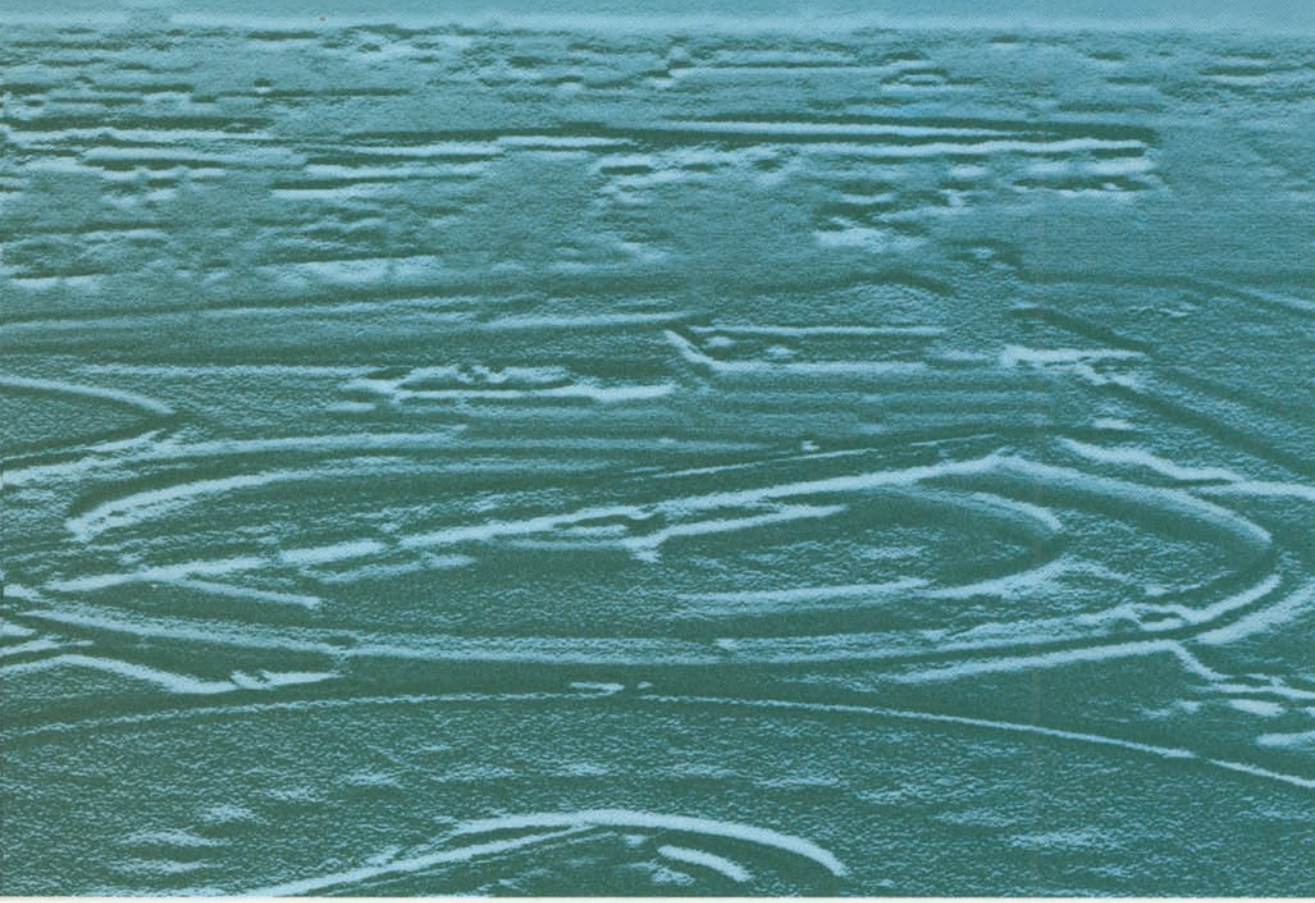
Damir Brđanović

Modeling Biological Phosphorus Removal in Activated Sludge Systems



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MODELING BIOLOGICAL PHOSPHORUS REMOVAL IN ACTIVATED SLUDGE SYSTEMS

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, 9 September 1998 at 10:30 h

by

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CRC Press
Taylor & Francis Group
Boca Raton London New York

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Dr ir M.C.M. van Loosdrecht of Delft University of Technology and Dr ir C.M.H. Hooijmans of International Institute for Infrastructural Hydraulic and Environmental Engineering had as mentors invaluable contribution to this research.

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This research was sponsored by the Dutch Foundation for Applied Water Research (STOWA) and The World Bank in co-operation with the Government of Japan (JJ/WBGSP).

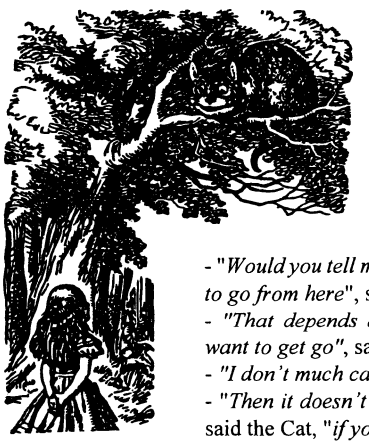
The project was carried out at the Department of Environmental Engineering, International Institute for Infrastructural Hydraulic and Environmental Engineering in Delft, and the Department of Biochemical Engineering, Delft University of Technology, Netherlands.

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

Published by:
CRC Press/Balkema
P.O. Box 447, 2300 AK Leiden, The Netherlands
e-mail: Pub.NL@taylorandfrancis.com
www.crcpress.com – www.taylorandfrancis.com

ISBN 13: 978-90-5410-415-5 (pbk)

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*".

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Contents

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	Embden-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_1	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}^{aer}	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^{aer}	duration of the aerobic phase	h
$Y_{i,j}$	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 $5°C \leq T \leq 20°C$ and $5°C \leq T \leq 30°C$), 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 (θ=1.085 and θ=1.078, respectively).Temperature had a moderate impact on the aerobic P-uptake process rate (θ =1.031) during long-term tests. However, a strong temperature effect on other metabolic processes of the aerobic phase, such as PHA consumption (θ=1.163), oxygen uptake (θ =1.090) and growth (θ>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 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.

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.

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Chapter 1

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).

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Contents

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.

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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 where 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 where 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 where 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* where 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 where 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 where 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).

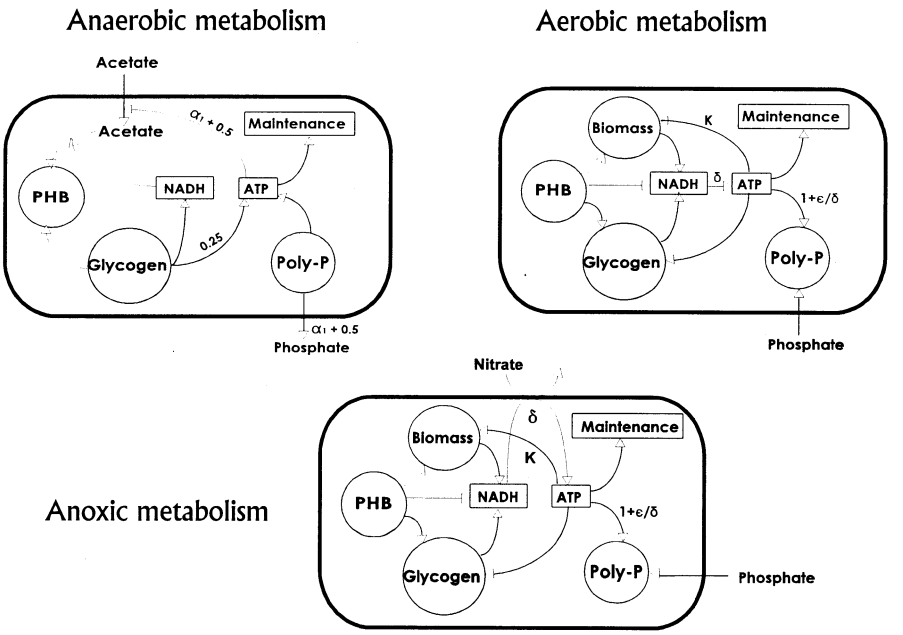

Figure 1.1 Metabolic processes of organisms involved in biological phosphorus removal.

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