients for reaction rates of BPR reliably simulate "long-term" experiments for modeling purposes. These temperature coefficients do provide the direct effect of temperature on the biomass specific metabolic process rates. However, they still only partly relate to the kinetic rate coefficients defined previously in the models for these metabolic processes.

The second important issue in process evaluation and modeling of BPR studied here is the proper characterization of wastewater and sludge. Since the essential role of the storage products in the BPR processes is now widely recognized, accurate measurement of the storage materials is needed. In comparison with poly-P and PHA, the glycogen determination is the least reliable method. So far, there are two methods for glycogen determination: the conventional one, which measures glycogen either as glucose extracted from the biomass or as carbohydrate other than glycogen; and the enzymatic method, which uses the enzyme amyloglucosidase to hydrolyze glycogen to glucose. However, these chemical methods are cumbersome and, in the case where exclusively the glycogen content of bio-P bacteria is of interest, often overestimate the glycogen content of the biomass due to the presence of glucose and/or carbohydrates other than glycogen in the cell material (conventional method), or due to inclusion of glycogen (glucose) of other bacteria (beside PAOs) present in the sludge (enzymatic method). In this part of the research a new biological method for glycogen determination in BPR systems was developed, tested and verified as more accurate and less labor-intensive alternative to chemical methods (chapter 4).

Presently, the general opinion is that the models for the simulation of activated sludge systems are sufficiently developed to be applied in full-scale WWTP. While already a considerable experience exists in using ASM1 as a tool for modeling the removal of organic matter and nitrification and denitrification processes, the situation is the opposite for the biological phosphate removal. There are two main reasons for such situation. Firstly, the models which include BPR became available only recently, and secondly, in the field of modeling wastewater treatment processes the compromise has to be met between complexity and simplicity, and between the many opinions on what THE correct model should look like. Consequently, the discussion on an optimal model for the BPR is still ongoing. However, the best way to determine the value of models is their practical application. Therefore, we selected the full-scale, COD-, N- and P-removing WWTP in Haarlem to be modeled using the combination of the part of ASM2 related to COD and N removal and the "Delft BPR model". This is one of the few applications of this combined activated sludge model on a full-scale plant, and is described in chapter 5.

The final section of the thesis comprises four chapters in which extreme conditions in BPR (such as limitation of potassium in the plant influent, full depletion of poly-P and PHA storage pools in the biomass, and the SRT limitation) were examined. A proper determination of the minimal SRT in activated sludge systems is of great importance for the design and operation of WWTP. The methodology for determination of the minimally required aerobic SRT in BPR systems as a function of process and operational factors is presented in chapter 6,

A full-scale WWTP designed for BPR may experience short or long-term shortage of

potassium in the influent. Potassium defines cell membrane permeability, plays a major role in the phosphate transport between the surrounding environment and the cell, is an essential counter ion for poly-P in the cell, and is an important factor in the cell's energy generation. The impact of severe shortage of potassium in the influent on dynamic behavior of BPR, and the response of the BPR system to variation in potassium concentration in the influent was studied and is presented in chapter 7.

There are two known microbial populations capable of anaerobic utilization of organic substrate in BPR processes: the PAOs and GAOs. With the aim to investigate in more detail the energy budget of PAOs and GAOs, the effect of poly-P limitation on the anaerobic metabolism of PAOs was investigated (chapter 8). In this chapter, the attempt was made to answer the following question: can PAOs perform the metabolism of GAOs under anaerobic conditions, or more specifically, can PAOs take up VFA in the absence of poly-P when sufficient glycogen is present?

It has been reported that BPR deteriorated at some WWTP after heavy rainfall or weekends. It was thought that the deterioration in BPR efficiency took place due to low plant loading during such events. Our hypothesis was that the cause of a decreased BPR efficiency at those WWTP may have been the excessive aeration due to inadequate control or adjustment of the aeration system after heavy rainfall and weekends. This hypothesis was examined and verified, and the underlying mechanisms of such deterioration were defined. The results of that study are presented in the chapter 9 of this thesis.

The final chapter comprises of the overall conclusions of this thesis, but also of the recommendations for further research concerning BPR. The research itself, in general, may not always provide complete answer on all of the given questions which were originally the driving force for that particular research. However, during its course, every research opens a new set of questions to be answered. Chapter 10 does not only provide new questions which arose from this research in particular, but also includes some interesting research topics brought up by other researchers in the field of BPR.

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2 Chapter

Temperature Effects on Physiology of Biological Phosphorus Removal

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Contents

2.1.	Introduction	29
2.2.	Materials and Methods	31
2.2.1.	Continuous Operation of Sequencing Batch Reactor	31
2.2.2.	Operation of the Batch Reactor	34
2.2.3.	Medium	35
2.2.4.	Analyses	35
2.2.5.	Anaerobic ATP Maintenance Coefficient	37
2.2.6.	Stoichiometry and Kinetics of the Anaerobic Phase of the SBR	37
2.2.7.	Stoichiometry and Kinetics of the Aerobic Phase of the SBR	37
2.2.8.	Aerobic ATP Maintenance Coefficient and the P/O Ratio	38
2.2.9.	Temperature Coefficients θ	38
2.3.	Results and Discussion	39
2.3.1.	Performance of BPR in the SBR	39
2.3.2.	Storage Compounds in the Biomass Used in the Batch Tests	39
2.3.3.	Anaerobic ATP Maintenance Coefficient	41
2.3.4.	Stoichiometry and Kinetics of the Anaerobic Phase of the SBR	41
2.3.5.	Stoichiometry and Kinetics of the Aerobic Phase of the SBR	43
2.3.6.	Aerobic ATP Maintenance Coefficient and the P/O Ratio	47
2.3.7.	Temperature Coefficients	47
2.4.	Conclusions	49

Abstract

P-removing sludge was enriched in an anaerobic-aerobic, acetate fed, sequencing batch reactor (SBR) at 20°C. Conversion of relevant compounds for biological phosphorus removal was studied at 5, 10, 20 and 30°C in separate batch tests (short-term temperature effects). The stoichiometry of the anaerobic processes was insensitive to temperature changes. Some effect on aerobic stoichiometry was observed. In contrast, temperature had a strong influence on the kinetics of the processes under anaerobic as well as aerobic conditions. The anaerobic P-release (or acetate-uptake) rate showed a maximum at 20°C. However, a continuous increase was observed in the interval 5-30°C for the conversion rates under aerobic conditions. Based on these experiments, temperature coefficients for the different reactions were calculated. An overall anaerobic and aerobic temperature coefficient θ was found to be 1.078 and 1.057 (valid in the ranges 5°C ≤ T ≤ 20°C and 5°C ≤ T ≤ 30°C), respectively.

2.1. Introduction

As wastewater treatment plants, including those operating with biological phosphorus removal (BPR), may experience a sewage temperature as low as 5°C, or as high as 30°C, there is a strong need for a systematic study of the impact of temperature on BPR systems, taking into account the specific requirements of mathematical models and their application in different climates. In this chapter, the results of the study for the effects of temperature changes on both the anaerobic and the aerobic stoichiometry and kinetics of BPR are presented. The chapter describes the effects of short-term (hours) temperature changes on the physiology of the BPR system. The influence of long-term (weeks) temperature changes on the ecology of the BPR system is presented in chapter 3.

It was thought some years ago that *Acinetobacter* sp. are the microorganisms most responsible for BPR, and consequently, most of the scientific research concerning the impact of temperature was related to these particular bacteria. However, it was recently shown that *Acinetobacter* has a limited role in BPR (Wagner et al., 1994) and, therefore, the information on the P-metabolism of *Acinetobacter* alone is to be considered less relevant.

There are several publications reporting the effect of temperature on the efficiency (the difference in the influent and the effluent quality) of BPR using activated sludge. The results are inconsistent. Improved BPR efficiency at higher temperatures (in the range 20-37°C) was observed by Jones et al., (1987); Yeoman et al., (1988); McClintock et al., (1993) and Converti et al., (1995). In contrast, good or even comparatively better P-removal efficiency at lower temperatures (in the range 5-15°C) was reported by Sell et al., (1981); Kang et al., (1985); Krichen et al., (1985); Barnard et al., (1985); Vinconneau et al. (1985) and Florentz et al., (1987). However, when the kinetics of BPR processes was studied, such inconsistencies did not exist. Increased P-release and/or P-uptake rate with increased temperature was reported by Shapiro et al., (1967), Boughton et al., (1971), Spatzierer et al., (1985), and Mamais and Jenkins, (1992). In addition to P-release and P-uptake rate, Mamais and Jenkins, (1992) also reported increased growth and substrate consumption rates with an increase in temperature (10-33°C). The effect of temperature on the stoichiometry of BPR processes has never been studied in great detail.

The different results on the temperature effect on BPR with activated sludge can be explained by the use of different substrates, activated sludge and measurement methods. Temperature influences a variety of processes in activated sludge systems (lysis, fermentation, nitrification etc...) which may influence BPR processes. These effects complicate the determination of the temperature effects on BPR. In addition, most of the findings presented earlier are based on a black-box approach, comparing the influent and effluent phosphorus concentrations of wastewater treatment plants at different sewage temperatures. So far, no structured study of the effect of temperature on stoichiometry and kinetics of the BPR processes under defined laboratory conditions is available. All this explains why we presently have conflicting results, which are difficult to interpret correctly. Therefore, a study of the temperature effects on stoichiometry and kinetics of the processes

in the anaerobic and aerobic phase of the BPR under defined laboratory conditions (i.e. using an enriched culture and synthetic medium) was performed.

With the application of mathematical modeling on biological processes, the role of temperature coefficients becomes more important. Mathematical models that include the presence of BPR in activated sludge systems, such as Activated Sludge Model no.2 (ASM2) (Henze et al., 1994), University of Cape Town Activated Sludge Model (UCTPHO) (Dold et al., 1994) or the metabolic model of the BPR (Smolders et al., 1994a) rely on stoichiometric and kinetic coefficients valid in a narrow temperature range or a single temperature value only. In ASM2, process coefficients are defined for two different temperature (10 and 20°C). In this model the stoichiometric coefficients are temperature independent, while the kinetic coefficients are affected by temperature change. The processes in ASM2 are classified into four groups based on their temperature dependency (namely zero, low, medium and high dependency). Identical values at 10 and 20°C were assigned to many of the coefficients. This was justified by the scarcity of data available or by the low sensitivity of the particular parameters to variations in temperature. In this classification, the BPR processes are considered to have a low degree of temperature dependency in comparison with other processes incorporated in ASM2. ASM2 is in general recommended for application on sewage treatment by activated sludge at temperatures between 10 and 25°C, and the authors of ASM2 are cautious about its applicability outside this interval. Similarly, the UCTPHO model has process parameters based on 20°C. For other operational temperatures the adjustment of the values is computed from the input temperature and Arrhenius temperature constants. In the metabolic model all parameters are determined at 20°C, but no information is available on their temperature dependency. Therefore, it can be concluded that the effects of temperature on BPR processes are insufficiently investigated. It has been suggested that the temperature impact on the polyphosphate accumulating organisms (PAOs) can be modeled with the same coefficients as heterotrophic organisms. However, due to large differences in metabolism and involvement of storage products, this can be erroneous.

This study builds on the work of Smolders et al., (1994c) who developed a metabolic model for the P-removal processes. In this model the conversions of the BPR processes are described by six reactions. Six kinetic relations are required to describe the reaction rates.

In this chapter, the simplified Arrhenius equation (in the form as used in UCTPHO, ASM1 and ASM2) was used to describe the effect of temperature on the rate of reactions in an activated sludge system.

A duplicate set of independent batch experiments was performed at 5, 10, 20 and 30°C, using sludge from an anaerobic-aerobic sequencing batch reactor (SBR) that operated in a steady state at 20°C. The following aspects of anaerobic and aerobic metabolism were studied with respect to temperature: (1) the anaerobic ATP maintenance coefficient m_{ATP}^{an} , (2) stoichiometry and kinetics of the anaerobic phase of the SBR, (3) stoichiometry and kinetics (including oxygen consumption) of the aerobic phase of the SBR, and (4) the aerobic ATP maintenance coefficient m_{ATP}^{acr} and the phosphate/oxygen (P/O) ratio.

2.2. Materials and Methods

2.2.1. Continuous Operation of Sequencing Batch Reactor

A double jacketed laboratory fermenter (2.5 L) with automated operation, control and monitoring (Smolders et al., 1994a) was used (figures 2.1 and 2.2). A temperature of 20°C and pH 7.00 ± 0.05 were maintained in the SBR. The fermenter operated as an anaerobic-aerobic-settling SBR with a cycle of six hours. At the beginning of the experiment the SBR was inoculated with P-removing sludge from a lab-scale SBR (Kuba et al., 1993). The inoculation sludge was already enriched with PAOs under anaerobic-anoxic conditions. The initial concentration of mixed liquor suspended solids (MLSS) in the inoculum was 2800 mg/L. The sludge was cyclically exposed to anaerobic (2.25 h), aerobic (2.25 h) and settling conditions (1.5 h) (figures 2.3 and 2.4). The cycle started with the introduction of N₂ gas into the SBR to remove all dissolved oxygen remaining after the previous settling phase. As effluent had already been removed from the reactor, the SBR contained, at this particular period, only 50% (1.25 L) of its maximal operating volume. Following the start of the anaerobic phase, 1.25 L of the medium was fed to the reactor over a period of 10 min. The aerobic phase began at minute 135. At the end of the aerobic phase 78 mL of mixed liquor (as excess sludge) was removed from the reactor, resulting in an sludge retention time (SRT) of eight days. Withdrawal of sludge for batch tests was compensated by adjusting the excess sludge withdrawal from SBR in order to maintain a constant SRT. At the end of the settling period 1.17 L of supernatant (effluent) was pumped out from the reactor, again leaving a working volume of 1.25 L at the start of the cycle, and resulting in an overall hydraulic retention time (HRT) of 12 hours. The off-gas was analyzed for CO₂ and O₂. The data acquisition program Biowatch (Applikon b.v. Schiedam) was used to continuously store monitored information about the system (pH, dissolved oxygen concentration, redox potential, CO₂ and O₂ concentration of the off-gas, and cumulative acid and base addition). The SBR operated for 26 days before the temperature experiments started. According to the measurements of P-release and P-uptake, and biomass concentration in the SBR (results not presented) in this period, the reactor achieved steady state operation. The last experiment was executed on day 64. Anaerobic as well as aerobic experiments were performed in a batch reactor (as explained in paragraph 2.2.2), and the SBR was used only as a sludge source. Sludge for each of the anaerobic experiments was taken from the SBR at the end of the aerobic phase and transferred to the batch reactor; similarly, sludge for the aerobic experiments was taken from the SBR and transferred to the batch reactor at the end of the anaerobic phase. The volume of mixed liquor to be taken out from the SBR varied and was determined as the sum of the minimal operating volume of the batch reactor (120 mL) and the total sampling volume for each experiment. This sum was limited to 312 mL, which is equal to the amount of sludge wasted daily from the SBR. The batch reactor's working temperature of 5, 10, 20 or 30°C was set prior to a sludge transfer.

Figure 2.1 An anaerobic-aerobic-settling sequencing batch reactor system

Figure 2.2 Schematic presentation of the operation, control and monitoring of the SBR system with respirometer.

Figure 2.3 Schematic presentation of an anaerobic-aerobic-settling cycle of SBR

Figure 2.4 The SBR system in full operation: (a) anaerobic/aerobic phase, (b) settling phase: idle, (c) settling phase: draw, and (d) start anaerobic phase: filling-up

2.2.2. Operation of the Batch Reactor

A double jacketed laboratory fermenter with a maximal operating volume of 0.5 L was used for the execution of anaerobic and aerobic batch experiments (figure 2.5). The experiments were performed at controlled temperatures and pH (7.00 ± 0.05). pH was maintained by dosing 0.1N HCl and 0.1N NaOH. At the beginning of each experiment sludge was manually transferred from the SBR to the batch reactor. The amount of sludge removed from the SBR depended on the minimal working and/or sampling volume for the particular experiment. Sludge used in the batch reactor was not returned to the SBR. N_2 gas was introduced to the reactor at flow rate of 30 L/h during anaerobic experiments. Compressed air was bubbled through the fermenter with a flow rate of 60 L/h in aerobic experiments. The dissolved oxygen concentration in the aerobic phase was always above 50% of the saturation concentration. Mixing was provided throughout the experiments at 500 rpm.

A respirometer (figure 2.5) was connected to the batch reactor for the measurement of oxygen consumption of the biomass in time (see also Smolders et al., 1994b). Sludge was pumped from the batch reactor to the double jacketed, thermostated respirometer with a volume of 10 mL. An oxygen electrode was introduced to the respirometer. The respirometer operated with a cycle of 5 min. During a period of 1 min the batch reactor's content was pumped (circulated) through the respirometer. The pump was automatically switched off and the dissolved oxygen depletion in the respirometer was measured for 4 min. For determination of the oxygen requirements for maintenance processes, the length of the cycle was 20 min with 5 min pumping per cycle. Measured data were continuously stored in the computer. The oxygen consumption rates were calculated using linear regression, taking into account that solubility of oxygen in water varies with temperature.

2.2.3. Medium

A synthetic medium prepared with demineralized water was used as SBR's influent. The medium contained per liter (Smolders et al., 1994a): 850 mg NaAc · 3H₂O (150 mg C/L), 107 mg NH₄Cl (28 mg N), 75.5 mg NaH₂PO₄ · 2H₂O (15 mg P), 90 mg MgSO₄ · 7H₂O, 14 mg CaCl₂ · 2H₂O, 36 mg KCl, 1 mg yeast extract, 3 mg EDTA, and 0.3 mL nutrient solution.

The nutrient solution contained per liter: 1.5 g FeCl₃ · 6H₂O, 0.15 g H₃BO₃, 0.03 g CuSO₄ · H₂O, 0.18 g KI, 0.12 g MnCl₂ · 4H₂O, 0.06 g Na₂MoO₄ · 2H₂O, 0.12 g ZnSO₄ · 7H₂O, 0.15 g CoCl₂ · 6H₂O.

2.2.4. Analyses

The performance of the SBR system was monitored on a daily basis by measuring phosphate, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and, occasionally, total organic carbon (TOC). In batch experiments phosphate (P), acetate (HAc), MLSS, MLVSS, poly-hydroxy-butyrate (PHB), poly-hydroxy-valerate (PHV) and ammonium (NH₄) concentrations were measured. Orthophosphate was determined by the ascorbic acid method, TOC by gas chromatography (GC) with Hayesep Q 80-100 mesh. column at 185°C and flame ionisation detector (FID), and ammonium by atomic spectrophotometry, all in accordance with Standard Methods (APHA, 1989). MLSS and MLVSS concentration measurements were performed after filtration of a 10 ml sample of mixed liquor on a Whatman glass micro fibre filter (GC/F) filter. Dry weight was determined after the filter was dried for 24 h at 105°C and weighted on a microbalance. The ash content was calculated after incinerating the dried filter in an oven for 1 h at 550°C. The elemental composition of biomass was measured with washed and freeze dried biomass. Poly-hydroxy-alkanoate: PHA (PHB and PHV) content was measured after extraction from biomass, and using a GC with FID (Smolders et al., 1994)

Figure 2.5 The system used for batch tests: (a) experimental set-up, (b) batch reactor, and (c) vessel for respirometry

0.5-2 mg benzoic acid in 1-propanol was added to 15-20 mg washed and freeze dried biomass in closed tubes of 15 mL. 1.5 mL of a mixture of 1-propanol and concentrated HCl (4:1) and 1.5 mL di-chloromethane 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 Na₂SO₄ and 0.4 µL was injected on a GC with a stabilwax (Restek) column at 200°C with FID 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 water bath at 100°C for 1 hour. After cooling and centrifugation the glucose concentration of the supernatant was measured on a HPLC.

2.2.5. Anaerobic ATP Maintenance Coefficient $m_{\text{ATP}}^{\text{an}}$

At the end of the aerobic phase, 312 mL of mixed liquor at 20°C was manually transferred from the SBR to the batch reactor. A working temperature in the batch reactor (5, 10, 20 or 30°C) was set 1 h before the sludge transfer. Phosphate concentration was zero, as phosphate was completely taken up in the aerobic phase of the SBR. Sludge was exposed to anaerobic conditions without substrate addition and endogenous P-release was measured after 60 min in the batch reactor.

2.2.6. Stoichiometry and Kinetics of the Anaerobic Phase of the SBR

This experiment was performed immediately after the endogenous P-release was measured. Therefore, the data obtained at the end of the experiment for determination of $m_{\text{ATP}}^{\text{an}}$ were used as an initial conditions of this set of experiments. Acetate was added to the batch reactor at the beginning of the experiment. Anaerobic conditions were maintained for a period of 135 min. The amount of HAc added to the batch reactor was less at lower temperatures (from 400 mg COD/L at 30°C to 250 mg COD/L at 5°C) in order to obtain a full HAc-uptake in the anaerobic phase at each temperature without changing its 135 min length.

2.2.7. Stoichiometry and Kinetics of the Aerobic Phase of the SBR

An hour before the end of the anaerobic phase, 312 mL of mixed liquor was transferred from the SBR to the batch reactor. HAc was already fully consumed in the SBR at this point. Working temperature in the batch reactor was set to the desired value prior to transfer. Sludge was exposed to anaerobic conditions for one hour in order to simulate the remaining part of the "normal" anaerobic phase in the SBR and to adjust the sludge to the new temperature. Then, compressed air was introduced to the batch reactor for 135 min, and an extensive sampling program of dissolved substances and storage polymers was performed. For the measurement of the oxygen uptake rate by activated sludge the respirometer was connected to the batch reactor, and the oxygen consumption was simultaneously measured throughout the aerobic experiment.

The rates of different processes under anaerobic and aerobic conditions were calculated as initial rates (during the first 40 min of experiments) using the linear regression method.

2.2.8. The aerobic ATP Maintenance Coefficient $m_{\text{ATP}}^{\text{aer}}$ and the P/O Ratio

This experiment followed the experiment on aerobic stoichiometry and kinetics. As the sludge was already exposed to the aerobic environment for 135 min, the experiment continued by extended aeration over 24 h without substrate addition. A steady respiration rate in the absence of a substrate indicated the oxygen requirements for cell maintenance. Calculation of the coefficient $m_{\text{ATP}}^{\text{aer}}$ was performed according to the metabolic model (Smolders et al., 1995b). The parameter δ (P/O, phosphate /oxygen ratio) was required for determination of ATP needed for maintenance under aerobic conditions. The P/O ratio was established by studying the effect of P-uptake and poly-P formation on the oxygen consumption in the batch reactor. The oxygen/phosphate yield was calculated from the oxygen consumption in the absence and presence of phosphate. Based on this yield, the P/O ratio (δ value) was established (a calculating method is described in detail by Smolders et al., 1995).

2.2.9. Temperature Coefficients θ

The effect of temperature on a rate constant relative to a standard temperature (here 293 °K) can, for small temperature differences, be expressed by the modified Arrhenius equation:

$$r_T = r_{293} \cdot \exp \{ [\Delta G_{\text{act}} \cdot (293-T)/R \cdot 293 \cdot T] \} \quad \dots (2.1)$$

In the literature this relation is often further simplified to:

$$r_T = r_{293} \cdot \theta^{(T-293)} \quad \dots (2.2)$$

where the r_T is reaction rate at temperature T , ΔG_{act} is energy of activation (J/mol), R is gas constant (J/mol. °K), T is temperature in °K, and θ is temperature coefficient.

In this study the simplified Arrhenius equation is used which allowed the results to be compared with the temperature coefficients of different processes in the mathematical models; the equation is also well suited for fitting the results. In spite of the fact that the modified Arrhenius equation is applicable for small temperature differences, and that the largest temperature difference in this study is 15°C (20°C - 5°C), the application of the simplified Arrhenius equation in this study gives only a 0.5% difference compared with the Arrhenius equation in its modified form.

2.3. Results and Discussion

2.3.1. Performance of BPR in the SBR

The system was inoculated on day 0 with concentrated sludge from an anaerobic-anoxic SBR. The initial concentration of MLSS was 2.8 g/L. After one week the MLSS and MLVSS concentration became stable at average of 2.65 and 2.0 g/L, respectively. This resulted in an average MLVSS/MLSS ratio of 0.75.

Phosphate monitoring showed that 100% removal was achieved immediately after inoculation. This pattern was maintained throughout the experiment. The maximum P-release in the anaerobic phase gradually increased from 68 mg P/L on day 0 to 80 mg P/L on day 28, and remained around that value to the end of experiment. Acetate was always fully consumed in the anaerobic phase and nitrification was absent throughout the experiments.

2.3.2. Storage Compounds in the Biomass Used in the Batch Tests

The biomass composition in batch experiments at four different temperatures is presented in tables 2.1 and 2.2.

Table 2.1 Biomass composition in the batch reactor at 5, 10, 20 and 30°C at the beginning and the end of anaerobic batch experiments.

Parameter	Unit	Start Anaerobic Phase					End Anaerobic Phase			
Temperature	°C	5	10	20	20 ^(b)	30	5	10	20	30
MLSS	mg/L	2820	2490	252	2620	2870	2538	2340	2400	2320
MLVSS	mg/L	2065	1790	858	1937	2170	2120	1967	2163	2140
MLVSS/MLSS		0.73	0.71	0.7	0.74	0.76	0.84	0.84	0.9	0.92
PHB	mg/L	61	52	59	61	54	245	301	407	372
PHV	mg/L	8	7	13	6	7	21	25	41	40
PHA ^(a)	mg/L	69	59	72	67	61	266	326	448	412
Glycogen ^(b)	mg/L	305	305	305	305	305	224	224	224	224
Active biomass ^(c)	mg/L	1691	1426	148	1498	1754	1630	1417	1491	1504

(a) PHA=PHB+PHV, (b) Smolders et al., (1995), and (c) Active biomass=MLVSS-PHB-Glycogen

As mentioned earlier, for the anaerobic experiments sludge was taken at the end of the aerobic phase of the SBR. The SBR operated close to steady-state conditions, therefore the

starting MLSS concentration should have been similar. However, a dilution effect occurred due to the addition of HAc in different amounts. Therefore the MLSS concentrations at the start of the experiments varied.

In addition to this, one could expect that the biomass composition at the end of the anaerobic experiment (table 2.1) and at the beginning of the aerobic experiment (table 2.2) should be equal. This is not the case here due to the fact that the aerobic experiments were not performed immediately after the anaerobic, and that the biomass concentrations varied slightly during the 36 days (duration of experiments) due to sludge extraction from the SBR.

Table 2.2 Biomass composition in the batch reactor at 5, 10, 20 and 30°C at the beginning and the end of aerobic batch experiments.

Parameter	Unit	Start Aerobic Phase				End Aerobic Phase			
Temperature	°C	5	10	20	30	5	10	20	30
MLSS	mg/L	2500	2470	2520	2540	2610	2600	2740	2540
MLVSS	mg/L	1950	1970	2020	2010	1990	1995	2080	1885
MLVSS/MLSS		0.81	0.79	0.8	0.79	0.76	0.76	0.76	0.74
PHB	mg/L	188	202	174	193	140	108	61	62
PHV	mg/L	41	22	32	37	22	12	6	8
PHA ^(a)	mg/L	229	224	206	230	162	120	67	70
Glycogen ^(b)	mg/L	224	224	224	224	318	318	318	318
Active biomass ^(c)	mg/L	1497	1522	1590	1556	1510	1557	1695	1497

(a) PHA=PHB+PHV, (b) Smolders et al., (1995), and (c) Active biomass=MLVSS-PHB-Glycogen

It was also assumed that the active biomass concentration during the anaerobic period should be almost constant. However, due to difficulties in measuring glycogen, the glycogen values reported by Smolders et al., (1995) were introduced in both anaerobic and aerobic experiments for all investigated temperatures.

These experiments and the experiments of Smolders et al. (1995) were performed under identical conditions, and the concentrations of other compounds were similar at 20°C between both researchers. The active biomass was calculated here as a difference between MLSS and PHB and glycogen. The inert material of the cells was neglected in this case.

Based on the values from tables 2.1 and 2.2 the anaerobic and aerobic conversions of relevant components were calculated and presented in table 2.3. A decrease in MLSS during anaerobic incubations is due to loss of poly-P and its comparatively higher molecular weight than utilized acetate.

Table 2.3 Production or consumption (-) of the components in the anaerobic and aerobic batch experiments at 5, 10, 20 and 30°C and SRT of 8 days.

Parameter	Unit	Conversions in the Anaerobic				Conversions in the			
Temperature	°C	5	10	20	30	5	10	20	30
MLSS	mg/L	-282	-150	-125	-550	110	130	220	0
MLVSS	mg/L	55	177	305	30	40	25	60	-125
PHB	mg/L	184	249	348	318	-48	-94	-113	-131
PHV	mg/L	13	18	28	33	-19	-10	-26	-29
PHA	mg/L	197	267	376	351	-67	-94	-139	-160
Glycogen	mg/L	-81	-81	-81	-81	94	94	94	94
Active biomass	mg/L	-61	-9	10	-250	13	35	105	-59

2.3.3. The Anaerobic ATP Maintenance Coefficient m_{ATP}^{an}

Based on the measurements of endogenous P-release in absence of substrate under anaerobic conditions, the specific maintenance coefficient m_{ATP}^{an} was calculated for each of the operating temperatures (table 2.4). The concentration of phosphorus in the solution measured after one hour increased from 0.4 mg P/L at 5°C to 7.6 mg P/L at 30°C. The release rate of phosphate during this period was taken as the energy requirements for the anaerobic maintenance (Smolders et al., 1994a). Consequently, m_{ATP}^{an} was in the range $0.3 \cdot 10^{-3}$ - $3.6 \cdot 10^{-3}$ mol ATP/C-mol act.biomass.h. At 20°C m_{ATP}^{an} was found to be $1.47 \cdot 10^{-3}$ mol ATP/C-mol act.biomass.h, 40% less than reported by Smolders et al, 1995, ($2.5 \cdot 10^{-3}$ mol ATP/C-mol act.biomass.h).

2.3.4. Stoichiometry and Kinetics of the Anaerobic Phase of the SBR

Patterns of all measured parameters in the experiments are displayed in figure 2.6 . The anaerobic stoichiometric and kinetic parameters are determined for each operating temperature and are summarized in table 2.4 and figure 2.7.

According to table 2.4, the P-release/HAc-uptake ratios (0.36-0.48 mg P/mg) were lower than experimentally observed by Smolders et al., (1994a) (0.52 mg P/mg). The temperature has little impact on this ratio. The measured value of endogenous P-release was subtracted from P-release achieved in the presence of HAc in order to obtain values of P-release exclusively as a result of HAc-uptake (net P-release). Ammonia concentrations remained almost unchanged during test (data not shown). PHA-formation/HAc-uptake ratios were in the range 0.73-1.01 mg/mg. At 20°C a good agreement was found between the value of 0.93 mg/mg reported by Smolders et al., 1994a and the value determined here 0.90 mg/mg. Again, the temperature has very little effect on this ratio.

Table 2.4 Summary of stoichiometric and kinetic parameters for anaerobic batch experiments at 5, 10, 20 and 30°C and SRT 8 days.

Parameter	Unit/°C	5	10	20	20 ^(a)	30
Stoichiometric parameters ^(b)						
P release/HAc uptake ratio	mg P/mg	0.48±0.21	0.48±0.03	0.38±0.05	0.52	0.36±0.11
PHA production/HAc uptake	mg/mg	0.73±0.24	0.94±0.29	0.90±0.12	0.93	1.01±0.15
Kinetic parameters ^(b)						
HAc uptake/active biomass	mg/mg.h	0.054±0.016	0.100±0.039	0.197±0.024	0.3	0.149±0.034
P release/active biomass rate	mg P/mg.h	0.028±0.002	0.053±0.017	0.076±0.022	0.1	0.055±0.019
PHA production/active	mg/mg.h	0.073	0.155	0.292	0.27	0.218
ATP maintenance coefficient	m ATP/mg.h	0.0003	0.0006	0.00147	0	0.00363

(a) Smolders et al., (1995), and (b) Where possible the confidence interval of 95% is associated with measured values.

Figure 2.6 Phosphate (■), acetate (●) and PHB (▲) concentrations in anaerobic batch experiments. At time ($t=0$ min) acetate was instantly added to the reactor.

Although the anaerobic stoichiometric coefficients appeared to be quite insensitive to temperature changes, a strong temperature dependency of anaerobic kinetic rates, acetate

uptake and anaerobic maintenance, was clearly observed. According to table 2.4, taking into account a confidence interval of 95%, the observed rates of HAc-uptake in general were close at 10 and 30°C, maximal at 20°C and minimal at 5°C. Thus, the temperature optimum for anaerobic HAc-uptake was found to be around 20°C. The anaerobic maintenance strongly increased with increased temperature.

2.3.5. Stoichiometry and Kinetics of the Aerobic Phase of the SBR

The measured parameters in the aerobic batch experiments are given in figures 2.8 and 2.9. Aerobic stoichiometric and kinetic parameters are summarized in table 2.5 and figure 2.10.

STOICHIOMETRIC PARAMETERS

KINETIC PARAMETERS

Figure 2.7 Stoichiometric and kinetic parameters of the anaerobic phase of BPR. The confidence interval of 95% (-) is associated with observed data (■). The simplified Arrhenius equation was fitted to the data with temperature coefficient θ (solid line) within the range of standard error (dashed lines).

Figure 2.8 Phosphate (■), ammonium (□) and PHB (▲) concentrations in aerobic batch experiments.

Figure 2.9 Oxygen respiration rates of the biomass in aerobic batch experiments at 5, 10, 20 and 30°C.

In this case figures 2.8 (a to d) can be directly compared, because the sludge concentration is the same in each incubation. P-uptake was completed in 60 minutes at 30°C while at 5°C only 40% of anaerobically released P was utilized in the same period of time.

According to table 2.5 and figure 2.10, the temperature had a moderate effect on aerobic stoichiometric coefficients, such as PHA, P and NH_4 -uptake, per amount of O_2 consumed. The PHA/oxygen ratio was rather steady in the temperature range 10-30°C. However, the value at 5°C is 45% higher than an average in the temperature range 10-30°C due to reasons still unknown. The NH_4 /oxygen ratio shows no consistent trend due to small differences in measured values and difficulties in measurement technique. Glycogen formed/oxygen consumed ratio of 1.08 mg C/mg O_2 at 20°C was 12% higher than the value found by Smolders et al., 1995. This is probably due to the assumption on glycogen described earlier, and a comparatively lower total O_2 consumption at 20°C. Other aerobic kinetic and stoichiometric parameters are in the range of the values reported by Smolders et al. (1994c).

Table 2.5 Summary of stoichiometric and kinetic parameters for aerobic batch experiments at 5, 10, 20 and 30°C and SRT 8 days.

Parameter	Unit\°C	5	10	20	20 ^(a)	30
Stoichiometric parameters ^(b)						
PHA consumption/ O_2 consumption ratio	mg/mg O_2	3.23±0.55	1.86±0.48	2.02±0.34	2	1.51±0.17
O_2/PO_4 ratio	mg O_2 /mg P	no data	no data	0.37	0.32	no data
P uptake/ O_2 consumption ratio	mg P/mg O_2	1.02±0.29	1.12±0.14	1.16±0.12	1.01	0.93±0.06
NH_4 consumption/ O_2 consumption ratio	mg/mg O_2	0.033±0.015	0.011±0.01	0.056±0.005	0.1	0.028±0.01
Glycogen formation/ O_2 consumption ratio	mg/mg O_2	no data	no data	1.08	0.96	no data
Glycogen formation/ PHA consumption ratio	mg/mg	no data	no data	0.68	0.51	no data
Kinetic parameters ^(b)						
P uptake/ active biomass rate	mg P/mg.h	0.012±0.004	0.022±0.004	0.044±0.007	0.1	0.055±0.005
O_2 consumption/ active biomass rate	mg O_2 /mg.h	0.012±0.002	0.020±0.002	0.038±0.004	0	0.059±0.006
NH_4 consumption/ active biomass rate	mg/mg.h	0.0004±0.003	0.0002±0.001	0.0021±0.004	0	0.0033±0.0033
PHA consumption/ active biomass rate	mg/mg.h	0.039±0.003	0.038±0.003	0.077±0.004	0.1	0.089±0.007
ATP maintenance coefficient	mol/mol.h	0.0019	0.0021	0.0032	0	0.0071

(a) Smolders et al., (1995), and (b) Where possible the confidence interval of 95% is associated with measured values

STOICHIOMETRIC PARAMETERS

KINETIC PARAMETERS

Figure 2.10 Stoichiometric and kinetic parameters of the aerobic phase of BPR. The confidence interval of 95% (-) is associated with experimentally observed data (■). The simplified Arrhenius equation was fitted to the data with temperature coefficient θ (solid line) within the range of standard error (dashed lines).

Similarly as concluded earlier for the anaerobic kinetics, the aerobic rates, such as P, NH_4 and PHA consumption per active biomass, were strongly influenced by temperature change. However, in contrast to the anaerobic kinetic rates, where an optimal temperature range was found, all aerobic kinetic rates for the tested temperature range increased with increase in the temperature (table 2.5 and figure 2.10).

It was found that the temperature has a large impact on the oxygen consumption rate in BPR system (figure 2.9). At 20 and especially at 30°C the overall trend of the oxygen consumption rate sharply changed after 75 and 60 min, respectively. This is attributed to the fact that complete P-uptake was observed at this time (figure 2.4c and 2.4d). This means that during the remaining part of the aerobic phase oxygen is not used for poly-P formation, but only for glycogen production, biomass growth and maintenance. At 5 and 10°C this pattern could not be observed due to incomplete P-uptake in the aerobic phase.

2.3.6. The Aerobic ATP Maintenance Coefficient $m_{\text{ATP}}^{\text{aer}}$ and the P/O Ratio

The oxygen consumption by the sludge during 24 h in absence of external substrate was measured. The oxygen utilization rate became stable after about 12 h at all temperatures. This is considered as the oxygen consumption rate for maintenance purposes. Observed oxygen respiration rates after approximately 12 h were in the range 4.06 mg $\text{O}_2/\text{L.h}$ (at 5°C) to 15.01 mg $\text{O}_2/\text{L.h}$ (at 30°C). The rate of 7.26 mg $\text{O}_2/\text{L.h}$ at 20°C was 10% lower than observed by Smolders et al., (1995). The oxygen consumption for maintenance purposes (m_o) was calculated for all working temperatures. The m_o increased with the temperature increase and was in the range $2.69 \cdot 10^{-3}$ – $9.83 \cdot 10^{-3}$ mg/mg.h. The maintenance PHA consumption per C-mol biomass (m_s) was within the range $1.60 \cdot 10^{-3}$ – $5.85 \cdot 10^{-3}$ mg PHA/mg.h. The δ value of 1.46 (1.85 reported by Smolders et al., 1995) needed for determination of $m_{\text{ATP}}^{\text{aer}}$ was calculated using experimentally observed O_2/PO_4 ratio at 20°C of 0.37 mg O_2/mgP . As the δ values for other temperatures are not known, the value of 1.46 for 20°C is used to calculate $m_{\text{ATP}}^{\text{aer}}$ for other operating temperatures. The ATP energy required for aerobic maintenance per C-mol active biomass of 0.012 mol ATP/Cmol.h is eight times higher than the anaerobic maintenance coefficient (0.0015 mol ATP/C-mol.h), due to reasons still unknown. An identical observation was reported earlier by Smolders et al., 1995.

2.3.7. Temperature Coefficients

Based on values from tables 2.4 and 2.5, using the simplified Arrhenius equation for the temperature dependency, the temperature coefficient θ was calculated for each reaction rate (table 2.6) by fitting the data to the equation (figure 2.7 and 2.10).

The rates of reaction in the anaerobic phase of BPR showed a maximum at 20°C. Therefore, only the rates at 5–20°C were fitted to the equation. Temperature coefficients are valid in the range 5–30°C for the rates of reaction in the aerobic phase.

Table 2.6 Temperature coefficients θ with standard error for the reaction rates of the anaerobic and aerobic BPR processes.

Anaerobic phase	$\theta \pm \text{st. error}$	Aerobic phase ^(b)	$\theta \pm \text{st. error}$
HAc uptake rate/ active biomass ^(a)	1.080±0.011	P uptake rate/ active biomass	1.048±0.012
P release rate/ active biomass ^(a)	1.055±0.019	O ₂ consumption rate/ active biomass	1.057±0.006
PHA formation rate/ active biomass ^(a)	1.081±0.017	NH ₄ consumption rate/ active biomass	1.081 ±0.026
ATP maintenance coefficient ^(b)	1.096±0.003	PHA consumption rate/ active biomass	1.035±0.00
		ATP maintenance coefficient	1.064±0.009
Average anaerobic temperature coefficient		Average aerobic temperature coefficient	

(a) Valid in the temperature range 5-20°C, and (b) valid in the temperature range 5-30°C

In the case of BPR, especially for the aerobic part, it is a very difficult task to establish the correct temperature coefficients, due to the complexity of the processes. According to the metabolic model, the anaerobic phase consists of two processes where acetate-uptake is more important than maintenance with respect to the overall metabolism. Therefore, the situation can be simplified by using only one temperature coefficient θ (for HAc-uptake rate) which consequently means that the temperature has no influence on stoichiometry. However, in the aerobic phase of BPR there are four independent rate temperature coefficients (growth, P-uptake, glycogen formation and maintenance), while for "normal" organisms there is only one rate coefficient (growth). If one or more of temperature coefficients vary, it will result in a complex interaction between processes. This makes it difficult to predict in which direction the situation will develop. Taking into account the standard errors, for the purpose of mathematical modeling, average anaerobic and aerobic θ of 1.078 and 1.057, respectively, are recommended.

According to the classification of temperature coefficients in ASM2, and the θ of BPR found here, BPR has a low to medium degree of temperature dependency. In comparison to the temperature coefficients in ASM2, an average θ of the anaerobic and aerobic phase of BPR determined in this study, indicates that the temperature has much less effect on BPR processes than on, for example, nitrification ($\theta=1.120$). Furthermore, it can be concluded that the temperature has a similar impact on BPR as on heterotrophs or fermentation ($\theta=1.070$). However, θ values given in ASM2 are based only on a general knowledge of temperature impact on various biological processes.

2.4. Conclusions

An exposure of the microorganisms responsible for BPR to temperature change from 20°C to 5, 10 and 30°C showed that the stoichiometry of the anaerobic processes was insensitive towards temperature changes. Some effect on aerobic stoichiometry was observed. In contrast, temperature had a strong influence on the kinetics of the processes under anaerobic as well as aerobic conditions. The anaerobic phosphorus-release (or acetate-uptake) rate showed a maximum at 20°C. However, a continuous increase was observed in the interval 5-30°C for the conversion rates under aerobic conditions. Based on these experiments, temperature coefficients for the different reactions were calculated. An overall anaerobic and aerobic temperature coefficient θ was found to be 1.078 (valid in the range 5°C < T < 20°C) and 1.057 (5°C < T < 30°C), respectively.

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3 Chapter

Influence of Temperature on Biological Phosphorus Removal: Process and Molecular Ecological Studies

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Contents

3.1. Introduction	55
3.2. Materials and Methods	56
3.2.1. Continuous Operation of SBR	56
3.2.2. Molecular Ecological Methods	57
3.2.2.1. Genomic DNA Extraction	58
3.2.2.2. PCR Amplification	58
3.2.2.3. Agarose Gel Electrophoresis	59
3.2.2.4. DDGGE Analysis	59
3.3. Results	59
3.3.1. Performance of BPR in the SBR at 5, 10, 20 and 30°C	59
3.3.2. Stoichiometry and Kinetics	62
3.3.3. Biomass Composition	64
3.3.4. Temperature Coefficients θ	68
3.3.5. Molecular Ecological Studies	69
3.4. Discussion	71
3.5. Conclusion	73

Abstract

This chapter describes the impact of long-term (weeks) temperature changes on stoichiometry and kinetics of the anaerobic and aerobic phases of BPR processes. Steady state conversion of relevant compounds for BPR was studied by adapting reactors in subsequent periods to 20, 30, 20, 10 and 5°C. Integrated in the process study, two methods (electron-microscopy and dry denaturing gradient gel electrophoresis) were applied to investigate the composition of the bacterial community of biological phosphorus removing sludge developing at those different temperatures. The temperature coefficient for metabolic conversions obtained from long-term temperature tests was similar to the temperature coefficient observed in short-term (hours) tests ($\theta=1.085$ and $\theta=1.078$, respectively). Temperature had a moderate impact on the aerobic P-uptake process rate ($\theta=1.031$) during long-term tests. However, a strong temperature effect on other metabolic processes of the aerobic phase, such as PHA consumption ($\theta=1.163$), oxygen uptake ($\theta=1.090$) and growth ($\theta>1.110$), was observed. Different temperature coefficients were obtained for the aerobic phase from long-term and short-term tests, probably due to a change in population structure. This change was also visible from molecular ecological studies. The different temperature coefficient found for P-uptake compared to the other metabolic processes of the aerobic phase underlines that in complex processes such as BPR, it is dangerous to draw conclusions from easily observable parameters (like phosphate) only. Such consideration can easily lead to underestimation of the temperature dependency of other metabolic processes of the aerobic phase of BPR..

3.1. Introduction

It is generally accepted that the biological reaction rate coefficients of biological wastewater treatment processes have temperature dependencies, which can be expressed by a simplified Arrhenius equation (Henze et al., 1994). Effect of temperature on biological phosphorus removal process (BPR) has been studied during recent years (i.e. Mamais and Jenkins, 1992, and McClintock et al., 1993), however, there has been no systematic study on the effect of temperature on both stoichiometry and kinetics of the anaerobic and aerobic metabolism of BPR processes under defined laboratory conditions.

In order to obtain detailed information about the effect of temperature on stoichiometry and kinetics of the anaerobic and aerobic BPR metabolic processes a study has been carried out, using an enriched culture cultivated in an anaerobic-aerobic-settling sequencing batch reactor (SBR) and synthetic substrate. The study was performed in two parts. The first part, described in chapter 2, investigated the effect of so-called short-term temperature changes on BPR processes in the anaerobic phase, such as acetate uptake, phosphate release, polyhydroxyalkanoate (PHA) synthesis and maintenance, and the BPR metabolic processes occurring in the aerobic phase, such as phosphate uptake, PHA and oxygen consumption, growth and maintenance.

It can be assumed that during short-term temperature experiments the BPR population structure did not change. However, if microorganisms are exposed to changes in temperature for a relatively long time (couple of weeks), the microbial population may adapt to the new process conditions and a change in population composition may occur. This change may lead to different temperature coefficients for the processes in the anaerobic and aerobic phases, as observed in short-term temperature experiments.

This chapter describes the second phase of a study in which the impact of long-term temperature changes, on stoichiometry and kinetics of anaerobic and aerobic BPR processes was investigated by monitoring the performance of phosphate accumulating organisms (PAOs) in a SBR at different temperatures (20, 30, 20, 10 and 5°C, following chronological order). The paper addresses the following questions: (1) what is the difference between temperature coefficients of BPR processes under short-term and long-term changes in temperature?, and (2) are the same groups of microorganisms responsible for the BPR at high and low temperatures? In other words, can temperature coefficients for reaction rates of BPR, obtained from short-term temperature experiments, be reliably used for modeling purposes, without execution of long-term experiments?

In this study the basic stoichiometric and kinetic parameters of the anaerobic and aerobic metabolism of the BPR processes, including their temperature dependency, were determined. Moreover, complete phosphorus removal was achieved under steady state SBR

operation in a relatively wide temperature range (5-30°C), providing the precondition for studying diversity of the microbial population developed in the reactor. All attempts so far to isolate microorganisms responsible for BPR failed (Van Loosdrecht et al., 1997, and Mino et al., 1997), and furthermore, there is no information available on the structure of the microbial population responsible for BPR at extreme temperatures. In this study the presence of different 16S rDNA genes as an indication of the complexity and composition of bacterial communities was analyzed by molecular ecological methods. By this molecular ecology approach it is possible to monitor how the microbial community composition changes depending on the operating temperature of the reactor.

3.2. Materials and Methods

3.2.1. Continuous Operation of SBR

A double jacketed laboratory anaerobic-aerobic-settling SBR (2.5 L) with automated operation, control and monitoring (Smolders et al., 1994), that operated in a cycle of six hours at controlled temperature and pH 7.00 ± 0.05 and sludge retention time (SRT) of 8, 16 and 32 days, was used in this study. A detailed description of the SBR cycle operation is given in paragraph 2.2.

Figure 3.1 Operating schedule of the SBR. The day in the boxes represent the day when the operating temperature (and in last three cases the SRT) was changed and when the "steady-state" and "the first cycle" measurements took place

The experimental procedure is presented in Figure 3.1. Following inoculation the SBR was operated at 20°C and SRT of 8 days for 22 days. Once steady state operation was achieved at 20°C, the operating temperature was switched to 30°C. The SBR operated at 30°C until, again, a steady state operation was achieved. Then the temperature was switched back to

20°C, and following the same criterium, to 10°C, and finally to 5°C. At each of these temperatures detailed measurements of the cycle under steady state operation was performed. In addition to this, each time when the SBR's operating temperature was changed, the first cycle at the new temperature was monitored too. Eight days SRT was maintained at 20 and 30°C, while the SRT had to be extended to sixteen days at 10°C, and thirty two days at 5°C, in order to achieve full BPR efficiency. For detailed description of media composition, respirometry and analytical procedures the reader is referred to chapter 2.

The simplified Arrhenius equation was used to describe the effect of temperature on the rate of reactions in an activated sludge system by fitting the equation to the data. This relation can be expressed as (see also eq. 2.2):

$$q_T = q_{293^{\circ}\text{K}} * \theta^{(T-293^{\circ}\text{K})} \quad \dots (3.1)$$

with q_T = active biomass specific reaction rate at temperature T; $q_{293^{\circ}\text{K}}$ = active biomass specific reaction rate at 293°K (20°C); T = temperature in°K; and θ = temperature coefficient. The reference $q_{293^{\circ}\text{K}}$, calculated as an average of $q_{293^{\circ}\text{K}}$'s obtained from long term and short-term tests, was used in a fitting procedure to obtain θ for each particular biomass-specific process rate.

3.2.2. Molecular Ecological Methods

The molecular ecological methods applied in this study consist of three major steps: (1) genomic DNA extraction from mixed bacterial population, (2) enzymatic amplification step (polymerase chain reaction - PCR) of the target 16S rRNA gene including a gel electrophoretical control of the reaction efficiency, and (3) analysis of this PCR fragments on dry denaturing gradient gel electrophoresis - DDGGE.

The DDGGE (modification of DGGE method developed by Myers et al., 1987) is a diagnostic tool to investigate differences in melting behavior of DNA fragments which were synthesized by PCR technique. The melting behavior of DNA fragments is directly depending on the DNA sequence. An increasing concentration of DNA denaturing agents (formamide and urea) in the gel leads to retardation of DNA fragments at a certain position within the gel. Differences in melting behavior result in an earlier or later denaturation of the particular DNA fragment and by that in a different position in a DDGGE gel. An extended form of this method (Sheffield et al., 1989) is currently introduced into the field of molecular ecology of bacteria (Muyzer et al., 1992 and Muyzer et al., 1995).

The usual target gene for such molecular ecological studies is the gene encoding the genetical information for the rRNA of the small subunit of ribosomes (translation

machinery of a cell). The partial sequence variation within the 16S rDNA genes in bacteria make them ideal targets for molecular ecological experiments such as DDGGE. Special characteristic of this particular gene is the fact, that it consists of conserved and variable sequence regions (Stackebrandt and Rainy, 1995). The conserved regions of the gene are very homologous in all bacteria, whereas the variable regions show a high degree of sequence difference. The 16S rRNA genes are identical for the same species of bacteria, but are different for different bacterial species. The degree of difference is used as a tool in bacterial phylogenetic (Ward et al., 1992).

PCR is a molecular technique which amplifies DNA sequences manyfold depending on specific primers (Saiki et al., 1988). Those primers are very small DNA pieces which are complementary to a certain stretch of DNA. PCR fragments synthesized from 16S rRNA genes, originating from a mix of different individuals (such as a bacterial community from activated sludge) consist of a mix of different PCR fragments, and each PCR fragment having (slightly) different DNA sequence displays a different electrophoretic mobility.

DDGGE checks the composition of sludge independent from any reactor parameters at the genetical level of the bacterial community. In the present study this method was applied to investigate firstly, whether the sludge consists of more than one type of bacteria and secondly, whether the bacterial community does change depending on reactor temperature. In general DDGGE does not lead to identification of bacteria involved, but results in a characteristic pattern on a DDGGE gel. Besides, it does not require information on the bacterial community, works very well for qualitative comparative analysis but should not be used for quantitative interpretations.

3.2.2.1. Genomic DNA Extraction

Bacterial cells from 2 ml of culture were harvested by centrifugation, resuspended in 250 µl of cocktail (0.1 M Tris/HCl, pH 8.0; 20 mg/mL RNase; 120 mg/mL lysozym; 40 mg/mL protease). After 10 min. at 37°C one volume of lysis buffer (50 mM Tris/HCl; 1 % SDS; 5 mM NaCl; 2.5 mM EDTA) was added and further incubated at 37°C for 10 min. This mixture was three times successively extracted with each one volume of phenol; phenol-chloroform-isoamyl alcohol [24:24:1 (by volume)] and chloroform. After centrifugation, the DNA was ethanol precipitated, pelleted, washed with 70 % ethanol, dried, dissolved in 50 µl TE buffer (10 mM Tris; 1 mM EDTA) and monitored by agarose gel electrophoresis (Sambrook et al., 1989).

3.2.2.2. PCR Amplification

PCR amplifications were performed in a RoboCycler® Gradient 40 cycler (Stratagene, United States) as follows: In 100 µl volume 100 ng template DNA, 200 µM of the primer

7-Bam (5'-CACGGATCCAGAGTTTGATYMTGGCTC-3') and reverse primer 907R (5'-CCGTCAATTCATTTGAGTTT-3'), 200 μ M dNTPs, 1x PCR buffer (supplier), 1 mM $MgCl_2$ and 2 U of Goldstar DNA Polymerase (Eurogentec, Belgium) overlaid with a drop of mineral oil (Sigma Chemicals Co., Ltd.). The amplification starts with 3 min denaturation continued by 30 cycles of: 1,5 min at 94°C for denaturation, 2 min at 48°C for annealing and 2 min at 72°C for extension. An additional synthesis step was performed for 5 min at 72°C. The PCR products were analyzed by agarose gel electrophoresis.

3.2.2.3. Agarose Gel Electrophoresis

Aliquot of extracted genomic DNA (2 μ l) and PCR amplification products (10 μ l) were analyzed by electrophoresis on 1% (w/v) agarose gels (Sea KEM®LE, FMC Bio Products, USA) containing 0,5 μ g/ml ethidium bromide in 1x TBE buffer. Lambda DNA/EcoRI and HindIII Marker (Promega) was used as a size standard. Bands were visualized on an UV transilluminator (Vilber Lourmat, France) and photographed with Polaroid 667 films.

3.2.2.4. DDGGE Analysis

DDGGE was performed using a Macrohor Electrophoresis Unit and a Thermostatic Plate (Pharmacia Biotech, Sweden) and a glass plate. PCR samples were concentrated by ethanol precipitation and electrophoretically separated on a 5.6 % polyacrylamide gel in 1xTBE buffer containing a linearly increasing gradient between 25% and 55% of denaturant [100% denaturant is 7 M urea and 40 % (w/v) form amide]. The gel was poured using a two chambers gradient mixer (2x13,5 ml). Electrophoresis was carried out in 1xTBE buffer at 50 mA for 155 min at 60°. After electrophoresis, the gel was stained for 15 min in ethidium bromide (0,5 mg/l) and bands were visualized as described above.

3.3. Results

3.3.1. Performance of BPR in the SBR at 5, 10, 20 and 30°C

The system was inoculated on day 0 with sludge from an anaerobic-anoxic SBR operated at a SRT of eight days. The initial concentration of mixed liquor suspended solids (MLSS) was 3.1 g/L. After two weeks MLSS and mixed liquor volatile suspended solids (MLVSS) concentration became stable at 2.55 and 1.9 g/L, respectively. This resulted in an average MLVSS/MLSS ratio of 0.75. Observed values are very similar to the results obtained under identical operating conditions by Kuba et al., (1993), Smolders et al., (1994), and Brdjanovic et al., (1996) and (1997a).

Phosphate monitoring showed that 100% removal was achieved immediately after

inoculation. The maximum P-release in the anaerobic phase gradually increased from 60 mgP/L on day 0 and became stable at 80 mgP/L on day 22, when the first cycle measurements were performed.

Dynamic patterns of phosphate and acetate in anaerobic/aerobic SBR cycles at different temperature are shown in figure 3.2. Results of cycle measurements under steady state SBR operation at 20°C and SRT of eight days (called standard cycle, figure 3.2a), are considered as typical for such an experimental set-up (Kuba et al., 1993, Smolders et al., 1994c, and Brdjanovic et al., 1997a). Complete phosphate removal was achieved, acetate (75 mgHAc-C/L) was fully consumed anaerobically, and nitrification was absent at all SRTs. The same pattern was maintained in the first cycle when the temperature was switched from 20 to 30°C (figure 3.2b), as well as under SBR steady state operation at 30°C (figure 3.2c). following the second temperature switch, the first cycle measurements at 20°C showed somewhat incomplete P-uptake at the end of the aerobic phase (figure 3.2d), however full BPR efficiency was already reestablished in the next cycle. Under steady state operation at 20°C (figure 3.2e) the system performed similarly as in a standard cycle. When the temperature was changed from 20 to 10°C incomplete P-uptake occurred again, however this time the BPR efficiency was not reestablished in the following cycles (figure 3.2f). At 10°C and SRT of eight days only a small portion of acetate (15%) was consumed anaerobically, the remaining part was utilized during aerobic phase resulting in further deterioration of BPR (figure 3.2g). As net P-removal was absent, phosphorus accumulated in the system resulting in a high effluent phosphate concentration (19 mgP/L). A high MLVSS/MLSS ratio (0.92) and a poor performance of the system suggested wash-out of PAOs from the reactor. When the SRT was prolonged from eight to sixteen days the system gradually recovered regaining full activity (figure 3.2h). Following a change of temperature from 10 to 5°C an identical scenario occurred as when the temperature was switched from 20 to 10°C (dramatic slow-down of process rates, incomplete anaerobic HAc-uptake and absence of net P-removal). Again the SRT had to be prolonged in order to achieve full BPR, this time from sixteen to thirty two days (figure 3.2k). During satisfactory performance of BPR the concentration of anaerobically released phosphate varied between 75 and 125 mgP/L. This is partly due to different P-release for maintenance as a result of temperature changes and different SRTs. Such variation was observed earlier by Smolders et al. (1995), Kuba et al (1996) and Brdjanovic et al (1996) who operated SBRs over a long period of time at a SRT of eight days and 20°C.

Respirometry was performed during the aerobic phase at each operating temperature and the results are presented in figure 3.3. A typical sharp drop in oxygen utilization rate (OUR) occurred at the moment when phosphate uptake was completed (figure 3.3a, b, c, e, g and i). From that moment on, oxygen was only used for glycogen synthesis, growth and maintenance [Smolders et al (1994c)]. In general, lowering of the temperature strongly decreased the OUR in the first cycles (figure 3.3d, f and h). The total oxygen consumption

in the experiments increased at lower temperature due to increase of biomass concentration in the reactor.

Figure 3.2 Acetate (■) and phosphate (●) concentrations during SBR cycle at 5, 10, 20 and 30°C

3.3.2. Stoichiometry and Kinetics

The anaerobic and aerobic stoichiometric and kinetic parameters observed at different operating temperatures are summarized in figure 3.4. In this figure three distinct groups of results obtained from SBR cycle measurements at different temperatures are given: (1) data from the steady state BPR operation during long-term experiments, (2) data from the first cycles following switch of temperature during long-term tests, and (3) data from the batch tests performed in short-term temperature experiments (Brdjanovic et al., 1997a). Comparison between the first and second group of results allows studying of change of temperature coefficients and possible change of population structure, while analysis of data of the first and third group allows comparison of temperature coefficients obtained from long-term and short-term tests. However, direct comparison of the results of the second and third group is not meaningful due to probable population shift in long term experiments, which did not occur in short-term tests. In general, the results from long-term test show that the anaerobic stoichiometry was not affected by temperature changes.

Figure 3.3 Oxygen Consumption Rate (●) and cumulative oxygen consumption (▼) during aerobic phase of SBR cycle at 5, 10, 20 and 30°C

Biomass-specific rates (process rate per amount of active biomass present in the SBR) of all monitored processes during long-term experiments showed a similar, increasing trend with increase in temperature in the range 5 to 20°C (see figure 3.4). However, for some processes, such as P-release, HAc-uptake, PHA and oxygen consumption, it is not clear where the optimal temperature lies, between 20°C and 30°C, or above 30°C (figure 3.4b, c, e, f, l and o). In contrast, the results from short-term temperature experiments showed that the highest anaerobic process rates occurred at around 20°C (figure 3.4h and i). This means that the different metabolic processes occurring in bio-P bacteria might have a different temperature optimum. The values of the kinetic parameters obtained from long-term tests are listed in table 3.1.

Table 3.1   Stoichiometric and kinetic parameters of the anaerobic and aerobic phase of SBR at 5, 10, 20, and 30°C.

ANAEROBIC PHASE					
Type of measurement	T [°C]	SRT [days]	Specific P release rate [g P/g act. bio. h]	Specific HAc uptake rate [g HAc-C/g act. bio. h]	PO ₄ /HAc [g P/g HAc-C]
Standard cycle	20	8	0.07	0.173	0.404
The first cycle	30	8	0.076	0.195	0.392
Steady-state cycle	30	8	0.068	0.18	0.376
The first cycle	20	8	0.077	0.199	0.388
Steady-state cycle	20	8	0.067	0.183	0.366
The first cycle	10	8	0.043	0.13	0.334
Steady-state cycle	10	16	0.041	0.08	0.508
The first cycle	5	16	0.024	0.05	0.486
Steady-state cycle	5	32	0.02	0.045	0.435
AEROBIC PHASE					
Type of measurement	T [°C]	SRT [days]	Specific P uptake rate [g P/g act. bio. h]	Specific OUR rate [g O ₂ /g act. bio. h]	Specific PHA consumption rate [g PHA/g act. bio. h]
Standard cycle	20	8	0.055	0.049	0.095
The first cycle	30	8	0.06	0.062	0.115
Steady-state cycle	30	8	0.057	0.071	0.108
The first cycle	20	8	0.046	0.041	0.117
Steady-state cycle	20	8	0.061	0.051	0.09
The first cycle	10	8	0.031	0.019	0.041
Steady-state cycle	10	16	0.044	0.02	0.031
The first cycle	5	16	0.03	0.012	0.011
Steady-state cycle	5	32	0.022	0.012	0.012

In comparison with the standard cycle at 20°C, the specific P-release rate was very similar in steady state at 30°C and more than threefold lower at 5°C. The same statement can also be applied on specific HAC-uptake rate. This similarity is expected due to direct stoichiometric coupling between those two metabolic processes, resulting in a fairly constant phosphate/acetate ratio observed in both steady state and first cycles at SRT of eight days (between 0.334 and 0.404 mg P/mg HAC-C). This ratio, however, increased up to 50% at higher SRT's (lower temperatures). This increase can be partly explained by the maintenance P-release at high SRT (high MLVSS).

According to table 3.1, the long-term specific P-uptake rate and specific OUR at 5°C equaled 40% and 25% of the value observed in standard cycle, respectively, while the specific PHA consumption rate at 5°C equaled only 13% of the value observed in standard cycle.

Two important comments are to be made: (1) Specific process rates were calculated as initial rates, with exception of the specific PHA consumption rate (due to only two PHA measurements per cycle available; see table 3.2). This rate was calculated using the kinetic expression for PHA consumption from Delft's metabolic model for the aerobic and denitrifying BPR (Murnleitner et al., 1997). (2) The SRT of 8 days at 20 and 30°C, 16 days at 10°C and 32 days at 5°C, was chosen arbitrarily; it might have been possible to achieve full BPR at somewhat lower SRT, therefore these values should not be taken as the minimum required SRT for a satisfactory performance of the BPR processes.

3.3.3. Biomass Composition

The biomass composition during SBR operation at different temperatures is presented in table 3.2. The biomass composition in the SBR at 20°C was similar to the composition observed earlier under identical operating conditions (Smolders et al., 1995, and Brdjanovic et al., 1997a,b). During steady state cycle measurements, around 15% of the mixed liquor was used for sampling. Therefore, the biomass concentration was always lower in the following cycle (the first cycle) due to this dilution effect. The biomass composition did not change much at a SRT of eight days and temperature of 20°C and 30°C. However, at 30°C around 10% less biomass was present in the reactor (table 3.2).

In general, energy requirements for maintenance is greater at higher temperature, thus at higher temperature less MLVSS is present in the reactor at the same SRT. Since the organic load remained constant, the HAC/MLVSS ratio increased resulting in a higher storage polymer (poly-P, PHA and glycogen) content at higher temperature. However, the extension of SRT from eight to sixteen days at 10°C, and further to thirty two days at 5°C, strongly increased the content of storage polymers (poly-P, PHA and glycogen) in the biomass, (fi, expressed as unit of polymer i per unit of active biomass). The reason for this

is the enhanced accumulation of storage polymers in the cell at higher SRT due to the fact that the observed conversion rate of storage compounds in one cycle (Δfi) showed, in general, a strong declining trend with decrease in operating temperature.

Figure 3.4 Stoichiometric and kinetic parameters of the anaerobic and aerobic phase of the SBR at 5, 10, 20 and 30°C, and SRT of 8 (■), □ relates to standard cycle), 16 (◆) and 32 (●) days. Simplified Arrhenius equation was fitted to experimentally observed data using temperature coefficient θ (bold line) within range of standard error for θ (thin line) (continued on the next page)

Figure 3.4

Stoichiometric and kinetic parameters of the anaerobic and aerobic phase of the SBR at 5, 10, 20 and 30°C, and SRT of 8 (■), 16 (◆) and 32 (●) days. Simplified Arrhenius equation was fitted to experimentally observed data using temperature coefficient θ (bold line) within range of standard error for θ (thin line).

Table 3.2 Biomass composition in the SBR at 5, 10, 20, and 30°C.

Cycle	Standard			First			Steady state			First			Steady state			First			Steady state		
	An.	Aer.	End	An.	Aer.	End	An.	Aer.	End	An.	Aer.	End	An.	Aer.	End	An.	Aer.	End	An.	Aer.	End
Temperature	20°C			30°C			30°C			20°C			20°C			10°C			10°C		
SRT	8 days			8 days			8 days			8 days			8 days			8 days			16 days		
Sampling Point	An. End			An. End			An. End			An. End			An. End			An. End			An. End		
Parameter (unit)																					
MLSS (mg/L)	2200	2540	1847	2180	2080	2480	1925	2100	2585	2760	1930	2175	5255	5590	4555	4855			12920		12010
MLVS (mg/L)	1947	1867	1647	1660	1735	1825	1590	1550	2135	2020	1675	1630	4165	4125	3710	3595			9320		8450
MLVSS/MLSS	0.89	0.74	0.89	0.76	0.83	0.74	0.83	0.74	0.83	0.73	0.87	0.75	0.79	0.74	0.81	0.74			0.72		0.70
Ash (mg ash/L)	253	673	200	520	345	655	335	550	450	740	255	545	1090	1465	845	1260			3600		3560
Poly-P (mg Poly-P/L)	171	575	113	433	254	559	251	468	338	634	167	518	871	1248	650	1071			3109		3115
PHB (mg PHB/L)	151	16	154	12	179	31	186	34	212	42	174	93	539	384	478	419			2491		2122
PHV (mg PHV/L)	43	4	41	2	54	9	45	5	47	6	28	12	82	42	89	76			211		157
PHA (mg PHA/L)	194	20	195	14	233	40	232	39	259	48	202	105	621	426	568	494			2702		2279
Glycogen (mg/L)	206	415	138	318	190	378	177	345	297	531	269	382	942	1226	859	907			520		684
Active biomass (mg a b/L)	1548	1432	1314	1328	1312	1407	1181	1166	1578	1441	1204	1144	2602	2473	2283	2194			6098		5486
f_{bp} (g Poly-P/g a b.)	0.110	0.401	0.086	0.326	0.193	0.397	0.213	0.402	0.214	0.440	0.139	0.453	0.335	0.505	0.285	0.488			0.510		0.568
f_{pha} (g PHA/g a b.)	0.125	0.014	0.149	0.11	0.178	0.029	0.196	0.033	0.164	0.033	0.168	0.091	0.239	0.172	0.249	0.255			0.443		0.415
f_{gly} (g glycogen/g a b.)	0.133	0.290	0.105	0.239	0.145	0.269	0.150	0.296	0.188	0.369	0.224	0.334	0.362	0.496	0.376	0.413			0.085		0.125
Δf_{bp} (g Poly-P/g a b.)	0.291		0.239		0.204		0.189		0.226		0.314		0.170		0.204				0.058		
Δf_{pha} (g PHA/g a b.)	0.111		0.138		0.149		0.163		0.131		0.076		0.067		0.023				0.028		
Δf_{gly} (g glycogen/g a b.)	0.157		0.134		0.124		0.146		0.180		0.110		0.134		0.037				0.039		

3.3.4. Temperature coefficients θ

Based on values from table 3.1, using a simplified Arrhenius equation for the temperature dependency, the temperature coefficients θ were calculated for biomass-specific process rates (P-release, HAc-uptake, P-uptake, OUR and PHA consumption rate) by fitting the equation to the data. Arrhenius equation was selected for easier comparison of temperature dependency of different processes investigated in this study with Arrhenius temperature coefficients available in the literature (for example in ASM2). Fitting was performed in a temperature range of 5 to 20°C because for most of the metabolic processes of bio-P bacteria, the process rates were similar at 20°C and 30°C. Since the optimum temperature is obvious in between these values inclusion of data obtained at 30°C would be erroneous.

Temperature coefficients θ are presented in figure 3.4 and summarized in table 3.3, along with modified temperature coefficients (valid between 5 and 20°C) of the relevant metabolic process rates from short-term temperature experiments (Brdjanovic et al., 1997a). Temperature coefficients presented in this study do provide the direct effect of temperature on the biomass-specific metabolic process rates. These temperature coefficients do not relate to the kinetic rate coefficients defined previously in mathematical models for these metabolic processes (Henze et al., 1994, Smolders et al., 1994c, and Murnleitner et al., 1997). The temperature coefficients for these kinetic rate coefficients are probably different because the level of storage polymers (which are part of the defined kinetic equations in the model) also change with temperature (table 3.3).

Table 3.3 Temperature coefficients θ with standard error for reaction rates of anaerobic and aerobic BPR processes obtained from short and long-term temperature experiments

Process rate	Long-term experiments Steady-state cycle	Long-term experiments The first cycle	Short-term experiments The first cycle	Average rate coefficient K at 20°C [g/g act. biomass.h]
	$\theta \pm \text{st. error}$	$\theta \pm \text{st. error}$	$\theta \pm \text{st. error}$	
P release	1.075±0.012	1.067±0.009	1.055±0.019	0.074
HAc uptake	1.095±0.013	1.063±0.019	1.080±0.011	0.19
P uptake	1.031±0.017	1.034±0.003	1.065±0.019	0.047
O ₂ consumption	1.082±0.006	1.090±0.004	1.075±0.004	0.042
PHA consumption	1.129±0.014	1.110±0.024	1.076±0.024	0.095

The values of temperature coefficients for the metabolic processes in the anaerobic phase (P-release and HAc-uptake), obtained in long-term steady state experiments (1.075 ± 0.012 and 1.095 ± 0.013), were only slightly higher than obtained from the first cycles (1.067 ± 0.009 and 1.063 ± 0.019) and from short-term experiments (1.055 ± 0.019 and 1.080 ± 0.011). For the aerobic phase the temperature effect on P-uptake rate was very similar in long-term tests (steady state and first cycle: 1.031 ± 0.017 to 1.034 ± 0.003), but rather strongly differed from short-term test (1.065 ± 0.019). The PHA consumption was stronger

differed from short-term test (1.065 ± 0.019). The PHA consumption was stronger influenced by temperature during long-term experiments (1.129 ± 0.014 versus 1.076 ± 0.024 obtained in short-term experiments). A similar statement can also be applied on OUR.

3.3.5. Molecular ecological studies

The sludge used in this research was cultivated under highly selective conditions (controlled pH and temperature, only acetate was the carbon source in the anaerobic phase). Therefore the first question was, whether enriched BPR sludge consist of mainly one type of bacteria, or whether the sludge consists of a bacterial community containing a range of different types of bacteria. Two methods were applied to investigate the bacterial composition of the BPR sludge: electron-microscopy (EM) and DDGGE. The EM photograph (figure 3.5) already suggests there are different morphological forms of microbes in the sludge. The presence of at least 5-6 bands on the DDGGE (figure 3.6, sample 1 on gel I) indicates the presence of at least as many bacteria. It should be notified that the DDGGE analysis is not quantitative, thickness of the bands has no relation with the relative presence of certain organisms in the culture.

Figure 3.5 Transmission electron microscopic photograph of the BPR biomass (x4800) cultivated on acetate as an external organic substrate in an A/O SBR at 20°C and SRT of 8 days ("standard cycle")

Figure 3.6 DDGGE analysis of the BPR sludge cultivated at different temperatures during short (*) and long-term temperature experiments. DDGGE analysis I did not succeed on sludge sample no.6 taken from the SBR in standard cycle at 20°C, therefore the new experiment was performed (DDGGE analysis II) using only samples taken from the SBR at 20°C (samples no. 1, 4 and 6)

The DDGGE allows to observe changes in bacterial population depending on changes in reactor temperatures. For that purpose, the DDGGE patterns originating from sludge samples taken from steady states at different reactor temperatures were compared (figure 3.6). At different temperatures different bands appear or disappear. This suggests that the composition of the microbial culture changed with temperature. Since the method is not quantitative it is not clear whether the changes in the population are major or marginal. The

only consistent band on all the gels appears at a denaturing agent concentration of 32%. It is tempting to relate this band to the bio-P bacteria, a clear conclusion is however not yet justified. From the gels several other interesting features can be seen. The samples 1 and 4 on gel II (figure 3.6) are operated with the same medium (and same researcher), but at different time and different laboratories in Delft. Clearly, there is a difference in the microbial population (at a gradient of 35-37%) due to unknown reasons. A comparison of sample 6 (standard cycle at 20°C) and 4 (sludge at 20°C after experiment at 30°C) indicates that the population in the reactor returned to the same composition.

3.4. Discussion

Taking into account the results from the chapter 2 (Brdjanovic et al., 1997a), combined with the findings of this study, it can be generally concluded that temperature impact on stoichiometry of BPR is marginal.

The temperature dependencies of P-release and HAc-uptake process rate, obtained from long-term tests, could be considered as similar, taking into account standard errors of temperature coefficients. A similar statement can also be applied on anaerobic process rates obtained from short-term tests. This is expected since these two processes are stoichiometrically coupled and theoretically should have identical temperature dependency.

In general it can be concluded that the temperature has a rather strong impact on anaerobic kinetics. Temperature coefficients obtained in short-term experiments (average anaerobic $\theta = 1.078$, Brdjanovic et al. 1997a) only very slightly deviate from the temperature coefficients of the anaerobic BPR processes obtained in a long-term experiments (average $\theta = 1.085$).

In contrast to the anaerobic phase, such a rather uniform temperature dependency of metabolic processes of the aerobic phase was not observed. This statement can be applied on both short-term and long-term tests. Such a heterogeneity of the temperature impact on the aerobic phase can be expected due to the fact that four more or less independent processes occur in the aerobic phase (growth, P-uptake, glycogen formation and maintenance), which are only linked with PHA consumption.

Temperature has a strong effect on most aerobic processes; a continuous increasing trend of all biomass-specific process rates was observed during long-term experiments in the full temperature range. A moderate impact was only observed on the P-uptake process during long term tests (θ around 1.031). In contrast, the temperature strongly affected the oxygen and PHA consumption. The observed temperature dependency of OUR and PHA biomass-specific consumption rate (average $\theta = 1.110$) corresponds to temperature dependency in

between heterotrophs and nitrifiers (1.070 - 1.120, Henze et al., 1994). During the aerobic phase around one third of the utilized oxygen is related to P-uptake and around two third is related to PHA consumption (Smolders et al., 1994c). The overall OUR is therefore the result of the OUR for those two processes. The combination of a moderate impact of temperature on the P-uptake process and rather strong impact on the PHA consumption process resulted in an intermediate dependency of respiration. Overall, the temperature dependency of OUR and PHA biomass-specific consumption rate, established in long-term temperature experiments, are higher than in short-term tests. Moreover, the process kinetic rates also have comparatively higher absolute values in long-term experiments.

The strong effect of temperature on BPR is also seen from the minimum required SRT. At 20°C the minimum SRT is around two days (Smolders et al., 1995b). At 10°C it was not possible to maintain stable BPR at a SRT of eight days, therefore the SRT was arbitrarily extended to 16 days. At 5°C, again it was not possible to operate the process at a SRT of sixteen days, and SRT extension to thirty two days was applied. This suggests a θ of at least 1.110 for the minimum SRT. Since the growth is a metabolic process in the aerobic phase which is mostly linked with PHA consumption of the biomass, the temperature coefficient of growth will be most close to the PHA biomass-specific consumption value (which is indeed around 1.130). This temperature dependency of the growth rate is comparable to the temperature dependency of nitrifiers.

It is important to note that in wastewater practice the aerobic phase of BPR is much less studied than the anaerobic phase, and of the aerobic metabolic processes the P-uptake process is generally the most considered. The bacteria responsible for BPR perform different metabolic processes (some of them are not directly stoichiometrically coupled), thus different temperature coefficients and optima for such processes can occur, as shown in this study. Therefore, isolated consideration of an easily observable parameter such as P-uptake (although very practical) might lead to underestimation of the temperature impact on, not only OUR and PHA consumption rate, but also on growth rate. Proper determination of the temperature dependency of growth requires studying of other metabolic processes in which the growth rate is related in a complex way to storage levels (fi), such as PHA, poly-P, and glycogen.

The composition of the SBR sludge was analyzed by electron-microscopy and on the genetical level. Those experiments indicated the presence of at least five to seven different types of bacteria. The author is aware of the fact, that there are most likely even more different types of microorganisms present. In comparable and more extended experiments with other wastewater sludges it was shown, that there are more different bacteria than DDGGE bands appear (Logemann S.- personal communication). From these experiments it is clear that even under selective conditions (one carbon source - HAc, controlled temperature and pH, and a long steady operation of the process) there is more than one type

of organism present.

It could be demonstrated that the bacterial population in BPR sludge shifts with temperature. This change in community composition coincided with a different temperature coefficient for short and long-term experiments for especially the PHA and oxygen consumption rate, and therefore also maximal growth rate. Since however the change in population here coincides not always with a change in observed kinetics, it is clear that no straight conclusions can be made based on observations of microbial composition of sludges solely.

DDGGE has been proven to be a good method to observe changes in microbial composition during the course of an experiment and as such it is a valuable tool in the study of microbial population dynamics. A drawback is formed by the fact that the method is not quantitative. The intensity of the bands is not related to the fraction of certain species in the population. The presented experiments give only a preliminary description of a given bacterial population. Further work in this direction could lead to more detailed knowledge or even to identification of the bacteria involved in BPR. It is possible to clone PCR fragments from 16S rDNA, to identify and sequence clones carrying inserts which comigrate with the permanently appearing DDGGE bands. This sequence information will at least show to which taxonomical group the BPR bacteria belong and will probably even lead to their identification. Probes derived from such sequences can later be used in fluorescent in situ hybridization (FISH) experiments to detect and to quantify these bacteria (Amann et al., 1995). It would be of great benefit if a pure bio-P culture could be obtained in order to study the temperature effect of stoichiometry and kinetics of BPR without change in bacterial population structure.

3.5. Conclusions

The results of the study showed that the process stoichiometry of the anaerobic phase was relatively insensitive to temperature changes. In contrast, the temperature impact on the kinetics of the anaerobic phase was rather strong and could be expressed by a single temperature coefficient. The coefficient obtained from long-term temperature tests was similar to the temperature coefficient observed in short-term tests ($\theta = 1.085$ versus $\theta = 1.078$, respectively).

Temperature had a moderate impact on the aerobic P-uptake process rate ($\theta = 1.031$) during long-term tests. However, a strong temperature effect on other metabolic processes of the aerobic phase, such as PHA consumption ($\theta = 1.129$), OUR ($\theta = 1.090$) and growth ($\theta > 1.110$), was observed. In contrast to the kinetics of the anaerobic phase, different temperature coefficients were obtained for the aerobic phase from long-term and short-term

tests, probably but not necessarily, due to a change in population structure.

The different temperature coefficient found for P-uptake compared to the other metabolic processes of the aerobic phase underlines that, in complex processes such as BPR, it is dangerous to draw conclusions from easily observable parameters (like phosphate) only. Such an approach can lead to underestimation of the temperature dependency of other metabolic processes of the aerobic phase of the BPR.

Molecular ecological techniques showed that the composition of the bacterial population structure shifted with temperature. The results of electron-microscopy and dry denaturing gradient gel electrophoresis show that, even under very selective conditions, as applied here, different type of bacteria (at least five to seven) were present in the reactor.

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4 Chapter

Bioassay for Glycogen Determination in Biological Phosphorus Removal Systems

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Contents

4.1. Introduction	81
4.2. Bioassay for Glycogen	82
4.2.1. Description	82
4.2.2. Testing	82
4.2.3. Verification	83
4.2.4. Application	83
4.2.5. The Conventional Procedure for Glycogen Determination	84
4.3. Results and Discussion	84
4.3.1. Bioassay Testing	84
4.3.2. Confirmation of Glycogen Limitation	86
4.3.3. Effect of pH	87
4.3.4. Bioassay Restrictions	88
4.4. Conclusions	88

Abstract

Glycogen plays an important role in BPR from wastewaters. Existing measurement techniques often overestimate the glycogen content of the biomass due to the presence of glucose and/or other carbohydrates than glycogen in the cell material. As an alternative to conventional methods a bioassay for glycogen determination in BPR systems was developed. The bioassay is based on the strict stoichiometric coupling between anaerobic acetate uptake and glycogen consumption. In other words, the glycogen concentration of the sludge was determined indirectly by measuring the maximal total acetate uptake by the activated sludge in anaerobic batch tests. The bioassay was successfully tested for the determination of glycogen content of the sludge taken from the lab-scale, acetate fed, anaerobic-aerobic-settling sequencing batch reactor operating at pH 7.0 ± 0.1 and temperature of 20°C . This determination of glycogen requires that glycogen (not poly-P) is the limiting factor for anaerobic acetate uptake. A method to verify this assumption based on the effect of pH on phosphate/acetate ratio is proposed and used. The bioassay is easy to apply and gives an indirect measure of the glycogen content of bio-P bacteria, but its reliability still needs to be verified at full-scale biological P-removal plants.

4.1. Introduction

Biological phosphorus removal (BPR) from wastewaters is based on the enrichment of activated sludge with phosphate accumulating organisms (PAOs). These microorganisms (also called P-removing organisms or poly-P bacteria) are able to store intracellular phosphorus as poly-phosphate (poly-P). The requirement to achieve a phosphorus removing bacterial population in an activated sludge system is exposure of activated sludge to anaerobic and aerobic (or anoxic) conditions. Under anaerobic conditions P-removing bacteria transport volatile fatty acids (VFA, e.g. acetate: HAc) into the cell and subsequently convert and store these as poly-hydroxy-alkanoates (PHA, e.g. poly-hydroxybutyrate: PHB). The energy for this transport and storage is supplied by hydrolysis of intracellularly stored poly-P to ortho-phosphate which is released from the cell to the liquid (figure 4.1). The anaerobic P-release is strongly influenced by pH (Smolders et al., 1994a). The reduction equivalents required for the conversion of acetate to PHB are supplied by conversion of intracellularly stored glycogen through the glycolytic pathway to PHB and CO_2 (Mino et al., 1987, Arun et al., 1988, and Smolders et al., 1994a). Glycogen plays an essential role in maintaining the redox balance in the cell. In absence of glycogen, anaerobic uptake of organic substrate by PAOs may not occur (Mino et al., 1996). Under aerobic conditions, the anaerobically formed PHB is used to generate energy for cell growth, poly-P synthesis, glycogen formation and maintenance, resulting in the uptake of phosphate. During the aerobic phase PAOs accumulate more phosphate than released during anaerobic phase, resulting in net uptake of phosphate. Phosphate is removed from the system by sludge wasting.

Figure 4.1 Anaerobic metabolism of biological phosphorus removal. The α_1 is dependent of pH and represents the energy (molATP/C-mol) required for acetate uptake (adopted from Smolders et al., 1994b)

An integrated metabolic model for aerobic and denitrifying BPR ("Delft Model for BPR"; Murnleitner et al., 1997) is based on the principle that the metabolism of the organism is composed of a small number of universal metabolic pathways. The metabolic pathways of BPR rely on three internally stored polymers: poly-P, PHB and glycogen. For the purposes of mathematical modeling and process evaluation of BPR an accurate measurement of the storage materials involved in the processes is required. Unfortunately, in comparison with poly-P, PHB and HAc, the glycogen determination is the least reliable method. So far the most common (bio) chemical analysis techniques for glycogen determination in the biomass are cumbersome and often give inaccurate results. Conventional methods measure glycogen either as glucose extracted from the biomass or as carbohydrates content of the biomass. The measured values may also include glucose of the cell's materials or carbohydrates other than glycogen. Consequently, the true glycogen concentration can be considerably lower than the measured one. This is especially a problem in activated sludge plants where a large fraction of non-poly-P bacteria is present, adding to a non-glycogen-glucose background. As an alternative to conventional methods bioassay for glycogen determination in BPR systems was developed, tested, verified and applied in this study.

4.2. Bioassay for Glycogen

4.2.1. Description

The bioassay is based on the strict stoichiometric coupling between the anaerobic acetate uptake by the activated sludge and the related glycogen consumption. According to the cell metabolism of BPR (Smolders et al., 1994b), for each C-mol of acetate taken up, 0.5 C-mol of glycogen is consumed (1.125 mg glycogen/mg HAc-C). Taking this ratio into account, the glycogen content of the biomass can be determined indirectly by measuring the total amount of acetate, taken up by the biomass in anaerobic batch test. The results obtained by this method presents the glycogen content of exclusively poly-P bacteria at the beginning of the batch test. These results do not include glucose (originated from cell material), other carbohydrates or glycogen content of bacteria other than poly-P. However, the bioassay is only valid under the assumption that glycogen is exhausted due to acetate uptake.

4.2.2. Testing

The bioassay was tested in the determination of the glycogen content of sludge from an anaerobic-aerobic-settling sequencing batch reactor (SBR) operating in steady-state (for full description of the SBR operation and medium composition the reader is referred to section 2.2). The sludge from the lab-scale SBR was selected due to its relatively high glycogen content, a large number of glycogen measurements by conventional method available and absence of carbohydrates in the medium. The sludge was taken from the SBR at two

characteristic points of the BPR process (at the end of the anaerobic and aerobic phase). At these points the biomass reaches its extreme concentrations of glycogen (minimum and maximum values, respectively). Two anaerobic batch tests (test I and II) with acetate addition in excess amounts were performed; in the first test the sludge was taken at the end of the anaerobic phase of the SBR, and in the second, at the end of the aerobic phase. At the beginning of each batch experiment 0.45 L of sludge was manually transferred from the SBR to a double jacketed laboratory fermenter (0.5 L) where the tests took place. The tests were performed at controlled temperature 20°C and pH (7.0±0.1). pH was maintained by dosing 0.1N HCl and 0.1N NaOH. Anaerobic conditions were achieved and maintained by continuous flushing of mixed liquor with N₂ gas (30 L/h). Mixing was provided throughout the tests at 500 rpm. Acetate (375 mgHAc-C/L as a final concentration) was added instantly at the beginning of the tests under completely anaerobic conditions. During the tests the acetate and phosphate concentrations were measured. The addition of high amounts of acetate ensured that surplus acetate remained in the solution at the end of the tests.

4.2.3. Verification

In order to verify that the poly-P was not limiting the anaerobic acetate uptake and P-release in bioassay application tests an additional test was performed in which the poly-P content of bacteria was artificially increased. Sludge (0.45 L) was taken from the SBR at the end of the anaerobic phase and transferred to the aerobic-anaerobic batch reactor. At the beginning of the aerobic phase phosphate (100 mgP/L) was instantly added to the batch reactor and mixed liquor was aerated for 2.75 hours in order to increase the polyphosphate level in the biomass. Subsequently, the sludge was exposed to anaerobic conditions. Once a complete anaerobic state was achieved, 450 mgHAc-C/L of acetate was added to the reactor and P-release and HAc-uptake were monitored over 4 hours. Acetate and phosphate concentrations were determined every 20 minutes, while MLSS, MLVSS, and PHB concentrations were measured at the end of each phase.

4.2.4. Application

The bioassay is valid only under glycogen limitations. Based on the fact that the P-release is dependent of pH, and acetate uptake (and glycogen consumption) not (Smolders et al., 1994a), the bioassay was applied on P-removing sludge in order to distinguish the shift from glycogen to poly-P limitation for anaerobic acetate-uptake as a function of pH. Glycogen limitation means that the P-release will stop due to the lack of glycogen for acetate uptake while poly-P limitation means that the biomass will completely release its poly-P content during the test.

Sludge (2.5 L) was taken from the end of the anaerobic phase of the SBR, split into equal parts and transferred to five anaerobic batch reactors. Each batch reactor operated at different pH values (from 6.5 in the first reactor to 7.0, 7.5, 8.0 and 8.5 in the fifth reactor).

Acetate (400 mgHAc-C/L) was instantly added to each reactor at the same moment and the anaerobic phosphate release and acetate uptake were monitored hourly over 5 hours. MLSS, MLVSS, PHB and glycogen (standard method) were measured at the beginning and the end of the test.

4.2.5. The Conventional Procedure for Glycogen Determination

Glycogen content was also measured conventionally as glucose extracted from the biomass (Smolders et al., 1994a and Kuba et al., 1996). 6 N HCl was directly added to samples to a final concentration of 0.6 N. The samples were placed in a water bath at 100 °C for 1 hour. After cooling and centrifugation the glucose concentration of the supernatant was measured on a HPLC.

4.3. Results and Discussion

4.3.1. Bioassay Testing

The results of bioassay testing (test I and II) on sludge from SBR are presented in figure 4.2. The biomass composition at the beginning and at the end of the tests is presented in table 4.1. An example of the calculation procedure to obtain the glycogen content of the biomass from the experimental observations is given in table 4.2.

Figure 4.2 Acetate (▲), phosphate(●), glycogen (□, measured by conventional method) and glycogen (■, determined by bioassay) concentration during anaerobic batch experiment for testing of bioassay for glycogen determination using the sludge taken from the SBR at (a) the end of the anaerobic phase, and (b) the end of the aerobic phase

Table 4.1 The composition of biomass used in the anaerobic batch experiments for testing of the bioassay

Parameter	Unit	Test I		Test II	
		Start	End	Start	End
MLSS	mg MLSS/L	2862	2321	3191	2231
MLVSS	mg MLVSS/L	2102	2212	2000	2125
MLVSS/MLSS		0.734	0.953	0.627	0.952
Ash	mg ash/L	760	109	1191	106
Poly-P	mg poly-P/L	684	98	1072	95
Phosphate	mg P/L	239	34	375	33
PHA	mg PHA/L	150	370	45	365
Glycogen _{HPLC}	mg glycogen/L	195	12	280	17
Glycogen _{Bioassay}	mg glycogen/L	180	0	264	0
Active biomass	mg active biomass/L	1722	1842	1691	1760

Table 4.2 Calculation procedure used in the bioassay for glycogen determination in BPR activated sludge systems

Acetate conversion	
Acetate concentration at the start of the test (HAc_{start})	375 mg HAc-C/L
Acetate concentration at the end of the test (HAc_{end})	140 mg HAc-C/L
Total HAc uptake ($HAc_{total} = HAc_{start} - HAc_{end}$)	235 mg HAc-C/L
Phosphate conversion	
Phosphate concentration at the start of the test (P_{start})	0 mg P/L
Phosphate concentration at the end of the test (P_{end})	321 mg P/L
Polyphosphate content of the biomass at the start of the test ($P_{b, start}$)	375 mg P/L
Released phosphate ($P_{rel} = P_{end} - P_{start}$)	321 mg P/L
Phosphate content of the biomass at the end of the test ($P_{b, end} = P_{b, start} - P_{rel}$)	54 mg P/L
Fraction of poly-P released ($P_{rel}/P_{b, start}$)	0.86
Glycogen conversion	
Glycogen/HAc ratio (fixed stoichiometry)	1.125 mg/mg HAc-C
Glycogen concentration determined by the bioassay ($G_{bioassay} = 1.125 \cdot HAc_{total}$)	264 mg/L
Glycogen concentration determined by conventional method (G_{HPLC})	280 mg/L
Basic glucose level ($G_b = G_m - G_{bioassay}$)	16 mg/L (6% of G_m)

This example is based on the test II where the sludge from the end of the aerobic phase was used (see also figure 4.2b)

In this procedure the terms “Glycogen_{bioassay}” and “Glycogen_{HPLC}” are related to the glycogen concentration determined using the bioassay and the conventional method, respectively. As stated earlier, the amount of glycogen present in the biomass (G_{bioassay}) can be calculated from the maximal acetate uptake and the fixed stoichiometric relation between anaerobic acetate uptake and related glycogen consumption (table 4.2). Since the bioassay is only valid under glycogen limitation, the calculated value $G_{\text{bioassay}} = 264 \text{ mg glycogen/L}$ (156 mg glycogen/g active biomass) is considered as the glycogen concentration of the bio-P bacteria in the SBR at the end of the aerobic phase.

The measured glycogen concentration using the conventional method (280 mg glycogen/L) was 6% (10 mg glycogen/g active biomass) higher than obtained by the bioassay, likely due to non-glycogen glucose, confirming the overestimating trend of conventional methods. The overestimation was 8% (8 mg glycogen/g active biomass), when sludge from the end of the anaerobic phase of the SBR was used. Sludge from full-scale sewage works is not as much enriched with bio-P bacteria, therefore larger deviations can be expected in such cases.

4.3.2. Confirmation of Glycogen Limitation

During the tests (I and II) not all poly-P was released, confirming the assumption that acetate uptake was limited by glycogen content of the biomass. In order to further confirm that the poly-P was not a limiting factor, an additional experiment was performed (figure 4.3). A higher poly-P level of the biomass was artificially achieved by phosphate addition at the beginning of the aerobic phase of the SBR.

Figure 4.3 Acetate (▲) and phosphate(●) concentrations measured in the aerobic-anaerobic batch test for the verification of the bioassay for glycogen using sludge taken from the SBR at the end of the anaerobic phase

During aerobic phase 129 mgP/L (80 mg P/g active biomass) was taken up, an approximately 30% increase in comparison with the amount of phosphate taken up by the biomass in the normal SBR cycle. Anaerobic consumption of acetate (220 mgHAc-C/L or 138 mg HAc-C/ g active biomass) resulted in a net P-release of 303 mgP/L (189 mg P/g active biomass) observed at the end of the test.

The amount of glycogen detected was very similar to the results of the anaerobic batch test with a normal (lower) initial poly-P content of the biomass. In other words, observed equal acetate uptake by sludge containing different amounts of poly-P strongly suggests glycogen limitation. Increased substrate consumption under identical conditions would have indicated poly-P limitation.

4.3.3. Effect of pH

The acetate/glycogen ratio is independent of pH, but the acetate/phosphate ratio decreases with increase in pH (Smolders et al., 1994a). This implicates that the limiting factor for HAc-uptake can shift from glycogen to poly-P at elevated pH levels. This is illustrated in figure 4.4. The glycogen was limiting at $\text{pH} \leq 7.0$, a transition zone was observed in the range $7.0 < \text{pH} < 8.0$ and poly-P became limiting at $\text{pH} \geq 8.0$. Taking into account that the bioassay was originally tested at pH 7.0, glycogen was confirmed as the limiting factor.

Figure 4.4 Specific phosphate release(●), specific acetate uptake (■), and acetate/phosphate ratio (▲) in anaerobic batch tests for determination of limitation conditions of the bioassay as a function of pH. Constant HAc-uptake indicates glycogen limitation, whereas constant P-release or/and decrease in HAc-uptake indicates poly-P limitation

4.3.4. Bioassay Restrictions

The bioassay was developed under the assumption of glycogen limitation. It was also shown that the poly-P may become the limiting factor for anaerobic acetate uptake. In such case the bioassay can not be used to determine the absolute glycogen content of the biomass, but can still be used to obtain the relative glycogen content of the cell.

The bioassay can be applied on both enriched and mixed cultures. In the case of simultaneous presence of PAOs and significant amount of glycogen accumulating non-poly-P bacteria (GAOs) the bioassay may not give accurate results due to, firstly, ability of GAOs to take-up organic substrate under anaerobic conditions, and secondly, due to different stoichiometric coupling between acetate uptake and glycogen consumption in PAOs and GAOs anaerobic metabolism (Mino et al., 1996). However, in practice such situation is quite rare and may only occur if BPR deteriorates from whatever reason, allowing GAOs population to dominate (e.g. Mino et al., 1997). The accuracy of the method might be affected by the products (acetate) of fermentation process. In laboratory systems where enriched PAO culture cultivated on acetate as a sole external organic substrate (as it is a case in this study) influence of fermentation on the method accuracy is expected to be marginal. At full-scale, the effect fermentation rate on the results of the method will depend on the location of the sampling point; for mineralized sludge this effect may be negligible.

The limitation zones established in the previous section are valid only for this particular sludge and may shift if sludge of a different origin is used.

4.4. Conclusions

A rather simple and straightforward bioassay for glycogen determination has been developed. The method was successfully tested, verified and applied on a lab-scale anaerobic-aerobic SBR system. It was shown that the conventional methods may overestimate the glycogen content of the biomass (in this case 6-8%). The method was applied to distinguish the region of glycogen or poly-P limitation in the anaerobic phase at different pH values. It was found that, for this particular sludge, either glycogen or poly-P may become the limiting factor for anaerobic acetate uptake at different pH levels. Clearly, the bioassay showed its potential, but the reliability of the method still needs to be verified at full-scale BPR plants.

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5 Chapter

Modeling COD, N and P Removal in a Full-scale WWTP Haarlem Waarderpolder

Chapter 5 is based on: "Modeling COD, N and P Removal in a Full-scale WWTP Haarlem Waarderpolder"
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Contents

5.1. Introduction	95
5.2. Materials and Methods	96
5.2.1. Configuration of WWTP "Haarlem Waarderpolder"	96
5.2.2. Influent Characterization	102
5.2.3. Performed Biological Methods for Sludge Characterization	102
5.2.4. Sampling Program and Analytical Methods	105
5.2.5. Modeling Tools	106
5.2.6. Modeling Strategy	107
5.3. Results	107
5.3.1. Sampling Program	107
5.3.2. Influent and Sludge Characterization	108
5.3.3. Hydraulic Set-up of the Plant Model	108
5.3.4. Model Calibration	111
5.3.5. Model Validation	114
5.3.6. Alternative Process Configurations of the Plant	118
5.4. Discussion	120
5.4.1. Influent characterization	120
5.4.2. Model Calibration	121
5.4.3. Operational Aspects	123
5.4.4. Process Configurations	124
5.5. Conclusions	124
Appendices	127

Abstract

An integrated activated sludge model was used to describe removal of organic matter, nitrogen and phosphorus in a full-scale wastewater treatment plant Haarlem Waarderpolder in The Netherlands. The plant consists of a conventional anoxic/aerobic activated sludge line, side-stream combined biochemical phosphorus removal line and sludge treatment line. In this integrated model, the "Delft model" for biological phosphorus removal (BPR) replaced the module for P removal of Activated Sludge Model No.2 (ASM2). With the adjustment of only three model parameters (fermentation rate constant, glycogen formation rate constant and the percentage of denitrifying activity of phosphorus accumulating organisms) the model proved well capable of describing the performance of the treatment plant (for both liquid phase and biomass). The model was validated using batch tests with the activated sludge and showed satisfactory results in describing the anaerobic P release and aerobic and anoxic P uptake processes. The model was to a lower extend capable of well predicting nitrification, endogenous P release and especially denitrification processes. Both model simulations and results of the sampling program suggested that the acetate dosage to the system could be reduced up to 30%. It was also shown that pH strongly affects observed phosphate/acetate ratio and possibly fermentation rate in the side-stream BPR process. Simulation of three alternative plant configurations showed that: (1) plant effluent quality would strongly deteriorate if the side-stream process is skipped and the rest of the plant is retained in its original state, (2) good effluent quality would be achieved in a main-stream process (modified UCT system) where an additional anaerobic tank is added in front of the activated sludge unit, however, the required acetate addition would be sevenfold higher in comparison with a present side-stream configuration.

5.1. Introduction

Several different mathematical models for the simulation of the activated sludge process in wastewater treatment are available. The most recent models allow for dynamic simulation of complex activated sludge systems and include carbon oxidation, nitrification, denitrification, chemical and biological phosphorus removal, and even attempts to model the formation of filamentous organisms (bulking) have been made. Besides different microbial conversion reactions, these models may also take into account dynamic feeding regime, influence of temperature, pH, dissolved oxygen, various hydraulic patterns, process control, and effects of gas/liquid mass transfer processes.

In order to promote the development and facilitate the application of models for design and operation of biological wastewater treatment systems, the IAWPRC (now IAWQ) formed in 1983 the Task Group on Mathematical Modeling for Design and Operation of Biological Wastewater Treatment. Four years later the Task Group proposed a general model for removal of organic matter, nitrification and denitrification, called Activated Sludge Model no. 1 - ASM1 (Henze et al. 1987). Since its appearance, this model has greatly encouraged the use of mathematical models. The increased requirement for nutrient removal during the last decade created a need to extend ASM1 by inclusion of biological phosphorus removal (BPR). In 1989, Wentzel et al. presented a kinetic model for aerobic BPR. This model served as a basis for development of the Activated Sludge Model no. 2 - ASM2 (Henze et al. 1994). Soon after, Mino et al. (1995) extended ASM2 by including the glycogen metabolism and BPR under anoxic conditions. Simultaneously, Smolders and co-workers (1994a) developed a structured metabolic model for BPR (also incorporating the glycogen metabolism) which served also as a basis for a metabolic model for denitrifying BPR (Kuba et al. 1996). In their integrated metabolic model for aerobic and anoxic BPR (so-called "Delft BPR Model"), Murnleitner et al. (1997), successfully described the two known BPR processes with the same kinetic equations and parameters. The metabolic model is based on the bioenergetics and stoichiometry of the bacterial metabolism, and describes all relevant metabolic reactions underlying the metabolism of phosphorus accumulating organisms (PAOs) by six independent reactions: two for the anaerobic metabolism and four for the aerobic/anoxic metabolism. In this model the ATP/NADH₂ ratio (called δ value) is the only model parameter that is different for aerobic and anoxic BPR. The process reaction rates are described by four kinetic relations and two maintenance terms. A comparison between the ASM2 and the "Delft BPR model" is discussed elsewhere (Van Loosdrecht, 1996). Very recently, the Task Group proposed ASM2d (Henze et al. 1998) - a minor extension of ASM2 by inclusion of two additional processes to account for denitrifying BPR. At the same time the Task Group moreover, proposed a structured model for simulation of oxygen consumption, sludge production, nitrification and denitrification: ASM3 (Gujer et al. 1998). ASM 3 replaces ASM1 and includes several improvements compared to ASM1, such as addition of storage of organic substrates as a new process, and replacement of lysis process with endogenous respiration process. This approach in ASM3 is more related to the Delft BPR model than to ASM1. Following this fast development of the activated sludge models, it can be expected that within few years the ASM4 may appear to describe COD, N, and P removal for aerobic and denitrifying conditions, possibly based

on a combination of ASM3 and a modified "Delft BPR model".

Presently, it is widely agreed that the models for simulation of activated sludge systems are suitable for application to complex full-scale wastewater treatment plants (WWTP). ASM1 has been used for more than a decade as a tool for modeling the removal of organic matter and for nitrification and denitrification processes; considerable experience with this model has been acquired. However, for ASM2, the situation is different. It has not been validated extensively due to the fact that the model became available only recently, and because its accuracy is being debated. While the information on the application of ASM2 is scarce, the "Delft BPR model" was already tested and verified over a range of SRT values (Smolders et al. 1995a) and oxygen or nitrate as electron acceptor (Murnleitner, 1997), as well as during both start-up and steady state conditions (Smolders et al. 1995b) - all in lab-scale SBR systems using BPR cultures enriched under laboratory conditions and at a temperature of 20°C. Recently, the "Delft BPR model" was for the first time applied on the full-scale installation WWTP "Holten" in The Netherlands (Van Veldhuizen et al. In press). The stoichiometry and to a less extend the kinetics of BPR processes are considered to be well defined by this model. In this model the part of ASM2 that is related to phosphate removal was replaced with the "Delft BPR model". This combined model was used in this study to simulate the operation of the WWTP "Haarlem Waarderpolder" to check how well this model predicts the measured concentrations of the components of both the liquid phase and the biomass.

5.2. Materials and Methods

5.2.1. Configuration of WWTP "Haarlem Waarderpolder"

The WWTP "Haarlem Waarderpolder" was built in 1969. In 1995 the installation was retrofitted and expanded to its present state (figure 5.1). The plant was designed for removal of organic matter, nitrogen and phosphorus from domestic and industrial wastewaters of Haarlem and five smaller nearby communities, in total 160.000 P.E. (table 5.1). The removal of organic matter and nitrogen is accomplished in a biological system which consists of four parallel activated sludge lines, while phosphate removal takes place in a combined biological-chemical side-stream process where the side-stream sludge from all four activated sludge lines is treated (description of the side-stream process is given in section 1.4.1). Due to its complexity from both the process and the operational point of view, this plant can be considered as one of the most complicated sewage works in the Netherlands.

The plant consists of three main lines: a conventional anoxic/aerobic activated sludge process line, a bio-P selection and P-precipitation line, and sludge treatment line.

Figure 5.1 Layout of the WWTP "Haarlem Waarderpolder"

Table 5.1 Designed and recorded data of the WWTP "Haarlem Waarderpolder"

Load	Unit	Designed 1994	Recorded 1996	Recorded 1997
Plant loading	P.E.	160.000	109.000	109.100
Hydraulic loading				
Average flow	m ³ /h		1.375	1.333
Dry weather flow	m ³ /h	2.175		
Storm weather flow	m ³ /h	7.000		
Sewage strength				
BOD (54 g BOD/P.E.d)	kg BOD/day	8.640 (54)	3.907 (39)	5.082 (47)
Kj-N (12 g Kj-N/P.E.d)	kg N/day	1.920 (12)	1.437 (14.2)	1.436 (13.2)
P _{total} (1.8 g P/P.E.d)	kg P/day	288 (1.8)	227 (2.2)	203 (1.9)
Effluent quality				
BOD	mg BOD/L	10	5	7
COD _{filtered}	mg COD/L		29	34
COD _{total}	mg COD/L		36	42
N _{total}	mg N/L	10	4.2	4.9
NO ₃ -N	mg N/L		3.5	3.4
NH ₄ -N	mg N/L		2.5	2.8
P _{total}	mg P/L	1	0.66	0.60
TSS	mg TSS/L	15	9	12

Figure 5.2 Simplified block process scheme of the WWTP "Haarlem Waarderpolder" (numbers in brackets indicate the number of process units)

5.2.1.1. Conventional Anoxic/Aerobic Activated Sludge Process Line

Raw sewage is transported to the pumping station of the plant via combined sewerage (figure 5.2). After screening, wastewater is elevated for a second time and divided into two primary settling tanks. Settled sewage from each primary settler is split into two, prior to being introduced to in total four activated sludge units. Each activated sludge unit comprises of three inter-connected compartments (figure 5.3 and 5.4), namely, a central compartment (non-bulking selector, $V=125 \text{ m}^3$), an inner non-aerated ring (denitrification tank, $V=4330 \text{ m}^3$), and an outer, aerated ring (nitrification tank with a total volume of 4330 m^3 of which around 85% is aerated). Beside the outer ring, also the non-bulking selector is equipped with an aeration facilities. However, the non-bulking selector is not aerated during normal plant operation. The aeration in the nitrification ring is provided by a set of twenty submerged small bubble aerators per unit. Activated sludge is circulated using four propellers located in each of four units. While the mixed liquor flows from the non-bulking selector to the denitrification ring and further to the non-aerated section of nitrification ring via weirs, a high internal recirculation ($5000\text{-}8000 \text{ m}^3/\text{h}$) from the non-aerated section of nitrification ring into the denitrification ring is obtained via a submerged tunnel located at the bottom of the structure (under the weir).

Figure 5.3 Activated sludge unit: (a) under full operation, (b) under construction, (c) central compartment (plug-flow non-bulking selector), (d) one of four propellers, (e) part of the diffused aeration system and weir to the secondary settlers and (f) connections between the denitrification and the nitrification ring (the weir and the tunnel for internal recirculation). *Photos by Paul Versteeg.*

Figure 5.4 Simplified scheme of activated sludge unit at the WWTP Haarlem Waarderpolder.

Recirculation is forced by the action of a propeller placed at the inlet of the tunnel (figure 5.3 and 5.4). A combination of diffused aeration, agitation by propellers and a high internal recirculation, makes the activated sludge in this unit well mixed (except in the comparatively small non-bulking selector which is designed as a plug-flow system). The activated sludge leaves the nitrification ring via the weir prior to splitting into two secondary settlers. After settling, the purified sewage is discharged into the nearby recipient.

A large fraction (95%) of the settled activated sludge (secondary sludge) is returned to the activated sludge unit in order to maintain the desired biomass content (currently around 6 g MLSS/L). Around 40% of this 95% is pumped to the non-bulking selector (recirculated sludge A) and remaining 60% to the denitrification ring (recirculated sludge B). The remainder (5%) of the settled sludge (side-stream sludge) is pumped to phosphorus treatment line.

5.2.1.2. Phosphorus Selection and Precipitation Line

Side stream sludge is introduced to two plug-flow agitated anaerobic tanks, each of $V=875 \text{ m}^3$, called the P selector tank (loaded with 10% of the side-stream sludge) and the P release tank (loaded with remaining 90%). P selector and P release tanks serve all four activated

sludge units and have an identical structure in which the same processes take place. However, there are two major differences between these two tanks: firstly, the source of VFA needed for P release in the P selector tank is the supernatant of the primary thickener, while in the P release tank, concentrated acetic acid is added to the sludge. About two thirds of the primary thickener's supernatant, (containing substantial amount of VFA as a result of fermentation process that takes place in this unit) is introduced to the P selector where the VFA were used by the PAOs in the sludge. Alternatively, there is a provision for sharing this two thirds of supernatant between the P selector and the P release tanks, which actually occurred at the time when sampling program at the plant was carried out. Due to the dilution effect caused by high thickener supernatant flow, a fivefold higher biomass content occurs in the P release tank in comparison with the P selector. Secondly, the function of the P selector and P release tanks in this plant is different; since there is no provision of an anaerobic tank in the main stream, the P selector is needed to achieve and maintain enough PAOs in the system by means of recirculation of the sludge from the P selector to denitrification ring. The purpose of the P release tank (as being a part of a side-stream process) is (beside selecting for PAOs) to achieve a maximal phosphate release at very high phosphate concentration from the biomass into the solution. The phosphate-rich mixed liquor flows into two belt thickeners where partial liquid-solid separation takes place (dry solids content in the thickened sludge is 6%). While 90% of the thickened sludge is returned to the denitrification ring, the remaining part (excess sludge) is wasted via sludge treatment line. The sludge wasting rate results in a total sludge retention time (SRT) in the system of 84 days. Phosphate-rich filtrate from belt thickeners is further treated by addition of calcium-hydroxide to precipitate phosphorus dissolved in the filtrate. Phosphate precipitation takes place in a small settler from which the phosphate-rich chemical sludge is withdrawn for further treatment.

5.2.1.3. Sludge Treatment Line

Three different types of sludge are produced at this plant: primary sludge, activated excess sludge collected in the secondary settlers, and phosphate-rich chemical sludge.

The sludge collected in primary settlers (primary sludge) is, after de-sanding, introduced into the primary gravity thickeners. In addition, the surplus activated sludge (5.360 kg DS/day) from the WWTP Schalkwijk (plant capacity of 80.000 P.E.) is pumped into the primary thickeners, mixed with primary sludge, and thickened.

Excess activated sludge is pumped and mixed with the primary sludge to anaerobic digesters, further thickened in a secondary gravity thickener and introduced to the homogenization tank. In this tank the phosphate-rich chemical sludge is also added and mixed with the already mineralized and thickened mixture of primary and excess sludge. The mixture of three sludges is then transported to band filter presses where the final liquid-solid separation occurs resulting in a sludge cake containing 20% DS. This sludge cake is

transported from the plant by means of trucks. All water streams resulting from the sludge treatment are via internal sewerage returned to the beginning of the plant.

5.2.2. Influent Characterization

To make the application of mathematical models for simulation of activated sludge systems (like ASM1 and ASM2) more uniform, the Dutch water authorities recently selected the simulation program SIMBA® as a modeling standard in the Netherlands. Furthermore, the influent characterization procedure was standardized as well (STOWA, 1996; Roeleveld and Kruit, submitted). The model variables, parameters to be measured and a set of equations needed for the influent characterization according to Dutch guidelines, are presented in table 5.2. A detailed derivation of the equations used in this characterization procedure is given in appendix 1.

There are two influent flows to the section of the WWTP "Haarlem Waarderpolder" which needed to be characterized: (1) the influent of the non-bulking selector (settled sewage) and, (2) the influent of the P selector and the P release tanks (supernatant of the primary thickeners). The term influent is further in the text used for these two flows; not for the raw sewage which enters the plant. The term effluent refers to the mixed supernatant of two secondary settlers which are part of the concerned activated sludge line, not to the total effluent of the plant (from all eight secondary settlers). Influent and sludge characterization was only performed using steady-state data obtained from sampling program and plant records (see figure 5.6 for sampling locations).

5.2.3. Performed Biological Methods for Sludge Characterization

A range of sludge batch tests were executed at the site in November 1996 and April 1997 as part of the experimental program. For this purpose, lab-scale sequencing batch reactors (SBRs) were transported and re-assembled at the on-site laboratory of the WWTP (figure 5.5).

Two double jacketed laboratory fermenters (each 1.5 L) with automated operation, control and monitoring were used in all batch tests. The equipment used in the tests is described elsewhere (Brdjanovic et al. 1997a). The activated sludge used in the tests was always the fresh sludge taken at the pumping station of recirculated sludge. Each test was repeated at least three times. The tests were performed at controlled temperature of 20°C and pH of 7.0±0.1. Process rates were determined as initial rates by using linear regression method.

Table 5.2 Influent characterization according to STOWA guidelines and combined ASM2 and Delft BPR model

Influent characteristics required by the combined ASM2 and Delft BPR model			Equations for determination of the influent characteristics according to STOWA, 1996.		Parameters needed to be measured for the influent characterization according to STOWA, 1996.	
Symbol	Name	Unit		Symbol	Name	Unit
Soluble Components						
S _{O2}	Oxygen (negative COD)	g COD/m ³	S _A	=	COD _{VFA}	Total influent COD
S _F	Readily biodegradable organics	g COD/m ³	S _I	=	0.9 · COD _{VFA}	Influent COD filtered
S _A	Volatile fatty acids	g COD/m ³	S _S	=	COD _{fil,inf} - S _I	Effluent COD filtered
S _{NH4}	Ammonium & ammonia nitrogen	g N/m ³	S _F	=	S _S - S _A	COD from VFAs
S _{NO3}	Nitrate & nitrite nitrogen	g N/m ³	S _{NH4}	=	K _J N - Σ(i ^N · X + i ^N · S)	influent BOD5
S _{PO4}	Inorganic soluble phosphorus	g P/m ³	S _{PO4}	=	P _{total} - Σ(i ^N · X + i ^N · S)	effluent BOD5
S _I	Soluble inert organic matter	g COD/m ³			NH ₄	Ammonia
S _{ALK}	Alkalinity	mol/m ³			PO ₄	Phosphate
Particulate Components						
X _I	Particulate inert organic matter	g COD/m ³	X _S	=	α · COD _{susp,inf}	BOD constant
X _S	Slowly biodegradable substrate	g COD/m ³	X _I	=	(1 - α) · COD _{susp,inf}	Yield factor for BOD
X _H	Active heterotrophic biomass	g COD/m ³	X _H	=	0	Note:
X _{PHA}	Phosphate accumulating organisms	g COD/m ³	X _{PHA}	=	0	In Dutch wastewater treatment practice the majority of the above parameters are measured as a part of sampling programs routinely performed at their biological nutrient removal plants.
X _{PAO}	Poly-phosphate	g COD/m ³	X _{AUT}	=	0.1 to 1.0	
X _{PP}	Poly-hydroxy-alkanoates	g P/m ³	X _{PAO}	=	0.1 to 1.0	
X _{PHA}	Glycogen	g COD/m ³	X _{PP}	=	0	
X _{GLY}	Active autotrophic biomass	g COD/m ³				
X _{AUT}	Mixed liquor suspended solids	g MLSSL/m ³	α	=	[(BOD _{tot} /(1 - Y _{H,BOD}) - S _S)/COD _{susp,inf}]	
X _{TSS}	Metal-hydroxides	g Fe(OH) ₃ /m ³	BOD _{tot}	=	COD _{susp,inf}	
X _{MeOH}	Metal-phosphate	g FePO ₄ /m ³	COD _{susp,inf}	=	BOD ₂ /(1 - e ^{-k₂})	
Q	Flow	m ³ /day		=	COD _{tot,inf} - COD _{fil,inf}	

Figure 5.5 Experimental set-up used for the batch tests at the WWTP "Haarlem Waarderpolder"

5.2.3.1. Anaerobic Phosphorus Release Batch Test

Acetate in excess (100-300 mgHAc-C/L or 266-800 mgCOD/L) was instantly added to the reactor under anaerobic conditions. During 3-4 h phosphate and acetate concentrations were measured to determine the maximal released phosphorus and the release rate. At the end of the test surplus acetate always remained in solution, therefore acetate did not limit phosphorus release.

5.2.3.2 Aerobic Phosphorus Uptake Batch Test

The sludge must be exposed first to anaerobic conditions in presence of acetate in order to deplete the internal poly-P pool and to increase the PHA level of the biomass so that PHB does not limit the P-uptake process. Acetate (20-25 mg HAc-C/L) was instantly added to the reactor under anaerobic conditions. During 3 h, acetate was fully consumed and phosphorus was released into solution. At this point half of the sludge was transferred to another, identical, reactor. The remaining part of the sludge was exposed to aerobic conditions to measure the maximal specific aerobic phosphorus uptake rate ($_{\max}q_P^{\text{aerobic}}$ in mgP/gVSS.h). In case the surplus acetate remains in solution after the anaerobic phase, the sludge should be washed and phosphate should be manually added to the mixed liquor prior to splitting.

5.2.3.3 Anoxic Phosphorus Uptake Test

The second part of the sludge cultivated in the foregoing batch test was exposed to anoxic conditions in order to determine the maximal specific anoxic phosphorus uptake rate ($_{\max}q_P^{\text{anoxic}}$ in mgP/gVSS.h). Anoxic conditions were maintained by addition of surplus amount of nitrate at the beginning of the test (28 mgN/L) and by the continuous flushing of the mixed liquor by N₂ gas.

5.2.3.4 Determination of the Fraction of Denitrifying Activity of PAOs

A bioassay for determination of the fraction of DPAOs in activated sludge has recently been proposed (Wachtmeister *et al.*, 1997). The method is based on the fact that DPAOs are active under both aerobic and anoxic conditions, whereas aerobic PAOs are inactive under anoxic conditions. From the comparison of the P-uptake rates under aerobic ($_{\max}q_P^{\text{aerobic}}$) and anoxic ($_{\max}q_P^{\text{anoxic}}$) conditions the relative proportion of denitrifying dephosphation activity ($_{\max}q_P^{\text{anoxic}} / _{\max}q_P^{\text{aerobic}}$) in the phosphorus removing organisms was calculated.

5.2.3.5 Nitrification Test

Ammonia was added under aerobic condition in excess amount (28 mg N/L) at the beginning of the batch test. During the test the conversion of ammonia to nitrate was monitored and the nitrification rate (q_{nitr} in mgN/gVSS.h) was determined.

5.2.3.6 Denitrification Test

Acetate (20 mg HAC-C/L) and nitrate in excessive amount (28 mg N/L) were added to the reactor in order to determine the denitrification capacity of the sludge. Acetate, phosphate, nitrate and nitrite concentrations were measured during the test. Denitrification rate (q_{denitr} in mgN/gVSS.h) was determined as initial nitrate utilization rate.

5.2.3.7 Endogenous Phosphorus Release Batch Test

The phosphate release under anaerobic conditions in absence of external organic substrate was measured during 4 h. The phosphorus release rate obtained from such test is considered as endogenous or secondary phosphorus release rate (q_P^{endog} in mgP/gVSS.h)

5.2.4. Sampling Program and Analytical Methods

The sampling and experimental program was executed in November 1996 and April 1997. During the course of the program, the weather conditions were favorable (there was no rain, and the plant operated under dry weather flow conditions at similar sewage temperature of

around 13°C). The on-site sampling locations are shown in figure 5.2. For influent characterization the following parameters were determined: COD_{total}, COD_{filtered}, BOD_{5, total}, PO₄, VFA (acetate, propionate, butyrate), NH₄, NO₃, pH, DO and temperature, while PHB, PHV, glycogen, MLSS and MLVSS were determined for sludge characterization (table 5.3).

Table 5.3 Overview of the parameters sampled at different locations of the WWTP "Haarlem Waarderpolder" as a part of the sampling program performed in November 1996 and April 1997

no.	Medium: sampling point	COD _{total}	COD _{filtered}	BOD ₅	PO ₄	Acetate	Propionate	Butyrate	NH ₄	NO ₃	MLSS	MLVSS	PHA	Glycogen	Temperature	DO	pH
1	Raw sewage: after screens				●												
2	Settled sewage: after primary settler	○	○	○	○	○	●	●	○	○					●	●	●
3	Activated sludge: after aerobic tank				●				●	●	●	●	●	●		●	
4	Effluent: after the secondary settlers				○				○	○							
5	Primary sludge: primary settler				●												
6	Schalkwijk sludge: outlet pipe				●	●											
7	Supernatant: primary thickener	●	●	●	●	●			●	●							
8	Side-stream sludge: P selector tank				●	●					●	●	●	●		●	●
9	Side-stream sludge: P release tank				●	●					●	●	●	●		●	●
10	Filtrate: belt thickener				●												

Spot sample
 24 h composite sample

The sampling points and frequency, and the choice of the parameters were governed by the specific requirements of mathematical models applied on this installation and by the content of the existing records. The analysis were performed according to procedures described in Brdjanovic et al. (1997a), and in the Dutch guidelines for sewage and sludge characterization (STOWA, 1996). The information obtained through the sampling program were combined with the data routinely collected (in weekly intervals) by the staff of the plant. In addition, the influent and the effluent flow records were used together with the internal flow rates obtained from the information on the capacities and operational time of selected pumps at the plant.

5.2.5. Modeling Tools

For the simulation of the WWTP "Haarlem Waarderpolder" the "Delft BPR model" replaced the module for P removal of ASM2. The stoichiometry and the kinetics of this combined model is presented in appendix 2 (after Van Veldhuizen et al. in press). This

combined model was incorporated in the software package SIMBA3.2+® (based on MATLAB 5.1® and SIMULINK 2®) which was used for the simulation of the plant operation. Simulations of plant operation were carried out (using static data) until a steady-state performance was achieved (usual simulation period was 365 days). The scope of modeling included one of the four, arguably identical, activated sludge lines and the biological part of the common section for combined biochemical phosphorus removal (see also figure 5.1). A personal computer (processor Pentium II® 233 MHz, 64 Mb memory) was used for simulation of this plant. Total computing time for present plant configuration as well as for each of three scenarios was up to 2 min from full start-up to steady-state (365 days chosen as simulation time). Computing time was up to 15 sec when the simulations were performed with previously calculated states. For simulation of batch test calculation time was as little as couple of seconds. All plant simulations were performed using the temperature of mixed liquor of 12.6°C as observed in the activated sludge unit, while in the batch sludge tests the temperature was controlled and was 20°C. Although the observed pH value at different locations at the treatment plant varied in the range 6.5 to 7.5, in the present version of SIMBA® it is still not possible to take into account different pH levels in the plant in one simulation.

5.2.6. Modeling Strategy

The simulation of the WWTP "Haarlem Waarderpolder" was performed in four steps of which the first three were interactive. In each step the characteristics of both liquid phase and activated sludge were compared with measured data. The steps are as follows:

- ☐ Simulation of the treatment plant operation (both liquid phase and biomass) and model calibration based on measured data (standard case Haarlem 1);
- ☐ Simulation of the batch tests using sludge properties as predicted in the previous step;
- ☐ Simulation of the performance of the treatment plant using feedback information from the batch test and their simulation;
- ☐ Simulation of different options for alternative process design and layout (Haarlem 2, 3 and 4).

5.3. Results

5.3.1. Sampling Program

The results of sampling program and a selection of routinely collected data by the plant staff are summarized in appendix 3. During the entire period of sampling the plant effluent quality was in accordance with Dutch standards.

5.3.2. Influent and Sludge Characterization

Results of the influent characterization performed by following the procedure described in appendix 1 and using data from appendix 3 are summarized in table 5.4. Sludge characteristics were obtained from sampling program (appendix 3) and by performing various batch tests as described in section 5.2.3.

5.3.3. Hydraulic Set-up of the Plant Model

Plant design documentation, existing process scheme and current operational mode of the plant were used to create the hydraulic flow scheme of the installation (called standard case Haarlem 1; figure 5.6) made in SIMBA®. More information about this scheme is given in table 5.5.

Table 5.5 Major components of the model scheme Haarlem 1

Component	Number of units	Total volume [m ³]	Modeled as (SIMBA® blocks)
☐ Non-bulking selector	1 ¹⁾	125	1 anaerobic reactor
☐ Denitrification ring	1 ¹⁾	4.380	1 anoxic reactor
☐ Nitrification ring	1 ¹⁾	4.380	1 aerobic reactor
☐ Secondary settlers	2 ¹⁾	6.000	1 mixed reactor with a loss of solids
☐ Sludge storage tanks	0 ²⁾	4.000 ⁴⁾	1 anoxic (anaerobic) reactor
☐ P selector tank ⁵⁾	1 ³⁾	875	1 anaerobic reactor
☐ P release tank ⁵⁾	1 ³⁾	875	1 anaerobic reactor
☐ Belt thickeners	2 ³⁾	n.a	1 ideal liquid/solid separator
1) Per line; in total there are four identical activated sludge lines 2) Introduced in the model to simulate sludge retention in secondary settlers 3) Serves all four activated sludge lines 4) Represents the volume of the sludge blanket 5) Made of eight identical compartments in serial			

Concerning the model hydraulic layout of the plant there are two points that need to be noted: (1) the sludge storage tank was introduced in the return sludge line to account for the conversions in the secondary settlers, allowing a sludge retention time similar to that in settler (sludge blanket only) and, (2) the P release and P selector tanks were each modeled as a one fully mixed anaerobic reactor, while, in fact, they consist of eight interconnected compartments in serial (plug-flow). Both actions were justified; the introduction of a sludge storage tank reduced the nitrate concentration in the sludge to the measured level (appendix 5), and modeling P release (and P selector tank) as one fully mixed tank instead of multiple tanks in series gave the same results for both liquid and solid phase (results of comparison not given).

Figure 5.6 Hydraulic scheme of the WWTP Haarlem Waarderpolder model

Table 5.4 Characterization of the settled sewage at the WWTP "Haarlem Waarderpolder". The characterization was based on the data collected during the period 3rd - 10th April 1998 at DWF and sewage temperature 12.6°C and pH 7.6

No.	Symbol	Name	Unit	Settled Sewage	Thickener's Supernatant	Comment
Soluble components						
1	S_{O_2}	Oxygen (negative COD)	g COD/m ³	2.1	0.05	Measured
2	S_F	Readily biodegradable organics	g COD/m ³	73	73	Calculated using eq. 5.9 in appendix 1
3	S_A	Volatile fatty acids (mainly)	g COD/m ³	32.4	49.9	Measured
4	S_{NH_4}	Ammonium & ammonia nitrogen	g N/m ³	46.5 ¹⁾	55 ²⁾	
5	S_{NO_3}	Nitrate & nitrite nitrogen	g N/m ³	0.1	0	
6	S_{PO_4}	Inorganic soluble phosphorus	g P/m ³	6.4 ³⁾	6 ⁴⁾	From eq. 5.8, $Y_{800} = 0.20$ and $k_{800} = 0.23d^{-1}$ Default value of the ASM2
7	S_I	Soluble inert organic matter	g COD/m ³	22	22	
8	S_{ALK}	Alkalinity	mol/m ³	5	5	
9	X_I	Particulate inert organic matter	g COD/m ³	63	50	Calculated using eq. 5.14 in appendix 1
10	X_S	Slowly biodegradable substrate	g COD/m ³	141	166	Calculated using eq. 5.13 in appendix 1
11	X_H	Active heterotrophic biomass	g COD/m ³	0.01	0.01	Assumed that there is very little biomass present in the settled sewage, see appendix 1
12	X_{PAO}	Phosphate acc. organisms	g COD/m ³	0.01	0.01	
13	X_P	Poly-phosphate	g P/m ³	0.001	0.001	
14	X_{PHA}	Poly-hydroxy-alkanoates	g COD/m ³	0.001	0.001	
15	X_{GLY}	Glycogen	g COD/m ³	0.001	0.001	
16	X_{AUT}	Active autotrophic biomass	g COD/m ³	0.01	0.01	Calculated using eq. 5.15 in appendix 1
17	X_{TSS}	Mixed liquor suspended solids	g MLSSL/m ³	153	162	
18	X_{MeOH}	Metal-hydroxides	g Fe(OH) ₃ /m ³	0	0	
19	X_{MeP}	Metal-phosphate	g FePO ₄ /m ³	0	0	Estimated zero
20	Q	Flow	m ³ /day	5760	5520	Measured

1) $K_1N = 55$ mg N/L   2) $K_1N = 65$ mg N/L   3) Total P = 9.1 mg P/L   4) Total P = 8.6 mg P/L

The results of simulation of the operation of WWTP Haarlem Waarderpolder (standard case Haarlem 1) are presented in appendix 5. The composition of liquid phase and the biomass was obtained at various locations throughout the plant (marked as T in appendix 4). The model described well the present operation of the plant in terms of both liquid phase and biomass concentrations. Predicted effluent concentrations, like ammonia, nitrate and phosphate, were similar to those observed at the plant. The performance of the P release and P selector tank was also predicted satisfactorily. In case Haarlem 1b, the acetate addition to the P release tank was adjusted to the actual process requirements resulting in a similar, satisfactory performance of the plant. According to the model, the acetate addition could be decreased by up to 30% without the effluent quality being affected. The possible optimization of acetate dosing will be discussed later in the section 5.4.3. Predicted SRT was 84 days.

5.3.4. Model Calibration

5.3.4.1. Calibration procedure

Calibration was based on a proper influent and sludge characterization and detailed evaluation of the flow scheme of the treatment plant. Calibration was done on static data from the treatment plant combined with separate batch experiments with sludge and wastewater. A step-wise calibration procedure was applied in which just a few specific parameters were calibrated on specific plant data according to procedure by Henze et al. (1998).

For the initial simulation of the plant performance a default set of parameters was used; the parameters related to COD and N conversions were default parameters of ASM 2, and the parameters for P removal originated from lab-scale SBR systems with an enriched culture of PAOs grown on acetate.

The first step was to calibrate the SRT and the activated sludge concentration (MLVSS). Since the plant was neither upgraded nor operated completely according to the project documentation, the plant manager indicated that the treatment plant operates at SRT which exceeds the designed value of 28 days at least two times. The biomass concentration in the activated sludge unit was adjusted to the observed level of around 4.5 gMLVSS/m³ by varying the sludge wasting rate and taking into account the amount of biomass leaving the plant via effluent. Proper biomass content of the P release and P selector tank was adjusted by equal distribution of the supernatant of the thickener between them (the amount of side-stream sludge was fixed by the maximal flow rate of four identical submerged pumps). Calculated SRT strongly exceeded the expectations, being 84 days. It is important to note that this first calibration step was based on volatile suspended solids, not on total suspended solids. Total suspended solids (in g/m³) were calculated from the MLVSS predicted by the model by using measured ash content (~25%) and a conversion factor of 1.37

gCOD/gMLVSS. The SRT was also calculated using a phosphorus balance over the treatment plant which indicated a SRT of 80 days, which is in accordance with the value of 84 days obtained from the calibration procedure.

When the SRT calibration is concerned, an accurate determination of the first order rate constant of the BOD measurement (k_{BOD}) used for influent characterization is of great importance (see further discussion in section 5.4.1). Although the value of k_{BOD} is the characteristic value of a specific plant (may vary from 0.15 to 0.60 1/d), the recommended value of 0.23 1/d (STOWA, 1996) appeared to be the correct one for this plant (there was no measured data on k_{BOD} available). Due to the reasons explained later in the text, it is likely that k_{BOD} needs to be calibrated for each plant.

Once the SRT and the activated sludge concentration were calibrated, the next steps were ammonium and nitrate calibration. By the adjustment of the value of dissolved oxygen concentration at the end of the nitrification ring in the model to the observed level of 1.8 mgO₂/L, very reasonable results were achieved (2.4 mgNH₄-N/L and 5.3 mg NO₃-N/L versus 2.5 mgNH₄-N/L and 4.6 mg NO₃-N/L in reality as average for April '97), hence there was no need for further calibration for nitrification and denitrification. In the context of wastewater treatment, it is almost impossible, but also unnecessary to take into account all uncertainties and to fit the model predictions exactly to the measured data. Here, the aim was to predict the observed data within the uncertainty of the measurements themselves.

As far as biological phosphate removal is concerned there was no need for further calibration of the phosphorus concentrations in the system. While P levels were predicted well, the acetate concentration was not fully correct in P selector and P release tanks. There could be at least three possible reasons for it: (a) the actual acetate dosage rate to the P release tank (at the time when the sampling was performed) was higher than the yearly average dosage rate of 22 L/h (this flow rate is estimated by the plant staff based on the records of annual purchase and consumption of 70% acetic acid), (b) default value for the maximal fermentation rate constant (q_{fe}) is not correct in the model, and (c) actual wastewater and sludge flows to P release and P selection tank were different from those obtained from the plant staff (and as such used in the hydraulic scheme of the plant model).

According to our measurements (appendix 3) a considerable fraction of acetate was not utilized in both P selector and P release tank, and therefore, was either wasted via excess sludge or returned to the activated sludge unit via return sludge (figure 5.2). Using the acetate dosing rate of 22 L/h the model predicted overall lower acetate levels in a comparison with the observed, and a complete acetate uptake in the P release tank was forecasted (contradictory to the measurements). To increase the acetate level in the P release tank and to obtain a better fit of the model prediction to the measured data a higher acetate dosage rate of 35 L/h was used. With this increased acetate dosage rate the level of acetate at the beginning of the P release tank was adjusted to the measured value of around

130 mgCOD/L. However, predicted acetate concentration at the end of this tank was still around 20 mgCOD/L lower than measured (data not shown). To further improve prediction of acetate levels in a side stream process, the maximal fermentation rate q_{fe} was tuned on P selector tank. By using a default value of q_{fe} in the model of 3 gCOD/gCOD.d the fermentation process was overestimated resulting in a higher predicted acetate concentration at the end of the P selector tank than at the beginning, which is again in disagreement with the observed situation. This clearly indicated a need to reduce q_{fe} (as it was also the case in a study of van Veldhuizen et al. in press). Reducing the q_{fe} improved a model fit to the measured data in the P selector tank. However, in the P release tank, reduced fermentation rate caused even further lowering of acetate level at the end of the tank. The best model prediction (but still not fully correct) was obtained with q_{fe} of 1.0 gCOD/gCOD.d. which makes the sum of the differences between predicted and measured acetate concentration at the end of the P release and P selection tank minimal. The wastewater and sludge flow rates are considered correct in this study.

The default and the observed phosphate/acetate ratio in this case could not be compared due to the fact that it was practically not possible to obtain adequate influent samples from the P release and selector tank. Therefore, it was decided to keep the default phosphate/acetate ratio ($Y_{PO_4}=0.36$ g P/g COD, Smolders et al. 1994a) of the model (see also section 5.3.5.1). The higher PO_4/HAc ratio observed in the P-selector tank in comparison with the P-release tank (1.34 and 0.27 gP/gCOD respectively) may be partially attributed to influence of pH on the anaerobic P release (Smolders et al, 1994b). The impact of pH on fermentation process, which can also influence the observed PO_4/HAc ratio, is not investigated yet. Measured pH was 6.5 in P release tank and 7.5 in P selector tank. Lower pH in P-release tank is caused by the addition of acetate and by the presence of CO_2 (produced in glycogen conversion to PHA) in a mixed liquor because there is no CO_2 stripping provided. SIMBA 3.2+ does not yet allow for simultaneous simulation of different pH values in different units. The other reasons beside influence of pH may be different fermentation and hydrolysis rates between these two tanks, and/or possible presence of glycogen accumulating non-poly-P bacteria (GAOs) in the systems (the later is discussed in section 5.3.5.1). Due to above complications no further attempt to have a better calibration was made.

Although the batch sludge experiments were used for model validation, some were also used for model calibration. Based on the results of aerobic and anoxic P uptake batch tests, the percentage of denitrifying activity of PAOs in this plant was determined to be as high as 80%. Therefore, the reduction factor under anoxic conditions $\eta_{NO_3}^P$ was changed from 0.50 to 0.80 to fit the ratio of phosphate removed in the aerobic ring to phosphate removed in the anoxic ring. After the first iteration, the calibration procedure was repeated in a second loop. It is striking that even with the default set of parameters the model predicted the measured parameters of the liquid phase throughout the plant reasonably well (the results not shown). This was to a less extend true for the observed internal biomass

concentrations, especially for the glycogen - active biomass ratio (f_{gly}) which was extremely high (2 gCOD/gCOD, results not shown) when the original value of k_{gly} (1.09 gCOD/gCOD.d) was used. Therefore, in the second loop the glycogen formation rate (k_{gly}) was changed to 0.15 gCOD/gCOD.d to fit the predicted data to the normal values of glycogen (~ 0.4 gCOD/gCOD, appendix 5). Change in k_{gly} influences f_{gly} which further affects f_{pp} and f_{pha} due to back coupling between these parameters in the bioP model. By decreasing k_{gly} , f_{gly} is reduced, and consequently X_{PAO} increased (this issue is discussed further in section 5.4.2). Based on the simulation results (appendix 5) it was judged that no further calibration is needed. The list of parameters adjusted in the calibration procedure is given in table 5.6.

Table 5.6 List of the model parameters which value was changed in the calibration procedure

Parameter	Symbo l	Unit	Old Value	New Value
Maximal fermentation rate	q_{fe}	gCOD/gCOD.d	3	1
Reduction factor under anoxic conditions	$\eta_{NO_3}^P$		0.5	0.8
Glycogen formation rate	k_{gly}	gCOD/gCOD.d	1.09	0.15

5.3.5. Model Validation

The model validation was based on a check of its capability to well describe (1) the liquid phase of the plant flows using static influent data, and (2) the sludge properties determined in batch tests. For this purpose batch experiments were simulated in SIMBA® (figure 5.7a-f) and simulation results are summarized in appendix 6.

5.3.5.1. Maximal Anaerobic Phosphate Release Batch Test

Observed phosphate and acetate concentrations obtained in sludge batch test were in general well predicted by the model (figure 5.7a). In case of activated sludge from Haarlem the maximal P release rate was 6 mgP/gVSS.h. According to model prediction total P release was limited by the poly-P content of the biomass. An average observed phosphate/acetate ratio from five repeated tests was 0.29 ± 0.04 gP/gCOD, similar to the value observed at the WWTP in P release tank (0.27 gP/gCOD), but lower than the default value of the model (0.36 gP/gCOD). The similarity between the PO_4/HAc ratio obtained in batch tests and observed PO_4/HAc ratio in the P release tank is expected due to the fact that the influence of temperature (in tests 20°C and in the plant 12.6°C) should have a marginal influence of anaerobic stoichiometry (Brdjanovic et al. 1997a) and that in both

Figure 5.7 Concentration of phosphate (■) acetate (●), ammonia (▲), and nitrate (◆) in batch tests performed with settled activated sludge at temperature of 20 °C and pH 7.0±0.1. The model prediction is represented with solid line. The experimental procedure is given schematically for each test

cases the same, external, acetate source was used. The model default value for phosphate/acetate ratio was determined from laboratory tests using enriched bio-P culture (Smolders et al, 1994b) and was confirmed in other tests using both enriched culture and activated sludge from full-scale plants. The reason for the comparatively lower PO_4/HAc ratio obtained in batch tests may either be the influence of extremely long SRT or that in WWTP in Haarlem GAOs (Cech and Hartman, 1990 & 1993; Liu et al., 1994; Satoh et al., 1994; Matsuo, 1994, Mino et al. 1996) have accumulated due to oversupply of acetate. GAOs are capable of anaerobic utilization of organic substrates which are converted and stored as PHAs, while the energy and reduction equivalents are provided by only glycogen degradation without involvement of poly-P (for more details the reader is referred to chapter 8). The fact that in all four maximal P release batch tests acetate was still utilized even after full poly-P depletion in the biomass suggests a significant presence of GAOs in the plant (Brdjanovic et al. 1997b). Because surplus acetate is available to both PAOs and GAOs, a competition for substrate, which PAOs would win under acetate limited conditions, did not take place here. There are three possible ways to proceed with this issue in this study: (1) to assume there is no GAOs in the system and therefore, to lower default PO_4/HAc ratio, (2) to believe there is significant presence of GAOs and include their metabolism in the model, or (3) to still presume there is significant presence of GAOs in the system, do not model their metabolism, retain the default PO_4/HAc ratio, and leave this issue for further research. The last option was adopted in this work.

5.3.5.2. Aerobic and Anoxic Phosphate Uptake Batch Tests

The P uptake tests (figure 5.7b and c) showed a very good agreement between predicted and observed data after adjustment of glycogen formation rate (k_{gly}) in the set of model parameters as described in section 5.3.4 and discussed in section 5.4.2. By comparison of P uptake rate under aerobic and anoxic conditions (2.2 and 1.7 mgP/gVSS.h, respectively) the fraction of denitrifying activity of PAOs was estimated to be 80%.

5.3.5.3. Nitrification Batch Tests

Nitrification process performance in the batch test (figure 5.7d) was not predicted very well by the model; observed ammonia conversion to nitrate was approximately 40% lower than predicted. There are two possible reasons for this discrepancy: either the amount of nitrifiers (X_{AUT}) predicted by the model in the plant is too high or nitrification rate in the model is too high (or maybe both). To check this assumption two actions were carried out: (1) the decay rate of autotrophs (b_{AUT}) was increased from 0.15 to 0.25 1/d, and (2) maximal growth rate of autotrophic biomass (μ_{AUT}) was reduced from 1 to 0.65 1/d. Each of these changes made very good fit of predicted data to measured ones in a batch test. However, when these new values were fed back into simulation of the treatment plant, nitrification was insufficient, and effluent ammonia and nitrate concentrations were not predicted well (32 mg $\text{NH}_4\text{-N/L}$ and 1.3 mg $\text{NO}_3\text{-N/L}$ when the b_{AUT} was increased, and 25 mg $\text{NH}_4\text{-N/L}$ and

1.7 mgNO₃-N/L when the μ_{AUT} was decreased). Phosphate effluent concentration changed only very marginally. Consequently, it was not possible to predict the nitrification process in the plant and in the batch test in the same time. The reason for this controversy remains unclear.

5.3.5.4. Denitrification Batch Tests

The model did not predict well observed concentrations in this test (figure 5.7e). Especially it is noted that in this denitrification test there is observed a very large release of phosphate and a rapid consumption of acetate. The model predicts no P release and much less acetate uptake. This can for the time being directly be explained by the fact that the model does not take simultaneous presence of VFA (acetate) and electron acceptors (in this case nitrate) into account. Under denitrifying conditions (as in this test) acetate is taken up and a part of the uptake results in P release, but now TCA cycle instead of glycogen is delivering reduction equivalents (Kuba et al. 1996). This is not taken into account in the present version of Delft BPR model. In general, simultaneous presence of acetate and nitrate at WWT plants does not occur often. However, in some cases, it will be necessary to take such situation also into account in the model. Although denitrification in sludge batch test was not described satisfactorily, the nitrate concentrations in the treatment plant were predicted well.

5.3.5.5. Endogenous Phosphorus Release Batch Test

The results of this test (figure 5.7f) suggest that the P release in absence of external substrate is overestimated by the model. This P release can be the result of: (1) maintenance process as used in Delft BPR model (real endogenous P release) and (2) endogenous processes like decay of heterotrophs by which an extra substrate is supplied (concept used in ASM2) leading to P release. We suggest that this decay of heterotrophs is overestimated. Possibly if endogenous respiration, as in ASM3, instead of decay would be used, model and experiments would give the same results.

It can be concluded that sludge batch tests are a useful tool for model validation. In the case of WWTP Haarlem Waarderpolder the maximal anaerobic P release, anoxic and aerobic P uptake were predicted quite well by the model, while there is still some room for improvement in case of nitrification and endogenous P release processes. Denitrification process was not described well in batch test, however, satisfactory results were obtained in the simulations of the treatment plant. The reason for this discrepancy is not known yet. Simultaneous presence of acetate and nitrate only rarely occurs in wastewater treatment practice. It is important to note the strong influence of pH on the observed PO₄/HAc ratio in P release and P selector tanks. It is well possible that pH also influences the fermentation rate. To check this, independent experiments should be performed with return activated sludge (in this case fed with settled sewage) at pH values in the range 6 to 8.

5.3.6. Alternative Process Configurations of the Plant

The model schemes of three alternative process configurations of the WWTP Harlem Waarderpolder (case Harlem 2, 3 and 4) are shown in appendix 4. The simulation results are given in appendix 5. The comparison between selected process configurations is given in tables 5.7a and 5.7b.

Table 5.7a Comparison between present and alternative process configurations of WWTP Harlem Waarderpolder.

Plant Configuration		Type of P-removal process	Acetate addition	Loading ²⁾	Comments
Standard Case Harlem 1	1a	Side stream	0.84 ¹⁾ m ³ /d	Total	Normal plant operation
	1b	Side stream	0.36 m ³ /d	Total	
Alternative Harlem 2	2a	Main stream	zero	25%	
	2b	Main stream	zero	25%	Non-bulking selector enlarged
Alternative Harlem 3	3a	Main stream	zero	25%	Extra anaerobic tank added
	3b	Main stream	2.50 m ³ /d	25%	Extra anaerobic tank added
Alternative Harlem 4	4	Side stream	0.84 m ³ /d	Total	Aerated zone reduced by 25%

1) Estimated acetate dosing rate during sampling period based on the measured acetate concentrations in the P release tank. The yearly average acetate dosage rate is 0.53 m³/d (22 L/h) as 70% acetic acid

2) Total loading to the plant (included all four lines) was applied in cases of Harlem 1 and 4, while in cases 2 and 3 only loading of one line was applied (see also figure 5.7b)

5.3.6.1. Case Harlem 2

The part concerning side-stream biochemical phosphorus removal of Harlem 1 was removed. The rest of the plant which remained unchanged was simulated as such (case Harlem 2a). Since Harlem 2a presents, in essence, a non-optimized conventional configuration for COD and N removal, the predicted effluent quality strongly deteriorated in comparison with the standard case. To achieve better process performance, including biological P removal, an anaerobic tank (larger than non-bulking selector) is needed. The design criteria was that the acetate entering this anaerobic tank is fully utilized. By increasing the volume of the non-bulking selector from 125 to 1500 m³, a main-stream BPR was obtained (Harlem 2b) resulting in improved process efficiency. However, predicted effluent quality was still less good in comparison with the standard case. According to the model, the plant operated at SRT between 34 and 37 days. Acetate was not added externally.

Table 5.7b Comparison of selected operational parameters of present and alternative process configurations of WWTP Haarlem Waarderpolder.

Parameter	Unit	Standard Case Haarlem 1		Alternative 2 Haarlem		Alternative 3 Haarlem		Alternative 4 Haarlem
		1a	1b	2a	2b	3a	3b	4
SRT total	day	85	85	34	37	37	37	83
MLVSS aeration tank	mg/L	5927	5814	2595	2598	2564	3043	5948
Sludge Wasting	kg/d	2979 ¹⁾	2927 ¹⁾	848 ²⁾	837 ²⁾	838 ²⁾	991 ²⁾	2950 ¹⁾
PO ₄ effluent	mg P/L	0.3	0.8	7.5	8.0	2.5	0.7	0.3
NH ₄ effluent	mg N/L	2.4	2.1	3.5	4.5	2.7	3.5	3.1
NO ₃ effluent	mg N/L	5.3	5.9	7.8	2.6	9.3	5.6	5.7

1) from the total plant (4 lines) 2) from one of four identical lines

5.3.6.2. Case Haarlem 3

Case Haarlem 3 builds on Haarlem 2a, where an additional anaerobic P release tank (2000 m³) was placed before the activated sludge unit (modified UCT process). Internal sludge recirculation - influent flow ratio between nitrification and denitrification rings was decreased from 1:13 (Haarlem 1 and 2) to 1:5 and between denitrification ring and P release tank was 1:1. Operating sludge age was 37 days. In case Haarlem 3a external addition of acetate to P release tank was not applied and the predicted effluent quality was similar to that from Haarlem 2b. However, still less good in comparison with the standard case. Comparatively good effluent quality was only achieved after external acetate dosing was introduced (Haarlem 3b). The amount of acetate needed to be added was sevenfold higher in comparison with optimized acetate addition in case Haarlem 1b. The increased acetate consumption in this case is not strange due to the main-stream process employed (Smolders et al., 1996).

5.3.6.3. Case Haarlem 4

In Haarlem 1 it was assumed that nitrification ring was fully aerated. In fact twenty aerators per unit only cover about 85% of the ring. Additionally, at the time when the sampling procedure was performed, the last three aerating sets were shut-off (figure 5.4), making the aerated part of about three quarters of the total volume provided for nitrification. This means that a non-aerated denitrifying section is larger than one used in Haarlem 1. In Haarlem 4 this increase in denitrification zone at the expense of the nitrification zone was

considered. Obtained simulation results of Haarlem 4 show only marginal difference in comparison with Haarlem 1.

5.4. Discussion

5.4.1. Influent characterization

As far as the WWTP Haarlem Waarderpolder is concerned, the procedure for wastewater characterization (STOWA, 1996) proved satisfactory. In this case, the critical part of this procedure was estimation of the coefficient k_{BOD} . The methodology for k_{BOD} determination is described elsewhere (Roeleveld and Kruit, submitted). This coefficient has a large influence on the determination of particulate inert organic matter (X_I) and slowly biodegradable substrate (X_S). The model is very sensitive towards change in k_{BOD} (and X_I); a relatively small change in k_{BOD} strongly affects the effluent quality and SRT or sludge concentration, especially in the systems with long SRT, as it is the case in Haarlem (simulation results not shown). The impact of estimated k_{BOD} on calculated SRT for WWTP Haarlem Waarderpolder is shown in figure 5.8.

Figure 5.8 Influence of estimated k_{BOD} on calculated SRT and fraction of X_I and X_S of suspended COD

Even only marginal change in this value (for example ± 0.01) changes the calculated SRT for 5 days. It is a very important, but also very difficult task to have k_{BOD} determined at this accuracy. It is expected that the k_{BOD} need to be adjusted in the model calibration for WWTP with long SRT due to high sensitivity of the model to this parameter and to its

relatively rough experimental estimation methodology (inaccuracy certainly higher than 0.01).

5.4.2. Model Calibration

The concentrations of the soluble components in the liquid phase in the WWTP were predicted quite well even without any calibration. This was true only to a lower extend when the polymer (PHB, glycogen and poly-P) concentrations are concerned. This strongly suggests that the model should be calibrated not only on the liquid phase (effluent) concentrations, but also on the polymer concentrations. One of three parameters which were changed in the model was the glycogen formation rate constant (k_{gly}). In the Delft BPR model k_{gly} directly influences glycogen-active biomass ratio (f_{gly}). If the value of f_{gly} is incorrectly determined by the model, it will lead to inaccurate prediction of the f_{pp} and f_{pha} , which may directly affect prediction of the observed rates, such as P uptake rate. The k_{gly} was already reduced from 1.09 to 0.45 g COD/g COD.d in the case of WWTP Holten (Van Veldhuizen et al. in press). To check the influence of change in k_{gly} on predicted aerobic P uptake rate (and also anoxic P uptake rate) performance of WWTP in Haarlem was simulated with three different values of k_{gly} (1.09, 0.45 and 0.15 g COD/g COD.d). Clearly, a change in k_{gly} strongly affects the ratio of storage pools to active biomass (table 5.8).

Table 5.8 An example of influence of variation in k_{gly} on fraction of storage materials in the biomass (source: simulation standard case Haarlem 1)

Parameter	Unit	k_{gly} [g COD/g COD.d]		
		0.15	0.45	1.09
		This study SRT = 84 days	van Veldhuizen et al. (in press) SRT = 50 days	Murnleitner et al. (1995) SRT = 20 days
f_{gly}	g COD/g COD	0.49	1.1	1.89
f_{pp}	g P/g COD	0.18	0.2	0.24
f_{pha}	g COD/g COD	0.3	0.24	0.18

The sludge, with different characteristics (obtained from three simulations), was used to simulate aerobic and anoxic P uptake batch tests. Each batch test simulation was performed again with three different k_{gly} . The results showed that this second variation in k_{gly} constant had no impact on predicted P uptake rates. However, a comparison (figure 5.9) between predictions of batch tests using sludge characteristics obtained from the simulation of the treatment plant performance clearly indicated the need for a lower k_{gly} . Lower k_{gly} results

in a higher formation of the PAOs (X_{PAO}), thus comparatively higher P uptake rate is predicted at lower k_{gly} . This indicates that k_{gly} either strongly depends on SRT or the kinetic expression for glycogen in the model (equation 14 in appendix 2) is not fully correct. It is interesting to note that in the systems with longer SRT (table 5.7), the k_{gly} needed to be lowered (from 1.09 g COD/g COD.d at 20 days SRT, to 0.45 at 50 days SRT and 0.15 at 84 days SRT) to obtain a good prediction of aerobic and anoxic P uptake rate.

Figure 5.9 Influence of glycogen formation rate constant on aerobic phosphate uptake

WWTP Haarlem Waarderpolder has no facilities for on-line measurements. All simulations were performed under steady-state conditions. It is recommended to perform an additional sampling program consisting of continuous flow measurements and collection of 1 h composite samples over a period of at least 24 h at selected locations at the treatment plant (settled sewage, effluent and to a less extend supernatant of the primary thickener). Although generally advisable, data collection between anaerobic, anoxic and aerobic zones is not possible, due to (for this purpose unfavorable) design of the activated sludge unit structure. Essentially, the same set of data as for non-dynamic calibration are needed, only now as a function of time. Whether dynamic influent modeling will provide much more useful and valuable information than non-dynamic influent modeling is the question to be answered in a course of future work. If the data obtained in this way show to be highly variable, the treatment plant should be also modeled using this dynamic data. However, even in case of highly dynamic influent it is still not certain that there will be a large difference between the calculated values of the static and the dynamic influent simulations. The study of van Veldhuizen et al. (in press) showed almost no difference in simulation results when the averaged daily data or the dynamic influent data were used.

5.4.3. Operational Aspects

According to project documentation and plant operational record (table 5.1), since its expansion in 1995, the WWTP Haarlem Waarderpolder has been under-loaded. Consequently, this resulted in a long SRT of 84 days. When the plant loading would be increased to its full capacity, according to the model prediction, the SRT would drop to 54 days with biomass concentration in the activated sludge unit of 6 g/L, and the plant would still perform well (data not shown: simulated NO_3 , NH_4 and P effluent concentrations were 3 mgN/L, 6.4 mgN/L and 0.4 mgP/L, respectively). If, in addition to a design load, also the designed biomass concentration in the activated unit would be taken into account (4 g/L), the SRT would be even further reduced to around designed value of 28 days. However, in this case model simulation indicate deterioration in effluent quality, with phosphate removal strongly affected (data not shown).

Another operational aspect deserves to be mentioned due to its potential economic value. Although acetate is relatively easy to measure, it is not included in routine sampling program at the plant, and therefore the acetate concentration in the effluent of P release tank probably has seldom been checked at this plant. According to our measurements and model prediction, a considerable fraction of acetate was not utilized in the P selector and the P release tank. This is especially important when relatively expensive (1.7 Dfl/kg) 70% acetic acid is continuously dosed to the tank at average yearly dosage rate of 22 L/h. Both predicted and observed acetate concentrations at the beginning and the end of the P release tank suggest that either the actual acetate dosage rate at the time when the sampling was performed was higher than 22 L/h, or that the flows to P release and P selection tank were different in comparison with these obtained from the plant staff. If the first scenario is true, there is obviously room for optimization of acetate dosing to the P release tank (reduction of up to 30% would be possible as shown in section 5.3.3 and appendix 5 - case Haarlem 1b) especially when the treatment of extra sludge production is included. The plant simulation results as well as the acetate measurements indicate that only a fraction of acetate, fermented in primary thickeners and added to the P selector tank, is utilized in this tank. During normal plant operation the thickener's supernatant is only introduced into the P selector. During the period of sampling this supernatant was delivered in equal proportions to both tanks. The additional shift of this flow into P release tank might further reduce the need for external dosing of acetate to P release tank. Additionally, the optimization of acetate dosing could bring an extra benefits in case the hypothesis of presence of significant amount of GAOs in this plant is true (section 5.3.5.1). If there would be less acetate available, the competition between PAOs and GAOs for substrate would intensify and, eventually, GAOs would be washed-out from the system. This might result in even further reduction in requirements for acetate in a side-stream process. Since this matter is highly speculative, it is recommended to perform an additional sampling program where the acetate dosage and acetate, phosphate and biomass concentrations would be more

closely monitored over a couple of days in the period of normal operating conditions.

5.4.4. Process Configurations

The present system configuration (standard case Haarlem 1a) was in general well described by the model. Simulation showed lowering acetate addition did not affect process performance, which supports the indication of excessive acetate addition at this treatment plant. The standard case Haarlem 1 is a simplification of configuration Haarlem 4 (see table 5.7 in section 5.3.6). The case Haarlem 4 is actually more comparable to the present situation. It is interesting that this simplification and the fact that the aerobic ring is only 75 % aerated gives no effect on the effluent quality. This means that a slightly higher prediction of ammonia in the effluent in comparison with the measured values is not due to kinetic limitation for nitrification.

The potential use of modeling was also illustrated in case of Haarlem 2 and 3, where a side-stream configuration for bio-chemical P removal was modified into a main-stream biological P removal system. It was shown that without an external addition of acetate to both original and enlarged non-bulking selector, the effluent quality seriously deteriorated. Comparatively good effluent quality (similar to that of the standard case Haarlem 1) was achieved only when an additional P release tank was added in front of the activated sludge unit and external acetate was added. It is important to note that without chemical removal, main-stream process required sevenfold more acetate for the removal of same P load. This can be explained by the essential difference between side- and main-stream P removal process. In side-stream process comparatively less acetate is needed due to the fact that it is a combined bio-chemical process where only a small fraction of the total phosphorus load must be removed biologically for which only small number of PAOs is needed, while in a main-stream system a full population of PAOs is active in BPR process to remove the total given phosphorus load (Smolders et al. 1996).

Practical aspects and author's experience with modeling the WWTP Haarlem Waarderpolder are given in appendix 7.

5.5. Conclusions

The combined ASM2 and Delft BPR model for COD, N and P removal proved well capable of describing the performance of WWTP Haarlem Waarderpolder with adjustment of only three parameters of the model. The model was to a less extend capable of well describing some of the batch tests performed with activated sludge from the treatment plant. To take into account extreme situations which may occur in practice a more refined and complex model would be needed. Despite of the fact that the selected model performed satisfactorily

in this study, there is still room for further optimization of the kinetic expressions and the integration of the COD, N and P conversions modules in the future. This modeling exercise was not only carried out to gain more experience from practical application of the model and to find out where the model should be improved, but was also beneficial to better understanding of plant operation and treatment processes, and to further optimize the plant performance.

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Appendix 1

**Influent characterization procedure according to Dutch
guidelines (STOWA, 1996)**

Characterization of the organic fractions of the influent

The total COD in the influent is a sum of the COD of soluble ($COD_{\text{filt,inf}}$) and particulate fractions ($COD_{\text{part,inf}}$):

$$COD_{\text{tot,inf}} = COD_{\text{filt,inf}} + COD_{\text{part,inf}} \quad (5.1)$$

Soluble fraction of the COD consists of following components,

$$COD_{\text{filt,inf}} = S_A + S_F + S_I \quad (5.2)$$

while the particulate fraction is represented by:

$$COD_{\text{part,inf}} = X_I + X_S + (X_H + X_{\text{PAO}} + X_{\text{PHA}} + X_{\text{GLY}} + X_{\text{AUT}}) \quad (5.3)$$

For simplicity the contribution of particulate components of the active biomass present in the settled sewage and the supernatant of the primary thickeners to the total COD is neglected. For the modeling purposes a very low value is assigned to these components (table 5.4). Therefore eq. 5.3 can be written as:

$$COD_{\text{part,inf}} = X_I + X_S \quad (5.4)$$

Volatile fatty acids COD is directly measurable organic fraction of the influent COD and is presented as S_A :

$$S_A = COD_{\text{VFAs}} \quad (5.5)$$

Readily biodegradable substrate S_S was introduced in the ASM1. In ASM2 it is replaced by:

$$S_F = S_S - S_A \quad (5.6)$$

Introducing eq. 5.6 to eq. 5.2, the fermentable, readily biodegradable organic substrates S_F can be expressed as:

$$S_F = COD_{\text{filt,inf}} - (S_A + S_I) \quad (5.7)$$

The S_I in the influent is not affected by the treatment processes and, therefore, leaves the treatment plant with the effluent. This inert fraction can be calculated using the effluent COD measured after filtration ($COD_{\text{filt,eff}}$). The $COD_{\text{filt,eff}}$ has to be corrected with a factor 0.9 due to some production of S_I during the treatment process. For presence of BOD in the effluent the S_I is further reduced by the factor 1.5 $BOD_{5,\text{eff}}$. The factor 1.5 is a conversion factor of BOD_5 to COD.

$$S_I = 0.9 \cdot COD_{\text{filt,eff}} - 1.5 BOD_{5,\text{eff}} \quad (5.8)$$

Finally, by combining eq. 5.7 with eq. 5.8, S_F can be calculated using directly measurable parameters:

$$S_F = \text{COD}_{\text{filt,inf}} - 0.9 \cdot \text{COD}_{\text{filt,eff}} + 1.5 \text{BOD}_{s,\text{eff}} - S_A \quad (5.9)$$

In ASM1, the slowly biodegradable particulate substrate X_S is given as the difference between the biodegradable fraction of COD (BCOD) and the readily biodegradable substrate S_S :

$$X_S = \text{BCOD} - S_S \quad (5.10)$$

The routine BOD measurements are not recommended to be used for the estimation of the BCOD due to the facts that the BOD_5 measurement underestimates the BCOD, and that the BOD_{20} measurement is not yet reliable enough. The Dutch guidelines recommend the estimation of BCOD via determination of the total BOD (BOD_{tot}) from the BOD measurement by the best fit of the BOD-curve to the measured data. The following equation is recommended:

$$\text{BOD}_{\text{tot}} = \text{BOD}_t / (1 - e^{-k_B t}) \quad (5.11)$$

where k_{BOD} is the constant which varies from plant to plant and is in the range 0.15-0.6 d^{-1} for domestic sewage (Roeleveld and Kruit, submitted). Description of the determination of the k_{BOD} from the BOD test is given elsewhere (Roeleveld and Kruit, submitted). It is now possible to calculate the biodegradable COD from the total BOD taking into account the initial value of RBOD will be somewhat higher than the BOD_{tot} calculated by eq. 5.11. This is due to the conversion of the fraction of BCOD into an inert fraction during the course of the BOD test. This is corrected by the factor Y_{BOD} which falls in the range of 0.1-0.2 (Roeleveld and Kruit, submitted). The BCOD can be expressed as follows:

$$\text{BCOD} = \text{BOD}_{\text{tot}} / (1 - Y_{\text{BOD}}) \quad (5.12)$$

The X_S can be calculated from eq. 5.10 and 5.12:

$$X_S = \{[\text{BOD}_t / (1 - e^{-k_B t})] / (1 - Y_{\text{BOD}})\} - S_S \quad (5.13)$$

Finally, the inert particulate organic material is calculated from the eq. 5.4 and 5.13:

$$X_I = \text{COD}_{\text{part,inf}} - X_S \quad (5.14)$$

The concentration of total suspended solids (X_{TSS}) in the influent is estimated from following equation:

$$X_{\text{TSS}} = 0.85 \cdot \text{COD}_{\text{part,inf}} \quad (5.15)$$

Characterization of the N and P fractions in the influent

In ASM2 the nitrogen compounds are for simplicity represented only with its soluble components which are directly measurable parameters: ammonium plus ammonia nitrogen plus Kjeldahl nitrogen (S_{NH_4}) and nitrate and nitrite nitrogen (S_{NO_3}). The phosphate compounds are represented also as soluble phosphorus (S_{PO_4}). A small correction has to be made for an eventual poly-P fraction (X_{pp}) in the influent.

Appendix 2

**Stoichiometry and kinetics of the combined ASM2 - Delft
BPR model**

Stoichiometric matrix for dissolved and particulate components of combined ASM 2 - Delft BPR model

Process	S_{O_2}	S_F	S_A	S_{NH_4}	S_{NO_3}	S_{PO_4}	S_i	S_{ALK}	X_i	X_S	X_H	X_{PAO}	X_{PP}	X_{PHA}	X_{GLY}	X_{AUT}	X_{FSS}
1. Aerobic hydrolysis		$1-f_g$		C_{1A}		C_{1P}	f_g		C_{1A}	-1							C_{1A}
2. Anoxic hydrolysis		$1-f_g$		C_{1A}		C_{1P}	f_g		C_{1A}	-1							C_{1A}
3. Anaerobic hydrolysis		$1-f_g$		C_{1A}		C_{1P}	f_g		C_{1A}	-1							C_{1A}
HETEROTROPHIC ORGANISMS: X_H																	
4. Growth on S_F	$1-1/Y_H$	$-1/Y_H$		C_{4A}		C_{4P}			C_{4A}		1						C_{4A}
5. Growth on S_A	$1-1/Y_H$		$-1/Y_H$	C_{5A}		C_{5P}			C_{5A}		1						C_{5A}
6. Denitrification with S_F		$-1/Y_H$		C_{6A}	$-(1-Y_H)/2.86 Y_H$	C_{6P}			C_{6A}		1						C_{6A}
7. Denitrification with S_A			$-1/Y_H$	C_{7A}	$-(1-Y_H)/2.86 Y_H$	C_{7P}			C_{7A}		1						C_{7A}
8. Fermentation		-1	1	C_{8A}		C_{8P}			C_{8A}								
9. Lysis				C_{9A}		C_{9P}			C_{9A}	$1-f_{lH}$	-1						C_{9A}
PHOSPHORUS ACCUMULATING ORGANISMS: X_{PAO}																	
Anaerobic			-1			Y_{PO_4}		C_{10A}					$-Y_{PO_4}$	Y_{PHA}	$-Y_{GLY}$		C_{10A}
10. Storage of PHA						1		C_{11A}					-1				C_{11A}
11. Maintenance						C_{12P}		C_{12A}				$1/Y_{PHA}^0$		-1			C_{12A}
12. Lysis of PHA	$1/Y_{PHA}^0-1$			C_{12A}	$1-Y_{PHA}^{NO}/2.86 Y_{PHA}^{NO}$												
13. Storage of poly-P	$-1/Y_{PP}^0$			C_{13A}	$-1/2.86 Y_{PP}^{NO}$	C_{13P}		C_{13A}				$-1/Y_{PP}^0$	1				C_{13A}
14. Glycogen formation	$1-1/Y_{GLY}^0$			C_{14A}	$-(1-Y_{GLY}^{NO})/2.86 Y_{GLY}^{NO}$	C_{14P}		C_{14A}				$-1/Y_{GLY}^0$		1			C_{14A}
15. Maintenance	-1			C_{15A}		C_{15P}		C_{15A}				$-m_{PAO}^0/m_{O_2}$					C_{15A}
16. Lysis of PHA				C_{16A}		C_{16P}		C_{16A}				$1/Y_{PHA}^{NO}$		-1			C_{16A}
17. Storage of poly-P				C_{17A}		C_{17P}		C_{17A}				$-1/Y_{PP}^{NO}$	1				C_{17A}
18. Glycogen formation				C_{18A}		C_{18P}		C_{18A}				$-1/Y_{GLY}^{NO}$		1			C_{18A}
19. Maintenance				C_{19A}	$-1/2.86$	C_{19P}		C_{19A}				$-m_{PAO}^{NO}/m_{NO}$					C_{19A}
NITRIFYING ORGANISMS: X_{AUT}																	
20. Growth	$-(4.57-Y_A)/Y_A$			C_{20A}	$1/Y_A$	C_{20P}		C_{20A}								1	C_{20A}
21. Lysis				C_{21A}		C_{21P}		C_{21A}	f_{lH}	$1-f_{lH}$						-1	C_{21A}

Definition of stoichiometric coefficients in the matrix

□ Stoichiometric coefficients for S_{NH_4} :

$$\begin{aligned}
 c_{1,n} &= i_{XS}^{N} - i_{SI}^{N} * f_{SI}^{N} - (1 - f_{SI}^{N}) * i_{SF}^{N} \\
 c_{4,n} &= i_{SF}^{N} / \gamma_{H-i}^{N} i_{BM}^{N} \\
 c_{5,n} &= -i_{BM}^{N} \\
 c_{6,n} &= i_{SF}^{N} / \gamma_{H-i}^{N} i_{BM}^{N} \\
 c_{7,n} &= -i_{BM}^{N} \\
 c_{8,n} &= i_{SF}^{N} \\
 c_{9,n} &= i_{BM}^{N} - i_{XI}^{N} * f_{XIH}^{N} - (1 - f_{XIH}^{N}) * i_{XS}^{N} \\
 c_{12,n} &= -i_{BM}^{N} / \gamma_{PP}^{O} \\
 c_{13,n} &= i_{BM}^{N} / \gamma_{PP}^{O} \\
 c_{14,n} &= i_{BM}^{N} / \gamma_{GLY}^{O} \\
 c_{15,n} &= i_{BM}^{N} * m_{PAO}^{O} / m_{O_2} \\
 c_{16,n} &= -i_{BM}^{N} / \gamma_{PP}^{NO} \\
 c_{17,n} &= i_{BM}^{N} / \gamma_{PP}^{NO} \\
 c_{18,n} &= i_{BM}^{N} / \gamma_{GLY}^{NO} \\
 c_{19,n} &= i_{BM}^{N} * m_{PAO}^{NO} / m_{NO_3} \\
 c_{20,n} &= -i_{BM}^{N} - 1 / \gamma_A \\
 c_{21,n} &= i_{BM}^{N} - i_{XI}^{N} * f_{XIA}^{N} - i_{XS}^{N} * (1 - f_{XIA}^{N})
 \end{aligned}$$

□ Stoichiometric coefficients for S_{PO_4} :

$$\begin{aligned}
 c_{1,p} &= i_{XS}^{P} - i_{SI}^{P} * f_{SI}^{P} - i_{SF}^{P} * (1 - f_{SI}^{P}) \\
 c_{4,p} &= i_{SF}^{P} / \gamma_{H-i}^{P} i_{BM}^{P} \\
 c_{5,p} &= -i_{BM}^{P} \\
 c_{6,p} &= i_{SF}^{P} / \gamma_{H-i}^{P} i_{BM}^{P} \\
 c_{7,p} &= -i_{BM}^{P} \\
 c_{8,p} &= i_{SF}^{P} \\
 c_{9,p} &= i_{BM}^{P} - i_{XI}^{P} * f_{XIH}^{P} - (1 - f_{XIH}^{P}) * i_{XS}^{P} \\
 c_{12,p} &= -i_{BM}^{P} / \gamma_{PP}^{O} \\
 c_{13,p} &= i_{BM}^{P} / \gamma_{PP}^{O} - 1 \\
 c_{14,p} &= i_{BM}^{P} / \gamma_{GLY}^{O} \\
 c_{15,p} &= i_{BM}^{P} * m_{PAO}^{O} / m_{O_2} \\
 c_{16,p} &= -i_{BM}^{P} / \gamma_{PP}^{NO} \\
 c_{17,p} &= i_{BM}^{P} / \gamma_{PP}^{NO} - 1 \\
 c_{18,p} &= i_{BM}^{P} / \gamma_{GLY}^{NO} \\
 c_{19,p} &= i_{BM}^{P} * m_{PAO}^{NO} / m_{NO_3} \\
 c_{20,p} &= -i_{BM}^{P} \\
 c_{21,p} &= i_{BM}^{P} - i_{XI}^{P} * f_{XIA}^{P} - i_{XS}^{P} * (1 - f_{XIA}^{P})
 \end{aligned}$$

□ Stoichiometric coefficients for S_{ALK} :

$$\begin{aligned}
 c_{1,a} &= c_{1,n} / 14 - c_{1,p} * (1.5/31); \\
 c_{4,a} &= c_{4,n} / 14 - c_{4,p} * (1.5/31); \\
 c_{5,a} &= c_{5,n} / 14 - c_{5,p} * (1.5/31); \\
 c_{6,a} &= c_{6,n} / 14 - c_{6,p} * (1.5/31) + (1 - \gamma_H) / (14 * 2.86 * \gamma_H); \\
 c_{7,a} &= c_{7,n} / 14 - c_{7,p} * (1.5/31) + (1 - \gamma_H) / (14 * 2.86 * \gamma_H); \\
 c_{8,a} &= c_{8,n} / 14 - c_{8,p} * (1.5/31) - 1/64; \\
 c_{9,a} &= c_{9,n} / 14 - c_{9,p} * (1.5/31) \\
 c_{10,a} &= -\gamma_{PO_4} * (1.5/31) + 1/64 \\
 c_{11,a} &= -1.5/31 \\
 c_{12,a} &= c_{12,n} / 14 - c_{12,p} * (1.5/31) \\
 c_{13,a} &= c_{13,n} / 14 - c_{13,p} * (1.5/31) \\
 c_{14,a} &= c_{14,n} / 14 - c_{14,p} * (1.5/31) \\
 c_{15,a} &= c_{15,n} / 14 - c_{15,p} * (1.5/31) \\
 c_{16,a} &= c_{16,n} / 14 - c_{16,p} * (1.5/31) + (1 - \gamma_{PHA}^{NO}) / (14 * 2.86 * \gamma_{PHA}^{NO}) \\
 c_{17,a} &= c_{17,n} / 14 - c_{17,p} * (1.5/31) + (1 - \gamma_{PP}^{NO}) / (14 * 2.86 * \gamma_{PP}^{NO}) \\
 c_{18,a} &= c_{18,n} / 14 - c_{18,p} * (1.5/31) + (1 - \gamma_{GLY}^{NO}) / (14 * 2.86 * \gamma_{GLY}^{NO}) \\
 c_{19,a} &= c_{19,n} / 14 - c_{19,p} * (1.5/31) + (1/2.86) / 14 \\
 c_{20,a} &= c_{20,n} / 14 - c_{20,p} * (1.5/31) + (1/\gamma_A) / 14 \\
 c_{21,a} &= c_{21,n} / 14 - c_{21,p} * (1.5/31) \\
 c_{22,a} &= 1.5/31 \\
 c_{23,a} &= -1.5/31
 \end{aligned}$$

□ Stoichiometric coefficients for X_{TSS} :

$$\begin{aligned}
 c_{1,t} &= -i_{XS}^{TSS} \\
 c_{4,t} &= i_{BM}^{TSS} \\
 c_{5,t} &= i_{BM}^{TSS} \\
 c_{6,t} &= i_{BM}^{TSS} \\
 c_{7,t} &= i_{BM}^{TSS} \\
 c_{9,t} &= i_{XI}^{TSS} * f_{XIH}^{TSS} + i_{XS}^{TSS} * (1 - f_{XIH}^{TSS}) - i_{BM}^{TSS} \\
 c_{10,t} &= -3.23 * \gamma_{PO_4} + 0.6 * \gamma_{PHA} - 0.84 * \gamma_{GLY} \\
 c_{11,t} &= -3.23 \\
 c_{12,t} &= i_{BM}^{TSS} / \gamma_{PHA}^{O} - 0.6 \\
 c_{13,t} &= -i_{BM}^{TSS} / \gamma_{PP}^{O} + 3.23 \\
 c_{14,t} &= -i_{BM}^{TSS} / \gamma_{GLY}^{O} + 0.84 \\
 c_{15,t} &= -i_{BM}^{TSS} * m_{PAO}^{O} / m_{O_2} \\
 c_{17,t} &= i_{BM}^{TSS} / \gamma_{PP}^{NO} - 0.6 \\
 c_{17,t} &= -i_{BM}^{TSS} / \gamma_{PP}^{NO} + 3.23 \\
 c_{18,t} &= -i_{BM}^{TSS} / \gamma_{GLY}^{NO} + 0.84 \\
 c_{19,t} &= -i_{BM}^{TSS} * m_{PAO}^{NO} / m_{NO_3} \\
 c_{20,t} &= i_{BM}^{TSS} \\
 c_{21,t} &= i_{XI}^{TSS} * f_{XIA}^{TSS} + i_{XS}^{TSS} * (1 - f_{XIA}^{TSS}) - i_{BM}^{TSS}
 \end{aligned}$$

Stoichiometric parameters of the activated sludge model valid for 20°C

Symbol	Definition	Value	Unit	Reference
$i_{S_i}^N$	N content of inert soluble COD (S_i)	0.01	gN/gCOD	Gujer et al, 1995
$i_{S_F}^N$	N content of soluble COD (S_F)	0.03	gN/gCOD	"
$i_{X_i}^N$	N content of inert particulate COD (X_i)	0.03	gN/gCOD	"
$i_{X_S}^N$	N content of particulate COD (X_S)	0.04	gN/gCOD	"
i_{BM}^N	N content of biomass (X_H , X_{PAO} end X_{AUT})	0.07	gN/gCOD	"
$i_{S_i}^P$	P content of inert soluble COD (S_i)	0	gP/gCOD	"
$i_{S_F}^P$	P content of soluble COD (S_F)	0.01	gP/gCOD	"
$i_{X_i}^P$	P content of inert particulate COD (X_i)	0.01	gP/gCOD	"
$i_{X_S}^P$	P content of particulate COD (X_S)	0.01	gP/gCOD	"
i_{BM}^P	P content of biomass (X_H , X_{PAO} end X_{AUT})	0.02	gP/gCOD	"
$i_{X_i}^{TSS}$	Ratio of TSS to X_i	0.75	gTSS/gCOD	"
$i_{X_S}^{TSS}$	Ratio of TSS to X_S	0.75	gTSS/gCOD	"
i_{BM}^{TSS}	Ratio of TSS to biomass (X_H , X_{PAO} end X_{AUT})	0.9	gTSS/gCOD	"
f_{S_i}	Fraction of S_i from hydrolysis	0	gCOD/gCOD	"
Y_H	Yield van heterotrophic biomass (X_H)	0.63	gTSS/gCOD	"
$f_{X_{IH}}$	Fraction of inert COD from lysis	0.1	gTSS/gCOD	"
Y_{PO4}	Yield coefficient (PO4/HAc)	0.36	gP/gCOD	Smolders et al, 1994
Y_{PHA}	Yield coefficient (PHA/HAc)	1.5	gCOD/gCOD	"
Y_{GLY}	Yield coefficient (glycogen/HAc)	0.5	gCOD/gCOD	"
$\bar{\theta}$	Amount of ATP produced per NADH	1.8	mol/mol	Murnleitner et al, 1995
m_{ATP}	ATP consumption for maintenance	0.019	mol/mol	"
Y_{PHA}^0	Yield coefficient (PHA/biomass)	1.44	gCOD/gCOD	"
Y_{PP}^0	Yield coefficient (PP/biomass)	4.52	gP/gCOD	"
Y_{GLY}^0	Yield coefficient (GLY/biomass)	1.28	gCOD/gCOD	"
Y_{PHA}^{NO}	Yield coefficient (PHA/biomass)	1.76	gCOD/gCOD	"
Y_{PP}^{NO}	Yield coefficient (PP/biomass)	3.11	gP/gCOD	"
Y_{GLY}^{NO}	Yield coefficient (GLY/biomass)	1.42	gCOD/gCOD	"
Y_{AUT}	Yield of autotrophic biomass (X_H)	0.24	gCOD/gN	Gujer et al, 1995

Kinetic expressions for the combined ASM2 -Delft BPR model

Process	Rate equation
1. Aerobic hydrolysis	$K_h \cdot M_{LO2} \cdot \frac{f_s \cdot X_H}{K_{LX} + f_s}$
2. Anoxic hydrolysis	$K_h \cdot \Gamma_{LO2} \cdot l_{LO2} \cdot M_{LO2} \cdot \frac{f_s \cdot X_H}{K_{LX} + f_s}$
3. Anaerobic hydrolysis	$K_h \cdot \Gamma_{LO2} \cdot l_{LO2} \cdot M_{LO2} \cdot \frac{f_s \cdot X_H}{K_{LX} + f_s}$
HETEROTROPHIC ORGANISMS: X_H	
4. Growth on S_F	$\mu_H \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot M_{HIH4} \cdot M_{HIPO4} \cdot M_{HULX} \cdot X_H$
5. Growth on S_A	$\mu_H \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_F}{K_F + S_F} \cdot M_{HIH4} \cdot M_{HIPO4} \cdot M_{HULX} \cdot X_H$
6. Denitrification with S_F	$\mu_H \cdot \Gamma_{HIH3} \cdot l_{HIH3} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot M_{HIH4} \cdot M_{HIPO4} \cdot X_H$
7. Denitrification with S_A	$\mu_H \cdot \Gamma_{HIH3} \cdot l_{HIH3} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot M_{HIH4} \cdot M_{HIPO4} \cdot X_H$
8. Fermentation	$q_{H2} \cdot l_{HIH3} \cdot \frac{S_F}{K_F + S_F} \cdot \frac{S_A}{K_A + S_A} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot X_H$
9. Lysis	$b_H \cdot X_H$
$f_s = X_S/X_H$ $f_{PH4} = X_{PH4}/X_{PH4}$ $f_{PP} = X_{PP}/X_{PH4}$ $f_{LO2} = X_{LO2}/X_{PH4}$ The terms M and l are so-called switching function with the function in the model to turn process rate equations on and off as environmental conditions are changed. For example, the l_{LO2} turns the reaction off in presence of oxygen in process 2 : $l_{LO2} = \frac{K_{LO2}}{S_{O2} + K_{LO2}}$	

Process	Rate equation
PHOSPHORUS ACCUMULATING ORGANISMS: X_{PH4}	
10. Anaerobic storage of PHA	$q_S^{max} \cdot \frac{S_A}{K_{PA} + S_A} \cdot l_{PH4} \cdot l_{PH4} \cdot M_{PH4} \cdot M_{PH4} \cdot X_{PH4}$
11. Anaerobic Maintenance	$m_{PH4} \cdot l_{PH4} \cdot l_{PH4} \cdot M_{PH4} \cdot X_{PH4}$
12. Aerobic lysis of PHA	$K_{PH4} \cdot f_{PH4}^{2/3} \cdot \frac{S_{O2}}{S_{O2} + K_{PH4}} \cdot M_{PH4} \cdot M_{PH4} \cdot X_{PH4}$
13. Aerobic storage of poly-P	$k_{PP} \cdot \frac{1}{f_{PP}} \cdot \frac{S_{O2}}{K_{PP} + S_{O2}} \cdot \frac{S_{PO4}}{K_{PP} + S_{PO4}} \cdot X_{PH4}$
14. Aerobic glycogen formation	$k_{GLY} \cdot \frac{1}{f_{GLY}} \cdot \frac{S_{O2}}{S_{O2} + K_{GLY}} \cdot X_{PH4}$
15. Aerobic maintenance	$m_{PH4} \cdot \frac{S_{O2}}{S_{O2} + K_{PH4}} \cdot X_{PH4}$
16. Anoxic lysis of PHA	$K_{PH4} \cdot \Gamma_{PH4} \cdot f_{PH4}^{2/3} \cdot \frac{S_{NO3}}{S_{NO3} + K_{PH4}} \cdot l_{PH4} \cdot M_{PH4} \cdot M_{PH4} \cdot X_{PH4}$
17. Anoxic storage of poly-P	$k_{PP} \cdot \Gamma_{PH4} \cdot \frac{1}{f_{PP}} \cdot \frac{S_{NO3}}{K_{PH4} + S_{NO3}} \cdot \frac{S_{PO4}}{K_{PP} + S_{PO4}} \cdot l_{PH4} \cdot X_{PH4}$
18. Anoxic glycogen formation	$k_{GLY} \cdot \Gamma_{PH4} \cdot \frac{1}{f_{GLY}} \cdot \frac{S_{NO3}}{S_{NO3} + K_{PH4}} \cdot X_{PH4}$
19. Anoxic maintenance	$m_{PH4} \cdot l_{PH4} \cdot \frac{S_{NO3}}{S_{NO3} + K_{PH4}} \cdot M_{PH4} \cdot X_{PH4}$
NITRIFYING ORGANISMS: X_{NUT}	
20. Growth	$\mu_{NUT} \cdot \frac{S_{O2}}{K_{NUT} + S_{O2}} \cdot \frac{S_{NH4}}{S_{NH4} + K_{NUT}} \cdot M_{NUT} \cdot X_{NUT}$
21. Lysis	$b_{NUT} \cdot X_{NUT}$

Kinetic parameters of the activated sludge model valid at 20 °C

Symbol	Definition	Value	Unit	Reference
K_h	Hydrolysis constant	3	1/d	Gujer et al, 1995
$\Gamma_{NO_3}^L$	Anoxic hydrolysis reduction factor	0.6		"
Γ_{le}	Anaerobic hydrolysis reduction factor	0.1		"
$K_{O_2}^L$	Saturation/inhibition coefficient for oxygen	0.2	qO_2/m^3	"
$K_{NO_3}^L$	Saturation/inhibition coefficient for nitrate	0.5	qN/m^3	"
K_Y	Saturation coefficient for particulate COD	0.1	$qCOD/qCOD$	"
μ_H	Maximal growth rate on substrate	6	1/d	"
q_{le}	Maximal fermentation rate	1	$qCOD/qCOD.d$	This study
$\Gamma_{NO_3}^H$	Reduction factor for denitrification	0.8		Gujer et al, 1995
b_H	Lysis rate constant	0.4	1/d	"
$K_{O_2}^H$	Saturation/inhibition coefficient for oxygen	0.2	qO_2/m^3	"
K_F	Saturation coefficient for growth on S_F	4	$qCOD/m^3$	"
K_{le}	Saturation coefficient for fermentation on S_F	20	$qCOD/m^3$	"
K_A^H	saturation coefficient for acetate	4	$qCOD/m^3$	"
$K_{NO_3}^H$	Saturation/inhibition coefficient for nitrate	0.5	qN/m^3	"
$K_{NH_4}^H$	Saturation coefficient for S_{NH_4} as nutrient	0.05	qN/m^3	"
K_P^H	Saturation coefficient for S_{PO_4} as nutrient	0.01	qP/m^3	"
K_{ALK}^H	Saturation coefficient for alkalinity	0.1	$molHCO_3/m^3$	"
q_{max}^S	Acetate consumption rate	9.67	$qCOD/qCOD.d$	Smolders et al, 1994
m_{AN}	Anaerobic maintenance coefficient	0.05	$qP/qCOD.d$	"
m_{NO_3}	Anoxic maintenance coefficient	0.02	$qN/qCOD.d$	Murnleitner et al, 1995
m_{O_2}	Aerobic maintenance coefficient	0.06	$qCOD/qCOD.d$	"
m_{PAO}^{NO}	Biomass consumption for anoxic maintenance	0.06	$qCOD/qCOD.d$	"
m_{PAO}^O	Biomass consumption for aerobic maintenance	0.07	$qCOD/qCOD.d$	"
K_A^S	Saturation coefficient for acetate	4	$qCOD/m^3$	Gujer et al, 1995
$K_{NO_3}^S$	Saturation coefficient for nitrate	1.4	qN/m^3	Murnleitner et al, 1995
$K_{O_2}^S$	Saturation coefficient for oxygen	0.2	$qCOD/m^3$	Gujer et al, 1995
K_{ALK}^S	Saturation coefficient for alkalinity	0.1	$molHCO_3/m^3$	"
$K_{NH_4}^S$	Saturation coefficient for ammonium	0.05	qN/m^3	"
$K_{PO_4}^S$	Saturation coefficient for phosphate	3.1	qP/m^3	Murnleitner et al, 1995
K_{PHA}^S	Saturation coefficient for PHA	0.01	$qCOD/m^3$	Gujer et al, 1995
K_{GLY}^S	Saturation coefficient for glycogen	0.01	$qCOD/m^3$	"
K_{PP}^S	Saturation coefficient for poly-P	0.01	$qCOD/m^3$	"
q_{PP}	Nitrate sensitivity factor for poly-P formation	0.1		Murnleitner et al, 1995
k_{PHA}	PHA decay rate	7.55	$qCOD/qCOD.d$	"
k_{PP}	Poly-P formation rate	0.11	$qP/qCOD.d$	Van Veldhuizen et al,
k_{GLY}	Glycogen formation rate	0.15	$qCOD/qCOD.d$	This study
$\Gamma_{NO_3}^P$	Reduction factor under anoxic conditions	0.8		This study
μ_{AUT}	Maximal growth rate of autotrophic biomass	1	1/d	Gujer et al, 1995
b_{AUT}	Decay rate	0.15	1/d	"
$K_{O_2}^N$	Saturation/inhibition coefficient for oxygen	0.5	qO_2/m^3	"
$K_{NH_4}^N$	Saturation coefficient for S_{NH_4}	1	qN/m^3	"
K_{ALK}^N	Saturation coefficient for alkalinity	0.5	$molHCO_3/m^3$	"
K_P^N	Saturation coefficient for S_{PO_4}	0.01	qP/m^3	"

Appendix 3

**Results of the sampling program and data routinely collected
by the plant staff during April 1997.**

Results of the sampling program performed at the WWTP Haarlem Waarderpolder in April 1997

Control Point	Medium: sampling point	Unit	Liquid phase										Biomass						Others				
			COD _{total} mg COD /L	COD _{filtered} mg COD /L	COD _{particulate} mg COD /L	BOD ₅ mg O ₂ /L	PO ₄ mg P/L	Acetate mg COD /L	Propionate mg COD /L	Butyrate mg COD /L	NH ₄ mg N/L	NO ₃ mg N/L	MLSS g /L	MLVSS g /L	MLVSS/MLSS	PHB mg COD /L	PHV mg COD /L	PHA mg COD /L	Glycogen mg COD /L	Temperature grad C	pH	DO mg O ₂ /L	
T1	Raw sewage: after screens Settled sewage: after the primary settler	8/4/97						6.7	45	9.1	2.5												
		7-8/4/97	329*	117*	212*	135*	6.7*	33*	4.7	0	42*	0.1*									12.6	6.7	2.1
		8-9/4/97	335*	138*	197*	134*	6.1*	32*			51*	0.1*											
		Average	332	128	205	135	6.4	33			46	0.1											
T8	Activated sludge: the end of the aerobic ring	8-9/4/97					0.5																
T10	Effluent: after the secondary settler	3-4/4/97		31*		4*	0.9*																
		9-10/4/97					0.8																
		10/4/97					6.5																
	Primary sludge: outlet of primary settler	9/4/97					6.5	11.7															
T12	Schalkwijk sludge: outlet pipe Supernatant: primary thickener	9/4/97	360*	144*	216*	157*	6.0*	50*			55*	0*											
		10/4/97					6	37.4	9	3.7													0.3
		8/4/97																					
T17	Side stream sludge: P selector tank: section 1 (start) section 2 section 3 (middle) section 4 section 5 (end)	8/4/97					5.7	33.2															
							7.1	32.1															
							9.6	30.4															
							10.5	29.5															
							11.1	29.2															
T18	Side stream sludge: P release tank: section 1 (start) section 2 section 3 (middle) section 4 section 5 (end)	8/4/97					22.9	129															
							25.2	109															
							26.6	107															
							31.3	96.1															
							33.2	92.5															
T15	Side stream sludge: P release tank: section 1 (start) section 2 section 3 (middle) section 4 section 5 (end)	8/4/97					1.1	0.9	0.78	77	17	94	156										
T16	Side stream sludge: P release tank: section 1 (start) section 2 section 3 (middle) section 4 section 5 (end)	8/4/97																					
T19	Side stream sludge: P release tank: section 1 (start) section 2 section 3 (middle) section 4 section 5 (end)	8/4/97																					

Control point refers to the scheme of standard case Haarlem 1 given in appendix 4

Numbers printed in bold are used for influent characterization (table 5.5)

Numbers with * refer to 24h composite samples, without *, to grab samples

Summary of the data collected at the WWTP Haarlem Waarderpolder by the plant staff on 12th April 1997 (concentration values are obtained from spot samples taken around 10 p.m.).

Sampling point	Parameter	Value	Unit
☐ RAW SEWAGE <i>Plant Influent</i>	☐ Q ☐ pH ☐ COD _{tot} ☐ BOD ₅ ☐ Kj-N ☐ (NO ₃ +NO ₂)-N ☐ P _{tot} ☐ TSS ☐ Load	27.580 7.75 495 190 56 0.05 7.5 220 109.002	m ³ /d gCOD/m ³ gBOD/m ³ gN/m ³ gN/m ³ gP/m ³ gTSS/m ³ P.E.
☐ SETTLED SEWAGE <i>Biological Unit Influent</i>	☐ COD ☐ BOD ☐ Kj-N ☐ P _{ortho} ☐ P _{tot} ☐ TSS	265 100 48 4.7 6.7 100	gCOD/m ³ gBOD/m ³ gN/m ³ gP/m ³ gP/m ³ gTSS/m ³
☐ ACTIVATED SLUDGE <i>Biological Unit Effluent</i>	☐ TSS ☐ SVI ☐ Inorganic matter	5.200 60 24	mgTSS/m ³ mL/g %TSS
☐ P-SELECTION TANK <i>Settled Sludge</i>	☐ TSS ☐ P _{ortho} ☐ pH	860 13.3 7.5	mgTSS/m ³ gP/m ³
☐ P-RELEASE TANK <i>Settled Sludge</i>	☐ TSS ☐ P _{ortho} ☐ pH	5.540 41.2 6.8	mgTSS/m ³ gP/m ³
☐ EFFLUENT <i>Secondary Settlers Effluent</i>	☐ pH ☐ COD _{tot} ☐ COD _{filtr} ☐ BOD ₅ ☐ Kj-N ☐ NH ₄ -N ☐ (NO ₃ +NO ₂)-N ☐ N _{tot} ☐ P _{tot} ☐ TSS ☐ Load	8.05 39 31 4 5.2 3.2 8.3 13.5 2.3 7 9.111	 gCOD/m ³ gCOD/m ³ gBOD/m ³ gN/m ³ gN/m ³ gN/m ³ gN/m ³ gP/m ³ gP/m ³ gTSS/m ³ P.E.
☐ OVERALL TREATMENT EFFICIENCY	☐ COD _{tot} ☐ BOD ₅ ☐ Kj-N ☐ N _{tot} ☐ P _{tot} ☐ TSS ☐ Load	92 98 91 76 69 97 92	% % % % % % %
☐ PLANT LOADING	☐ COD _{tot} ☐ BOD ₅ ☐ Kj-N ☐ P _{tot} ☐ TSS	13.652 5.240 1.544 207 5516	kgCOD/d kgBOD/d kgN/d kgP/d kgTSS/d

Appendix 4

**Configuration schemes of the WWTP Haarlem Waarderpolder
(case Haarlem 1, 2, 3 and 4)**

Haarlem 2

Haarlem 3

Appendix 5

**Results of the simulation of the WWTP Haarlem
Waarderpolder using SIMBA® and combined
ASM2 - Delft BPR model**

Sampling point		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24
Parameter		Unit																							
No.		SRT = 85 days																							
1	SO ₂	g COD/m ³	2.1	0.0	0.9	0.0	1.5	1.4	0.0	1.7	0.0	1.7	0.0	0.1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7
2	SF	g COD/m ³	73.0	0.4	33.2	30.0	0.9	4.1	0.7	0.5	4.0	0.5	0.4	73.0	0	0.4	29.4	18.0	66.4	60.9	18.0	18.0	18.0	54.9	0.5
3	SA	g COD/m ³	32.4	0.0	14.6	9.1	0.3	1.3	0.2	0.0	2.6	0.0	0.0	49.9	750000	175.0	38.2	45.4	38.8	38.2	38.2	38.2	38.7	0.0	0.0
4	SNH ₄	g N/m ³	46.5	3.4	22.8	22.8	2.8	5.0	4.9	2.4	6.2	2.4	3.4	55.0	0	3.4	24.0	25.3	50.3	50.8	25.3	25.3	47.3	2.4	3.4
5	SNO ₃	g N/m ³	0.1	0.5	0.3	0.1	4.8	4.2	2.8	5.3	0.5	5.3	0.5	0.0	0	0.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3
6	SPO ₄	g P/m ³	6.4	0.4	3.1	5.0	0.4	0.9	0.6	0.3	1.3	0.3	0.4	6.0	0	0.4	2.6	38.8	5.5	11.1	38.8	38.8	14.9	0.3	0.4
7	SI	g COD/m ³	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
8	SALK	mol/m ³	5.0	8.7	7.0	7.0	8.4	8.2	8.3	8.3	8.5	8.3	8.7	5.0	0	8.7	7.2	6.9	5.3	5.2	6.9	6.9	6.9	5.4	8.3
9	Xi	g COD/m ³	63	6452	3569	3569	5122	4951	4952	4952	6511	10	6452	50	0	6450	3890	3892	632	633	0	48659	48659	7364	6447
10	Xs	g COD/m ³	141	61	97	97	49	54	52	45	79	0	61	166	0	61	103	111	156	157	0	1384	1384	329	59
11	XH	g COD/m ³	0	812	445	447	657	634	636	638	816	1	812	0	0	811	487	470	74	68	0	5881	5881	883	831
12	XPAO	g COD/m ³	0	334	183	183	265	256	256	256	336	1	334	0	0	333	200	199	30	30	0	2490	2490	375	334
13	XPP	g P/m ³	0	60	33	31	47	45	45	46	56	0	60	0	0	60	36	0	5	0	0	0	0	0	59
14	XPHA	g COD/m ³	0	98	54	63	82	80	79	77	119	0	98	0	0	98	59	218	9	34	0	2724	2724	411	100
15	XGLY	g COD/m ³	0	165	91	88	130	125	125	126	160	0	165	0	0	165	99	46	15	7	0	577	577	87	164
16	XAUT	g COD/m ³	0	97	53	53	78	75	75	76	98	0	97	0	0	97	58	58	9	9	0	723	723	109	99
17	XMeOH	g Fe(OH) ₃ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	XMeP	g FePO ₄ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	m ³ /d	6000	7296	13296	13296	107712	121008	121008	25008	11712	5808	960	4800	0.84	3601	6001	6001	2640	2640	5521	430	50	768	19200
20	COD _{part}	g COD/m ³	204	8019	4492	4499	6383	6176	6175	6171	8120	12	8019	216	0	8017	4897	4994	925	937	0	62436	62436	9557	8034
21	VSS _{total}	g VSS/m ³	149	5853	3279	3284	4659	4508	4507	4504	5927	9	5853	158	0	5852	3574	3645	675	684	0	45574	45574	6976	5864
22	MLSS _{total}	g MLSS/m ³	196	7701	4314	4321	6130	5931	5931	5927	7798	12	7701	207	0	7700	4703	4797	889	900	0	59966	59966	9178	7716

		Simulation results: Case Haarlem 1b																				SRT = 85 days			
Sampling point		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24
No.	Parameter	Unit																							
1	SO ₂	2.1	0.0	0.9	0.0	1.6	1.4	0.0	1.8	0.0	1.8	0.0	0.1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0
2	SF	73.0	0.3	33.1	29.4	0.8	4.0	0.7	0.5	3.8	0.5	0.3	73.0	0	0.3	29.4	16.5	66.4	59.8	16.5	16.5	16.5	53.7	0.5	0.3
3	SA	32.4	0.0	14.6	9.4	0.2	1.2	0.2	0.0	1.5	0.0	0.0	49.9	75000	75.0	65.0	4.3	45.4	26.4	4.3	4.3	4.3	23.3	0.0	0.0
4	SNH ₄	46.5	3.1	22.7	22.6	2.6	4.8	4.7	2.1	6.0	2.1	3.1	55.0	0	3.1	23.9	25.0	50.3	51.1	25.0	25.0	25.0	47.4	2.1	3.1
5	SNO ₃	0.1	0.8	0.5	0.1	5.3	4.7	3.4	5.9	0.8	5.9	0.8	0.0	0	0.8	0.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	5.9	0.8
6	SPO ₄	6.4	0.7	3.3	5.1	0.9	1.4	1.1	0.8	1.8	0.8	0.7	6.0	0	0.7	2.8	32.0	5.5	14.7	32.0	32.0	32.0	17.1	0.8	0.7
7	SI	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
8	SALK	5.0	8.9	7.1	7.1	8.5	8.3	8.4	8.5	8.6	8.5	8.9	5.0	0	8.9	7.3	7.0	5.4	5.3	7.0	7.0	7.0	5.5	8.5	8.9
9	XI	63	6482	3585	3586	5146	4975	4975	4976	6542	10	6482	50	0	6481	3909	3911	635	635	0	48888	48888	7398	6478	6482
10	Xs	141	57	95	95	47	52	50	44	75	0	57	166	0	57	101	108	156	156	0	1350	1350	323	57	57
11	XH	0	824	452	454	666	643	645	646	829	1	824	0	0	824	494	480	75	71	0	5995	5995	901	842	824
12	XPAO	0	301	165	165	240	232	232	232	304	0	301	0	0	301	181	181	27	22	0	2261	2261	336	302	301
13	XPP	0	111	61	59	87	84	85	85	108	0	111	0	0	111	67	38	10	1	0	472	472	67	111	111
14	XPHA	0	23	12	20	21	21	20	19	39	0	23	0	0	23	14	131	2	44	0	1640	1640	268	25	23
15	XGLY	0	80	44	41	62	60	60	60	75	0	80	0	0	80	48	9	7	0	0	107	107	15	79	80
16	XAUT	0	98	54	54	79	76	76	76	99	0	98	0	0	98	59	58	9	9	0	727	727	109	99	98
17	XMeOH	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	XMeP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	6000	7296	13296	13296	107712	121008	121008	25008	11712	5808	960	4800	0.36	3600	6000	6000	2640	2640	5520	430	50	768	19200	19200
20	COD _{part}	204	7865	4408	4415	6261	6058	6057	6053	7962	12	7865	216	0	7864	4805	4877	911	937	0	60968	60968	9350	7881	7865
21	VSS _{total}	149	5741	3217	3222	4570	4422	4421	4418	5812	9	5741	158	0	5740	3507	3560	665	684	0	44502	44502	6825	5752	5741
22	MLSS _{total}	196	7553	4233	4240	6013	5818	5818	5814	7647	11	7553	207	0	7553	4615	4684	875	900	0	58555	58555	8980	7569	7553

Simulation results: Case Haarlem 2a													SRT = 34 days		
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	SO ₂	g COD/m ³	2.1	0.1	1.8	0.7	0.0	1.6	0.0	2.0	1.9	2.0	2.0	2.0	0.0
2	SF	g COD/m ³	73.0	73.0	73.0	28.5	23.4	3.9	0.7	0.4	0.4	0.4	0.4	0.4	0.3
3	SA	g COD/m ³	32.4	49.9	35.3	13.7	11.5	1.8	0.3	0.1	0.1	0.1	0.1	0.1	0.0
4	SNH ₄	g N/m ³	46.5	55.0	47.9	21.3	21.1	6.2	6.1	3.5	3.6	3.5	3.5	3.5	4.4
5	SNO ₃	g N/m ³	0.1	0.0	0.1	1.6	0.7	6.4	5.3	7.8	7.5	7.8	7.8	7.8	2.6
6	SPO ₄	g P/m ³	6.4	6.0	6.3	7.2	7.2	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.8
7	Si	g COD/m ³	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
8	SALK	mol/m ³	5.0	5.0	5.0	7.2	7.3	8.1	8.2	8.2	8.2	8.2	8.2	8.2	8.6
9	Xi	g COD/m ³	63	50	61	1651	1651	1947	1948	1948	2000	4	2653	2653	2658
10	Xs	g COD/m ³	141	166	145	87	86	48	45	40	41	0	54	54	51
11	XH	g COD/m ³	0	0	0	530	535	644	646	648	664	1	882	882	866
12	XPAO	g COD/m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
13	XPP	g P/m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
14	XPHA	g COD/m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
15	XGLY	g COD/m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
16	XAUT	g COD/m ³	0	0	0	54	54	66	66	66	68	0	90	90	89
17	XMeOH	g Fe(OH) ₃ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
18	XMeP	g FePO ₄ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	m ³ /d	6000	1200	7200	18576	18576	122160	122160	122160	103584	6960	240	18960	18960
20	COD _{part}	g COD/m ³	204	216	206	2323	2327	2705	2705	2702	2772	6	3680	3680	3663
21	VSS _{total}	g VSS/m ³	149	158	150	1696	1699	1974	1975	1972	2024	4	2686	2686	2674
22	MLSS _{total}	g MLSS/m ³	196	207	198	2231	2235	2598	2598	2595	2663	5	3534	3534	3518

Simulation results: Case Haarlem 2b													SRT = 37 days		
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	SO ₂	g COD/m ³	2.1	0.1	1.8	0.7	0.0	1.6	0.0	2.1	1.9	2.1	2.1	2.1	0.0
2	Sf	g COD/m ³	73.0	73.0	73.0	28.5	13.9	2.5	0.6	0.5	0.4	0.5	0.5	0.5	0.3
3	SA	g COD/m ³	32.4	49.9	35.3	13.7	1.3	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
4	SNH ₄	g N/m ³	46.5	55.0	47.9	21.8	22.0	7.2	7.1	4.5	4.6	4.5	4.5	4.5	5.2
5	SNO ₃	g N/m ³	0.1	0.0	0.1	1.8	0.1	6.5	5.5	8.0	7.6	8.0	8.0	8.0	2.9
6	SPO ₄	g P/m ³	6.4	6.0	6.3	3.2	10.0	3.6	3.1	2.6	2.5	2.6	2.6	2.6	1.2
7	Si	g COD/m ³	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
8	SALK	mol/m ³	5.0	5.0	5.0	7.1	7.0	7.8	7.9	7.9	8.0	7.9	7.9	7.9	8.4
9	Xi	g COD/m ³	63	50	61	1551	1552	1828	1828	1829	1877	4	2490	2490	2494
10	Xs	g COD/m ³	141	166	145	82	84	43	41	36	36	0	48	48	42
11	XH	g COD/m ³	0	0	0	401	402	486	487	488	501	1	665	665	655
12	XPAO	g COD/m ³	0	0	0	177	177	212	213	213	219	0	290	290	289
13	XPP	g P/m ³	0	0	0	88	81	103	104	105	107	0	142	142	144
14	XPHA	g COD/m ³	0	0	0	6	34	14	13	11	11	0	15	15	11
15	XGLY	g COD/m ³	0	0	0	25	16	27	28	28	29	0	38	38	41
16	XAUT	g COD/m ³	0	0	0	50	50	61	61	61	63	0	83	83	82
17	XMeOH	g Fe(OH) ₃ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
18	XMeP	g FePO ₄ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	m ³ /d	6000	1200	7200	18576	18576	122160	122160	122160	103584	6960	240	18960	18960
20	COD _{part}	g COD/m ³	204	216	206	2293	2314	2671	2669	2666	2735	5	3630	3630	3614
21	VSS _{total}	g VSS/m ³	149	158	150	1674	1689	1950	1948	1946	1997	4	2650	2650	2638
22	MLSS _{total}	g MLSS/m ³	196	207	198	2202	2223	2566	2564	2560	2627	5	3487	3487	3471

Simulation results: Case Haarlem 3a											SRT = 37 days				
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	SO ₂	g COD/m ³	2.1	1.8	0.9	0.0	0.0	0.0	2.2	0.0	4.2	4.2	3.5	4.2	4.2
2	SF	g COD/m ³	73.0	73.0	36.8	20.1	11.4	7.8	3.2	0.5	0.4	0.4	0.4	0.4	0.4
3	SA	g COD/m ³	32.4	35.3	17.7	1.7	1.0	0.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0
4	SNH ₄	g N/m ³	46.5	47.9	27.9	28.3	17.2	17.1	8.1	7.9	2.7	2.7	2.8	2.7	2.7
5	SNO ₃	g N/m ³	0.1	0.1	2.1	0.1	2.1	1.5	5.9	4.1	9.3	9.3	8.5	9.3	9.3
6	SPO ₄	g P/m ³	6.4	6.3	4.9	13.5	8.0	8.0	4.4	3.4	2.5	2.5	2.3	2.5	2.5
7	Si	g COD/m ³	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
8	SALK	mol/m ³	5.0	5.0	6.4	6.4	7.3	7.3	7.8	7.9	7.9	7.9	8.0	7.9	7.9
9	Xi	g COD/m ³	63	61	944	945	1628	1628	1826	1827	1828	4	1944	2489	2489
10	Xs	g COD/m ³	141	145	94	94	70	69	47	43	33	0	34	45	45
11	XH	g COD/m ³	0	0	245	247	429	431	488	489	492	1	521	670	670
12	XPAO	g COD/m ³	0	0	106	106	187	187	211	212	213	0	226	290	290
13	XPP	g P/m ³	0	0	52	44	89	89	104	105	105	0	112	144	144
14	XPHA	g COD/m ³	0	0	7	42	28	28	17	14	10	0	10	14	14
15	XGLY	g COD/m ³	0	0	14	3	21	21	28	29	30	0	32	41	41
16	XAUT	g COD/m ³	0	0	32	31	55	55	63	63	64	0	68	87	87
17	XMeOH	g Fe(OH) ₃ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
18	XMeP	g FePO ₄ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	m ³ /d	6000	7200	14400	14400	25776	25776	69360	69360	62160	6960	43584	240	18960
20	COD _{part}	g COD/m ³	204	206	1441	1467	2417	2419	2681	2677	2670	5	2835	3636	3636
21	VSS _{total}	g VSS/m ³	149	150	1052	1071	1764	1766	1957	1954	1949	4	2069	2654	2654
22	MLSS _{total}	g MLSS/m ³	196	198	1384	1409	2321	2323	2575	2571	2564	5	2723	3492	3492

Simulation results: Case Haarlem 3b											SRT = 37 days				
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	SO ₂	g COD/m ³	1.8	0.9	0.0	0.0	0.0	1.9	0.0	3.7	3.1	3.7	3.7	3.7	0.0
2	SF	g COD/m ³	73.0	37.0	28.9	16.3	14.9	5.9	0.9	0.5	0.5	0.5	0.5	0.5	0.4
3	SA	g COD/m ³	35.3	50.5	1.5	0.8	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	SNH ₄	g N/m ³	47.9	28.4	28.9	18.0	18.0	9.0	8.8	3.5	3.6	3.5	3.5	3.5	4.2
5	SNO ₃	g N/m ³	0.1	0.3	0.0	0.4	0.1	3.1	0.5	5.6	4.8	5.6	5.6	5.6	0.8
6	SPO ₄	g P/m ³	6.3	4.5	25.9	14.6	14.8	5.9	2.7	0.7	0.6	0.7	0.7	0.7	0.3
7	Si	g COD/m ³	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
8	SALK	mol/m ³	5.0	6.5	6.3	7.3	7.3	7.9	8.1	8.2	8.2	8.2	8.2	8.2	8.5
9	Xi	g COD/m ³	61	928	929	1601	1601	1795	1796	1797	1911	4	2447	2447	2450
10	Xs	g COD/m ³	145	100	102	80	80	57	55	42	44	0	57	57	52
11	XH	g COD/m ³	0	230	224	401	401	459	460	466	493	1	634	634	624
12	XPAO	g COD/m ³	0	274	274	483	483	547	549	551	585	1	750	750	746
13	XPP	g P/m ³	0	68	47	109	109	132	136	138	146	0	187	187	188
14	XPHA	g COD/m ³	0	56	145	139	140	119	113	102	107	0	139	139	132
15	XGLY	g COD/m ³	0	67	37	107	107	133	134	139	149	0	189	189	195
16	XAUT	g COD/m ³	0	30	30	53	53	61	61	62	65	0	84	84	83
17	XMeOH	g Fe(OH) ₃ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
18	XMeP	g FePO ₄ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	m ³ /d	7200	14401	14401	25777	25777	69361	69361	62161	43584	6961	240	18960	18960
20	COD _{part}	g COD/m ³	206	1687	1742	2864	2865	3172	3168	3158	3353	6	4300	4300	4283
21	VSS _{total}	g VSS/m ³	150	1231	1272	2090	2091	2315	2312	2305	2448	5	3139	3139	3126
22	MLSS _{total}	g MLSS/m ³	198	1620	1673	2750	2751	3046	3043	3033	3221	6	4130	4130	4114

Simulation results: Case Haarlem 4																									SRT = 83 days			
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24		
No.	Parameter	Unit																										
1	SO ₂	g COD/m ³	2.1	0.0	1.0	0.0	0.0	1.1	1.9	1.4	2.3	2.3	0.0	0.1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0		
2	SF	g COD/m ³	73.0	0.3	33.1	29.4	3.9	4.0	0.5	0.5	0.5	0.5	0.3	73.0	0	0.3	29.4	17.9	66.4	60.9	17.9	17.9	17.9	54.9	0.5	0.3		
3	SA	g COD/m ³	32.4	0.0	14.6	9.0	2.5	1.3	0.1	0.0	0.0	0.0	0.0	49.9	750000	175.0	124.9	34.3	45.4	38.3	34.3	34.3	34.3	37.7	0.0	0.0		
4	SNH ₄	g N/m ³	46.5	4.0	23.2	23.1	6.9	5.9	3.5	3.4	3.1	3.1	4.0	55.0	0	4.0	24.4	25.7	50.4	50.9	25.7	25.7	25.7	47.4	3.1	4.0		
5	SNO ₃	g N/m ³	0.1	0.8	0.5	0.1	0.8	4.4	5.3	5.4	5.7	5.7	0.8	0.0	0	0.8	0.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	5.7	0.8		
6	SPO ₄	g P/m ³	6.4	0.4	3.1	4.9	1.3	1.0	0.4	0.4	0.3	0.3	0.4	6.0	0	0.4	2.6	38.6	5.5	11.0	38.6	38.6	38.6	14.9	0.3	0.4		
7	SI	g COD/m ³	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0		
8	SALK	mol/m ³	5.0	8.8	7.1	7.1	8.6	8.3	8.5	8.5	8.5	8.5	8.8	5.0	0	8.8	7.3	7.1	5.3	5.2	7.1	7.1	7.1	5.5	8.5	8.8		
9	XI	g COD/m ³	63	6497	3593	3594	6557	4986	4987	4987	4987	10	6497	50	0	6495	3918	3919	636	637	0	48997	48997	7418	6493	6497		
10	XS	g COD/m ³	141	55	94	94	73	52	44	43	42	0	55	166	0	55	100	107	156	156	0	1340	1340	322	55	55		
11	XH	g COD/m ³	0	812	445	447	816	633	636	636	637	1	812	0	0	811	487	470	74	68	0	5881	5881	883	829	812		
12	XPAO	g COD/m ³	0	332	182	182	335	255	255	255	255	0	332	0	0	332	199	198	30	30	0	2471	2471	372	332	332		
13	XPP	g P/m ³	0	59	33	31	56	45	45	45	45	0	59	0	0	59	36	0	5	0	0	0	0	0	59	59		
14	XPHA	g COD/m ³	0	102	56	64	123	83	80	80	80	0	102	0	0	102	61	226	9	35	0	2824	2824	426	104	102		
15	XGLY	g COD/m ³	0	153	84	81	148	116	117	117	117	0	153	0	0	153	92	37	14	5	0	464	464	70	152	153		
16	XAUT	g COD/m ³	0	95	52	52	96	74	74	74	74	0	95	0	0	95	57	57	9	8	0	708	708	107	97	95		
17	XMeOH	g Fe(OH) ₃ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
18	XMeP	g FePO ₄ /m ³	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
19	Q	m ³ /d	6000	7296	13296	13296	11712	121008	793008	697008	25008	5808	960	4800	0.84	3601	6001	6001	2640	2640	5521	431	49	768	19200	19200		
20	COD _{part}	g COD/m ³	204	8046	4507	4515	8148	6198	6193	6193	6193	12	8046	216	0	8044	4913	5014	928	940	0	62685	62685	9598	8062	8046		
21	VSS _{total}	g VSS/m ³	149	5873	3290	3295	5947	4524	4521	4521	4520	9	5873	158	0	5872	3586	3660	677	686	0	45756	45756	7006	5885	5873		
22	MLSS _{total}	g MLSS/m ³	196	7728	4329	4336	7825	5953	5948	5948	5948	12	7728	207	0	7726	4719	4816	891	903	0	60205	60205	9218	7743	7728		

Appendix 6

Results of the simulation of the batch tests performed with the activated sludge from WWTP Haarlem Waarderpolder using SIMBA® and combined ASM2 - Delft BPR model

No.	Parameter	Unit	Test 1				Test 2				Test 3				Test 4				Test 5				Test 6			
			a) Anaerobic maximal phosphate release test				b) Aerobic phosphate uptake test				c) Anoxic phosphate uptake test				d) Nitrification test				e) Denitrification test				f) Endogenous phosphate release test			
			Start	End	T1	T2	Start	End	T1	T2	Start	End	T1	T2	Start	End	T1	T2	Start	End	T1	T2	Start	End	T1	T2
1	SO ₂	g COD/m ³	0.0	0.0			7.0	8.6			0.0	0.0			7.0	8.2			0.0	0.0			0.0	0.0		
2	SF	g COD/m ³	0.2	3.3			2.6	1.3			2.6	0.4			0.2	0.3			0.2	19.1			0.2	8.8		
3	SA	g COD/m ³	400	334			9	0			9.0	0.0			0	0			8.0	23			0	0		
4	SNH ₄	g N/m ³	1.7	2.6			1.6	0.0			1.6	0.9			28.0	8.5			1.3	0.0			2.0	12.4		
5	SNO ₃	g N/m ³	0.3	0.0			0.0	1.0			28.0	17.3			0.3	20.1			28.0	20.6			0.3	0.0		
6	SPO ₄	g P/m ³	0.2	25.5			19.2	2.7			19.2	6.9			0.2	0.0			0.1	1.2			0.2	1.7		
7	SI	g COD/m ³	11.0	11.0			7.0	7.0			7.0	7.0			11.0	11.0			8.5	8.5			13.3	12.4		
8	SALK	mol/m ³	4.4	4.2			2.7	3.5			2.7	3.3			4.4	4.4			3.4	3.9			5.3	5.0		
9	Xi	g COD/m ³	3225	3227			2049	2051			2049	2044			3226	3215			2501	2502			3910	3653		
10	Xs	g COD/m ³	31	44			27	6			27	13			31	7			24	14			37	48		
11	XH	g COD/m ³	405	384			245	259			245	253			406	409			315	333			492	435		
12	XPAO	g COD/m ³	167	167			106	112			106	115			167	163			129	127			202	189		
13	XpP	g P/m ³	25	0			0	16			0	12			30	30			23	22			36	32		
14	XPHA	g COD/m ³	49	152			109	87			109	80			49	47			38	49			60	56		
15	XGLY	g COD/m ³	83	48			26	32			26	29			83	86			64	62			100	93		
16	XAUT	g COD/m ³	49	47			30	30			30	29			49	52			38	37			59	54		
17	XMeOH	g Fe(OH) ₃ /m ³	0	0			0	0			0	0			0	0			0	0			0	0		
18	XMeP	g FePO ₄ /m ³	0	0			0	0			0	0			0	0			0	0			0	0		
19	COD _{part}	g COD/m ³	4009	4069			2593	2575			2593	2564			4009	3979			3108	3123			4860	4527		
20	VSS _{total}	g VSS/m ³	2926	2970			1892	1880			1892	1872			2927	2905			2269	2280			3547	3305		
21	MLSS _{total}	g MLSS/m ³	4009	4069			2628	2575			2628	2564			4065	4034			3066	3081			4820	4590		

Appendix 7

Practical aspects related to modeling of a full-scale WWTPs

This work is the first case where the validation of a model for combined COD, N and P removal was performed on number of batch tests with fresh activated sludge, in an on-site well equipped laboratory. Although some batch tests were not well predicted by the model, they were very valuable as a source of feedback information used for model calibration. The batch tests also yielded useful information which could not be obtained otherwise for this particular plant. If planned well and possibly already performed elsewhere, the time spend on their execution can be reduced. If the time and the technical conditions allow, it is worthwhile to include them in a modeling process of existing WWT plants.

Finally there are some practical recommendations which may help in modeling tasks such as the one presented in this chapter. For users less familiar with SIMBA® simulation software it is recommended to first thoroughly study the software itself and work-out a couple of examples, prior to undertaking any further work on this matter. Good understanding of mathematical models to be used is a must. Once this is done, the next steps should be to study in depth project documentation, investigate the present state of the plant, and to compare the differences between these planned and actual situation (which usually will exist). One should make sure that the most recent and true information is obtained, because it may well happen that due to complexity of the plant some changes occurred which are considered not important to the plant employees, but could well be essential for the modeling. It is crucial that all flow rates and their fate are known, and that the hydraulic scheme is complete and fully correct. In case that a plant consists of two or more parallel lines and only one is chosen to be modeled, one should make sure that there is no difference between them, or if there is, be aware of them or take this into account, if significant. Once a proper layout and hydraulics is obtained influent characterization should be done. Here, it is important to select a good characterization protocol, making certain that the required model inputs and the parameters determined by a protocol match. One must be certain which data are needed to be measured for influent characterization. It is advisable to get a good quality data set routinely measured by the staff of the plant. These data should be studied prior to making selection of the items and frequency of parameters to be included in, very likely always required, an additional sampling program. The design of a sampling program will also be governed by the decision on whether static or dynamic influent data will be used for modeling. The sampling program should be designed in a way to provide sufficient data for confident influent characterization, model calibration and evaluation. Samples should be analyzed as soon as possible, otherwise stored immediately and transported and handled properly. A longer period without rain or snow is desirable for execution of sampling providing easier working conditions and evaluation of data. If data obtained from the plant staff are used, the way how they are analyzed should be known. One needs to be sure to choose a period of very normal plant operation. Obtained data from sampling program should be worked out and evaluated as soon as possible, while there is still possibility to relatively easy repeat some sampling or analyses. Sludge characterization can be performed as a part of the sampling program and/or by executing batch experiments using preferably fresh sludge from the treatment plant. Care should be taken that the sludge

is taken from the locations at the treatment plant which best correspond with the purpose of batch test. If possible, batch tests should be performed at the treatment plant itself, if not, sludge could be transported and stored in a dark and cold place for up to a couple of days. It should be checked if there is any difference using fresh and stored sludge. Batch tests should be performed at the same temperatures and pH as observed in the process. Once influent and sludge characterization is done simulation and calibration steps can be performed (for recommendations concerning this work phase the reader is referred to Henze et al. 1998).

Figure 5.10 Time (in %) spent on various stages of modeling WWTP Haarlem Waarderpolder.

The time needed for the execution different phases of modeling the WWTP in Haarlem is given below. This time distribution is subject to many factors such as experience and skills of researcher, availability, quality and quantity of existing information, configuration, size and complexity of the treatment plant, enthusiasm of the local staff, capacity and analytical diversity of the lab, availability of equipment for batch experiments, weather conditions etc. Therefore, figure 5.10 can be used as indication only.

6 Chapter

Minimal Aerobic Sludge Retention Time in Biological Phosphorus Removal Systems

Chapter 6 is based on: "Minimal Aerobic Sludge Retention Time in Biological Phosphorus Removal Systems" by Damir Brdjanovic, Mark C.M. van Loosdrecht, Christine M. Hooijmans, Guy J. Alaerts, and Joseph J. Heijnen. *Biotechnology & Bioengineering* (in press).

Contents

6.1. Introduction	169
6.2. Equations for Determination of Minimal Aerobic SRT	169
6.3. Materials and Methods	171
6.4. Results and Discussion	172
6.4.1. Model Validation	172
6.4.2. Sensitivity Analysis	174
6.4.3. Factors Affecting the Minimal Aerobic SRT	175
6.4.3.1. Temperature	175
6.4.3.2. Maximal PHA Level in the Cell	176
6.4.3.3. Number of Anaerobic-Aerobic Sequences	178
6.4.3.4. Type of Electron Acceptor	179
6.5. Conclusions	179

Abstract

The methodology for determination of the minimally required aerobic sludge retention time (SRT_{min}^{aer}) in BPR systems is presented in this chapter. Contrary to normal biological conversions, the BPR process is not limited by a SRT_{min} , which results from a maximum growth rate of the organisms. This is because the aerobic SRT should be long enough to oxidise the amount of PHA stored in the anaerobic phase. This makes that the SRT_{min}^{aer} will primarily depend on the PHA conversion kinetics and the maximal PHA content in the cell (storage capacity), and on other operational parameters, such as temperature (the SRT_{min}^{aer} exhibited very high temperature dependency similar to that of nitrifiers) and process configuration. It is expected that in denitrifying BPR systems the minimally required SRT will be around 35% longer in comparison with aerobic BPR. A model equations for determination of the minimal aerobic SRT as a function of above kinetic and process parameters were developed and the model predictions were compared with experimental data used to evaluate several operational aspects of BPR. The model was proved to be capable of describing these data satisfactorily.

6.1. Introduction

Biological phosphorus removal (BPR) from wastewater is characterised by the recirculation of activated sludge through a phase without external electron acceptor (anaerobic conditions) and a phase with oxygen or nitrate present (aerobic or anoxic conditions). Under anaerobic conditions volatile fatty acids (VFAs, like acetate) are taken up and stored as intracellular poly-hydroxy-alkanoates (PHA). In sequencing batch reactor (SBR) systems with acetate as sole organic substrate in the feed, the PHA consists mostly of poly-hydroxy-butyrate (PHB, up to 90%) and poly-hydroxy-valerate (PHV) (Sato et al., 1992; Smolders et al., 1995a; Kuba et al., 1993; and Pereira et al., 1996). During aerobic or anoxic periods no external substrate is present and internally stored PHB is primarily used for poly-phosphate (poly-P) synthesis. The remaining PHB is used for glycogen formation, maintenance requirements and growth (Murnleitner et al., 1997). In a BPR system the behaviour of the three storage pools in the cells (PHA, poly-P and glycogen) is highly dynamic and is determined by their conversion during the anaerobic and aerobic (or anoxic) phase. The PHA content of the biomass depends on the biomass concentration in the reactor. The biomass concentration can be easily controlled by the manipulation of substrate loading (noted further as acetate: HAc) and sludge retention time (SRT). While the anaerobic PHA production depends on the substrate loading to the SBR, the aerobic PHA consumption depends on the PHA level inside the biomass and on the kinetics of four PHA utilizing processes. The PHA formed under anaerobic conditions must be consumed during the aerobic phase. Otherwise, the PHA level in the cells will increase until a maximal level is reached. From that moment on, no acetate uptake will occur under anaerobic conditions leading to deterioration of BPR.

In activated sludge systems designed for the removal of organic matter and nitrogen the SRT is directly linked to the growth rate of the microorganisms; the minimally required SRT corresponds to the maximal growth rate ($SRT_{min} = 1/\mu_{max}$). However, in the BPR systems where storage materials play an important role in bacterial metabolism, the determination of the total SRT_{min} (defined as a sum of the minimally required anaerobic and aerobic SRT: $SRT_{min}^{total} = SRT_{min}^{anacr} + SRT_{min}^{aer}$) depends on a process kinetic rates and on a number of process conditions, notably the time needed for anaerobic acetate conversion to PHA, the time required for PHA consumption under aerobic or anoxic conditions, the biomass specific acetate loading rate, the temperature, the operation of the cycle, and the cell maximal PHA content. Since growth only occurs under aerobic conditions only the aerobic BPR process (and therefore only the SRT_{min}^{aer}) will be considered in this study. Clearly there does exist a minimal aerobic oxidation time below which the anaerobically produced PHA can not be further oxidised. The objective of this study is to develop a calculation procedure for determination of SRT_{min}^{aer} in BPR systems.

6.2. Equations for Determination of SRT_{min}^{aer}

In the BPR process the aerobic period is needed in order to convert the anaerobically accumulated PHA. The required aerobic period needed for this conversion directly

determines the aerobic SRT of a system. The calculation procedure is based on the hypothesis that the aerobic PHA conversion rate is a function of PHA concentration in the cell. The PHA conversion rate (and therefore the biomass production) is maximal when the active biomass specific PHA ratio, f_{pha} is maximal. This means that the f_{pha}^{max} and the PHA conversion kinetics strongly affect the SRT_{min}^{aer} .

The PHA consumption rate under aerobic conditions (r_{pha}^{aer}) can be expressed by the following equation (Smolders et al., 1995a):

$$r_{pha}^{aer} = -q_{pha}^{aer} \cdot C_x \quad \dots(6.1)$$

where q_{pha}^{aer} is the active biomass specific aerobic PHA reaction rate (g PHA/g active biomass, h) and C_x is the active biomass concentration (mg active biomass/L).

Murnleitner et al. (1997) proposed the following expression for q_{pha}^{aer} :

$$q_{pha}^{aer} = -k_{pha} \cdot (f_{pha})^{0.667} = df_{pha} / dt \quad \dots(6.2)$$

where k_{pha} is the kinetic coefficient for PHA conversion (g PHA/g active biomass) and f_{pha} is active biomass specific PHA fraction (g PHA/g active biomass). The effect of temperature on the kinetic coefficient can be described by the simplified Arrhenius equation (Brdjanovic et al. 1997):

$$k_{pha} = k_{pha,20^\circ C} \cdot q_{pha}^{(T-20^\circ C)} \quad \dots(6.3)$$

where $k_{pha,20^\circ C}$ presents the kinetic coefficient for PHA conversion at $20^\circ C$, q_{pha} is the temperature coefficient for PHA, and T is the operating temperature ($^\circ C$).

After integration of eq. 6.2 over the length of time of the aerobic phase ($t^{aer} = t - t_0$) taking into account eq. 6.3, the decreased PHA content of the cell at the end of the aerobic phase of the SBR, $f_{pha,t}$, can be calculated using eq. 6.4:

$$f_{pha,t} = [(f_{pha,to})^{0.333} - 0.333 k_{pha,20^\circ C} \cdot q_{pha}^{(T-20^\circ C)} \cdot t^{aer}]^3 \quad \dots(6.4)$$

Based on eq. 6.4 the aerobic PHA conversion (Δf_{pha}^{aer}) can be calculated as the difference between the PHA content of the cell at the beginning ($f_{pha,to}$) and at the end ($f_{pha,t}$) of the aerobic phase:

$$\Delta f_{pha}^{aer} = f_{pha,to} - f_{pha,t} = f_{pha,to} - [(f_{pha,to})^{0.333} - 0.333 k_{pha,20^\circ C} \cdot q_{pha}^{(T-20^\circ C)} \cdot t^{aer}]^3 \quad \dots(6.5)$$

During anaerobic conditions the acetate is transported into the cell and converted to PHA. The active biomass specific PHA formation based on acetate ($\Delta f_{pha,HAc}^{anaer}$) can be expressed as follows (Smolders et al., 1995b):

$$\Delta f_{pha,HAc}^{anaer} = C_{HAc} \cdot Y_{pha,HAc} / C_{x,to} \quad \dots(6.6)$$

The ΔC_{HAc} is the acetate addition per cycle (g HAc/L), $Y_{\text{pha,HAc}}$ is the PHA yield based on HAc, (g PHA/g HAc) and $C_{x,10}$ is the active biomass concentration during the anaerobic phase (mg active biomass/L).

In steady state the formation of PHA under anaerobic conditions is equal to the aerobic PHA consumption, i.e., $\Delta f_{\text{pha,HAc}}^{\text{anaer}}$ equals $\Delta f_{\text{pha}}^{\text{aer}}$. The PHA in sludge wasted per cycle (0.8-3.2% of the PHA present in the reactor) is for simplicity neglected. The initial biomass concentration $C_{x,10}$ in steady state can now be obtained by combining eq. 6.5 and eq. 6.6:

$$C_{x,10} = \Delta C_{\text{HAc}} \cdot Y_{\text{pha,HAc}} \cdot \{f_{\text{pha},10} - [(f_{\text{pha},10})^{0.333} - 0.333 k_{\text{pha},20^\circ\text{C}} \cdot q_{\text{pha}}^{(T-20^\circ\text{C})} \cdot t^{\text{aer}}]^3\}^{-1} \quad \dots(6.7)$$

The growth of biomass takes place only during aerobic conditions and therefore inclusion of a definition for the aerobic SRT is required (Smolders et al., 1995b):

$$\text{SRT}^{\text{aer}} = \frac{C_{x,10} \cdot (t^{\text{aer}}/24)}{\Delta C_{\text{HAc}} \cdot Y_{x,\text{HAc}}} \quad \dots(6.8)$$

In eq. 6.8 the ΔC_{HAc} is the amount of substrate added per cycle (g HAc/L) and $Y_{x,\text{HAc}}$ is the biomass yield based on HAc (g active biomass/g HAc).

It should be realised (according to eq. 6.2) that the maximal consumption rate of PHA is obtained when the cells have stored the maximal amount of PHA. The $\text{SRT}_{\text{min}}^{\text{aer}}$ can now be directly obtained by combining eq. 6.7 and eq. 6.8, where the $f_{\text{pha},10}$ is substituted with the maximal PHA content of the biomass ($f_{\text{pha}}^{\text{max}}$):

$$\text{SRT}_{\text{min}}^{\text{aer}} = \frac{(Y_{\text{pha,HAc}}/Y_{x,\text{HAc}}) \cdot (t^{\text{aer}}/24)}{f_{\text{pha}}^{\text{max}} - [(f_{\text{pha}}^{\text{max}})^{0.333} - 0.333 k_{\text{pha},20^\circ\text{C}} \cdot q_{\text{pha}}^{(T-20^\circ\text{C})} \cdot t^{\text{aer}}]^3} \quad \dots(6.9)$$

6.3. Materials and Methods

This study is based mainly on the results of the study of temperature influence on BPR, presented in chapter 3 [Brdjanovic et al., (1998a)]. Therefore, the reader is referred to that chapter for further details about the experimental set-up and the SBR operation. The analytical procedures used in this study can also be found elsewhere (see Smolders et al., 1994).

The linear sensitivity analysis was applied to determine the sensitivity factor $\lambda_{\text{SRT},p}$:

$$\lambda_{\text{SRT},p} = (d\text{SRT}^{\text{aer}}/\text{SRT}^{\text{aer}})/(dp/p) \quad \dots(6.10)$$

This factor $\lambda_{\text{SRT},p}$ gives the relative change of variable SRT^{aer} per relative change of the parameter p . For the sensitivity analysis the calculation is performed three times (for a

temperature of 20, 10 and 5°C). Each parameter was varied in the range $\pm 10\%$ by the step of 2% (in total 10 calculated data per parameter). Only one parameter was varied per calculation.

6.4. Results and Discussion

6.4.1. Model Validation

The dependence of SRT_{min}^{acr} on temperature (via θ_{pha}), maximal PHA content of the biomass (via f_{pha}^{max}), amount of acetate added (via both Y values) and operating pattern (via t^{acr}) can be expressed in a graphical form (figure 6.1). Figure 6.1, based on eq. 6.9 and typical parameter values (table 6.1), allows the SRT_{min}^{acr} of the BPR system to be estimated for chosen f_{pha}^{max} and operating temperature.

Figure 6.1 Minimally required aerobic SRT as a function of maximal storage capacity of the cell (0.4–0.7 gCOD-PHA/g COD-active biomass) and temperature. The symbols indicate the aerobic SRT of several laboratory scale SBR systems (Smolders et al., 1996, Brdjanovic et al., 1998a). Numbers indicate the total SRT of the experiments. A good BPR was achieved at SRTs marked as ■, while the BPR failed at SRTs marked as □ due to too short SRT.

Table 6.1 Characteristic values of the variables used for the calculation of SRT_{min}^{aer}

Parameter	Value	Unit	Reference
$k_{pha,20^{\circ}C}$	0.346	gCOD-PHA/gCOD-act.biomass	Brdjanovic et al., 1998a
θ_{pha}	1.163		Brdjanovic et al., 1998a
$Y_{x,HAc}$	0.3	gCOD-act.biomass/gCOD-HAc	Smolders et al., 1995b
$Y_{pha,HAc}$	1.5	gCOD-PHA/gCOD-HAc	Smolders et al., 1995b
ΔC_{HAc}	500	gCOD-HAc/L.cycle	Smolders et al., 1994
t^{aer}	2.25	h	Smolders et al., 1994

The method was validated using measured values of f_{pha} in an anaerobic-aerobic (A/O) SBR operated at different temperatures and SRTs (Brdjanovic et al., 1998a). Clearly, two zones can be distinguished in figure 6.1 as a function of SRT and temperature: the washout zone associated with the deterioration of the BPR, and the zone of stable BPR operation. The results of the method validation are summarised in table 6.2.

Table 6.2 Comparison of measured concentrations of f_{pha} and operating SRT in the A/O SBR cycle with the results predicted by the model. The difference between actual and predicted values are given in brackets.

Parameter	Unit/Temperature	5°C	10°C	20°C
actual ^{a)} $f_{pha,to}$	g PHA/g active biomass	0.443	0.239	0.125
actual ^{a)} $f_{pha,t}$	g PHA/g active biomass	0.415	0.172	0.014
actual ^{a)} SRT^{total}	day	32	16	8
actual ^{a)} SRT^{aer}	day	12	6	3
predicted ^{b)} $f_{pha,t}$	g PHA/ g active biomass	0.398 (-4%)	0.179 (+4%)	0.014 (0%)
predicted ^{c)} SRT^{aer}	day	10.4 (-13%)	7.7 (+28%)	3.2 (+7%)
predicted ^{d)} SRT_{min}^{aer}	day	9	4.4	1.25

a) Brdjanovic et al., 1998a
b) Using eq. 6.4. and $k_{pha,20^{\circ}C} = 0.346$ g PHA/g active biomass.h (gCOD/gCOD.h) and $q_{pha} = 1.163$
c) Using eq. 6.9 and $f_{pha}^{max} = f_{pha,to}$
d) Using eq. 6.9 and $f_{pha}^{max} = 0.55$ g PHA/g active biomass (gCOD/gCOD)

The model describes the change in PHA consumption during the aerobic phase very well over the wide range of SRT and temperature (maximal deviation $\pm 4\%$). The SRT^{aer} primarily depends on PHA conversion rate. Therefore, it is expected that the model also predicts the SRT^{aer} with similar accuracy. In general, the model prediction could be considered acceptable although at 10°C the deviation from the actual value is somewhat

larger (28%). This discrepancy could be explained by other reasons than by the inaccuracy of the model, such as fluctuation in growth rate under aerobic conditions, change in bacterial population or, more likely, by the slight variation in the rate of sludge wastage. Using a value of 0.55 g PHA/g active biomass as a f_{pha}^{max} (Brdjanovic et al., 1998a) the predicted SRT_{min}^{aer} deviates up to 55% from actual SRT^{aer} . This means that in the above mentioned study good and stable BPR process could be still achieved at somewhat lower SRT. The error made by not taking into account the PHA in wasted sludge in the model is less than 3.5% over the entire SRT range studied.

6.4.2. Sensitivity Analysis

The results of the sensitivity analysis of the parameter values towards the predicted SRT are presented in figure 6.2. The analysis showed that a change of aerobic time, t^{aer} caused only a marginal impact on the predicted SRT_{min}^{aer} . The sensitivity factor $\lambda_{SRT,p}$ of $Y_{x,HAc}$, $k_{pha,20^\circ C}$ and f_{pha}^{max} was around -1 and of $Y_{pha,HAc}$ around +1, which implies a proportional effect of change in the parameter towards the predicted SRT_{min}^{aer} . The SRT_{min}^{aer} was exceptionally sensitive to the temperature coefficient q_{pha} ; at 5°C the change of 5% of q_{pha} increases the SRT_{min}^{aer} already by a factor two. The main factors affecting the SRT_{min}^{aer} are elaborated more in detail below.

Figure 6.2 Relative sensitivity of the result ($dSRT/SRT$) due to change in the parameter (dp/p) in the range $\pm 10\%$ at 5°C (dotted line), 10°C (dashed line), and 20°C (solid line).

6.4.3. Factors Affecting the SRT_{min}^{aer}

6.4.3.1. Temperature

The influence of temperature (30, 20, 10 and 5°C) on the BPR process was experimentally investigated in chapter 3 (Brdjanovic et al., 1998a). In that study an anaerobic/aerobic-settling SBR was used operating in steady state under controlled operating conditions of temperature, pH and SRT. The acetate feed to the reactor was kept constant throughout the study (187.5 mgHAc-C/cycle in 2.5 L reactor volume). An interesting observation was made during the study. In order to achieve and maintain full P-removal the SRT was extended from 8 days (at 30 and 20°C) to an arbitrarily chosen value of 16 days at 10°C, and finally to 32 days at 5°C (see also figure 6.1). Obviously at low temperatures biomass failed to grow fast enough and phosphate accumulating organisms (PAOs) were washed out. It might have been possible to achieve full P-removal at each temperature at shorter SRT. The results of that study confirmed that biomass concentration increases with increase of SRT (Smolders et al., 1995b).

Figure 6.3 Comparison of a) specific active biomass PHA concentration (f_{pha}^{acr}) and b) PHA conversion under aerobic conditions (Δf_{pha}^{acr}) between data from the study of Brdjanovic et al., (1998a) obtained at different temperature (●) and predicted values by the metabolic model for aerobic BPR at 20°C (solid line, Smolders et al., 1995b).

However, figure 6.3a shows that the f_{pha} would strongly increase with the increase of biomass (and SRT) which disagrees with the study by Smolders et al., (1995b). Since the

experiments in the study of Smolders et al., (1995b) were performed at 20°C the temperature changes in the study of Brdjanovic et al., (1998a) were the main factor affecting the process. The experimental results showed strong temperature dependency of the kinetics of the metaboloc processes of BPR. For example, a decrease of temperature caused a strong decrease of the kinetic constant k_{pha} in eq. 6.2. In order to keep q_{pha}^{aer} (and SRT) constant the fraction of the internal PHA storage, f_{pha} , increased with decreasing temperature. This is an internal control mechanism established by the microorganisms in order to maintain a steady state operation where the anaerobic PHA production equals the aerobic PHB consumption. Additionally, measured PHA aerobic conversion (Δf_{pha}^{aer}) in a steady state cycle at different temperatures showed a decreasing trend with increasing SRT (figure 6.3b). These values correspond with the data given by Smolders et al., (1995), which suggests that the temperature history has only a marginal impact on the overall aerobic PHA conversion (Δf_{pha}^{aer}). This can be explained by the experimental evidence that the stoichiometry of growth is temperature independent (Brdjanovic et al., 1998a) which leads to constant yield of biomass on PHA.

The temperature dependency of the SRT_{min}^{aer} can be determined using the values predicted by the model (table 6.2). In order to express this dependency a simplified Arrhenius equation was applied (analog to eq. 6.3) on the reciprocal value of the SRT_{min}^{aer} (from table 6.2):

$$\frac{1}{SRT_{min(T)}^{aer}} = \frac{1}{SRT_{min(20^{\circ}C)}^{aer}} \cdot \theta_{1/SRT_{aer,min}}^{(T-20^{\circ}C)} \quad \dots(6.11)$$

The temperature coefficient $\theta_{1/SRT_{aer,min}}$ was obtained as result of fitting the eq. 6.11 to the values of SRT_{min}^{aer} predicted by the model. The calculated value of $\theta_{1/SRT_{aer,min}} = 1.137 \pm 0.002$ is in between that of nitrifiers (1.120, Henze et al., 1994) and aerobic PHA conversion (1.163, Brdjanovic et al., 1997). This indicates a very high temperature dependency of SRT_{min}^{aer} .

6.4.3.2. Maximal PHA Level in the Cell

In order to calculate the SRT_{min}^{aer} the value of the f_{pha}^{max} needs to be known (see eq. 6.9); the SRT_{min}^{aer} is defined as the minimum time required to aerobically consume the anaerobically stored PHA. Since this depends on the PHA concentration, the SRT_{min}^{aer} depends on the maximal reachable active biomass specific PHA ratio under the most optimal conditions for storage. A maximal PHA level in the cell will not be reached during limiting substrate availability. However, if the microorganisms can not aerobically convert all the PHA accumulated under anaerobic conditions then the PHA level will gradually increase over a few anaerobic-aerobic sequences until saturation occurs at f_{pha}^{max} . This limitation is likely of physical nature and depends on the available storage space in the cell for which also poly-P and glycogen pools compete. Since these storage pools in a BPR

system can be manipulated by changing the operating conditions [e.g. the length of the anaerobic and aerobic phases and the feeding regime (Brdjanovic et al., 1998b), the temperature and SRT (Brdjanovic et al., 1998a), SRT (Smolders et al., 1995b), the electron acceptor from oxygen to nitrate and SRT (Kuba et al., 1993) and the number of anaerobic-aerobic sequences (Kuba et al., 1996)], there might exist no universal saturation level for PHA.

Representative values of f_{pha} obtained from different activated sludge systems under wide range of operating and process conditions are summarised in table 6.3.

Table 6.3 The measured PHA content of the cells in bacterial populations cultivated under laboratory conditions

f_{pha}	Unit	Sludge	Comments	Reference
0.58	g PHA/ g act.biomass	enriched BPR culture in A2 system	anoxic batch test	Murnleitner et al., (1997)
0.55	g PHA/ g act.biomass	enriched BPR culture in A/O SBR system	at 10°C, 8 days SRT	Brdjanovic et al., (1998a)
0.47	g PHA/ g act.biomass	enriched BPR culture in A2 SBR system	at 20°C, 8 days SRT	Murnleitner E., (1995)
0.44	g PHA/ g act. biomass	enriched BPR culture in A/O SBR system	at 5°C, 32 days SRT	Brdjanovic et al., (1998a)
0.28	g PHA/ g act. biomass	enriched BPR culture in A2 SBR system	at 20°C, 14 days SRT	Murnleitner E., (1995)
0.25	g PHA/ g act. biomass	enriched BPR culture in A/O SBR system	at 20°C, 5 days SRT	Murnleitner E., (1995)
0.08	g PHA/ g act. biomass	enriched BPR culture in A/O/A SBR system	at 20°C, 20 days SRT	Murnleitner E., (1995)
0.19	g PHA/ g act. biomass	enriched BPR culture in A/O s SBR system	at 20°C, 8 days SRT	Smolders et al., (1995)
0.18	g PHA/ g act. biomass	enriched BPR culture in A/O SBR system	at 20°C, 8 days SRT	Brdjanovic et al., (1996)
0.34	g PHA/ g act. biomass	enriched BPR culture in A/O SBR system	acrobic P-release batch test	Brdjanovic et al., (1998b)
0.48	g PHA/ g act. biomass	enriched BPR culture in A/O SBR system	at 20°C, 4 days SRT	Smolders et al., (1995b)
0.8	g PHA/ g TSS	pure culture of <i>A. eutrophus</i>	Commercial PHA production	Byron, (1992)
0.62	g PHA/ g TSS	mixed culture in MAA system	Commercial PHA production	Satoh et al., (1997)

Clearly, reported PHA content of the cells is highly variable. The PHA content of the biomass during commercial PHA production by pure culture of *A. Eutrophus* can be as high as 80% (based on TSS) which corresponds to 4 g PHB/g act. biomass (Byron, 1992). The maximal PHA content observed in a microaerophilic-aerobic sludge was 0.62 g PHA/g TSS or 1.5 g PHA/g active biomass (Satoh et al., 1997). However, these values are much higher than what can be achieved by bio-P bacteria because these have more than one storage pool.

The highest reported value of f_{pha} achieved in laboratory scale BPR systems was 0.5-0.6 g PHA/g act. biomass. The value of f_{pha}^{max} of 0.55 g PHA/g act. biomass was used in this study for the calculation purposes. According to figure 6.1 the effect of change in f_{pha}^{max} between 0.4 and 0.7 g PHA/g act. biomass on SRT_{min}^{acr} is minor.

6.4.3.3. Number of Anaerobic-Aerobic Sequences

Kuba et al., (1997) showed that at the same SRT the average PHA content of the biomass increased with increasing number of anaerobic-aerobic (anoxic) cycles. Since the PHA content of the biomass is linked to SRT^{acr} , it is likely that the SRT_{min}^{acr} also depends on the number of anaerobic-aerobic sequences.

The SRT_{min}^{acr} can be determined as a function of the operating regime by keeping the substrate loading rate constant and by varying the number of anaerobic-aerobic (A/O) sequences in comparison with the standard SBR operation (in this case the SBR with four A/O sequences per day was used). Detailed description of the standard SBR cycle can be found elsewhere, i.e. Kuba et al., (1993), Smolders et al, (1994), Brdjanovic et al., (1996). The value of $f_{pha}^{max} = 0.55$ g PHA/g act. biomass (obtained from Brdjanovic et al. 1998a) is assumed to be the maximal PHA content of the biomass obtained for the standard SBR cycle. The time needed for aerobic degradation of anaerobically accumulated PHA (SRT^{acr}) can be calculated from eq. 6.5, assuming that Δf_{pha} of small cycles is equal to Δf_{pha}^{acr} of standard cycle divided by the number of small cycles, $f_{pha,to} = f_{pha}^{max}$, and t^{acr} is the time needed for complete utilization of Δf_{pha} under aerobic conditions.

Calculations showed that increasing the number of A/O sequences relative to standard SBR operation would reduce SRT^{acr} up to 1% at 5 °C and 7% at 20 °C. The effect of decreasing the number of A/O sequences would result in substantially longer SRT^{acr} at higher temperature (figure 6.4). For example, decrease of the number of A/O sequences from four to one per day will lead to increase of SRT^{acr} by 84% at 20 °C, 16% at 10 °C and 6% at 5 °C.

These results suggest marginal influence of the operational pattern, i.e. number of anaerobic-aerobic sequences, at lower temperature on SRT_{min}^{acr} . However, this parameter might be considered when operating plants in warm climates with long hydraulic retention time (and therefore small number of A/O sequences per day). It is also interesting to note that an increase in a number of cycles per day over the value of around 4 will have no effect on SRT_{min}^{acr} .

Figure 6.4 Dependence of minimal aerobic SRT on number of anaerobic/aerobic sequences in BPR systems at different temperature.

6.4.3.4. Type of Electron Acceptor

It was shown above that the PHA level in the cell together with the PHA consumption rate and biomass yield allows determination of SRT_{min}^{acr} . However, in the case of denitrifying BPR it is expected that the SRT_{min}^{anox} will be 30 to 40% longer than the SRT_{min}^{acr} in aerobic BPR due to the fact that the Y_{pha,HA_c} is considerably lower in anaerobic-anoxic (A2) systems than in A/O system (Kuba et al., 1996). Unfortunately, no experimental data are available to support the evaluation of the model on denitrifying BPR systems.

6.5. Conclusions

Contrary to normal biological conversions the BPR process is not limited by a minimal SRT, which results from a maximum growth rate of the organisms. This is because the aerobic SRT should be long enough to oxidize the amount of PHA stored in the anaerobic phase. This makes that the minimally required aerobic SRT will primarily depend on the PHA conversion kinetics and the maximal PHA content in the cell (storage capacity) but also on the other operational parameters, mainly on temperature (very high temperature dependency - in the range of nitrifiers). The length and the number of the anaerobic-aerobic sequences, and sludge organic loading are less important. It is expected that in denitrifying BPR systems the minimally required SRT will be around 35% longer in comparison with aerobic BPR. The developed model for the prediction of the minimally required aerobic SRT was well capable of describing the experimentally obtained data.

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7

Chapter

Effect of Potassium Limitation on Biological Phosphate Removal

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Contents

7.1. Introduction	185
7.2. Experimental Design	186
7.3. Materials and Methods	187
7.3.1. Continuous Operation of SBR	187
7.3.2. Medium	187
7.3.3. Analyses	187
7.4. Results and Discussion	188
7.5. Conclusions	189

Abstract

A full-scale sewage treatment plant designed for BPR may experience short or long-term shortage of potassium in the influent. In this chapter, using an anaerobic-aerobic SBR system, inoculation sludge from laboratory, pilot and full-scale P-removal plants was exposed to different potassium-phosphorus ratios in the influent. By simulating the conditions which may occur in practice, it was shown that potassium is an essential factor in BPR processes. When the system was exposed to severe shortage of potassium in the influent (a) phosphorus removal was nil, (b) poly-P concentration in the biomass decreased exponentially due to sludge wasting, and (c) the anaerobic P release and the related acetate uptake were only affected after several days of potassium absence, likely due to insufficient content of poly-P in the biomass to allow full acetate uptake under anaerobic conditions. In contrast, the system achieved complete P removal when potassium was present in excess amounts in the feed (in this case 1.25 and 2.5 g K/g P).

7.1. Introduction

Microorganisms require various nutrients in the form of cations. Cations as potassium, magnesium, calcium and iron must be present above critical concentrations in culture media. The need for potassium in the influent of biological wastewater treatment systems, in quantities appropriate for the specific requirements of microorganisms involved in the processes, has been identified earlier (van Groenestijn, 1988). Potassium, as one of the principal inorganic cations in cells, ranks seventh among the elements in order of abundance, and constitutes approximately 1% of the dry weight of the microbial cell (Stainer et al., 1983). As a cofactor for some enzymes, potassium stimulates enzyme reactions associated with the synthesis of cell materials. The importance of potassium for microorganisms responsible for biological phosphorus removal (BPR) is even more significant. This cation defines cell membrane permeability (Medveczky and Rosemberg, 1971) and plays a major role in the phosphate transport between surrounding environment and cell. Moreover, potassium is an essential counterion for poly-phosphate in the cell (Groenestijn, 1988), and is in general an important factor in the cells' energy generation (Mulder et al., 1986). Consequently, this cation is necessary for poly-phosphate accumulation in BPR systems.

There is only limited information available on the influence of potassium concentration on BPR system operation. It was shown (van Groenestijn, 1988) that in batch experiments using media without potassium, phosphate uptake by a pure *Acinetobacter* strain 210A culture was significantly decreased. The same study suggested an interdependency of the P-uptake and P-release cycles of potassium and phosphate, and stated that maximum P-uptake by the pure culture system and by selected activated sludge requires a potassium content in the reactor of >10 and 5 mg/L, respectively. However, in the meantime, it has been recognized that *Acinetobacter* sp. has a limited role in BPR (Wagner, 1994, Smolders, 1995).

As potassium is not a parameter that is routinely measured in wastewater, relevant information on potassium content in domestic sewage is limited. Annual average values in The Netherlands for potassium and phosphate (as total P) concentration in typical crude domestic sewage are 10 and 7.5 mg/L, respectively. In the absence of measured data, a rough estimation of the potassium concentration in domestic sewage can be based on human production of the potassium excreted with faeces and urine and the potassium content of the tap water used in the household, taking into account a specific potable water consumption and wastewater production in the area. On average, an adult human excretes 225 g of faeces with 440 mgK/d, and 1000 mL of urine with 2260 mgK/d, in total 2700 mgK/d (Bitton, 1994). In 1994, potassium content in Dutch drinking waters was in the range 0.6-11.3 mg/L (VEWIN, 1994). EC regulations and the standards for drinking water quality allow a maximum potassium content in water supply networks of 12 mg/L.

Wastewater treatment plants for phosphorus removal from domestic sewage may be exposed to regional and temporal variations in potassium concentration in the plant influent (chemical composition of potable and process water, pollution type, rainwater dilution,

runoff contribution etc.). For example (Marais, 1995, personal communication), the performance of a full scale sewage plant Bandigo in Australia, designed for BPR, deteriorated due to shortage of potassium in the influent.

This work addresses the questions, (1) how significant the impact of severe potassium shortage in the influent on dynamic behaviour of BPR is, and (2) how the BPR system behaves when one switches the potassium concentration from K absence to increased K content in the influent.

7.2. Experimental Design

A sequenced batch reactor (SBR) system operating in consecutive anaerobic-aerobic-settling mode, as described in chapter 2, was used in this study to simulate the treatment process. In total three experiments were performed. Inoculation sludge was obtained from different activated sludge systems designed for BPR (table 7.1).

Table 7.1 Overview of the experiments with the variable potassium content in the feed, using fresh P-removing activated sludge obtained at different locations.

Test no.	Duration (days)	Sludge source	Location	Influent K/P ratio (mg K/mg P)
IA	10 (day 0 to day 10)	Full scale WwTP	Heemstede	0.0009
IB	20 (day 11-day 31)	Anaerobic/Aerobic SBR	Delft	0.0009
IC	12 (day 32-day 44)	Anaerobic/Anoxic SBR	Delft	0.0009
ID	30 (day 45-day 75)	Anaerobic/Anoxic SBR	Delft	0.0009
IE	11 (day 76-day 87)	Pilot scale WwTP	Wageningen	0.0009
II	24 (day 88-day 112)	Pilot scale WwTP	Wageningen	1.25
IIIA	23 (day 113-140 day)	Anaerobic/Anoxic SBR	Delft	1.25
IIIB	13 (day 141-day 154)	Anaerobic/Anoxic SBR	Delft	2.5

In Experiment I the effect of K-absence was tested. The system was inoculated and started-up five times (IA-IE). Previously used sludge was removed from the SBR prior to each inoculation. Influent potassium content was 0.013 mg/L. The influent K/P ratio was 0.0009 mgK/mgP.

In Experiment II the SBR was supplied with substrate containing potassium in excess

amount (K/P ratio 1.25 mgK/mgP). The inoculation sludge was from the same source as used in the Experiment IE.

The strategy applied in Experiment III differed from before. At the beginning of Experiment IIIA, the reactor was inoculated with the same sludge as in Experiment ID, and a medium with a K/P ratio of 1.25 mgK/mgP was used. However, the SBR in Experiment IIIB was not inoculated again, as the reactor continued to operate after Experiment IIIA, now with the K/P ratio set at 2.5 mgK/mgP.

7.3. Materials and Methods

7.3.1. Continuous Operation of Sequencing Batch Reactor

A double jacketed laboratory fermenter with automated operation, control and monitoring (Smolders et al., 1994) with an operating volume of 2.5 L was used in the experiment. The experiment was performed at controlled temperature (20°C) and pH (7.00 ± 0.05). pH was maintained by dosing 1N HCl and 1N NaOH. The fermenter operated as an anaerobic-aerobic-settling SBR with a cycle of 6 hours (see also section 2.2).

At the beginning of each experiment the SBR was inoculated with phosphorus-removing sludge. Fresh inoculum was used in each experiment. The only exception was made in Experiment IIIB, when a new inoculation was not performed. Otherwise, 2.25L of resuspended sludge was transferred to the empty reactor followed by the addition of 0.25 L of substrate. The initial concentration of mixed liquor suspended solids (MLSS) in inoculum varied from 2.5 to 3.5 g/L and depended on the sludge source.

7.3.2. Medium

Synthetic medium, as described in paragraph 2.2.3, was used in this experiment, except of the potassium content. In Experiment I, potassium was added to the system through the nutrient solution in a trace amount only (0.013 mg/L). In Experiment II the medium contained 18.9 mgK/L as an influent concentration. Experiment III was performed with medium containing 18.9 mgK/L (IIIA) and 37.8 mgK/L (IIIB).

7.3.3. Analysis

The performance of the system was monitored on a daily basis by measuring orthophosphate (PO_4^{3-}), total organic carbon (TOC), polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHV), MLSS and mixed liquor volatile suspended solids (MLVSS).

Samples for the determination of P-release and P-uptake in the cycle were taken at the end of the anaerobic and aerobic phase, respectively. TOC measurements were performed at the end of the anaerobic, and MLSS and MLVSS at the end of the aerobic phase. Samples for PHV and PHB content determination were taken at the beginning of each phase in the cycle. Analyses were performed according to procedures described in section 2.2.4.

7.4. Results and Discussion

Experiments were continuously run for 154 days in total. The length of each experiment is listed in table 7.1. The phosphate concentration at the end of the anaerobic phase and in the SBR's effluent are presented in figure 7.1.

During Experiment I (A-E) the net P-elimination was not achieved because the influent and effluent phosphate concentrations were nearly equal. Although the inoculation sludges were obtained from four different BPR plants (full-, pilot- and lab-scale), they all originally contained a high level of the P-removing bacteria and poly-phosphate. The initial P-release was within the expected range of 50-60 mg/L (according to HAc addition and complete anaerobic HAc uptake), depending on the type of sludge used as inoculum. However, anaerobic P-release rapidly declined in each case. Within a week from the start-up, P-release was only 50% of the initially observed value. After 10-15 days there was no or very modest P-release noticed in the system. Such a pattern was best observed in Experiments IB and ID (see figure 7.1).

Figure 7.1 Phosphate concentration in the SBR cycle at the end of the anaerobic phase (■) and at the end of the aerobic phase (□) in Experiments I, II and III.

This behaviour can be explained as follows. Because potassium was only available in trace amounts in the influent, poly-P production was halted and, consequently, the poly-P content in the biomass was depleted. Initially, poly-P was present in the biomass to allow complete HAc uptake under anaerobic conditions. The amount of phosphate released remained constant and so did the amount of poly-P organisms. Due to potassium and poly-P washing-out by the excess sludge, the poly-P content gradually decreased below the concentration needed for full anaerobic HAc uptake. From this moment on the amount of P-release steadily decreased and the amount of HAc entering the aerobic phase increased. This led to a gradual shift in the population from poly-P organisms to normal heterotrophic organisms. The decrease in poly-P concentration in the reactor was the direct result of the removal of poly-P and potassium by sludge wasting. Therefore the following exponential equation should be capable of describing the observed pattern:

$$C_{pp} = C_{pp(0)} * \exp (-t/SRT) \quad \dots(7.1)$$

where C_{pp} is the poly-P concentration in the biomass (in mg P/L), $C_{pp(0)}$ is the initial concentration of the poly-P in the biomass at the end of the aerobic phase (in mgP/L), t is the time of the experiment, and SRT is the biomass retention time in the SBR (in 1/day).

$C_{pp(0)}$ was calculated using the equation:

$$C_{pp(0)} = \text{Ash} * 0.95 * P/K_{0.333} \text{Mg}_{0.333} \text{PO}_3 \quad \dots(7.2)$$

assuming that 95% of ash is polyphosphate and there is a K/P ratio of 0.42 mg K/mg P (0.33 molK/molP) in the biomass at the end of the aerobic phase. The molar K/P ratio of 0.33 is most often reported in literature (Comeau et al., 1987; van der Velde, 1992). Miyamoto-Mills et al., (1983); Arvin et al., (1985) and Richard et al., (1992) reported molar K/P ratios in BPR systems of 0.27, 0.23 and 0.21, respectively. The ash content was calculated as the difference between MLSS and MLVSS concentration measured at the end of the aerobic phase in SBR. The poly-P concentration in the biomass, required for complete anaerobic HAc uptake, was calculated (71.54 mgP/L), using known P-HAc stoichiometry (Smolders et al., 1995). The model was used to simulate Experiment IB. The result of the simulation is presented in figure 7.2.

There is a good agreement between simulated and experimentally observed poly-P values. Indeed, the poly-P is washed out from the reactor by sludge wasting rate. The cross-point of the simulation curve of C_{pp} , and the line marking C_{pp} needed for full HAc uptake, fall in the range of 4-5 days, which matches the end of the period of stable P-release and the start of wash-out of the poly-P from SBR, observed in Experiment IB (see figure 7.1).

In contrast, during Experiments II and III when the influent contained a sufficient amount of potassium, instead of deteriorating, the system performance improved significantly. At

the beginning of these experiments, the initial maximal P-release in a cycle was in the range 50-70 mg/L. From the start, P-uptake was 100%, resulting in complete P-removal from the system. In Experiment II, when the medium contained 18.9 mgK/L (influent K/P ratio 1.25 mg K/mg P), P-release gradually increased up to an average value of 60 mg/L. Experiment IIIA was executed with the same K/P ratio as in Experiment II. An initial P-release of 70 mg/L was maintained. Exposed to the increased potassium content in the influent (K/P ratio 2.5 mg K/mg P) over the following 13 days (Experiment IIIB), P-release further increased by 13% and became steady at 80 mg/L. A modest increase in P-release with increased potassium concentration in the medium was observed earlier (Groenestijn, 1988). However, in this case, a higher P-release could also be attributed to the improved efficiency of the system, as the system was not fully in steady-state. The average P-release values in Experiments II and III corresponded with data reported earlier by Smolders et al. (1994) for "normal" BPR without K-deficiency.

Figure 7.2 Simulation of polyP concentrations in the biomass during Experiment IB. K/P ratio of 0.333 molK/molP in the bacteria and SRT of 8 days were used. Experimentally observed values measured at the end of the aerobic phase of SBR are presented by (■)

Anaerobic HAc consumption was monitored by measuring the TOC concentration in the liquid at the end of the anaerobic phase (results not shown). Throughout the experiment HAc was added at an amount of 400 mg COD/L (influent). During Experiment I, acetate was not consumed completely at the end of the anaerobic phase, likely due to limited amounts of polyphosphate in the inoculated sludge. This means that, in the aerobic phase, the remaining part of HAc became available to the microorganisms, possibly out-competing

the organisms responsible for P-removal in the aerobic phase. In contrast, when complete P-removal was achieved (Experiments II and III), the acetate was fully consumed by P-removing bacteria. The observed phosphate/acetate ratio of 0.49 mgP/mgCOD agrees with a value of 0.48 mgP/mgCOD reported by Smolders *et al.* (1994).

During the experiments, MLVSS and MLSS concentrations in the SBR were monitored. The MLVSS/MLSS ratios are presented in figure 7.3. A low MLVSS/MLSS (less than 0.75) indicates a high polyphosphate content in the biomass. An increasing MLVSS/MLSS ratio in Experiment I was found. This again indicated the depletion of polyphosphate. When potassium was present in the system in excess amounts (Experiments II and III), full P-removal was achieved. The system stabilized at 2.3 g MLSS/L and 1.7 g MLVSS/L, and 2.7 g MLSS/L and 2 g MLVSS/L, respectively. In both cases, the MLVSS/MLSS ratio became steady and averaged 0.74 which matches the range in "normal" operation.

When the reactor was fed with influent with K/P ratios of 1.25 and 2.5 mg K/mg P, the average potassium consumption during one cycle was 3.16 and 7 mg/L, respectively. As full P-removal was achieved in both cases, observed K/P ratios were 0.21 and 0.47 mg K/mg P, respectively.

Figure 7.3 MLVSS/MLSS ratio at the end of the aerobic phase of the SBR in Experiments I, II and III.

The PHB and PHV content of the biomass was measured on two occasions: on day 75 (Experiment ID) and day 113 (experiment IIIA). The results are given in table 7.2. On day

75 (Experiment ID), only 35 mg/L of PHB accumulated in cells during the anaerobic phase, indicating the lack of HAc uptake and P-release. The situation was very different on day 113 (Experiment IIIA), when the system achieved full P-removal and when 256 mg/L of PHB accumulated anaerobically. In Experiment ID, the PHV/PHB ratio was high (0.35). This may indicate the presence of G-bacteria (Cech and Hartman, 1993; Mino et al., 1993) in the SBR. In Experiment IIIA the PHV/PHB ratios are in accordance with information provided by Smolders et al.(1994), for "normal" BPR in the SBR where PHV makes up only about 10% of the PHB formed in a cycle.

Table 7.2 PHB and PHV measurements (mg/L) in the SBR cycle at the beginning and the end of the anaerobic phase, and the end of the aerobic phase, in the Experiment ID (day 75) and IIIA (day 113).

Test no.	Start anaerobic phase (prior to feeding)		End anaerobic phase/ Start aerobic phase		End aerobic phase		
	PHB	PHV/PHB ratio	PHB	PHV/PHB ratio	PHB	PHV/PHB ratio	MLVSS/MLSS ratio
ID	215	0.35	250	0.35	215	0.3	0.97
IIIA	44	0.14	300	0.13	46	0.09	0.74

The observations in Experiment I suggested that full-scale sewage treatment plants (STPs), designed to biologically remove phosphate from sewage, can be affected if severe shortage of potassium in the plant influent occurs. In general, severe shortage of potassium in the influent of STPs designed to treat exclusively domestic sewage is unlikely to occur, due to the fact that an average K/P ratio in excreted faeces and urine of humans is 0.8 and 1.9 mgK/mgP, respectively (Bitton, 1994). However, if the plant influent is partially or fully constituted of industrial wastewater which contains high amounts of phosphate and no potassium, the risk of impact on BPR increases. Assessment of the performance of an STP designed for BPR should take into account that, in case of a lack of potassium in the plant influent, P-removal will be absent, but this does not necessarily mean that P-removing bacteria are until that moment lost from the system. Therefore, if simply a comparison of P concentration in the STP's influent and effluent is applied, indicating a zero P-removal efficiency, it might give a false impression that poly-P bacteria are washed out from the installation. However, if HAc is not taken up totally by poly-P bacteria over a longer period of time than a couple of days, a wash-out of poly-P bacteria begins and HAc becomes available to other bacteria which may deteriorate the system performance in the long run. It is therefore recommended that STP operators check the K/P ratio in the influent, if the BPR is failing.

7.5. Conclusions

When the BPR system was exposed to a severe shortage of potassium in the influent: (a) phosphorus removal was absent, (b) polyphosphate concentration in the biomass decreased exponentially due to sludge wasting and, (c) the anaerobic phosphorus release and the related acetate uptake was only affected after several days of potassium absence, likely due to insufficient content of polyphosphate in the biomass to allow full acetate uptake under anaerobic conditions. In contrast, the SBR system achieved complete phosphorus removal when potassium was present in excess amounts.

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8

Chapter

Effect of Poly-phosphate Limitation on the Anaerobic Metabolism of Bio-P Bacteria

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Contents

8.1. Introduction	199
8.2. Materials and Methods	200
8.3. Results	201
8.4. Discussion	203
8.5. Conclusions	205

Abstract

There are two types of microbial populations described in the literature as being capable of anaerobic storage of acetic acid in activated sludge processes: the poly-phosphate accumulating organisms (PAOs) and the glycogen accumulating non-poly-phosphate organisms (GAO). Both groups use the conversion of glycogen to poly-hydroxy-alkanoate (PHA) to produce ATP and NADH; however, the first group can also produce ATP from poly-phosphate (poly-P). No representative pure cultures are available from either group. The question arises: is the observed activity of GAO due to PAO that are depleted in poly-P? In this study, using a laboratory sequencing batch reactor containing an enriched culture, the ability of the enriched PAO to utilize organic substrate (acetate) anaerobically was investigated under conditions of poly-P limitation and surplus glycogen content of the biomass. This study showed clearly that, under these conditions, almost no acetate was taken up. Furthermore, this strongly suggests that PAO can not use glycogen conversion to PHA as the sole energy source under anaerobic conditions, which seems to be restricted to a separate group of GAO. On the basis of the results and literature data, an improved scheme for the anaerobic acetate accumulation is presented.

8.1. Introduction

Biological phosphorus removal is based on the enrichment of activated sludge with poly-phosphate accumulating organisms (PAO). Under anaerobic conditions PAO take up organic substrates (preferably volatile fatty acids; VFAs) and store them as poly-hydroxy-alkanoates (PHAs), while the reduction equivalents are provided by the degradation of internally stored glycogen (Mino et al. 1987; Arun et al. 1988; Smolders et al. 1994; Pereira et al. 1996). The energy is generated by the conversion of glycogen and poly-phosphate (poly-P) resulting in phosphate release into the solution. In the subsequent aerobic or anoxic phase, the internal pool of PHAs is oxidized and used for growth, phosphate uptake, glycogen synthesis and maintenance (Arun et al. 1988; Smolders et al. 1995). It has been reported that glycogen accumulating non-poly-P organisms (GAO, also called G bacteria) may appear when biological P removal deteriorates (Cech and Hartman 1990, 1993; Liu et al. 1994; Satoh et al. 1994; Matsuo 1994). These organisms are also capable of anaerobic utilization of organic substrates that are converted and stored as PHAs, while the energy and reduction equivalents are provided only by glycogen degradation without involvement of poly-P. In the absence of glycogen, anaerobic substrate uptake by PAO or GAO can not occur (Mino et al. 1996; Brdjanovic et al. 1998).

During anaerobic acetate metabolism in both PAO' and GAO' energy is required for maintenance, transport of external carbon-substrate into the cells and its conversion to PHAs. The PAO obtain the energy from poly-P degradation and glycolysis [glycogen conversion to poly-hydroxy-butyrate (PHB) via acetyl-CoA and carbon dioxide; no glycogen conversion to propionyl-CoA is needed for PHA synthesis], (Mino et al. 1996). According to Mino et al. (1996), the anaerobic metabolism of GAO resembles that of PAO (glycolysis of stored glycogen and substrate conversion to PHAs through either acetyl-CoA or propionyl-CoA, and propionyl-CoA production by the succinate-propionate pathway), except that there is no involvement of poly-P. They hypothesised that the energy generated by glycolysis is not exclusively used by GAO, but can also be a complementary energy source for PAO. The hypothesis was based on the assumption that both PAO and GAO have a glycolysis pathway (figure 8.1). This was recently proven by in-vivo NMR measurements (Pereira et al. 1996).

In PAO, glycogen is only converted to deliver the NADH required for HAc reduction to PHB, while in GAO, more glycogen is converted in order to deliver enough ATP for HAc conversion to acetyl-CoA. The surplus in reduction equivalents obtained in this way in GAO, is balanced by formation and polymerisation of propionyl-CoA into PHB or PHV. Although PAO could be expected to possess the anaerobic metabolism of GAO, there is no experimental evidence to confirm this. With the aim of investigating in more detail the energy budget of PAO and GAO, this work addresses the question: can PAO perform the

Figure 8.1 Simplified metabolic pathways of acetate and glycogen conversion to PH(B/V) by (a) PAO and (b) GAO under anaerobic conditions (after Mino et al. 1997).

metabolism of GAO under anaerobic conditions? Or more specifically, can PAO take-up VFAs in the absence of poly-P when sufficient glycogen is present? Underlying this question is whether PAO and GAO are possibly one group of organisms. This question can be answered by observing the behaviour of bio-P bacteria with a sufficient level of glycogen, but low or no poly-P under anaerobic conditions with acetate present. In this case only the glycolysis can supply the energy and reduction equivalents needed for acetate uptake.

8.2. Materials and Methods

In order to answer the above questions anaerobic substrate (acetate) uptake by sludge highly enriched with PAO was monitored under poly-P limitation and glycogen surplus. The poly-P level in the enriched sludge was depleted by enhanced P-release at high pH in the presence of surplus acetate under anaerobic conditions (Smolders et al. 1994). At this point the glycogen content of the biomass may become extremely low due to anaerobic acetate uptake (Brdjanovic et al. 1998). Application of aeration was therefore required for the recovery of the glycogen pool where PHB was converted to glycogen in the absence of phosphate. After the extended phase of anaerobic P-release the sludge was washed twice with medium containing no acetate or phosphate in order to prevent the aerobic consumption of surplus acetate, phosphate uptake and its accumulation as poly-P. This sludge-handling procedure (anaerobic phosphate release with acetate uptake, washing away phosphate and remaining acetate, and subsequent aerobic conversion of PHB to glycogen) resulted in sludge containing high glycogen, low PHB and little poly-P as required for the experiment. The final, and most important part of the experiment consisted of monitoring

of the anaerobic acetate consumption by the poly-P depleted sludge containing surplus glycogen.

A double jacketed, anaerobic-aerobic-settling laboratory sequencing batch reactor (SBR) with automated operation, control and monitoring, was used in the experiments. The only organic substrate was acetate (HAc) which was fully consumed during the anaerobic phase (a full description of the SBR and medium composition can be found elsewhere (Smolders et al. 1994). This ensured that a highly enriched sludge with poly-P microorganisms was obtained. The sludge was cyclically exposed to anaerobic (2.25 h), aerobic (2.25 h) and settling conditions (1.5 h), in a 6 h cycle ("standard" SBR operation). The fermenter operated under steady-state conditions in standard mode for 250 days at a temperature of 20°C, a pH value of 7.0 ± 0.1 and a sludge retention time (SRT) of 8 days before the experiment was performed.

Following the influent addition to the SBR, the "standard" cycle measurement (consisting of so-called anaerobic phase I, aerobic phase I and the settling phase) was performed (figure 8.2). The pH was increased from 7.0 to 8.0 and acetate (400 mgHAc-C/L as a final SBR concentration) was added to the SBR under anaerobic conditions (anaerobic phase II) in order to achieve the maximum phosphorus release. During this phase (3.5 h) the phosphorus release and acetate uptake were closely monitored. After the end of the anaerobic phase II, the pH was returned to 7.0 and the sludge was washed twice. The aim of the sludge washing was to maintain a very low poly-P content in the cells (achieved before the washing step occurred) until the end of the experiment. Following dilution with synthetic medium without phosphate and acetate, the sludge was excessively aerated (18 h, aerobic phase II) in order to regenerate the glycogen-storage pool of the biomass by oxidation of stored PHAs. After the aerobic period, acetate (200 mg HAc-C/L as a final SBR concentration) was added to the reactor under anaerobic conditions. During this anaerobic phase III (3 h) the acetate uptake, phosphate release and PHAs formation were closely monitored.

8.3. Results

The results of the experiment are presented in figure 8.2 and table 8.1. The measurements of all parameters during a "standard" SBR cycle showed the typical pattern observed earlier under similar operating conditions (Kuba et al. 1993; Smolders et al. 1995). During anaerobic phase I the acetate (75 mg HAc-C/L) was fully utilized resulting in a P-release of 87 mg P/L. The aerobic P-uptake was completed within one hour. During the subsequent enhanced P-release phase at pH 8.0 (anaerobic phase II) 210 mg HAc-C/L was utilized, resulting in phosphate release of 208 mg P/L and almost total depletion of poly-P and glycogen storage pools (table 8.1). After washing, acetate was absent but some phosphorus

Figure 8.2 Acetate (○), phosphate (●), glycogen (■), PHB (□) and PHV (◆) concentrations during the experiment performed at the temperature of 20°C, pH 7.0±0.1 and SRT of 8 days. Synthetic influent of the "standard" SBR cycle contained per litre: 400 mg COD of acetate as a carbon source, 15 mg P, 28 mg N, plus other necessary macro and micronutrients (Smolders et al. 1994).

(28 mg P/L) still remained in solution. This phosphorus was quickly taken-up in aerobic phase II. At the beginning of anaerobic phase III (the end of aerobic phase II) the biomass contained very low poly-P and PHAs levels and a substantial amount of glycogen (table 8.1). Approximately 90 % of the total acetate consumption (63 mg HAc-C/L) and phosphate release (37 mg P/L), associated with rapid formation of PHAs, occurred during the first hour of this phase (figure 8.1). Initially fast HAc-uptake and P-release are the result of the presence of poly-P (0.02 g P/g VSS) and glycogen in the cells (table 8.1). This relatively small poly-P pool was due to the uptake of phosphorus in the aerobic phase (figure 8.2). After depletion of the poly-P pool a dramatic slowing-down occurred of all the

processes involved. Only marginal conversions of acetate, phosphate and PHAs were observed until the end of the experiment. During anaerobic phase III, glycogen was not depleted; a decrease of glycogen concentration from 184 mg glycogen/L, observed at the beginning of the phase, to 112 mg glycogen/L at the end of the phase, was mainly attributed to the initial acetate uptake. The ratio of newly formed PHV to newly formed PHB (dPHV/dPHB ratio in figure 8.2) of 0.09 g PHV/g PHB observed at the end of the anaerobic phase I (standard SBR cycle), is similar to the value of 0.10 gPHV/gPHB, obtained by Smolders et al. (1995) under identical operating conditions. This ratio increased to 0.46 g PHV/g PHB in the last phase of anaerobic phase III.

Table 8.1 Biomass composition during the experiment

Parameter	Unit	End anaerobic I	End aerobic I	End anaerobic II	After washing	End aerobic II	End anaerobic III
MLSS	mg/L	3047	3287	2297	1917	1920	1670
MLVSS	mg/L	2253	2227	2030	1833	1737	1550
MLVSS/MLSS		0.74	0.68	0.88	0.96	0.9	0.93
Ash	mg/L	794	1060	267	84	183	120
Poly-P (as P)	mg P/L	236	330	56	~0	32	13
Glycogen	mg/L	169	236	17	12	194	112
PHB	mg/L	141	46	410	311	25	182
PHV	mg/L	10	2	24	16	1	27
PHA	mg/L	151	48	434	327	26	209
dPHV/dPHB	g/g	0.09	n.a.	0.05	n.a.	n.a.	0.46
Active biomass	mg/L	1933	1943	1579	1494	1517	1229

MLSS (Mixed Liquor Suspended Solids)

MLVSS (Mixed Liquor Volatile Suspended Solids)

Active Biomass = MLVSS - (PHA + Glycogen)

PHB (Poly-Hydroxy-Butyrate)

PHV (Poly-Hydroxy-Valerate)

PHA (Poly-Hydroxy-Alkanoate)

8.4. Discussion

On the basis of these results, it is clear that the PAO are not responsible for the observed GAO activity in activated sludge. Although, in theory, PHV production is not required in the anaerobic metabolism of PAO, in practice some PHV is always formed in BPR systems. In general, higher PHV levels were observed; for example, in a systems where G bacteria dominated. Since, in this experiment, no acetate was taken up after depletion of the internal poly-P pool of bacteria, we believe that the proportion of G bacteria in the sludge was very

low. Recently, Pereira et al. (1996), in the light of in-vivo NMR experiments, proposed the metabolism of anaerobic acetate take-up by PAO in which a small flux of substrate goes through the tricarboxylic acid (TCA) cycle, which consequently leads to the formation of PHV. In that study PHV accumulation is attributed to the enzymatic activity through the full TCA cycle, which occurs in PAO under anaerobic conditions, rather than to the presence of the G bacteria. However, Pereira et al. (1996) placed succinate in two pathways that run in different directions. This can not take place in one cell. Furthermore, it was shown that the succinate-malate conversion cannot occur in PAO under anaerobic conditions (Mino et al. in press). In order to explain the results we propose a modified metabolism of anaerobic acetate taken up by PAO where PHV is formed via enzymatic activity in a part of TCA cycle, but where the glyoxylate cycle also remain active (figure 8.3). The activity of these routes (needed under aerobic conditions) is probably caused by the fact that the enzymes involved are not repressed under anaerobic conditions. The very slow conversion of glycogen to PHB/PHV when poly-P is depleted can be due to the maintenance ATP need of the cells. Normally this would be satisfied by poly-P hydrolysis (Smolders et al. 1994).

Figure 8.3 Modified metabolism for conversion of acetate and glycogen to P(HB/HV) by bio-P bacteria under anaerobic conditions on the basis of the model of carbon metabolism in BPR of Pereira et al., 1996

8.5. Conclusions

It can be concluded that the marginal microbial activity observed under conditions where poly-P was completely depleted strongly indicates that acetate uptake stops, since PAO are not capable of the anaerobic uptake of carbon sources based solely on the glycolysis of glycogen. Available observations suggest that PAO and GAO do not belong to the same group of microorganisms. The reason why PAO cannot perform a metabolism similar to GAO, although they seem to have the necessary enzymes, remains unclear.

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9

Chapter

Impact of Excessive Aeration on Biological Phosphorus Removal

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Contents

9.1.	Introduction	211
9.2.	Materials and Methods	212
	9.2.1. Operation of the SBR	212
	9.2.2. Experimental Set-Up	213
	9.2.3. Analyses	214
9.3.	Results	215
	9.3.1. Experiment E1	215
	9.3.2. Experiment E2	218
	9.3.3. Experiment E3	220
9.4.	Discussion	220
	9.4.1. Excessive Aeration	220
	9.4.2. Other Factors Affecting Phosphate Uptake Rate	222
	9.4.3. Maintenance Versus Decay	222
	9.4.4. Practical Implications	223
9.5.	Conclusions	224

Abstract

It has been reported that deterioration of biological phosphorus removal efficiency at some wastewater treatment plants regularly occurred after heavy rainfall or weekends. The deterioration has been attributed to low plant loading that took place during such events. However, it is hypothesized in this study that the cause of such deterioration may have been the excessive aeration that took place due to inadequate control of aeration system during weekends and rainfall periods. In order to test this hypothesis, the influence of excessive aeration (aeration during starvation conditions) on BPR processes was studied using a laboratory anaerobic-aerobic-settling sequencing batch reactor. It was clearly demonstrated that the phosphorus uptake stops due to a gradual depletion of poly-hydroxy-butyrate (PHB) in an over-aerated process. If organic substrate is introduced to the system, phosphorus release is immediately at its maximal rate. However, the released phosphorus cannot be taken-up fully again because the PHB content limits the uptake rate. Consequently, incomplete phosphorus uptake leads to temporarily reduction of BPR efficiency. This causal effect can explain the deterioration of BPR efficiency after heavy rainfall or weekends. Since excessive aeration clearly negatively affects the BPR processes, the aeration should be properly controlled at sewage treatment plants. Some other findings of this study deserve to be mentioned. It was confirmed that the presence of acetate under aerobic conditions provokes phosphorus release. This may also contribute to deterioration of the BPR efficiency. The aerobic phosphate uptake was found to depend not only on the PHB but also on polyphosphate (poly-P) content of the cells. A maximal poly-P (0.18 g P/g VSS) and minimal PHB content of the cells (2.11 mg COD/g VSS) were observed in the enriched sludge during excessive aeration experiments. It was shown that under aerobic starvation conditions glycogen can not replace PHB for phosphate uptake and is only used for maintenance. During this period no oxygen consumption due to decay processes has been observed.

9.1. Introduction

Biological phosphorus removal (BPR) is a complex wastewater treatment process due to the fact that the main part of the metabolism takes place on internally stored substrates and products. The metabolism is based on the anaerobic consumption of volatile fatty acids (VFAs) and subsequent storage as poly-hydroxy-butyrate (PHB) while energy and reduction equivalents are provided by the degradation of internally stored poly-phosphate and glycogen. During anoxic or aerobic conditions the internally stored PHB is oxidized and used for growth, phosphate uptake, glycogen formation and maintenance (Arun et al., 1988, and Smolders et al., 1994c). The net phosphorus removal is achieved when the aerobic phosphorus uptake is higher than the sum of the phosphorus present in the influent and the phosphorus released anaerobically.

The stability and efficiency of BPR processes can be disturbed by several factors. It has been reported that, for example, deterioration of BPR efficiency regularly occurred at some wastewater treatment plants (WWTPs) after heavy rainfall or weekends. The phenomenon was attributed to low plant loading that took place during such events. In a case of heavy rainfall the WWTP temporarily receives low concentrated sewage and high hydraulic loading. According to Henze (1996) prolonged exposure to storm water conditions will negatively affect BPR processes. It is still not clear whether this is due to inhibition of P-removing bacteria or that the low COD concentrations in the influent are causing the problem. A temporary deterioration of the BPR efficiency in the study of Temmink et al., (1996) has been explained by partial or complete depletion of the internal PHB stores of the poly-P bacteria. As a control strategy to counteract such negative effects on BPR they recommended adjustable aeration times to avoid unnecessary PHB consumption and/or to maintain a certain minimum level of PHB in the cells by the addition of an external carbon source.

Some reports refer to a quite regular increase of the effluent phosphate concentration after weekends, so-called "Monday P-peaks". Pitman et al., (1983) and Wolf and Telgemann, (1991) attributed this phenomena to the low organic load during weekends which resulted in a high nitrate input to the anaerobic tank and deterioration in BPR efficiency observed on Mondays.

The deterioration of BPR efficiency observed at activated sludge WWTPs with BPR which temporarily experience a period of low organic loading may be explained by the following hypothesis:

- *The main reason for deterioration of BPR under low COD loading regime is excessive aeration of activated sludge. Excessive aeration might lead to changes in internal storage pools, especially in PHB. In that case PHB can become partially*

or completely depleted. As the phosphate uptake rate is kinetically controlled by the fraction of PHB in the biomass (Henze et al., 1995; Smolders et al., 1994c; Temmink et al., 1996; Murleitner et al., 1997) this depletion in PHB will lead to a lower phosphate uptake rate. After restoration of normal loading conditions phosphorus release is not affected, but phosphorus uptake is comparatively slower, resulting in deterioration of BPR.

There are two common causes of excessive aeration of activated sludge which occur in practice. Firstly, a combination of a heavy rain event and sewerage with large hydraulic gradient may result in a relatively high input of air into the sewer. The dissolved oxygen (DO) input to the sewage can further increase if screw pumps and/or aerated grit chambers are employed at the WWTP. This means that during rainfall events the anaerobic hydraulic retention time of BPR units may become temporarily substantially shortened, making the aerobic phase longer than designed for and the activated sludge excessively aerated. The second cause of over-aeration lies in the fact that some WWTPs have inadequate aeration control. In such plants, like in the study of Wolf and Telgmann, (1991), the control of aeration system could not be adjusted to the process requirements which made the activated sludge excessively aerated during weekends.

In order to investigate the above hypothesis in detail the dynamic behavior of internal storage products in BPR during periods of excessive aeration was studied. The aim of the study was to investigate: (1) the response of the system during and after an excessive aeration period, (2) the fate of PHB and glycogen pools of the P-removing microorganisms under starvation conditions and, (3) the effect of substrate (acetate) presence under aerobic conditions. For this purpose an anaerobic-aerobic-settling SBR with enriched phosphorus removing sludge was used. In the first part of the study (experiment E1) the effect on storage polymer content and phosphorus behavior during the "prolonged" SBR cycle and the "subsequent standard" cycle was monitored and compared with the observations during "standard" operation of the SBR. In the second part of the study (experiment E2 and E3) the levels of internal storage pools were manipulated by varying the length of the anaerobic and aerobic phase and by applying different feeding strategies.

9.2. Materials and Methods

9.2.1. Operation of the SBR

A double jacketed, anaerobic-aerobic-settling laboratory fermenter (2.5 L) with automated operation, control and monitoring was used in the experiments (see also section 2.2.1). The only carbon substrate was acetate (HAc) which was fully consumed during the anaerobic phase. This ensured that a highly enriched sludge with poly-P microorganisms was

obtained. The sludge was cyclically exposed to anaerobic (2.25 h), aerobic (2.25 h) and settling conditions (1.5 h), in total six hours per cycle (standard SBR operation). The fermenter operated under steady state conditions for 200 days at a temperature of 20°C, pH value of 7.0 ± 0.1 and sludge retention time (SRT) of eight days. This standard operation was changed in three occasions (in experiment E1, E2 and E3) where the length of the phases, substrate concentration and the time of substrate addition to the SBR were changed during **one** cycle only. The differences in operational conditions of the SBR are summarized in table 9.1.

Table 9.1 Operational conditions of the SBR under "standard" operation and during experiments E1, E2 and E3.

Parameter	Unit	Standard Operation	Experiment E1	Experiment E2	Experiment E3
Cycle	h	6	30	27	26
Anaerobic phase	h	2.25	2.25	3	2.25
Aerobic phase	h	2.25	26.25	7.5 + 15	19.25 + 3
Settling phase	h	1.5	1.5	1.5	1.5
Total P-load	mg P/cycle	18.75 ^(a)	18.75 ^(a)	375 ^(a) + 375 ^(b)	18.75 ^(a) + 100 ^(c)
Total C-load	mg HAC-C/cycle	187.5 ^(a)	187.5 ^(a)	375 ^(a) + 375 ^(b)	187.5 ^(a)

P and/or HAC were added to the SBR at: (a) the beginning of the cycle, (b) the middle of the prolonged aerobic phase and, (c) 3 h before the end of the prolonged aerobic phase

9.2.2. Experimental Set-up

Experiment E1 consisted of three consecutive cycle measurements (the "standard", "prolonged" and "subsequent standard" cycle). During the prolonged cycle the duration of the aerobic phase was extended from 2.25 (standard length) to 26.25 hours. In comparison with standard cycle the SBR operated during prolonged cycle under low loading conditions. The phosphorus uptake capacity of the biomass exposed to excessive aeration was studied in nine separate aerobic batch tests with sludge taken (140 mL each time) from the SBR. A single batch test was executed each hour during the first seven hours of the prolonged aeration phase and two batch tests were performed during the last two hours of the phase. For this purpose a double jacketed laboratory fermenter with a maximal operating volume of 0.15 L was used. The tests were performed at a controlled temperature (20°C) and pH (7.0 ± 0.1). The pH was maintained by dosing 0.1N HCl and 0.1N NaOH. During aerobic tests compressed air was bubbled through the batch fermenter with a flow rate of 15 L/h. The dissolved oxygen concentration in the batch reactor was always above 50% of the saturation concentration. Mixing was provided throughout the tests at 500 rpm. Following

manual transfer of the sludge from the SBR to the batch reactor, phosphate was instantly added to the sludge (final concentration 100 mgP/L). Aerobic P-uptake was monitored during 30 minutes. It was ensured that at the end of the experiments a surplus of phosphate remains in solution. Sludge used in the batch tests was not returned to the SBR.

During the prolonged cycle about 50% of mixed liquor was used for sampling and batch tests. Therefore the operating volume and consequently all pumping rates were reduced by 50% in the "subsequent standard" SBR cycle. This has been done in order to maintain identical operating conditions in the cycles taking place before and after prolonged cycle. After the experiment the recovery of the system was monitored for a week and the SBR was refilled after that week to full operating volume with collected excess sludge.

In experiment E2 the PHB content of the biomass was increased by doubling the HAC influent concentration in one cycle. The initial anaerobic phosphorus content in the SBR was also increased from 7.5 to 150 mg P/L in order to secure a surplus phosphorus in the solution at the end of the first 7.5 hours of the prolonged aeration period. Then acetate and phosphorus (plus necessary minerals) were added to the SBR for a second time under aerobic conditions in a prolonged cycle, increasing the SBR's acetate and phosphorus concentration by 150 mg C/L and 150 mg P/L respectively. Aerobic conditions were maintained for 15 hours (22.5 h in total). At the end of the experiment 0.90 L of freshly collected excess sludge was added to the SBR to compensate for the amount of mixed liquor used in sampling procedure, and the SBR operation was switched again to standard operation.

An anaerobic-aerobic-settling sequence with prolonged aeration phase was also performed in experiment E3. After the end of the anaerobic phase mixed liquor was centrifuged, sludge was separated from liquid and diluted with synthetic medium without phosphate. This procedure was repeated twice. The length of the aerobic phase was prolonged from 2.25 h to 22.25 h. The phosphorus (100 mg P/L as a final concentration) was added separately to the SBR under aerobic conditions three hours before the end of the phase. During these three hours the phosphate and glycogen concentrations in the reactor were closely monitored. To check whether a lack of important cations limit P uptake in the system, an hour after phosphate was added, potassium and magnesium (15 mg K/L and 30 mg Mg/L as a final concentration) were at the same time added to the SBR as well.

9.2.3. Analyses

The efficiency of the SBR system was monitored on a daily basis by measuring phosphate (as P), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and occasionally total organic carbon (TOC) at the end of the anaerobic and aerobic phase. In experiments E1, E2 and E3 phosphate, HAC, MLSS, MLVSS, glycogen, PHB and poly-hydroxy-valerate (PHV) concentrations were measured according to the

sampling schedule for each experiment. Poly-hydroxy-alkanoate (PHA) was determined as a sum of PHB and PHV. The active biomass was calculated by subtracting the glycogen and PHA from MLVSS concentration. During the aerobic batch tests in experiment E1, the phosphate concentration was measured every 6 minutes and PHB, PHV, MLSS and MLVSS at the start and the end of each experiment. The data acquisition program Biowatch (Applikon b.v. Schiedam) was used to continuously store monitored information of the system (pH, dissolved oxygen concentration and redox potential). Analyses were performed as described in chapter 2. Respirometry was performed during the aeration phase of the experiments. A respirometer was connected to the SBR for the measurement of oxygen consumption of the biomass as a function of time (see also section 2.2).

9.3. Results

Immediately after inoculation on day 0 the system achieved 100% P-removal efficiency. This performance was maintained throughout 200 days of operation with the exception of several cycles after execution of the experiments. On day 112 the first experiment was performed. Analyses showed that the system could be considered in a steady state. The average concentration of MLSS and MLVSS at the end of the aerobic phase was in this period 2.61 and 1.89 g/L, respectively (ratio 0.73). The average P-release at the end of the anaerobic phase throughout the operation of the SBR was 86.1 mgP/L. Nitrification was absent and acetate was always fully utilized anaerobically.

9.3.1. Experiment E1

The dynamic pattern of the parameters monitored during a standard cycle in steady state is presented in figure 9.1a and the biomass composition is shown in table 9.2. The results obtained from the standard cycle are considered as typical for the particular operating system (i.e. Kuba et al., 1993; Smolders et al., 1995). Acetate (75 mgHAc-C/L) was fully consumed anaerobically and full P-removal efficiency was achieved. The oxygen utilization rate showed a characteristic shape with a sharp bend associated with the termination of the phosphorus uptake (similarly as in figure 3.3). The total oxygen consumption by the biomass, as obtained from respirometry, was 81 mgO₂/L (46 mgO₂/gVSS). The pattern and concentrations of all monitored parameters of the normal cycle and the first 4.5 h of the prolonged cycle (2.25 h of the anaerobic phase and the first 2.25 h of the extended aerobic phase) were highly similar (figure 9.1b and table 9.2). After 2.25 h of the prolonged aerobic phase the PHB concentration was already low (30 mg/L) and glycogen content was 395 mg/L. Within the next few hours of the excessive aerobic phase the PHB content depleted to 5 mg/L (2.11 mg COD/g VSS). This seemingly minimum PHB level was maintained until the end of the aerobic phase. From the moment when PHB became very low or even fully utilized (complete P-uptake was already achieved) until the end of the excessive

aeration phase the glycogen consumption equaled 190 mg/L (an overall consumption rate of 3.93 mg glycogen/gVSS.h or 4.66 mg COD/g VSS.h). During the excessive aeration phase the glycogen content was reduced by 50% with respect to its maximum level observed in the prolonged aeration phase (398 mg/L). After 10 h of aeration the oxygen consumption rate stabilized at 4.74 mg O₂/g VSS.h. During the prolonged aerobic phase the total oxygen consumption was 290 mg O₂/L.

Figure 9.1 Concentration of relevant parameters in (a) "standard", (b) "prolonged" and (c) "subsequent standard" SBR cycle of experiment E1.

The results of the aerobic batch tests for the determination of P uptake during the excessive aeration phase showed a negligible or zero uptake rate in all tests. In the subsequent standard cycle (figure 9.1c) the acetate (75 mg HAC-C/L) was fully consumed anaerobically within 90 minutes resulting in a net phosphate release of 79.3 mgP/L (PO₄/HAC ratio of 1.00 mg P/mg HAC-C). The anaerobic and aerobic conversions of glycogen and PHB were balanced. Under aerobic conditions the phosphate present in the solution was only partially taken up by the biomass resulting in only 24% P-removal efficiency. Within a day the BPR had fully recovered. The total oxygen consumption by the biomass was 84 mg O₂/L. The kinetic rates of selected parameters are summarized in table 9.3.

Table 9.2 Biomass composition during "standard", "prolonged" and "subsequent standard" SBR cycle of experiment E1.

Parameter	Unit	"Standard" cycle			"Prolonged" cycle			"Subsequent standard" cycle		
		Start anaerobic phase	Start aerobic phase	End aerobic/ start anaerobic phase	Start aerobic phase	End aerobic/ start anaerobic phase	Start aerobic phase	End aerobic/ start anaerobic phase	Start aerobic phase	End aerobic phase
MLSS	mg MLSS/L	2650	2215	2643	2200	2050	1785	2085		
MLVSS	mg MLVSS/L	1820	1770	1812	1750	1410	1465	1435		
MLVSS/ML		0.69	0.8	0.69	0.8	0.69	0.82	0.69		
Ash	mg ash/L	830	445	831	450	640	320	650		
Poly-P	mg poly-P/L	734	352	736	358	566	243	574		
Glycogen	mg glycogen/L	383	162	388	160	206	84	205		
PHB	mg PHB/L	39	185	35	182	5	156	36		
PHV	mg PHV/L	8	44	7	43	1	29	4		
PHA	mg PHA/L	47	229	42	225	6	185	40		
Act. Biomass	mg active biomass/L	1390	1379	1382	1365	1198	1196	1190		
f _{pp}	g P/g active biomass	0.18	0.09	0.19	0.09	0.17	0.07	0.17		
f _{phb}	g PHB/g active	0.03	0.13	0.03	0.13	~0	0.13	0.03		

Ash=MLSS-MLVSS, Poly-P=(MLSS-MLVSS)-MLVSS*5/95, PHA=PHB+PHV, Active biomass=MLVSS-Glycogen-PHA, f_{pp}=Poly-P*0.35/active biomass, f_{phb}=PHB/active biomass

Table 9.3 Rates* of selected processes measured during anaerobic and aerobic phase of "standard", "prolonged" and "subsequent standard" SBR cycle of experiment E1.

Type of the cycle	"Standard"	"Prolonged"	"Subsequent Standard"
Parameter			
Anaerobic P-release rate [mg P/mg active biomass.h]	0.092	0.093	0.059
Anaerobic HAc-uptake rate [mg HAc-C/mg active biomass.h]	0.086	0.087	0.055
Aerobic P-uptake rate [mg P/mg active biomass.h]	0.075	0.076	0.060

* Initial process rates (first 35 min of the phase)

9.3.2. Experiment E2

Following an instant addition of acetate and phosphate at the beginning of the experiment E2 (table 9.1) the acetate was anaerobically consumed (period I) by the biomass within 90 minutes, resulting in a total phosphorus concentration of 293 mgP/L at the end of the phase (figure 9.2a). The glycogen was utilized (228 mg glycogen/L) for acetate transport and converted to PHB (237 mg PHB/L was formed). Consequently the PHB/active biomass ratio (f_{phb}) increased while the ratio poly-P/active biomass ratio (f_{pp}) decreased (table 9.4).

During the first part of excessive aerobic phase (period II) 75% of the PHB pool was oxidized, glycogen was formed and the phosphate was partially (67%) taken up by the biomass. Consequently the f_{phb} ratio decreased and f_{pp} ratio increased in value and became high and stable at the end of period II (figure 9.2b and table 9.4). At the same point of time the specific phosphate uptake rate (q_{pur}) equaled zero and the MLVSS/MLSS ratio was very low (0.66). At the beginning of period III the acetate and phosphorus concentration increased due to instant addition to the SBR (table 9.1 and figure 9.2a). During aerobic acetate consumption the phosphate was released into solution, PHB was formed and glycogen was utilized. Similar observations have been reported for anoxic P-removing sludge (Kuba et al., 1994).

Figure 9.2 (a) conversion of relevant parameters of BPR during "prolonged" SBR cycle and, (b) dynamics of f_{pp} and f_{phb} fraction and specific phosphorus uptake rate during experiment E2.

Table 9.4 Biomass composition during "standard" SBR cycle of experiment E3.

Parameter	Unit	Start period I	Start period II	Start period III	Start period IV	End period IV
MLSS	mg MLSS/L	2250	1860	2500	2305	2406
MLVSS	mg MLVSS/L	1550	1605	1650	1685	1530
MLVSS/MLSS		0.69	0.86	0.66	0.73	0.64
Ash	mg ash/L	700	255	850	620	876
Poly-P	mg poly-P/L	618	171	763	531	795
Glycogen	mg glycogen/L	302	74	395	243	450
PHB	mg PHB/L	75	312	77	294	36
PHV	mg PHV/L	14	97	15	23	5
PHA	mg PHA/L	89	409	92	317	41
Active biomass	mg active biomass/L	1159	1122	1163	1125	1039
f_{pp}	mg P/mg active biomass	0.187	0.033	0.230	0.165	0.268
f_{phb}	mg PHB/ mg active biomass	0.065	0.278	0.066	0.261	0.035

Ash=MLSS-MLVSS, Poly-P=(MLSS-MLVSS)-MLVSS*5/95, PHA=PHB+PHV, Active biomass=MLVSS-Glycogen-PHA, f_{pp} =Poly-P*0.35/active biomass, f_{phb} =PHB/active biomass

After the acetate was fully taken-up by the biomass an incomplete and very slow phosphorus uptake occurred simultaneously with PHB utilization and glycogen production (period IV). Only 27 % of the available phosphate was taken-up until the end of the experiment. At the end of the prolonged aerobic phase the f_{phb} ratio became close to zero as well as the q_{pur} while the f_{pp} ratio was extremely high (0.268 mg P/mg active biomass).

9.3.3. Experiment E3

The results of experiment E3 are shown in figure 9.3. All parameters measured at the beginning and at the end of the anaerobic phase were very similar to the measurements of the standard anaerobic phase presented in the section 9.3.1. Phosphorus and acetate were absent in the solution after the biomass was washed two times and resuspended in the mineral medium. As a result of the instant phosphate addition under aerobic conditions the phosphate concentration increased from 0 to 103 mgP/L. After one hour the phosphorus concentration remained unchanged while the glycogen content of the biomass only slightly decreased. It was checked whether potassium or magnesium limitation could explain the absence of phosphorus uptake (Brdjanovic et al., 1996). Potassium and magnesium were added in excess amount. After addition no significant phosphorus uptake was observed.

Figure 9.3 Concentration of relevant parameters during "prolonged" SBR cycle of experiment E3.

9.4. Discussion

9.4.1. Excessive Aeration

The patterns and concentrations of all monitored parameters of the standard cycle and of the first part (4.5 h) of the prolonged cycle were almost identical (experiment E1 and presumably in E2). This was expected since the operating conditions were identical. Observed data (figure 9.1 and table 9.2) are in the range reported by Kuba et al., (1993), Smolders et al., (1995b) and Brdjanovic et al., (1997) under similar operational conditions.

The PHB concentration after 2.25 h of the aerobic phase was already relatively low (35.9 mg COD/g VSS). Such relatively low PHB content of the biomass was also regularly observed at the end of the aerobic phase when experiments at different SRT are compared (Smolders et al., 1995c and Kuba et al., 1996). It seems that under normal operational procedures the PHB content of the biomass at the end of the aerobic phase is minimized. Further aeration rapidly depletes the PHB content of the biomass to 2.1 mg COD/g VSS - seemingly the minimum level in the cell. This value corresponds to the minimum PHB content of a mixed culture of 2.6 mg COD/g VSS reported by Temmink et al., (1996). After depletion of the PHB no phosphorus uptake could be observed in separate batch tests, showing the dependance of poly-P formation on PHB conversion. Similar observations were described by Temmink et al., (1996).

In the "subsequent standard" cycle, the acetate was fully consumed anaerobically since glycogen and poly-P remained in the cells after the prolonged aeration period. A comparison of the "standard" and "subsequent standard" cycle indicates two major differences between them, (1) an incomplete phosphorus uptake in the subsequent standard cycle and therefore deterioration in BPR efficiency and, (2) all rates before disturbance were comparatively higher (anaerobic 35% and aerobic 20%) than the rates observed after excessive aeration (table 9.3). These differences are attributed to the impact of the excessive aeration period leading to changed contents of storage polymers. It seems that the anaerobic rates are influenced by the decreased glycogen content. Under aerobic conditions the decreased PHB content could lead to a lower phosphate uptake rate. Temmink et al., (1996) also pointed to a relation between the PHB content of the cells and the phosphate uptake rate. The decreased P-removal is however not only due to a lower uptake rate but also to depletion of PHB during the aerobic phase. After 3.5 h the oxygen uptake rate is at the level of the maintenance oxygen uptake rate, indicating full PHB conversion at that point (experiment E1)

It has been shown that extra aeration compared to the standard SBR operation leads to a quick full depletion of the already relatively low PHB content of the bio-P cells present at the end of the standard aerobic phase. After the system is returned to normal operation the phosphate uptake is strongly affected due to the dependence of phosphate uptake on the PHB content of the cells (full depletion of PHB during the aerobic phase). Since the phosphate release is hardly affected, the net result is a decreased phosphorus removal efficiency after a period of excessive aeration.

9.4.2. Other Factors Affecting Phosphate Uptake Rate

It was shown in experiment E1 that PHB is required for phosphorus uptake. In order to investigate in more detail the relationship between phosphorus uptake and PHB content of the biomass, the PHB level was artificially increased by doubling the acetate influent concentration during one cycle. In experiment E1 it was found that the phosphate uptake stopped due to PHB limitation. However, in experiment E2 (e.g. at the end of the period II) the phosphate uptake stopped in spite of the presence of PHB in the cells. At the same time the f_{pp} ratio became quite high (0.23 mg P/mg active biomass and a VSS/SS ratio of 0.66). This strongly indicates that the aerobic phosphorus uptake is not only dependent on the PHB content of the biomass, but also on the maximum poly-P storage capacity of the cells (see also figure 9.2b). The maximum poly-P content of the cell (f_{pp}^{max}), observed at the end of period IV, was 0.766 mg poly-P/mg active biomass or 0.18 g P/g VSS. This value is in between the values of f_{pp}^{max} observed by Smolders et al., (1996) and Wentzel et al., (1989).

Simultaneous presence of acetate and oxygen results in phosphate release (figure 9.2a). Similar observations were made by Kuba et al., (1994) for the simultaneous presence of substrate and nitrate. Obviously a discrepancy between ATP need for substrate uptake and conversion and ATP generation due to oxidative phosphorylation can under aerobic/anoxic conditions be supplemented by phosphate release. This means that if under certain conditions substrate becomes available in the aerobic phase the phosphate uptake will be decreased leading to increased effluent concentrations. This situation may probably occur during excessive rainfall or weekend conditions.

9.4.3. Maintenance Versus Decay

Another explanation of deterioration of BPR at some WWTP during or after heavy rainfalls or weekends can be formulated as follows. Low loading periods that occur during heavy rainfall and weekends which cause starvation of the microorganisms. The shortage in food supply consequently leads to an overall higher death than growth rate, resulting in a decrease of the net amount of bio-P bacteria present in the system. Once the normal loading rate is reestablished the BPR efficiency deteriorates due to slower and incomplete phosphate uptake caused by wash-out of bio-P bacteria from the installation.

There are two main concepts describing the fate of microorganisms under starvation conditions. In the Activated Sludge Model no.2, (Henze et al., 1995) it is assumed that the cells degenerate and are recycled as substrate under starvation conditions (the decay concept). However, in the metabolic model (Smolders et al., 1995b and Murleitner et al., 1996) it is assumed that the organic substrate (acetate) is used for growth and maintenance processes (the maintenance concept). The results of experiment E1 allow both approaches to be evaluated.

A stable oxygen utilization rate of $5.9 \text{ mgO}_2/\text{gVSS}\cdot\text{h}$ observed in experiment E1 under starvation conditions agrees with the oxygen utilization rate for maintenance of $5.6 \text{ mg O}_2/\text{g VSS}\cdot\text{h}$ and $5.4 \text{ mg O}_2/\text{g VSS}\cdot\text{h}$ observed under similar conditions by Smolders et al., (1994b) and Brdjanovic et al., (1997), respectively. This maintenance rate can adequately predict the relation between SRT and net sludge production (Smolders et al., 1995c). A comparison between the glycogen ($4.66 \text{ mg COD/g VSS}\cdot\text{h}$) and the oxygen utilization rates ($4.74 \text{ mg O}_2/\text{g VSS}\cdot\text{h}$) during the period of PHB limitation, indicates that during the excessive aeration period all consumed glycogen is fully oxidized to CO_2 .

The energy derived from glycogen oxidation is only used for maintenance purposes and not for phosphate uptake nor growth processes. This means that no significant oxygen consumption due to decay processes has been observed and that the oxygen consumption can be mainly attributed for the maintenance purposes. These observations support the application of the maintenance concept for the description of BPR processes.

In order to confirm that glycogen can not substitute PHB as a substrate used for phosphorus uptake, extra phosphate was added to the SBR under starvation conditions (experiment E3) when the biomass PHB content was at its minimum value (2.1 mg COD/g VSS) and when the polyphosphate content was very low ($f_{pp}=0.042 \text{ mg P/mg active biomass}$). The f_{pp} ratio was deliberately decreased in order to eliminate eventual polyphosphate inhibition for the phosphate uptake. According to the results, presented in figure 9.3, phosphate consumption was absent. The glycogen was still consumed by the rate assumably needed for maintenance.

9.4.4. Practical Implications

The results from the laboratory tests showed that excessive aeration can negatively affect BPR. Therefore it is suggested that WWTPs should have an adjustable and flexible aeration system. In order to cope with the events like low COD loading of the plant, the aeration should be controlled. An adequate and flexible control of oxygen input at WWTPs does not only save the energy and keep the operational costs to a minimum, but also (and maybe more important) contributes to the stability of the biological processes. Moreover the overall nitrogen removal could be improved. Under low loading conditions ammonium is fully oxidized and nitrate is accumulated in the system. By reducing the aerated volume nitrate can be reduced by endogenous substrate. Controlling the nitrate level has also the advantage that after a low loading period minimum nitrate content is present in the return sludge of the system. In this case substrate competition between bio-P bacteria and denitrifying bacteria is minimized and the fraction of PHB in the cells is more rapidly increased.

9.5. Conclusions

The results from the laboratory experiments confirmed the hypothesis that excessive aeration (aeration during starvation conditions) of activated sludge can lead to deterioration in BPR efficiency. It was demonstrated that excessive aeration of activated sludge can cause deterioration in BPR efficiency (phosphorus uptake stops) due to gradual depletion of PHB and/or saturation of the biomass by polyphosphate. If COD is added to the system phosphorus release occurs, but the released phosphate can not be taken-up fully again, because the PHB content limits the phosphorus uptake rate. This causal effect can explain the deterioration of BPR efficiency during heavy rainfalls or weekends. Since excessive aeration negatively affects the BPR process, the aeration should be properly controlled at sewage treatment plants.

It was confirmed that the presence of acetate under aerobic conditions provokes phosphate release, which may also contribute to deterioration of the BPR efficiency. The aerobic phosphate uptake was found to depend not only on the PHB but also on the poly-P content of the cells. The maximal poly-P and seemingly minimal PHB content of the cells were observed in the enriched sludge during excessive aeration experiments. Under aerobic starvation conditions glycogen can not replace PHB for phosphate uptake and is only used for maintenance. During this period no oxygen consumption for decay processes has been observed. The latest finding favors the application of maintenance concept versus decay concept for description of BPR processes.

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10 Chapter

Evaluation and Outlook

Contents

10.1. On the Status of Mathematical Modeling of Activated Sludge Systems	231
10.2. On the Status of Assays	232
10.3. On the Boundaries of Model Application	234
10.4 Final Summation	237

Abstract

Biological phosphate removal has been discovered in wastewater treatment plants by accident, and has developed from an interesting observation to an established biotechnological process implemented widely at full-scale. Presently, biological phosphorus removal models are developed sufficiently to be successfully applied for the description of activated sludge processes. The combined COD and N module of the activated sludge model no. 2 (ASM2) and the Delft BPR model proved well capable of describing the performance of a full-scale treatment plant in Haarlem. The next step should be the application of this model using also dynamic plant data, since all simulations described in this thesis were performed with static, steady-state, data. Furthermore, the model capability to predict well start-up conditions should be investigated. This research showed that modeling of wastewater treatment plants does not only bring more experience from practical application of the model, but also provides better understanding of plant operation and treatment processes and gives the opportunity to find out where the model should be improved. Additionally, it was demonstrated that the activated sludge models can be used for process design as well as for the optimization of performance of wastewater treatment installations. Following the rapid development of the activated sludge models, it can be expected that within few years the ASM4 may appear to describe COD, N and P removal for aerobic and anoxic conditions, possibly based on combination of ASM3 and a modified Delft BPR model. At that stage of model development it may be reasonable to let the model be applied and tested for a certain number of years (as was the case with ASM1) prior to any further serious changes in the model take place.

10.1. On the Status of Mathematical Modeling of Activated Sludge Systems

An activated sludge model was used in this thesis to describe removal of organic matter, nitrogen and phosphorus in a full-scale wastewater treatment plant (WWTP) Haarlem Waarderpolder which consists of a conventional anoxic/aerobic activated sludge line, side-stream biochemical phosphorus removal line and sludge treatment line. In this model, an integrated metabolic model for the aerobic and denitrifying biological phosphorus removal from wastewater (Delft BPR model, Murnleitner et al. 1997) replaced the module for phosphorus removal of Activated Sludge Model No.2 (ASM2, Henze et al. 1994), and was combined with the equations for chemical oxygen demand (COD) and nitrogen (N) conversion of the ASM2. In general, this combined activated sludge model proved well capable of predicting accurately the complex conversions of COD, N and P in this full-scale wastewater treatment plant. It is remarkable that performance of the full-scale treatment plant were in general well described for both liquid phase and biomass with the adjustment of only three model parameters (fermentation rate constant, glycogen formation rate constant and percentage of denitrifying activity of phosphorus removing bacteria), although all model parameters of the Delft BPR model were determined using an enriched culture with phosphorus accumulating organisms (PAOs) grown on acetate in a lab-scale sequencing batch reactor. The combined model was validated using batch tests with the activated sludge and showed satisfactory results in describing the anaerobic phosphorus release and aerobic and anoxic phosphorus uptake processes. The model was to a less extend capable of well predicting nitrification, endogenous phosphorus release and especially denitrification in a batch tests. The standardized influent characterization procedure (STOWA, 1996) proved adequate for characterization of the organic, nitrogen and phosphorus fractions in the incoming sewage to the treatment plant in Haarlem.

Despite of the fact that the selected model performed satisfactorily in this study, there is still room for further optimization of the kinetic expressions (for example aerobic glycogen formation) and better integration of the COD, N and P conversions modules in the future. For example, in ASM2 it is assumed that the cells degenerate and are recycled as substrate under starvation conditions (the decay concept). Therefore, decay combined with growth is used in ASM2 to describe the lumped effects of endogenous respiration of e.g. lysis, predation, death, decay, maintenance, motility, storage compounds etc. of the biomass. However, in Delft BPR model the maintenance concept is adopted and is assumed that the organic substrate is used for growth and maintenance processes. In the combined activated sludge model applied on WWTP in Haarlem both concepts were used (decay in COD and N removal part and maintenance in BPR part). Since it is recognized that the decay concept for modeling purposes is less accurate, it should be replaced in ASM2 by maintenance (as in Delft BPR model) or endogenous respiration (as in ASM3, Gujer et al. 1998).

A strong influence of pH on the anaerobic P release process and, thus, on phosphate-acetate ratio was observed at WWTP Haarlem Waarderpolder. Contrary to P release process, the effect of pH on fermentation process, which can also influence the observed phosphate-acetate ratio, is not yet known. Separate activated sludge batch tests using, in one case settled sewage (containing volatile fatty acids: VFAs) and, in another acetate, could bring more information on this matter by comparing acetate (HAc) concentrations at different pH. The Delft metabolic model predicts a variable, pH dependent, phosphate-acetate ratio. However, the present version of simulation package SIMBA® does not allow different pH at different units of the plant. Since pH is clearly an important environmental factor in activated sludge process and may significantly vary along the treatment plant, it is desirable to incorporate the possibility of simultaneous occurrence of different pH values at the treatment plant in the next version of SIMBA®.

In the present Delft BPR model the effect of simultaneous presence of external organic substrates (such as VFAs) and electron acceptor (oxygen or nitrate) is not taken into account. This research showed that simultaneous presence of phosphate and organic substrates under aerobic or anoxic conditions has negative effects on phosphorus uptake process, since carbon sources (in this case acetate) will be primarily used for poly-hydroxy-alkanoates (PHA) formation, resulting in phosphorus release. The phosphorus will be taken up only after the external substrates are exhausted. Although simultaneous presence of VFAs and electron acceptor does not occur in wastewater treatment practice very often, in some cases it may be necessary to include such a situation in the model.

The stoichiometry of the Delft BPR model was developed on acetate as a sole external organic source in the influent. Since the possible presence of substrates other than acetate (like butyrate and propionate) in the plant influent may influence the accuracy of model predictions (due to a different anaerobic stoichiometry), it is recommended to check the model in cases where acetate is not an exclusive substrate to bio-P bacteria. In the case of WWTP Haarlem Waarderpolder the VFAs in the influent consisted almost completely of acetate and, therefore, the acetate stoichiometry was used. To take into account situations in which a large proportion of organic matter in the sewage reaches the treatment plant before acid fermentation is completed, a more refined and complex model is required. Further research is needed to investigate the influence of different type of carbon sources (beside VFAs) present in the plant influent on BPR, including the fermentation processes, the identification of storage polymers (other than PHA) and the metabolic pathways for their formation.

10.2. On the Status of Assays

A crucial role of storage polymers in BPR has been recently widely accepted. Consequently, the storage polymers such as PHA, poly-P and glycogen are included in the latest models

for simulation of activated sludge systems. It has also been recently recognized that PAOs can be considered to consist of two fractions, one of which can denitrify (DPAOs). The scientific interest in the other known microbial population, beside PAOs, capable of anaerobic utilization of organic substrate in BPR processes, i.e. glycogen accumulating non-poly-P organisms (GAOs), increases due to the fact that their interaction with PAOs in BPR systems is still not fully understood. All this urged for a development of innovative sludge characterization methods for determination of: (a) the storage polymers of bio-P bacteria, and (b) the fraction of PAOs, DPAOs and GAOs in BPR sludge.

So far, the chemical method for PHA determination (Smolders et al. 1994, Brdjanovic et al. 1997a) has been proven as very accurate. This method is based on a dry weight basis, which underlines the importance of accurate measurements of mixed liquor suspended solids (MLSS). Calculation of poly-P as the 95% ash content is sufficiently accurate in enriched culture by PAOs. However, in mixed culture systems, it is recommended to calculate the poly-P content of the biomass based on the measurement of the total phosphorus content of bacteria. Although not completely correct from a microbiological point of view, a definition of the active biomass as the difference between MLVSS and the sum of PHA and glycogen (as used in this dissertation) was satisfactory, as far as an enriched culture of PAOs is concerned. For mixed cultures, it is preferred to use mixed liquor volatile suspended solids (MLVSS) to express biomass specific reaction rates. It was shown that the conventional methods for glycogen determination overestimate the glycogen content of the biomass in an enriched bio-P culture (Brdjanovic et al. 1997b). In a case of mixed cultures, where a large fraction of non-PAOs is present, the inaccuracy is expected to be even larger due to non-glycogen-glucose (or cell wall carbohydrates) background. The new glycogen bioassay presented in this dissertation showed its potential when applied in laboratory enriched culture systems, but its validity on full-scale installations still needs to be verified. It must be noted that the method is valid only under glycogen limitation. Recently, Wentzel (personal communication) proposed an additional, rather simple way to test glycogen limitation. The proposal is actually an extension of the bioassay (see figure 4.2). Originally, the anaerobic batch test was terminated when acetate uptake by PAOs stopped. An extension of Wentzel consists of the addition of glucose to the system at that point of the test, and follow-up of the acetate uptake (since Sathasivan et al. 1993 and Liu et al. 1997 reported that a BPR sludge took up glucose and stored it as glycogen accompanied by P-release under anaerobic conditions). If additional acetate uptake and/or phosphate release is observed, the glycogen is indeed a limiting factor for acetate uptake.

The experiments described in this dissertation were performed with both enriched culture, cultivated under laboratory conditions, and mixed culture, obtained from a full-scale treatment plant. While it is assumed that phosphate-removing enriched culture contains virtually only PAOs, the situation is different in a full-scale systems where PAOs make up only a fraction of the total sludge present in the system. The fraction of PAOs in the activated sludge can be roughly determined from the measurement of: (a) the specific

anaerobic P release due to acetate uptake, and (b) the anaerobic P release due to maintenance in absence of external substrates, relative to the reference values obtained from the same tests in which enriched culture was used. Both methods were applied over the course of this research and proved as a good estimation of the fraction of bio-P bacteria in the system.

A bioassay for determination of the fraction of DPAOs in activated sludge (Wachtmeister et al. 1997) was already successfully applied at full-scale WWTPs (Kuba et al. 1997, Van Veldhuizen et al. in press, and Brdjanovic et al. in preparation). Meinhold et al. (1998) recently tested four different modifications of the above mentioned method and concluded that all of them give essentially the same result, indicating that they are sufficiently accurate for further use.

In well operating BPR plants the fraction of GAOs in the sludge is probably negligible. However, it has been reported that GAOs may appear when BPR deteriorates (Cech and Hartman, 1990 & 1993, Liu et al., 1994, Satoh et al., 1994, Matsuo, 1994). For the purpose of process evaluation and mathematical modeling of BPR it is interesting to know the GAOs-PAOs ratio in activated sludge. The method for determination of GAOs-PAOs ratio in activated sludge was proposed (Brdjanovic et al. 1997a, not included in this dissertation), but not tested yet. The method is based on measurement of acetate consumption with and without depletion of poly-P pool in the cells in an anaerobic batch test. Acetate in surplus amount is added to the activated sludge and acetate (or/and glycogen) consumption and phosphate release are frequently measured (say each 15 min over 3-4 hours). It is expected that the poly-P pool of the PAOs will be exhausted due to the acetate uptake. From this moment on, the PAOs will not be able to take-up acetate due to their inability to utilize glycogen as the sole energy source (Brdjanovic et al. in press). This implies that further acetate uptake will occur solely due to presence of GAOs. The ratio GAOs-PAOs can be therefore obtained from the plot of acetate consumption and phosphate release with and without depletion of poly-P in the cell. It is worthwhile trying this method on the activated sludge systems where higher GAOs/PAOs is expected (say >10%) as it may be the case of WWTP Haarlem Waarderpolder (see figure 5.7a). So far the only feasible strategy to obtain stable GAOs enriched cultures is to limit the phosphorus feeding regime to the growth requirement only (Mino, in press). Having a stable GAOs enriched culture could help to clarify some microbial ecology aspects related to the metabolic and physiological characteristics of GAOs, and to the competition between PAOs and GAOs for organic substrates in the anaerobic phase of BPR process. This could be a very interesting subject for further research.

10.3. On the Boundaries of Model Application

Activated sludge models do not yet take into account the extreme situations that can

occasionally take place in wastewater treatment practice. At the present level of development of activated sludge models and their practical application, it is still not necessary to have the model capable of describing plant operation outside the range of normal operating conditions. Nevertheless, from academic, but also from a practical point of view it is needed to better define the mechanisms which take place when the microorganisms experience, for example, extreme sewage temperature, excessive aeration, limitation of internal storage pools (poly-phosphate, glycogen and PHA), lack of potassium in the influent etc. In this research several limiting factors for BPR were explored. The most important findings are given below.

Overall, stoichiometry and kinetics of BPR processes can be considered as well defined, however only for temperature of 20°C. A structured study of the short and long term temperature effects on stoichiometry and kinetics of the anaerobic and aerobic phases of BPR processes performed at temperature of 5, 10, 20 and 30°C showed that the stoichiometry of the anaerobic and aerobic processes was in general insensitive towards temperature changes. In contrast, strong temperature effects on the kinetics of most of the BPR processes were observed. The results of the short and long term experiments showed that the anaerobic process conversion rates have a maximum in the interval between 20 and 25°C, while for the aerobic process rates a continuous increase was observed. Different temperature coefficients derived from short and long term observations strongly suggest that for proper evaluation of temperature dependency of BPR processes long term experiments are needed. The explanation is that different temperature generates different populations. Such a change in population requires at least five sludge retention times (SRTs) in time. Since mathematical models for simulation of activated sludge systems rely on stoichiometric and kinetic coefficients valid in a narrow temperature range or a single temperature value of 20°C, it is recommended to incorporate the temperature coefficients derived from long term experiments into these models. The observed discrepancy between the temperature coefficients obtained in short and long term tests occurs likely due to (a) change in a level of storage polymers at different temperature during long term tests and (b) shift in a population structure. The application of molecular ecological techniques such as dry denaturing gradient gel (DDGGE) demonstrated that the bacterial population in long term temperature experiments indeed shifted with temperature. Furthermore, both electron microscopy and DDGGE showed that even under very selective conditions there was more than one type of organism present in an enriched culture used in long term tests. This strongly suggests that PAOs do not consist of one single dominant bacterium but consist of several different bacterial groups. No definite conclusions can be made yet concerning the microbial community structure of the BPR process. Although, a DDGGE has been proven to be a promising method for the study of microbial population dynamics, it is not quantitative, and therefore, further work in this direction is required. Cloning of polymerase chain reaction (PCR) fragments from 16S rDNA, their identification and sequencing, and application of the fluorescent in-situ hybridization (FISH) technique may at least show to which taxonomical group the PAOs belong, and will probably even lead to their

identification. So far, no single pure cultures have ever been proven to be one of the predominant microorganisms in the BPR process (Mino et al. in press), thus isolation and identification a pure PAO culture is still an interesting challenge worth undertaking up. Among other benefits, a pure bio-P culture could be used to determine temperature coefficients for BPR processes in the experiments without change in bacterial population structure. An ecological aspect (the interspecies relations) between PAOs and other microbial populations may be important to the work related to isolation of PAOs and should be, therefore, further studied. However, from the modeling aspects, identification of the exact organisms responsible for BPR is of minor importance. Although isolation of PAOs may provide information valuable for modeling purposes, the activated sludge models are not based on the behavior of the specific microorganisms, but rather on the observed behavior of groups of organisms identified by their function. The research has shown that under dynamic process conditions bacteria show a complex ecophysiological behavior, which is difficult to observe in standard pure-culture work. Although the physiology has been quite well established, there is still a need to isolate the responsible organisms in a pure culture. For this purpose innovative enrichment and isolation techniques need to be developed. Traditional techniques are usually rather static, which is probably the reason why the phosphate removing organisms have not been cultured yet. Obviously only under dynamic enrichment conditions (similar as those experienced by the organisms in the treatment plant) it will be possible to enrich bacteria relying on these conditions for their competitive advantage. A better study of microbial competition in the real (continuously changing) environment, rather than in steady-state pure cultures, might reveal a range of new and interesting capacities of new microorganisms. These are most likely not only of academic interest, but can lead to new biotechnological processes.

Furthermore in this research, an important role of storage polymers in BPR was proven and fully established. It was demonstrated that in BPR processes extreme concentrations (either maximal, when saturation conditions are reached, or minimal, where full depletion of storage materials related to BPR is achieved) clearly negatively affect the process performance and efficiency.

In activated sludge systems designed for COD and N removal the SRT is directly linked to the growth rate of the bacteria; the minimal SRT corresponds to the maximal growth rate ($SRT_{min} = 1/\mu_{max}$). It is striking that in the BPR systems the SRT_{min} is not related to μ_{max} but primarily depends on the PHA conversion kinetics, the maximal PHA content of the cell, and number of process and operational parameters. This later discovery is not only important for bio-P bacteria, but also for other microorganisms who are capable of accumulation of PHA. Therefore, the competition phenomena in organisms who have storage polymers (which is quite different in comparison with "normal" organisms who do not have storage pools, but compete for extracellular substrate) should be further investigated.

10.4. Final Summation

Biological phosphate removal has been discovered in wastewater treatment plants by accident, and has developed from an interesting observation to an established biotechnological process implemented widely at full-scale. Presently, biological phosphorus removal models are developed enough to be successfully applied for the description of activated sludge processes. The combined ASM2 and Delft BPR model proved well capable of describing performance of a full-scale treatment plant in Haarlem. The next step should be the application of this model using also dynamic plant data, since all simulations described in this thesis were performed with static, steady-state, data. Furthermore, the model's capability to predict start-up conditions should be investigated. This research showed that modeling and simulation of wastewater treatment plants does not only bring more experience from practical application of the model, but also provides better understanding of plant operation and treatment processes and gives the opportunity to find out where the model should be improved. Additionally, it was demonstrated that the activated sludge models can be used for process design as well as for the optimization of performance of wastewater treatment installations. Following the rapid development of the activated sludge models, it can be expected that within few years the ASM4 may appear to describe COD, N and P removal for aerobic and anoxic conditions, possibly based on combination of ASM3 and a modified Delft BPR model. At that stage of model development it may be reasonable to let the model be applied and tested for a certain number of years (as it was the case with ASM1, Henze et al. 1987) prior to any further serious changes in the model take place.

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Samenvatting

Proefschrift

Modeleren van Biologische Fosfaatverwijdering in Actieve Slib Systemen

van

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Biologische fosfaatverwijdering (BPR) uit afvalwater is een gevestigd proces geworden in de dagelijkse afvalwater zuivering. Ondanks de brede kennis en praktische ervaringen die zijn opgebouwd in de laatste twee decennia, zijn er nog steeds bepaalde aspecten van BPR die nader onderzoek vereisen. Deze thesis behandelt geselecteerde microbiologische, biochemische, procestechnologische, en modelmatige aspecten; het onderzoek was zo gestructureerd dat de hiaten in operationele en fundamentele kennis van kinetisch modelleren van BPR werden opgevuld.

Momenteel is er een sterke behoefte aan het gebruik van mathematische modellen voor het simuleren van COD-, N- en P-verwijdering op bestaande rioolwaterzuiveringsinstallaties (RWZI). Hiervoor is de 'Delftse BPR model' ontwikkeld die de bestaande ASM2 model voor P verwijdering vervangt. Dit gecombineerde model werd gebruikt om een van de meest complexe zuiveringsinstallaties van Nederland, de rioolwaterzuiveringsinstallatie "Haarlem Waarderpolder" te simuleren met als doel na te gaan hoe goed het model in staat is de concentraties van de diverse componenten in de vloeibare fase als ook de biomassa te voorspellen. Dit vereiste een juiste influent en actief slib karakterisering en dus een intensief influent monsternamprogramma en de uitvoering van een serie biologische batch testen. Gelijktijdig moest een nieuwe bioassay worden ontwikkeld om het glycogeen

gehalte van bio-P bacteriën te bepalen, omdat de huidige methodieken zeer omslachtig waren en onnauwkeurig. Omdat BPR processen toegepast worden in koude en warme seizoenen en klimaten, of omdat ze geconfronteerd kunnen worden met temperatuurschokken ten gevolge van industriële lozingen, werd besloten om de korte- en langere-termijn temperatuureffecten te bestuderen die van invloed zijn op de stoichiometrische en kinetische parameters van de anaerobe en aerobe fase van het BPR proces.

Tevens werden kritische, maar tegelijkertijd minder goed begrepen kinetische aspecten van BPR, die gerelateerd waren aan het effect van extreme omstandigheden op BPR (zoals onder andere de minimale slibleeftijd, kalium tekorten in afvalwater, het gedrag van BPR onder poly-fosfaat, poly-hydroxy-alkanoate, of glycogeen tekort, en excessieve beluchting in BPR systemen) onderzocht. Een samenvatting van deze onderzoeksonderwerpen volgt hierna.

Hoofdstuk 1

Biologische Fosfaat Verwijderings (BPR) Processen

Biologische fosfaatverwijdering is een betrouwbaar en goed begrepen proces geworden in rioolwaterzuivering. Dit hoofdstuk beschrijft de historische ontwikkeling van het proces en de meest belangrijke microbiologische en procestechnische, als ook modelmatige aspecten. Vanuit microbiologisch standpunt bekeken, zijn de rol van poly-hydroxy-alkanoate (PHA) als reserve materiaal in een dynamisch proces, en het gebruik van poly-fosfaat (poly-P) als energiereserve de meest belangrijke aspecten. Vanuit de procestechnologie toont dit onderzoek aan dat zeer gecompliceerde biologische processen adequaat kunnen worden ontworpen en beheerst, wanneer voldoende kennis van de microbiologische processen ontwikkeld is. Voor wat het modelleren betreft, zijn er twee verschillende methoden om het proces van biologische fosfaatverwijdering te simuleren: de ene is gebaseerd op het metabolisme van de bacteriën (het 'Delftse model'), terwijl het andere gebaseerd is op een 'black box' benadering (ASM2). Op dit moment zijn de modellen voor het beschrijven van biologische fosfaatverwijdering voldoende ontwikkeld om te worden toegepast in het beschrijven van actiefslibprocessen. De combinatie van ASM2 en het 'Delftse model' lijkt voor het beschrijven van aerobe en anoxische BPR en het ermee simuleren van actiefslibsystemen veelbelovend.

Hoofdstuk 2

Temperatuureffecten op de Fysiologie van BPR

In een anaëroob-aëroob alternerende batch reactor, gevoed met een acetaat-oplossing, werd een fosfaatverwijderende biomassa gekweekt. In afzonderlijke batch reactoren werd de conversie van de componenten relevant voor het proces

van de biologische fosfaatverwijdering bestudeerd en dit bij verschillende omgevingstemperaturen, met name bij 5, 10, 20 en 30°C. Deze testen worden ondergebracht onder de benaming 'korte-termijn temperatuureffecten'. De stoichiometrie van het anaërobe proces bleek ongevoelig voor temperatuurveranderingen, terwijl een lichte invloed op de stoichiometrie van het aërobe proces werd waargenomen. De temperatuur had daarentegen een belangrijke invloed op de kinetiek van het proces en dit zowel onder anaërobe als aërobe omstandigheden. De anaërobe fosfaat-vrijstellings-snelheid (of de azijnzuuropnamesnelheid) vertoonde een maximum bij 20°C. Voor de conversiesnelheden onder aërobe omstandigheden daarentegen werd een continue stijging waargenomen. De temperatuurcoëfficiënten voor de verschillende reacties werden op basis van de uitgevoerde experimenten bepaald. Een globale anaërobe en aërobe temperatuurcoëfficiënt werd verkregen, met als respectievelijke waarden 1.078 en 1.057 (geldig in de temperatuurbereiken 5 tot 20°C en 5 tot 30°C).

Hoofdstuk 3

Invloed van de Temperatuur op BPR: Proces en Moleculair Ecologische Studie

Dit hoofdstuk beschrijft de invloed van lange-termijn temperatuureffecten op de stoichiometrie en de kinetiek van de anërobe en aërobe fasen van het BPR proces. De conversie van de relevante componenten onder stationaire toestand werd bestudeerd in een reactor en dit achtereenvolgens bij 20, 30, 20, 10 en 5°C. Voor deze studie werden tevens twee methoden (electronen-microscopie en droge denaturerende gel gradient electroforese) gebruikt om de samenstelling te bestuderen van de bacteriële populatie bij de verschillende temperaturen. De temperatuurcoëfficiënten die werden verkregen in deze lange-termijn temperatuurexperimenten waren gelijkaardig aan deze verkregen in de korte-termijn temperatuurexperimenten ($=1.085$ en $=1.078$ respectievelijk). De temperatuur had een gematigde invloed op de aërobe fosfaatopnamesnelheid (1.031) gedurende de lange termijn-experimenten. Er werd daarentegen wel een sterke temperatuurinvloed geobserveerd op de overige metabole processen van de aërobe fase, zoals met name de PHA-opname ($=1.163$), de zuurstofopname ($=1.090$) en de groei (> 1.110). De temperatuurcoëfficiënten voor de aërobe fase verkregen uit lange- en korte- termijnexperimenten bleken verschillend, waarschijnlijk te wijten aan een veranderde microbiële populatie. Deze verandering werd bevestigd door de moleculair ecologische studies. Het feit dat in de aërobe fase een andere temperatuurcoëfficiënt werd verkregen voor fosfaat-opname in vergelijking tot de andere metabole processen, geeft aan dat het gevaarlijk is conclusies te trekken uit eenvoudig waarneembare parameters (zoals fosfaat) alleen. Zulke onderstellingen kunnen snel aanleiding geven tot een onderschatting van de temperatuurafhankelijkheid van andere metabole processen van de aërobe fase van het BPR proces.

Hoofdstuk 4

Bioassay voor Glycogeenbepaling in BPR Processen.

Glycogeen blijkt een belangrijke rol te spelen in biologische fosfaatverwijdering uit afvalwater. Bestaande meettechnieken overschatten dikwijls de glycogeenconcentratie van de biomassa, en dit door de aanwezigheid van glucose en/of andere koolwaterstoffen dan glycogeen in het celmateriaal. Als een alternatieve methode ten opzichte van de conventionele meettechnieken werd een bioassay ontwikkeld voor de glycogeenbepaling in biologische fosfaatverwijderingsprocessen. De techniek is gebaseerd op een strikt stoichiometrische koppeling tussen anaërobe acetaatopname en glycogeenverbruik. Met andere woorden, de glycogeenconcentratie van het slib wordt indirect bepaald uit de maximale acetaat-opname van de biomassa tijdens een anaërobe test. De techniek werd met succes uitgetest voor de glycogeenbepaling van slib afkomstig uit een laboschaal, acetaatgevoede, anaërobe aërobe alternerende batch reactor bedreven bij een pH van 7.00 ± 0.01 en een temperatuur van 20°C . Deze glycogeen bepalingstechniek vereist dat glycogeen, en dus niet polyfosfaat, de limiterende factor is voor de anërobe acetaatopname. Een methode om deze onderstelling te controleren gebaseerd op het effect van pH op de fosfaat/acetaat verhouding wordt voorgesteld en gebruikt. De techniek is eenvoudig en geeft een directe meting van de glycogeenconcentratie van bio-P organismen, maar haar betrouwbaarheid moet evenwel nog geverifieerd worden voor biologische fosfaatverwijderende installaties in praktijk

Hoofdstuk 5

Modeleren COD, N and P verwijdering in de RWZI "Haarlem Waarderpolder"

Een geïntegreerd actief slib model was gebruikt om de verwijdering van organische materie, nitrogen en fosfaat te beschrijven in de RWZI 'Haarlem Waarderpolder' in Nederland. De waterzuiveringsinstallatie bevat een conventioneel anaërobe-aërobe actieve slib lijn, gecombineerde biochemische fosfaat side-stream verwijderingslijn, en een slibbehandelingslijn. In dit geïntegreerde model, het 'Delft model' voor biologische fosfaat verwijdering (BPR) vervangt de module voor P verwijdering van Activated Sludge Model Nr. 2 (ASM2), en is gecombineerd met de veigelijkingen voor COD en N conversie van de ASM2. Met het bijstellen van alleen drie modelparameters (fermentatiesnelheid constant, glycogeen-formatie-snelheid constant en het percentage denitrificatie activiteit van bio-P bacterieën). Bleek het model heel goed de werking van de waterzuiveringsinstallatie te kunnen beschrijven voor zowel de vloeibare fase als de biomassa. Het model is gevalideerd gebruik makend van batch experimenten met actief slib en liet bevredigende resultaten zien bij de

beschrijving van anaërobe P verwijdering en aerobe en anoxic P-opname processen. Het model was minder geschikt om een goede nitrificatie te voorspellen, endogene fosfaatafgifte en vooral denitrificatie processen. Zowel de model simulatie als het resultaat van het voorbeeldprogramma suggereerden dat de acetaatdosering naar het systeem zal kunnen worden verminderd tot 30%. Het is ook aangetoond dat de pH sterk de geobserveerde phosphate-acetaat ratio beïnvloedt als ook mogelijke fermentatiesnelheid in de side-stream BPR proces. Simulatie van drie alternatieve zuiveringsinstallaties heeft aangetoond dat: (1) kwaliteit rioolwater zuivering zal sterk achteruitgaan als het side-stream proces wordt overgeslagen en de rest van de waterzuiveringsinstallatie zal worden behouden in zijn originele conditie, (2) goede waterkwaliteit zal worden bereikt in het main-stream proces (aangepast UCT systeem) waar een extra anaëroob bassin is toegevoegd aan het begin van de actieve slib eenheid, alhoewel, de verlangde acetaat dosering zeven maal zal hoger zijn in vergelijking met de huidige side-stream configuratie.

Hoofdstuk 6

De Minimale Aerobe SRT in een BPR Systeem

In dit hoofdstuk wordt een methode beschreven waarmee de minimale aerobe slibverblijftijd (SRT_{min}^{aer}) in een BPR systeem kan worden bepaald. In tegenstelling tot normale biologische omzettingen is het BPR proces niet gelimiteerd door een SRT_{min} , die volgt uit de maximale groeisnelheid van de biomassa. In het BPR proces moet de aerobe SRT lang genoeg zijn om het in de cel aanwezige PHB te kunnen oxyderen. Dit zorgt er voor dat SRT_{min}^{aer} voornamelijk wordt bepaald door de PHB omzettingssnelheid en PHB fractie in de cel. Hiernaast wordt de SRT_{min}^{aer} bepaald door de temperatuur (er is een vergelijkbare temperatuurafhankelijkheid met die van nitrificeerders) en de procesconfiguratie waaronder wordt gewerkt. Het is de verwachting dat onder denitrificerende omstandigheden de SRT 35% langer zal zijn in vergelijking met aerobe omstandigheden. Er wordt in dit hoofdstuk een model ontwikkeld die de minimale SRT voorspelt als functie van de eerder genoemde kinetische en procesparameters. Hiermee zijn verschillende operationele aspecten van BPR onderzocht. Het is goed mogelijk met het model de experimentele data te beschrijven.

Hoofdstuk 7

Het Effect van een Kalium Limitatie op BPR

Het is mogelijk dat een full-scale rioolwaterzuivering ontworpen voor de verwijdering van fosfaat, voor lange of kortere tijd een tekort aan kalium in het

influent te verduren krijgt. Met gebruik van een anaeroob-aeroob SBR systeem is inoculatie slib uit laboratorium, pilot en full-scale P-verwijderende systemen blootgesteld aan verschillende kalium-fosfaatverhoudingen in het influent. Door deze condities te simuleren kan worden aangetoond dat de kalium concentratie een belangrijke factor is voor BPR. Bij een tekort aan kalium in het influent was (a) de fosfaatverwijdering nul, (b) nam de poly-p concentratie in het systeem exponentieel af door spui van slib en (c) werd en pas na enkele dagen de anaerobe fosfaatafgifte en de acetaatopname beïnvloed. Dit laatste komt waarschijnlijk doordat pas na enkele dagen de fractie poly-p in de biomassa limiterend wordt voor de opname van acetaat. Bij aanwezigheid van kalium (1.25 en 2.5 gK/gP) in het influent was het systeem in staat te herstellen waarbij de initiële P-verwijderende capaciteit werd herwonnen.

Hoofdstuk 8

Het Effect van Poly-P Limitatie op het Anaërobe Metabolisme van Bio-P Bacteriën

Er zijn twee microbiële populaties die onder anaerobe omstandigheden organisch substraat kunnen benutten: de fosfaat accumulerende organismen (PAO's) en glycogeen ophopende non-poly-P organismen (GAO's). In deze experimenten is met behulp van een SBR en een PAO verrijkte cultuur de anaerobe opname van substraat gevolgd onder omstandigheden waarbij er een tekort is aan poly-p en een overschot van glycogeen in de cellen. Er kan worden aangetoond dat onder deze omstandigheden er bijna geen acetaat wordt opgenomen. Hieruit kan worden afgeleid dat de dominante microbiële populatie in het slib bestaat uit PAO's en dat de aanwezigheid van GAO's marginaal is. Verder wijst dit er op dat PAO's niet in staat zijn de omzetting van glycogeen naar PHB als enige energiebron te gebruiken onder anaerobe omstandigheden. Dit in tegenstelling tot GAO's die hier wel toe instaat zijn. Om deze experimentele waarnemingen te kunnen verklaren wordt een metabolisme voor de anaerobe acetaatopname beschreven. Ook is een batch-test ontwikkeld om de verhouding tussen de populatie GAO/PAO in slib te bepalen. Deze test is gebaseerd op metingen van de acetaatopname waarbij poly-P is in uitgeput en glycogeen in overmaat in de cel aanwezig is.

Hoofdstuk 9

Effecten van Uitbundige Beluchting op BPR

De literatuur is beschrijft dat de BPR rendementen van sommige rioolwater-zuiveringsinstallaties regelmatig verslechteren na zware regenval of tijdens weekends. Deze verslechtering wordt toegeschreven aan de verminderde organische belasting die plaats vindt tijdens deze gebeurtenissen. Echter, in dit onderzoek wordt verondersteld dat de oorzaak van deze verslechtering de uitbundige beluchting is die plaats vindt op sommige van bovengenoemde

installaties ten gevolge van onjuiste afstelling van de beluchtingscapaciteit ten tijde van zware regenval en/of weekends.

Om deze hypothese te bewijzen is het effect van uitbundige beluchting (beluchting ten tijde van endogene afsterving) op de BPR processen bestudeerd met behulp van een laboratorium opstelling van een SBR systeem. Het werd overduidelijk aangetoond dat de fosfaat opname stopt ten gevolge van een graduele vermindering van PHB in een overbelucht proces. Wanneer organisch materiaal wordt toegevoegd aan het systeem komt de fosfaat afgifte onmiddellijk terug tot haar maximale omzettingssnelheid. Echter, het vrijgekomen fosfaat kan niet volledig worden opgenomen omdat de PHB concentratie deze opname belemmert. Daarom leidt onvolledige fosfaat opname tot een tijdelijke reductie van de BPR efficiëntie. Dit causale effect kan de verslechtering van de BPR efficiëntie verklaren dat optreedt na zware regenval of weekends.

Daar uitbundige beluchting zo duidelijk negatief de BPR processen beïnvloedt, moet de beluchtingscapaciteit op rioolwaterzuiveringsinstallaties beter worden afgesteld (in dit geval: verminderd).

Enkele andere bevindingen van dit onderzoek die het vermelden waard zijn zijn: 1) een bevestiging was verkregen van het feit dat de aanwezigheid van acetaat onder aërobe omstandigheden de fosfaat afgifte opwekt. Dit kan ook bijdragen aan de vermindering van de BPR efficiëntie, 2) de aërobe fosfaatopname blijkt niet afhankelijk te zijn van de PHB, maar van de poly-P concentratie in de cellen, 3) het is aangetoond dat onder omstandigheden van aërobe afsterving, glycogeen het PHB niet kan vervangen voor fosfaat opname maar dat het slechts gebruikt wordt voor onderhoud. Gedurende deze periode werd er geen zuurstof verbruikt waargenomen ten gevolge van afbraak processen.

Hoofdstuk 10

Evaluatie en Overzicht

Biologische fosfaatverwijdering is bij toeval ontdekt in RWZI en heeft zich ontwikkeld van een interessante waarneming tot een wetenschappelijk aanvaard biotechnologisch proces dat algemeen op grote schaal wordt toegepast. Momenteel zijn BPR modellen voldoende ontwikkeld om met succes te worden toegepast voor de beschrijving van actieve slib processen. De gecombineerde COD en N module van het actieve slib model nr. 2(ASM2) en het 'Delft BPR model' bleek heel goed op grote schaal de werking van de afvalwaterzuivings installatie Waarderpolder in Haarlem te kunnen beschrijven. De volgende stap zal de toepassing van dit model moeten zijn hierbij gebruik makend van dynamische zuiveringsinstallatie gegevens, omdat alle simulaties die in dit proefschrift worden beschreven zijn uitgevoerd met

vaststaande gegevens. Verder zou het vermogen van het model dat de opstart condities voorspelt, moeten worden onderzocht. Dit onderzoek heeft aangetoond dat modellering en simulatie van afvalwaterzuiveringsinstallaties niet alleen meer ervaring met zich meebrengt door de praktische toepassing van het model maar ook een beter beeld geeft van de zuiveringsinstallatie en de behandelingsprocessen en geeft aan waar het model zou moeten worden verbeterd. Bovendien, is aangetoond dat de actieve slib modellen kunnen worden gebruikt voor proces ontwerp en tevens voor de optimalisering van de uitvoering van RWZI. De snelle ontwikkeling volgend van de actieve slib modellen, mag worden aangenomen dat in een paar jaar tijd de ASM4, COD, N en P verwijdering kan beschrijven voor aerobe en anaerobe omstandigheden, waarschijnlijk gebaseerd op een combinatie van ASM3 en een aangepast Delft BPR model. Op dat punt van model ontwikkeling zou het redelijk zijn om het model toe te passen en het een paar jaar lang te testen (zoals bij ASM1) voordat er serieuze andere veranderingen in het model zouden plaats vinden.

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Curriculum Vitae

Damir Brdjanovic was born in Sarajevo, Bosnia and Herzegovina on October 11th, 1962. He graduated in 1988 as a Civil Engineer from the Faculty of Civil Engineering, University of Sarajevo, and received the master's degree in Pollution and Environmental Control in 1993 at School of Biological Sciences, University of Manchester. For outstanding academic achievement during his studying Damir was awarded a numerous prizes at both an national and international level. Brdjanovic was also granted awards of high academic and professional value, e.g. The Soros Foundation Scholarship and The World Bank Scholarship.

Brdjanovic started his professional career in water industry in 1988 with Unioninvest in Sarajevo, where he was responsible for the design of water treatment plants and water supply systems. In 1990 he joined the Institute for Water Resources Development in Sarajevo and managed process design on a variety of wastewater treatment projects. Brdjanovic's international career started in 1993 with North West Water Engineering in Risley as a wastewater process engineer working on large wastewater treatment projects in United Kingdom and the Czech Republic. In the 1997 Brdjanovic earned a summer internship with The World Bank, Washington D.C. where he managed the prognostic study of water supply and sanitation in East India. From 1994 until beginning of 1998 Damir worked as Ph.D. researcher at the International Institute for Infrastructural, Hydraulics and Environmental Engineering (IHE) and the Faculty of Chemical Technology and Materials Science of Delft University of Technology (TUD). After completing his Ph.D research he worked shortly as lecturer at IHE.

The aim of the International Institute for Infrastructural, Hydraulic and Environmental Engineering, IHE Delft, is the development and transfer of scientific knowledge and technological know-how in the fields of transport, water and the environment.

Therefore, IHE organizes regular 12 and 18 month postgraduate courses which lead to a Masters Degree. IHE also has a PhD-programme based on research, which can be executed partly in the home country. Moreover, IHE organizes short tailor-made and regular non-degree courses in The Netherlands as well as abroad, and takes part in projects in various countries to develop local educational training and research facilities.

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