1999-08_modellering-biologische-fosfaatverwijdering

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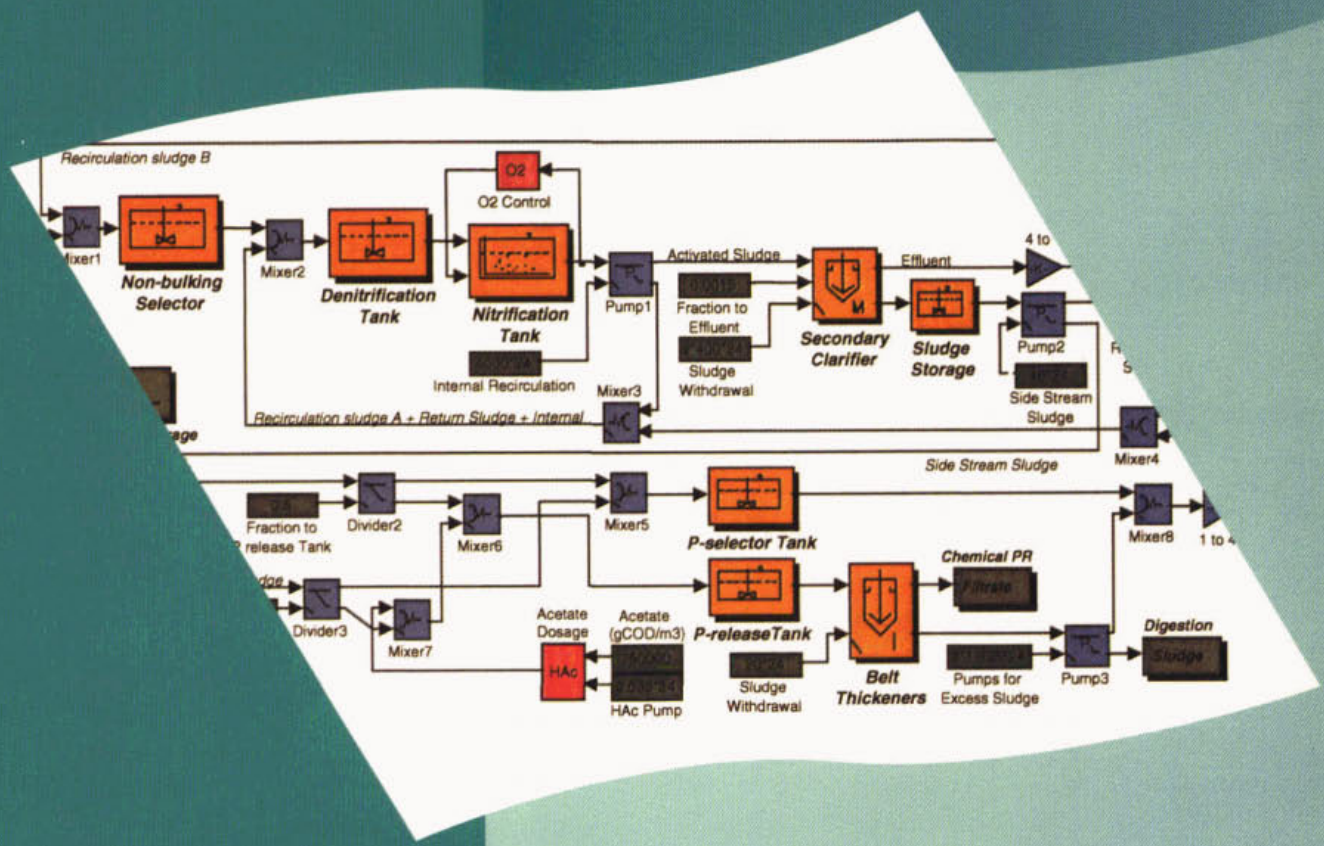
Modellering van biologische fosfaatverwijdering in actiefslibsystemen



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Modellering van biologische fosfaatverwijdering in actiefslibsystemen

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Publicaties en het publicatie-overzicht van de STOWA kunt u uitsluitend bestellen bij:
Hageman Verpakkers BV
Postbus 281
2700 AC Zoetermeer
tel. 079 - 361 11 88
fax 079 - 361 39 27
o.v.v. ISBN- of bestelnummer en een duidelijk afleveradres.
ISBN 90.5773.057.x

Ten geleide

Op 9 september 1998 promoveerde ir. Damir Brdanovic aan de Technische Universiteit Delft op het proefschrift "Modeling Biological Phosphorus Removal in Activated Sludge Systems". In dit wetenschappelijke werk werden geselecteerde microbiologische, biochemische, procestechnologische en modelmatige aspecten behandeld.

Het onderzoek was daarbij zo gestructureerd dat de hiaten in operationele en fundamentele kennis van kinetisch modelleren van de biologische fosfaatverwijdering in het IAWQ actiefslibmodel 2 konden worden opgevuld. Het verkregen model is getoetst aan de werking van de rwzi Haarlem-Waarderpolder.

Met het onderhavige rapport wordt een verkorte en op de praktijk gerichte versie aangeboden van voornoemde dissertatie. Het rapport is in het Engels gesteld en voorzien van een uitgebreide Nederlandse samenvatting.

Utrecht, juli 1999

De directeur van de STOWA

ir. J.M.J. Leenen

Contents

Samenvatting

Modelleren van biologische fosfaatverwijdering in actief slib systemen

Biologische fosfaatverwijdering is een standaardproceswijze geworden voor huishoudelijke afvalwaterzuivering. De complexiteit van zowel de onderliggende microbiologische processen alswel de procesuitvoering zelf (met anaërobe, anoxische en aërobe compartimenten) maakt het niet eenvoudig om het proces te doorgronden. Mathematische modellen gebaseerd op de basale microbiële fysiologie maken het mogelijk om meer inzicht in de biologische defosfatering te verwerven en processen te optimaliseren.

In het verleden is, mede op initiatief van STOWA, een biologisch model opgesteld wat de fosfaatverwijdering in ophopingcultures van defosfaterende micro-organismen beschrijft. Dit 'delftse bio-P' mode is een alternatief voor het door de IAWQ voorgestelde ASM no.2. Beide zijn in staat om processen voor biologische P-eliminatie te beschrijven. ASM no.2 is echter slecht voor een deel gebaseerd op de onderliggende biologische processen. In het 'delftse bio-P' model worden deze expliciet meegenomen. Het aantal benodigde parameters in het model wordt hierdoor gereduceerd en de gebruikswaarde verhoogd. In dit onderzoek is met name aandacht gegeven aan het gebruik van het eerder opgestelde model voor het beschrijven van praktijk zuiveringsprocessen.

Het onderzoek heeft geresulteerd in een proefschrift en een aantal wetenschappelijke artikelen. Deze staan vermeld op pagina 119 en zijn verkrijgbaar via Prof.dr.ir. M.C.M. van Loosdrecht van de TU-Delft. In het proefschrift en de artikelen staat het onderzoek uitgebreid beschreven. Dit rapport behandelt de voor de praktijk relevante aspecten van het onderzoek.

Het onderzoek is deels op het lab, middels ophopingcultures van bio-P organismen, en deels op de rwzi Haarlem Waarderpolder uitgevoerd.

FUNDAMENTELE ASPECTEN

Effect van temperatuur op de biologische defosfaterende micro-organismen

Temperatuur heeft een zeer complexe invloed op het biologische defosfateringsproces. Dit is zowel een direct effect op de betrokken bio-P organismen als een indirect effect op andere organismen waardoor het bio-P proces wordt beinvloed (b.v. denitrificeerders). Om het directe effect van de temperatuur op de bio-P organismen te onderzoeken werd labonderzoek verricht aan ophopingscultures die vrijwel volledig uit bio-P organismen bestaan. Wanneer deze effecten in

een model worden ondergebracht tezamen met het effect van de temperatuur op andere organismen worden het indirecte effecten goed beschreven.

Het effect van een temperatuursvariatie tussen 5-30 C werd onderzocht, waarbij zowel een korte termijn effect als naar lange termijn effecten is gekeken. In het laatste geval is het mogelijk dat de populatie van organismen wijzigt. Lange termijn effecten zijn het meest relevant voor de rwzi. Door de graduele temperatuur verandering past de populatie zich ook gradueel aan. Indien echter slib uit een rwzi aan een activiteitstest wordt onderworpen kan sprake zijn van een korte termijn effect.

In het algemeen werd er geen effect van de temperatuur op de stoichiometrie van het proces waargenomen. Het anaërobe proces (acetaatopname en P-afgifte) werd in het korte en lange termijn temperatuur experiment in gelijke mate beïnvloed. De waarde van de temperatuurcoëfficiënt (1.08) is gelijkwaardig aan die van andere heterotrofe organismen. Voor de aërobe processen werd wel een duidelijk verschil tussen korte en lange termijn experimenten waargenomen. Dit duidt op een verandering in populatiesamenstelling. Deze verandering werd ondersteund door moleculaire microbiologische onderzoeksmethoden. Klaarblijkelijk had de verandering in samenstelling van de bio-P populatie dus geen effect op de anaërobe fysiologie maar wel op de aërobe fysiologie.

In de korte termijn temperatuur effect experimenten werd een gemiddeld effect van de temperatuur op de omzetsnelheden waargenomen (coëfficiënt 1.06). Bij de lange termijn experimenten werd waargenomen dat de fosfaatopname slechts zeer gering werd beïnvloed door de temperatuur (coëfficiënt 1.03). De groeisnelheid en de vorming van glycogeen werden echter sterk negatief beïnvloed, het temperatuur effect lijkt daar ongeveer net zo groot te zijn als voor nitrificatieprocessen (coëfficiënt 1.12). Dit betekent dat de verschillende processen in de organismen verschillend gevoelig zijn voor de temperatuur en dat dit in het model ook expliciet moet worden meegenomen. Tevens betekent dit dat voor bio-P processen de minimale slibleeftijd bij lage temperatuur sterk toeneemt. Aangezien doorgaans ook nitrificatie in het systeem moet optreden, wat een nog langere slibleeftijd vereist, leidt dit doorgaans niet tot problemen.

Het geconstateerde verschil in temperatuureffecten tussen korte en lange termijn experimenten betekend dat activiteitstesten het best kunnen worden uitgevoerd bij de temperatuur van het zuiveringsproces. Indien een andere temperatuur wordt gebruikt, dient men de potentiële verschillen in acht te nemen.

Bioassay voor glycogeenbepaling

Glycogeen speelt naast polifosfaat en PHB een belangrijke rol als opslagstof in de fysiologie van de bio-P organismen. In een eerder onderzoek was geconstateerd dat glycogeen de limiterende factor kan zijn voor substraatopname en P-afgifte in de anaërobe fase. Voor diagnose van slecht functionerende systemen is het dus noodzakelijk deze component ook goed te kunnen meten. Standaardanalyses zijn niet voorhanden, glycogeen is een polymeer van glucose, maar veel andere polisachariden kunnen ook glycogeen bevatten. Door gebruik te maken van het feit dat bij een lage pH doorgaans glycogeen de P-afgifte en substraatopname limiteert, en er een directe relatie is tussen acetaat opname en glycogeen verbruik, is het mogelijk gebleken een bioassay op te

zetten waarmee het glycogeen gehalte kan worden bepaald. Tevens is een verificatie methode voor het vaststellen of de vooronderstelling dat glycogeen limiterend is voorgesteld.

ACTIEFSLIBMODELLERING

Modeleren van de COD, N en P verwijdering in de rwzi Haarlem Waarderpolder

Een combinatie van actiefslib model no 1 (ASM no. 1, voor conversie van COD en N) en het Delftse bio-P model is gebruikt om de rwzi Haarlem Waarderpolder te modeleren. Hierbij bleek dat voor een goede modellering de primaire procesdata (debieten, flowschema etc.) goed gecontroleerd dienen te worden. Vaak zijn deze slechts uit het ontwerp bekend, of is door aanpassingen in het verleden (op een complexe zuiveringsproces) het procesverloop niet meer goed bekend. Voor de dagelijkse procesvoering is dit vaak niet relevant, in een modelleringstudie kunnen ze echter grote invloed hebben.

Nadat het processchema goed was vastgesteld en het influent volgens de STOWA standaard procedure geanalyseerd, kon het volledige actiefslibmodel met aanpassing van slechts 3 parameters worden 'gekalibreerd'. Het model is gevalideerd op basis van batch experimenten met slib uit de rwzi, die daarna met het gekalibreerde model werden beschreven. Dit is in feite een veel strengere validatie dan de traditionele waar twee meetcampagnes aan het systeem worden gebruikt. Een voor de calibratie en een voor de validatie. In de batch experimenten wordt het slib namelijk aan sterk andere condities bloot gesteld.

Met het procesmodel zijn ook een aantal proces alternatieven onderzocht. Hierbij bleek dat het bestaande zijstroom defosfateringsproces zeven keer minder acetaat nodig heeft dan een traditioneel hoofdstroomproces om een gelijke effluent P te behalen. Indien echter gebruik wordt gemaakt van een UCT-proces met in-line P stripper (BCFS proces) is geen acetaat nodig en kan toch een vergelijkbare P effluent worden behaald.

PROCESMATIGE ASPECTEN VAN BIO-P PROCESSEN

Minimale SRT

Een theoretische afleiding van de minimale SRT in bio-P processen is afgeleid. Probleem is dat het PHB metabolisme niet toelaat om via de traditionele maximale groeisnelheid de minimale slibleeftijd te bepalen. De minimale aerobe SRT is ongeveer 10 dagen bij 5 C en ongeveer 1.5 dag bij 20 C. Indien er ook een anoxische fase kan de aërobe verblijftijd korter zijn. De minimale verblijftijd in een volledig anoxisch proces liggen ongeveer 35 % boven de minimale aërobe SRT.

Effect poly-P limitatie op bio-P bacterien

In een bio-P proces dat wordt ondersteund door chemische precipitatie kan de hoeveelheid poly-P in de cellen limiterend worden voor substraat opname onder anaërobe condities. In theorie kunnen

de bio-P organismen ook substraat opnemen via het mechanisme beschreven voor "glycogen accumulating organisms", in dat geval zal een overdosering van chemicaliën niet snel tot problemen leiden. Het blijkt echter dat de bio-P organismen wanneer het poly-P is uitgeput geen ander mechanisme hebben om substraat op te nemen. Dit betekent dat wanneer chemicaliën worden gedoseerd de dosering niet te groot mag worden, omdat anders het bio-P proces snel verstoord kan worden.

Effect overbeluchting op de biologische P-eliminatie

In de literatuur is regelmatig beschreven dat bio-P processen na zware regenval of periodes van onderbelasting (b.v. weekends) ernstig zijn verstoord. Het mechanisme is onderzocht. Hieruit bleek dat het niet een onderbelasting maar eerder een overbeluchting van het systeem is. Installaties met een goede beluchtingregeling hebben doorgaans geen last van verstoringen t.g.v. onderbelasting. Wanneer er teveel wordt belucht verdwijnt de PHB snel uit de cellen. Hierna gaat het organisme glycogeen oxideren. Dit leidt tot een verminderd vermogen om substraat op te nemen onder anaërobe condities, waardoor daarna de aërobe P-opname negatief wordt beïnvloed. Er is dus sprake van een soort van domino-effect.

Chapter 1

State of art on bio-P removal

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. 1999) followed by a detailed discussion on the process engineering and modelling aspects.

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 that 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).

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 poly-phosphate. 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).

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, is 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).

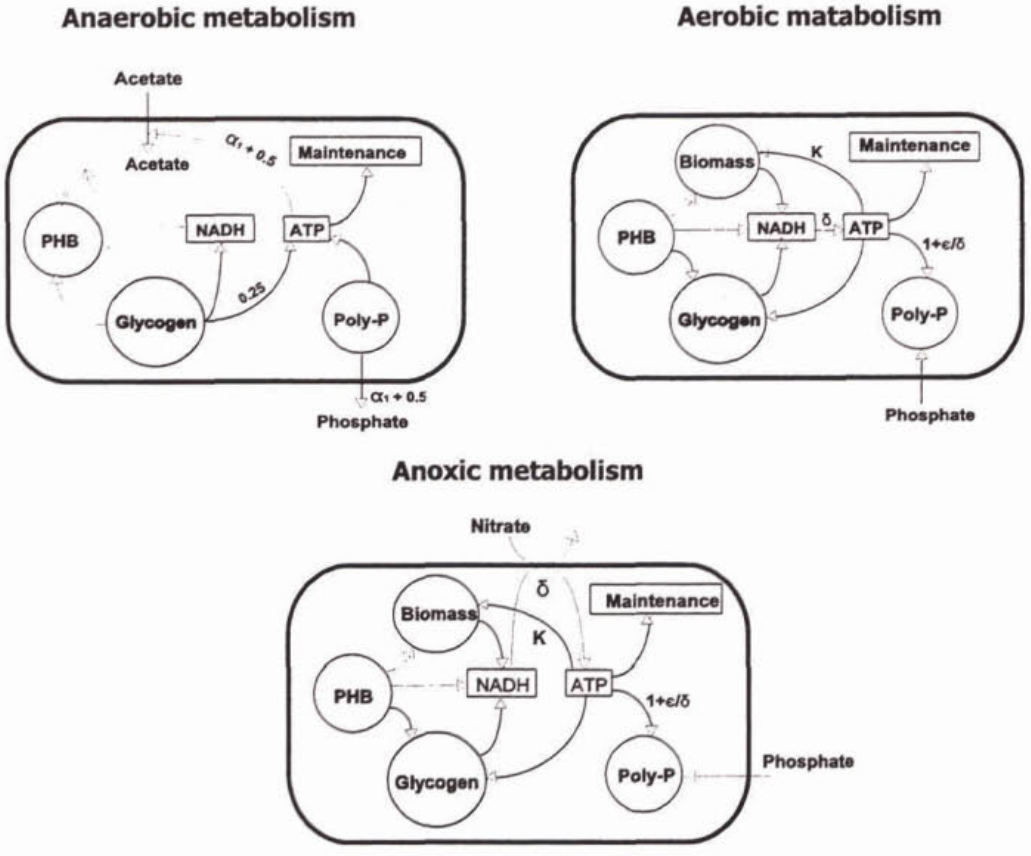


Figure 1.1 Metabolic processes of organisms involved in biological phosphorus removal.

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.

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.

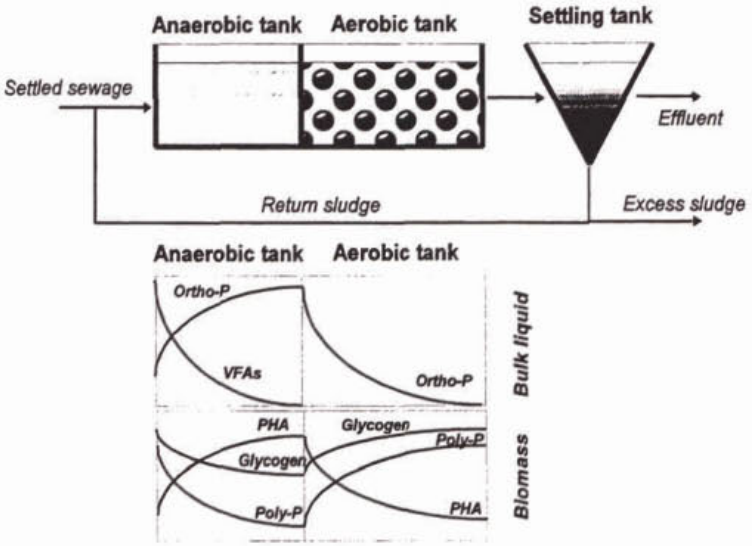


Figure 1.2 Schematic representation of a BPR process.

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.

From the process design point of view, it is essential to have a sewage properly characterised, 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 characterisation, the more reliable the design will be. Proper sewage characterisation is not only of extreme importance for the design of new plants, but also for the optimisation, 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 characterisation 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 season 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 hypothesised 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 depleted PHA levels may limit the aerobic phosphate uptake.

This hypothesis still needs to be examined.

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 loose 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.

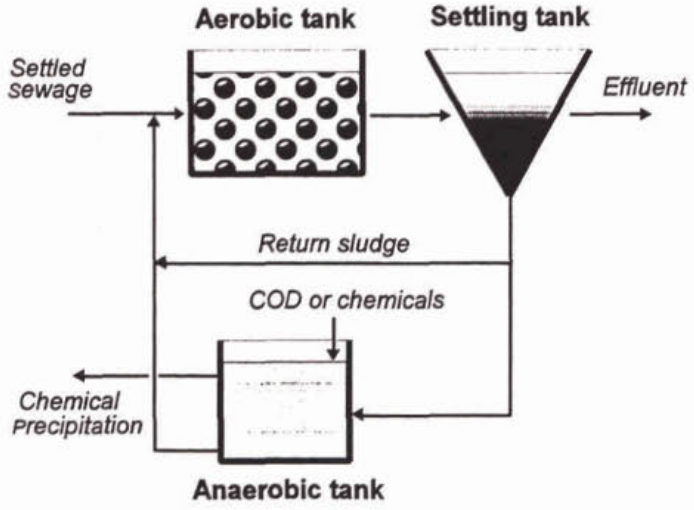


Figure 1.3 Schematic representation of the Phostrip® process.

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. Flocculation or crystallisation (Eggers et al. 1991) can treat this flow. 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. The Dutch water board 'Groot Salland' has developed an elegant solution 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.

Combination of biological phosphate and nitrogen removal

It has already been mentioned that biological phosphate and nitrogen removal requires 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.

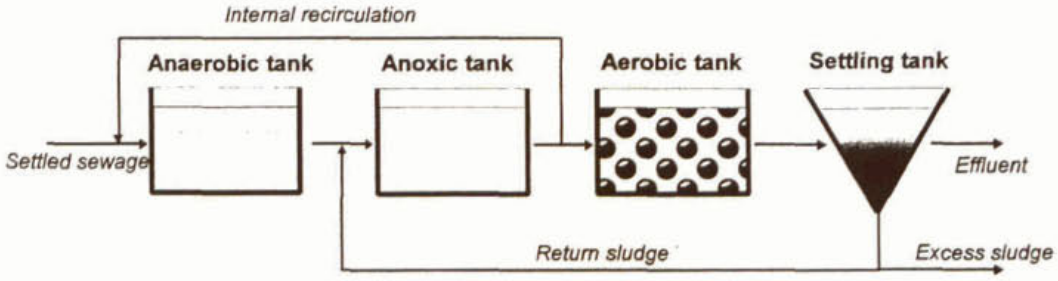


Figure 1.4 Schematic representation of a UCT-type process.

The negative effect of nitrate on phosphate release is caused by a direct substrate competition between heterotrophic denitrifyers and PAOs. The denitrifyers win this competition. 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 optimise the units sizing and, therefore, minimise the related investments. In activated sludge systems designed for COD and N removal, the SRT is directly linked to the growth rate of the micro-organisms. 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.

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 PAOs did not use COD (which was removed during pre-settling) 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 sulfide levels in the methane gas.

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 - ASM no.1 (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 ASM no.1 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 - ASM no.2 (Gujer et al. 1995). Soon after, Mino et al. (1995) extended ASM no.2 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 bio-P 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 PAOs by six independent reactions: two for the anaerobic metabolism and four for the aerobic/anoxic metabolism. In this model the $ATP/NADH_2$ ratio (called δ value) is the only model parameter that is different for aerobic and anoxic BPR. Four kinetic relations and two maintenance terms describe the process reaction rates. The comparison between the ASM no.2 and the Delft bio-P model is discussed elsewhere (Van Loosdrecht, 1996). Very recently, the Task Group proposed the ASM no.2d (Henze et al. 1998) - a minor extension of ASM no.2 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: ASM no.3 (Gujer et al. 1998). ASM no.3 is related to ASM no.1 and includes several improvements compared to ASM no.1, 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 ASM no.4 may appear, possibly as a combination of ASM no.3 and a slightly modified Delft bio-P model.

So far, the Delft bio-P mode 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 bio-P model was successfully applied for the first time on the full-scale installation WWTP Holten in The Netherlands (Van Veldhuizen et al. accepted). For this application the Delft bio-P model for P removal was combined with the equations for COD and N conversions of ASM no.2 following the structure of ASM no.2 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.

CONCLUSIONS

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.

Chapter 2

Temperature effects on BPR

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 first part 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 the second part. The results obtained from the second section served as a basis for development of calculation procedure for determination of minimal required sludge retention time (SRT) in BPR systems, presented in the third part of this chapter.

Part I: SHORT-TERM TEMPERATURE EFFECTS ON BPR

There are several publications reporting the effect of temperature on the efficiency of BPR using activated sludge. The results are inconsistent. Presently results are conflicting and difficult to interpret correctly. The findings of literature study indicated that the effects of temperature on BPR processes are insufficiently investigated. 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.

Materials and methods

A duplicate set of independent batch experiments (in duration of couple of hours - short term temperature effects) 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) stoichiometry and kinetics of the anaerobic phase of the SBR, (2) stoichiometry and kinetics (including oxygen consumption) of the aerobic phase of the SBR, (3) the anaerobic ATP maintenance coefficient m_{ATP}^{an}, and (4) the aerobic ATP maintenance coefficient m_{ATP}^{aer} and the phosphate/oxygen (P/O) ratio. Detailed description of experimental setup, operation of SBR and batch reactor, batch tests, medium and analyses used in this study is given elsewhere (Brdjanovic et al., 1997).

Results and discussion

Stoichiometry and kinetics of the anaerobic phase of the SBR

The anaerobic stoichiometric and kinetic parameters are determined for each operating temperature and are summarized in table 2.1 and characteristic example given in figure 2.1.

According to table 2.1, 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 2.1 Summary of stoichiometric and kinetic parameters for anaerobic batch experiments at 5, 10, 20 and 30 °C and SRT 8 days.

Parameter	Unit	5°C	10°C	20°C	20°C [a]	30°C
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	mg ATP/mg.h	0.0003	0.0006	0.00147	0	0.00363

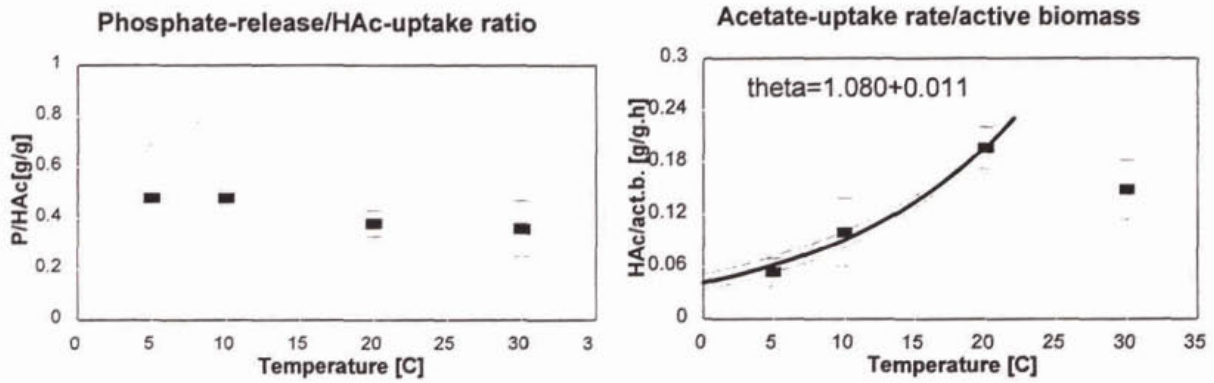
(a) Smolders et al., (1995a), and (b) Where possible the confidence interval of 95% is associated with measured values.

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 2.1, 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.

Stoichiometry and kinetics of the aerobic phase of the SBR

Aerobic stoichiometric and kinetic parameters are summarized in table 2.2 and characteristic example given in figure 2.1.

Anaerobic phase



Aerobic phase

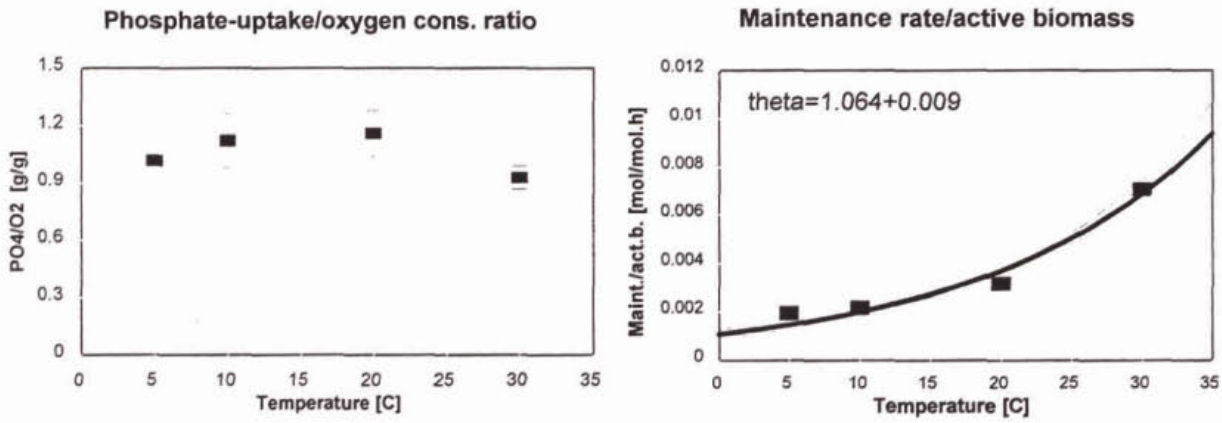


Figure 2.1 Characteristic examples of stoichiometric and kinetic parameters of the anaerobic and aerobic phase of BPR. The confidence interval of 95% (-) is associated with observed data (■). The simplified Arrhenius equation was fitted to the data with temperature coefficient θ (solid line) within the range of standard error (dashed lines).

According to the results given in table 2.2, 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., 1995a. 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 2.2 Summary of stoichiometric and kinetic parameters for aerobic batch experiments at 5, 10, 20 and 30 °C and SRT 8 days.

Parameter	Unit	5°C	10°C	20°C	20°C [a]	30°C
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	0.079	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	0.053	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	0.03	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	0.0023	0.0033±0.0033
PHA consumption/ active biomass rate	mg/mg.h	0.039±0.003	0.038±0.003	0.077±0.004	0.059	0.089±0.007
ATP maintenance coefficient	mol/mol.h	0.0019	0.0021	0.0032	0.004	0.0071

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

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 2.2 and figure 2.1).

It was found that the temperature has a large impact on the oxygen consumption rate in BPR system (table 2.2). 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 (results not shown). 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.

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 2.2). 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 $1.47 \cdot 10^{-3}$ molATP/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).

The aerobic ATP maintenance coefficient m_{ATP}^{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 O_2/L.h (at 5 °C) to 15.01 mg O_2/L.h (at 30 °C). The rate of 7.26 mg O_2/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., 1995a) needed for determination of m_{ATP}^{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_{ATP}^{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.,1995a.

Temperature coefficients

Based on values from tables 2.1 and 2.2, using the simplified Arrhenius equation for the temperature dependency, the temperature coefficient θ was calculated for each reaction rate (table 2.3) by fitting the data to the equation (figure 2.1).

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.

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.

Table 2.3 Temperature coefficients θ with standard error for the reaction rates of the anaerobic and aerobic BPR processes.

Anaerobic phase	θ ± st. error	Aerobic phase [b]	θ ± 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 1.078		**Average aerobic temperature coefficient 1.057**	

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

According to the classification of temperature coefficients in ASM no.2, and the θ of BPR found here, BPR has a low to medium degree of temperature dependency. In comparison to the temperature coefficients in ASM no.2, 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 (θ=1.120). Furthermore, it can be concluded that the temperature has a similar impact on BPR as on heterotrophs or fermentation (θ=1.070). However, θ values given in ASM no.2 are based only on a general knowledge of temperature impact on various biological processes.

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.

Part II: LONG-TERM TEMPERATURE EFFECTS ON BPR

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 part 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 part 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.

Materials and methods

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 Brdjanovic et al., 1998a.

The experimental procedure is presented in figure 2.2. 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 Brdjanovic et al., 1998a.

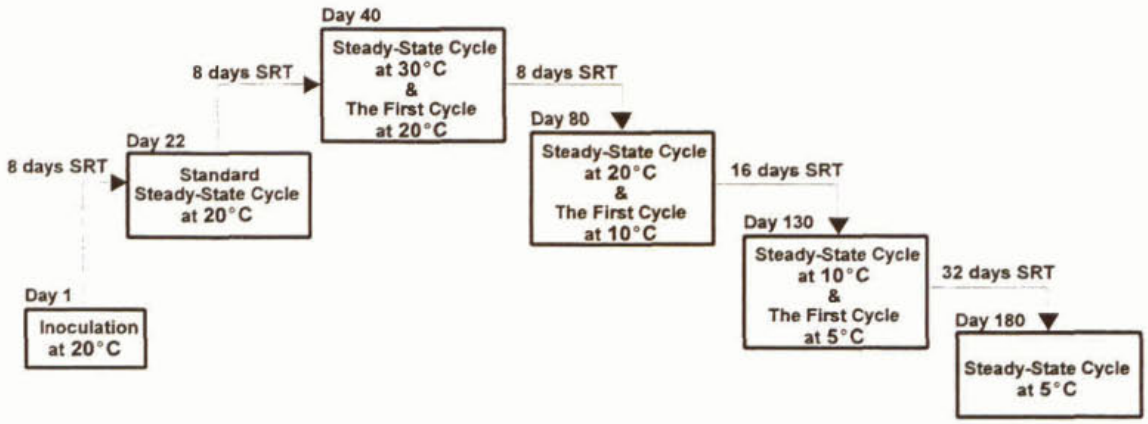


Figure 2.2 Operating schedule of the SBR. The day in the boxes represent the day when the operating temperature (and in last three cases the SRT) was changed and when the "steady-state" and "the first cycle" measurements took place

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. Detailed description of these methods is given in Brdjanovic et al., 1998a.

Results

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 (1997).

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 2.3. Results of cycle measurements under steady state SBR operation at 20°C and SRT of eight days (called standard cycle, figure 2.3a), are considered as typical for such an experimental set-up (Kuba et al., 1993, Smolders et al., 1994c, and Brdjanovic et al., 1997). 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 2.3b), as well as under SBR steady state operation at 30°C (figure 2.3c). 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 2.3d), however full BPR efficiency was already reestablished in the next cycle. Under steady state operation at 20°C (figure 2.3e) 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 2.3f). 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 2.3g). 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 2.3h). 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 2.3k). 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. (1995a), 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. A typical sharp drop in oxygen utilization rate (OUR) occurred at the moment when phosphate uptake was completed (Smolders et al., 1995b; Brdjanovic et al., 1997). 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. The total oxygen consumption in the experiments increased at lower temperature due to increase of biomass concentration in the reactor.

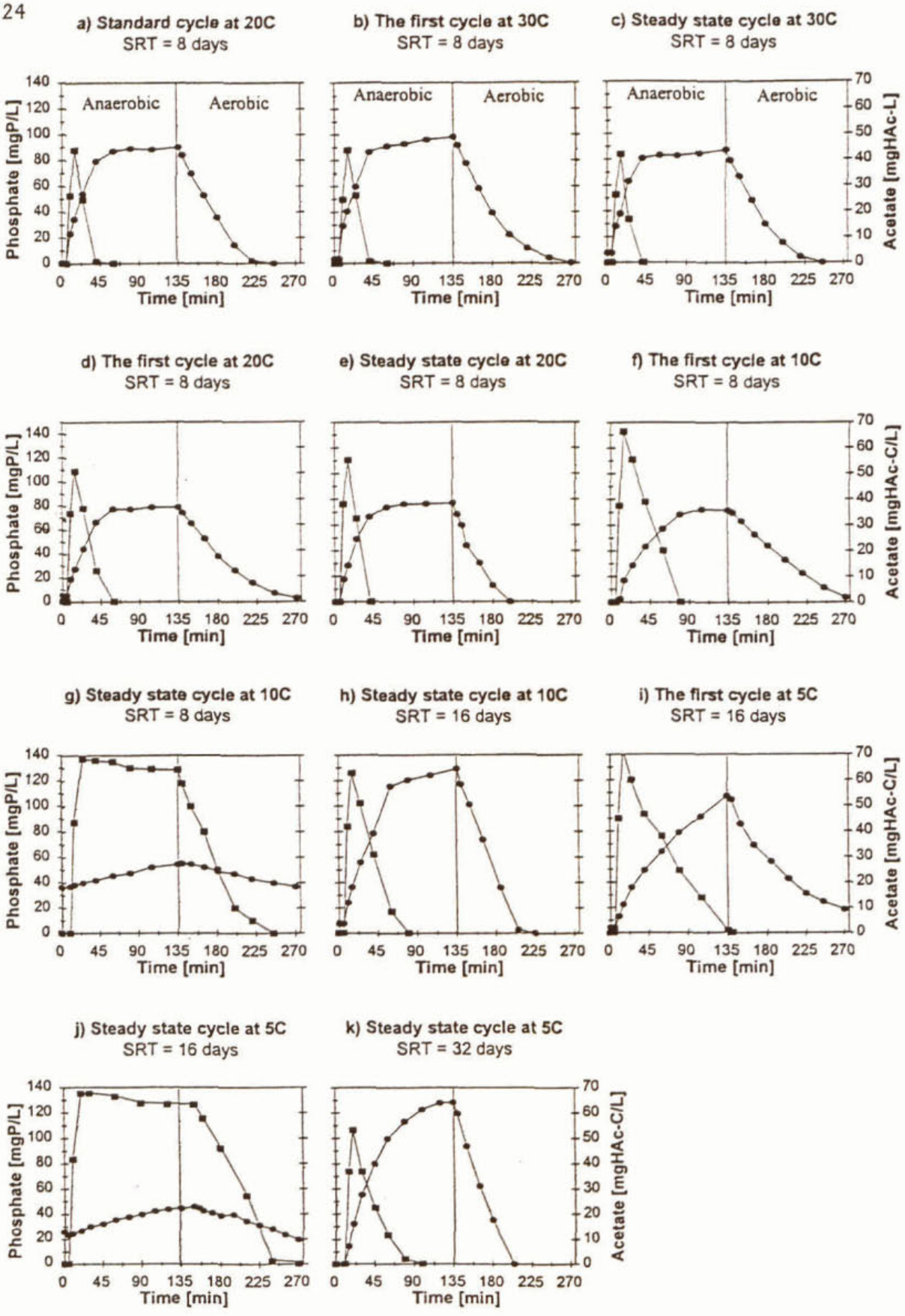
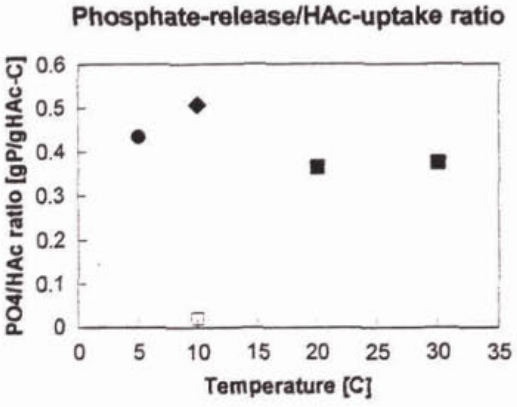


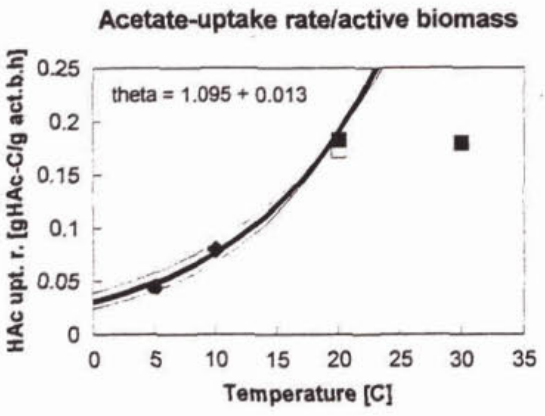
Figure 2.3 Acetate (■) and phosphate (●) concentrations during SBR cycle at 5, 10, 20 and 30 °C

Stoichiometry and kinetics

The anaerobic and aerobic stoichiometric and kinetic parameters observed at different operating temperatures are summarized in table 2.4. Characteristic examples are given in figure 2.4.

Anaerobic phase





Aerobic phase

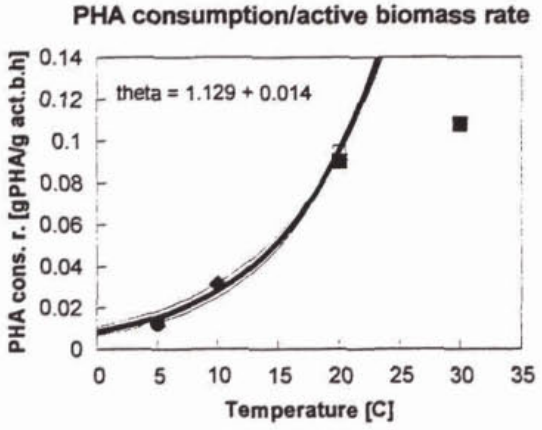


Figure 2.4 Stoichiometric and kinetic parameters of the anaerobic and aerobic phase of the SBR at 5, 10, 20 and 30 °C, and SRT of 8 (■, □ relates to standard cycle), 16 (◆) and 32 (●) days. Simplified Arrhenius equation was fitted to experimentally observed data using temperature coefficient θ (bold line) within range of standard error for θ (thin line).

In general, the results from long-term test show that the anaerobic stoichiometry was not affected by temperature changes. 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 2.4). 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. In contrast, the results from short-term temperature experiments showed that the highest anaerobic process rates occurred at around 20°C. 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 2.4.

Table 2.4 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 2.4, 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). 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).

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.

Biomass composition

The biomass composition during SBR operation at different temperatures is presented in table 2.5. The biomass composition in the SBR at 20 °C was similar to the composition observed earlier under identical operating conditions (Smolders et al., 1995a, and Brdjanovic et al., 1997). 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.

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.

Temperature coefficients θ

Based on values from table 2.4, 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.

Table 2.5 Biomass composition in the SBR at 5, 10, 20, and 30°C.

Cycle	Standard		First		Steady state		First		Steady state		First		Steady state		First		Steady state	
Temperature	20°C		30°C		30°C		20°C		20°C		10°C		10°C		5°C		5°C	
SRT	8 days		8 days		8 days		8 days		8 days		8 days		16 days		16 days		32 days	
Sampling Point	An. End	Aer. End	An. End	Aer. End	An. End	Aer. End	An. End	Aer. End	An. End	Aer. End	An. End	Aer. End	An. End	Aer. End	An. End	Aer. End	An. End	Aer. 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_{pp} (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_{pp} (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	

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 ASM no.2).

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 summarized in table 2.6, along with modified temperature coefficients (valid between 5 and 20°C) of the relevant metabolic process rates from short-term temperature experiments. Temperature coefficients presented in this chapter 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 2.5).

Table 2.6 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)
	θ ± st. error	θ ± st. error	θ ± 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 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.

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 (not shown) already suggests there are different morphological forms of microbes in the sludge. The presence of at least 5-6 bands on the DDGGE (not shown) 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.

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 . 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% (not shown). 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 (for more details the reader is referred to Brdjanovic et al., 1998b).

Discussion

Taking into account the results from both short- and long-term temperature experiments, 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 θ = 1.078, table 2.3) only very slightly deviate from the temperature coefficients of the anaerobic BPR processes obtained in a long-term experiments (average θ = 1.085, table 2.6).

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 θ = 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 nitrifyers.

It is important to note that in wastewater practice the aerobic phase of BPR is much less studied then 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. 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.

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.

Part III: DETERMINATION OF ANAEROBIC SRT IN BPR SYSTEMS

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) (Satoh 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: $SRT_{min}^{total} = SRT_{min}^{anaer} + SRT_{min}^{aer}$) 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.

Equation 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 detailed derivation of the equations for determination of SRT_{min}^{aer} is given

elsewhere (Brdjanovic et al., 1998d), therefore only the final equation is given below:

$$SRT_{min}^{aer} = \frac{(Y_{PHA,HAc}/Y_{x,HAc})\cdot(t^{aer}/24)}{f_{PHA}^{max} - [(f_{PHA}^{max})^{0.333} - 0.333\, k_{PHA,20°C}\cdot q_{PHA}^{(T-20°C)}\cdot t^{aer}]^3} \quad ...(2.1)$$

It should be realised that the maximal consumption rate of PHA is obtained when the cells have stored the maximal amount of PHA.

Materials and methods

This study is based mainly on the results of the study of temperature influence on BPR, presented in part I and II of this chapter. 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 $\lambda_{SRT,p}$:

$$\lambda_{SRT,p} = (dSRT^{aer}/SRT^{aer})/(dp/p) \quad ...(2.2)$$

This factor $\lambda_{SRT,p}$, 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 ±10% by the step dp of 2% (in total 10 calculated data per parameter). Only one parameter was varied per calculation.

Results and discussion

Model validation

The dependence of SRT_{min}^{aer} 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^{aer}) can be expressed in a graphical form (figure 2.5). Figure 2.5, based on eq. 2.1 and typical parameter values (table 2.7), allows the SRT_{min}^{aer} of the BPR system to be estimated for chosen f_{PHA}^{max} and operating temperature.

Table 2.7 Characteristic values of the variables used for the calculation of SRT_{min}^{aer}

Parameter	Value	Unit	Reference
$k_{PHA,20°C}$	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., 1994b
t^{aer}	2.25	h	Smolders et al., 1994b

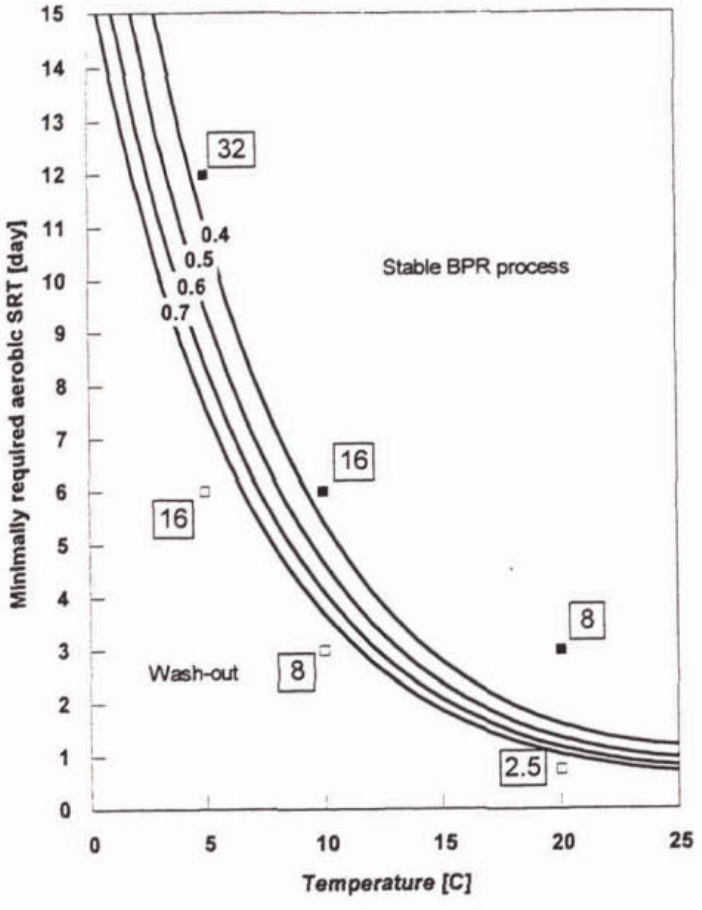


Figure 2.5 Minimally required aerobic SRT as a function of maximal storage capacity of the cell (0.4-0.7 gCOD-PHA/g COD-active biomass) and temperature. The symbols indicate the aerobic SRT of several laboratory scale SBR systems (Smolders et al., 1996, Brdjanovic et al., 1998a). Numbers indicate the 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.

The method was validated using measured values of f_{PHA} in an anaerobic-aerobic (A/O) SBR operated at different temperatures and SRTs (table 2.5). Clearly, two zones can be distinguished in figure 2.5 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 2.8.

The model describes the change in PHA consumption during the aerobic phase very well over the wide range of SRT and temperature (maximal deviation ±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} (table 2.5) 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 then 3.5% over the entire SRT range studied.

Table 2.8 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	0.014 (0%)
predicted[c] SRT^{aer}	day	10.4 (-13%)	7.7	3.2 (+7%)
predicted[d] SRT_{min}^{aer}	day	9	4.4	1.25

a) Long-term temperature effect study (Brdjanovic et al., 1998a)
b) Using eq. 6.4. and $k_{PHA,20°C}$ = 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)

Sensitivity analysis

The results of the sensitivity analysis of the parameter values towards the predicted SRT 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 $Y_{x,HAc}$, $k_{PHA,20°C}$ 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.

Factors affecting the SRT_{min}^{aer}

Temperature

The influence of temperature (30, 20, 10 and 5°C) on the BPR process was experimentally investigated in part I and II of this chapter 3. An interesting observation was made during the long-term temperature experiments. 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 2.5). 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).

However, figure 2.6a 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 long-term temperature experiment study were the main factor affecting the process.

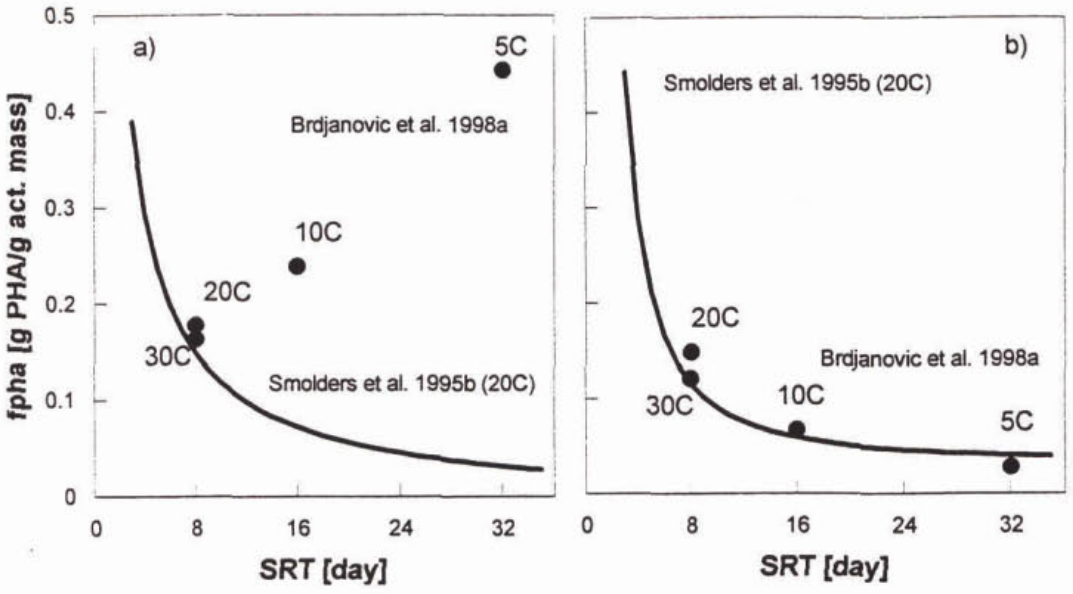


Figure 2.6 Comparison of: (a) specific active biomass PHA concentration (f_{PHA}^{aer}) and (b) PHA conversion under aerobic conditions (Δf_{PHA}^{aer}) between data from the study on long-term temperature experiments (Brdjanovic et al., 1998a) obtained at different temperature (●) and predicted values by the metabolic model for aerobic BPR at 20°C (solid line, Smolders et al, 1995b).

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. 2.1. 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 2.6b). These values correspond with the data given by Smolders et al., (1995a), 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 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 2.8). In order to express this dependency a simplified Arrhenius equation was applied on the reciprocal value of the SRT_{min}^{aer} (from table 2.8):

$$\frac{1}{SRT_{min(T)}^{aer}} = \frac{1}{SRT_{min(20°C)}^{aer}} \cdot \theta_{1/SRTaer,min}^{(T-20°C)} \qquad ...(2.3)$$

The temperature coefficient $\theta_{1/SRTaer,min}$ 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 $\theta_{1/SRTaer,min} = 1.137 \bullet 0.002$ 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}.

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. 2.1); 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 exists no universal saturation level for PHA.

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}^{aer} is minor.

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^{aer}, it is likely that the SRT_{min}^{aer} also depends on the number of anaerobic-aerobic sequences.

The SRT_{min}^{aer} 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, (1994a), 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^{aer}) can be calculated from eq. 2.1, assuming

that Δf_{PHA} of small cycles is equal to $\Delta f_{PHA}{}^{aer}$ of standard cycle divided by the number of small cycles, $f_{PHA,to} = f_{PHA}{}^{max}$, and t^{aer} 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^{aer} 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^{aer} at higher temperature. For example, decrease of the number of A/O sequences from four to one per day will lead to increase of SRT^{aer} 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 $SRT_{min}{}^{aer}$. 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 $SRT_{min}{}^{aer}$.

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 $SRT_{min}{}^{aer}$. However, in the case of denitrifying BPR it is expected that the $SRT_{min}{}^{anox}$ will be 30 to 40% longer than the $SRT_{min}{}^{aer}$ in aerobic BPR due to the fact that the $Y_{PHA,HAc}$ 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.

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 equations for the prediction of the minimally required aerobic SRT were well capable of describing the experimentally obtained data.

Chapter 3

The role of storage polymers in BPR

INTRODUCTION

In wastewater treatment processes microorganisms are subjected to a feast and famine regime. For highly dynamic systems such as sequencing batch processes this is even more pronounced. In general, microorganisms respond to these feast-famine regimes by accumulating storage polymers (for example PHA) when substrate is present. The storage polymers are used for growth and other processes when the external substrate is depleted. Microorganisms which are capable to quickly store external substrate (that is available only for relatively short period of time) and consume this stored substrate in a more balanced way, have a strong competitive advantage over organisms without the capacity of substrate storage. Several types of storage polymers have been reported in literature, both organic (PHB, glycogen and lipids) and inorganic (poly-P). Although storage processes are often neglected in activated sludge research and practice they play a significant role in activated sludge systems, especially in BPR process. While the role of lipids in bio-P removal is unclear (Van Loosdrecht et al., 1997a), several recent studies confirm essential role of PHA, glycogen and poly-P in BPR (see chapter 1 and excellent reviews of Van Loosdrecht et al., 1997d and Mino et al., 1999).

Biological phosphorus removal (BPR) is based on the enrichment of activated sludge with poly-phosphate accumulating organisms (PAOs). Under anaerobic conditions PAOs 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., 1994b; Pereira et al., 1996). The energy is generated by the conversion of glycogen and poly-phosphate (poly-P) resulting in phosphate release into 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., 1995a). It has been reported that glycogen accumulating non-poly-P organisms (GAOs) may appear when BPR 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 absence of glycogen anaerobic substrate uptake by PAOs or GAOs can not occur (Mino et al., 1996; Brdjanovic et al., 1998b).

In both PAOs' and GAOs' anaerobic acetate metabolism, energy is required for maintenance, transport of external carbon-substrate into the cells and its conversion to PHAs. The PAOs 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 GAOs resembles that of PAOs (glycolysis of stored glycogen and substrate conversion to PHAs through either acetyl-CoA or propionyl-CoA, and propionyl-CoA production by succinate-propionate pathway), except there is no involvement of poly-P. They hypothesised that the energy generated by glycolysis is not an exclusive privilege of GAOs, but can also be a complementary energy source for PAOs. The hypothesis was based on the assumption that both PAOs and GAOs have a glycolysis pathway (figure 3.1). This was recently proven by in-vivo NMR measurements (Pereira et al., 1996).

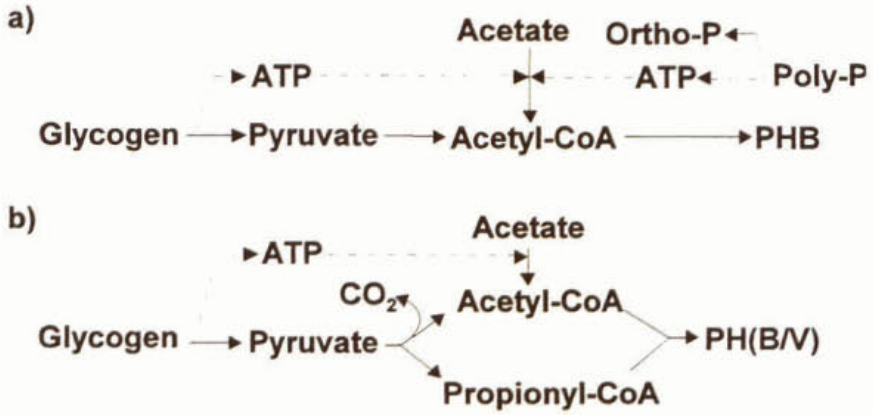


Figure 3.1 Simplified metabolic pathways of acetate and glycogen conversion to PH(B/V) by (a) PAOs and (b) GAOs under anaerobic conditions (after Mino et al., 1997).

In PAOs, glycogen is only converted to deliver the NADH required for HAc reduction to PHB, while in GAOs, 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 GAOs, is balanced by formation and plymerisation of propionyl-CoA into PH(B/V). Although PAOs could be expected to posses the anaerobic metabolism of GAOs, there is no experimental evidence to confirm this statement.

It is clear that for bio-P bacteria storage polymers form a crucial part of the bacterial metabolism. Recognition of the fact that the storage processes are an intrinsic aspect of microbial physiology and ecology of activated sludge processes makes it possible to optimize existing or design new processes.

This chapter explores two subjects related to bacterial behaviour under depletion or full exhaustion of internal storage materials such as poly-P and PHB. Bacterial metabolism under poly-P limited conditions was studied at rather academic level, while the study of bacterial behaviour under PHB limiting conditions (due to excessive aeration) was more practically orientated. The results of these two studies are given below.

Part I: EFFECT OF POLY-P LIMITATION ON ANAEROBIC METABOLISM OF BIO-P BACTERIA

With the aim to investigate more in detail the energy budget of PAOs and GAOs this work address the question: can PAOs perform the metabolism of GAOs under anaerobic conditions, or more specifically, can PAOs take-up VFAs in the absence of poly-P when sufficient glycogen is present? Underlying this question is whether PAOs and GAOs are possibly one group of organisms.

In order to answer above question anaerobic substrate (acetate) uptake by sludge highly enriched with PAOs 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 presence of surplus acetate under anaerobic conditions (Smolders et al., 1994b). At this point the glycogen content of the biomass may become extremely low due to anaerobic acetate uptake (Brdjanovic et al., 1998b). 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 anaerobic P-release phase the sludge was washed two times 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 the most important part of the experiment consisted of monitoring of the anaerobic acetate consumption by the poly-P depleted sludge containing surplus glycogen. Detailed description of the experiment is given in Brdjanovic et al., 1998c.

Results

This sludge handling procedure resulted in almost total depletion of poly-P and glycogen storage pools (2 and 12 mg/L respectively) at the end of extended anaerobic phase (II) at pH 8.0. After washing acetate was absent but some phosphorus (28 mg P/L) still remained in solution. This phosphorus was quickly taken-up in the subsequent aerobic phase. At the beginning of the main anaerobic experiment phase the biomass contained very low poly-P and PHAs level and a substantial amount of glycogen (32, 26 and 194 mg/L respectively). Approximately 90 % of total acetate consumption and phosphate release, associated with rapid formation of PHAs, occurred during the first hour of the main experiment. Initially fast HAc-uptake and P-release are the result of the presence of some poly-P (0.02 g P/g VSS) and glycogen in the cells. This relatively small poly-P pool was due to uptake of phosphorus in aerobic phase. After depletion of the poly-P pool a dramatic slow-down of all involved processes occurred (figure 3.2). Only marginal conversions of acetate, phosphate and PHAs were observed until the end of the experiment. During main experiment 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. Based on these results, it is clear that the PAOs

are not responsible for the observed GAO activity in activated sludge.

Although in theory PHV production is not required in PAO's anaerobic metabolism, in practice some PHV is always formed in BPR systems. The ratio of newly formed PHV per newly formed PHB (dPHV/dPHB ratio in figure 4.2) 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 g PHV/g PHB obtained by Smolders et al. (1995a) under identical operating conditions. This ratio increased to 0.46 g PHV/g PHB in the last phase of the main experiment.

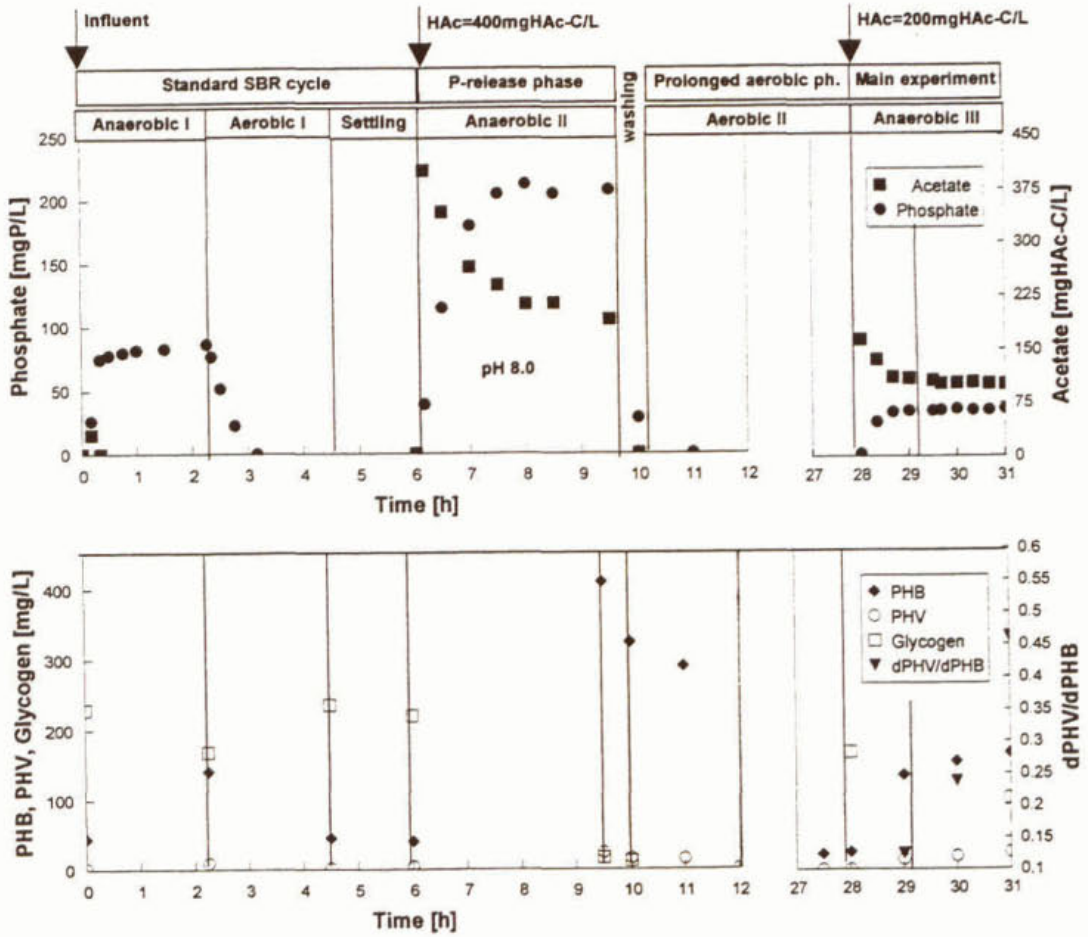


Figure 3.2 Acetate (■), phosphate (●), glycogen (□), PHB (◆) and PHV (○) concentrations during the experiment.

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) proposed, based on in-vivo NMR experiments, the metabolism of anaerobic acetate uptake by PAOs in which a small flux of substrate goes trough the TCA cycle which consequently leads to formation of PHV. In that study PHV accumulation is attributed to the enzymatic activity through the full TCA cycle which occur in PAOs under anaerobic conditions rather than to presence of the G bacteria. However, Pereira et al. placed succinate in two pathways

which run in different directions. This can not take place in one cell. Furthermore, it was shown that the succinate-malate conversion cannot occur in PAOs under anaerobic conditions (Mino et al., 1996). In order to explain the results we propose a modified metabolism of anaerobic acetate uptake by PAOs where PHV is formed via enzymatic activity in a part of TCA cycle, but where also the glyoxylate cycle remain active (figure 3.3). 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., 1994b).

Both PAOs and GAOs are capable of anaerobic utilization of organic substrate in activated sludge processes. In well operating BPR plants the fraction of GAOs in the sludge is probably negligible. However, as mentioned earlier, GAOs may appear when BPR deteriorates. For the purposes of process evaluation and mathematical modeling of BPR it is interesting to know the GAOs/PAOs ratio in activated sludge. The results of this study strongly indicates that PAOs do not utilize acetate under anaerobic conditions when the internal poly-P storage is exhausted (even though glycogen was not limiting). This served as a platform for the development of the method for determination of GAOs/PAOs ratio in activated sludge. The method is proposed as described in appendix 1.

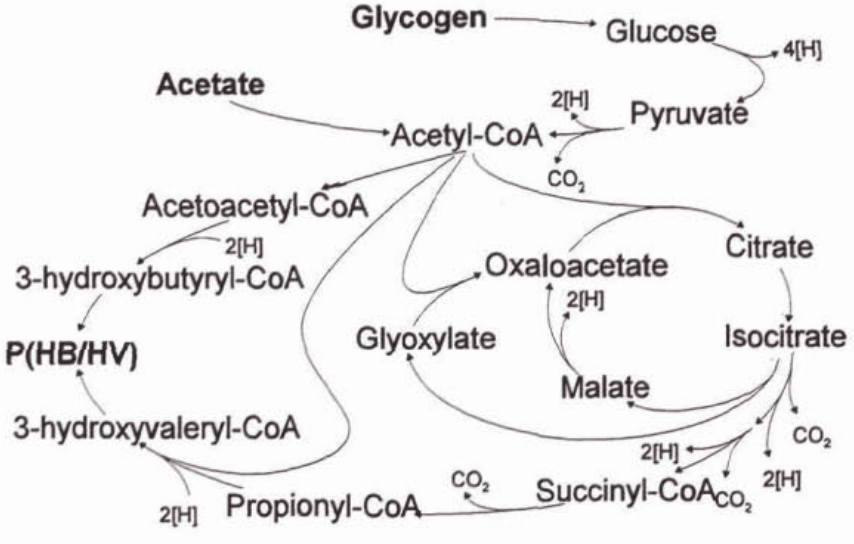


Figure 3.3 Proposed metabolism for conversion of acetate and glycogen to P(HB/HV) by bio-P bacteria under anaerobic conditions (after Pereira et al., 1996).

Conclusions

The marginal microbial activity observed under the conditions where poly-P was completely depleted strongly indicates that acetate uptake stops since PAOs are not capable of anaerobic uptake of carbon sources based on glycolysis of glycogen solely. The reason why PAOs cannot perform a metabolism similar to GAOs (although they seem to have the necessary enzymes) remains unclear.

Part II: IMPACT OF EXCESSIVE AERATION ON BIO-P REMOVAL

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 wwtp 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 temporarily 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 wwtp 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 wwtp have inadequate aeration control. In such plants, like in the study of Wolf and Telgemann, (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 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 the levels of internal storage pools were manipulated by varying the length of the anaerobic and aerobic phase and by applying different feeding strategies.

A double jacketed, anaerobic-aerobic-settling laboratory fermenter operated under steady state conditions for 200 days at a temperature of 20 °C, pH value of 7.0 ± 0.1 and SRT of eight days. This standard operation was changed to adopt specific requirements of experiments, when the length of the phases, substrate concentration and the time of substrate addition to the SBR were changed during **one** cycle only.

The first 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 from the SBR.

In the second 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 twentyfold in order to secure a surplus phosphorus in the solution at the end of the first period (7.5 h)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. Aerobic conditions were maintained for 15 hours (22.5 h in total). At the end of the experiment the SBR operation was switched again to standard operation. Detailed description of the experiments is given in Brdjanovic et al., 1998e.

Results

Experiment E1

The SBR operated for 200 days and therefore it can be considered in a steady-state.The dynamic pattern of the parameters monitored during a standard cycle in steady state is presented in figure 4a (biomass composition is given in Brdjanovic et al., 1998e). 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., 1995a). 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. The total oxygen consumption by the biomass, as obtained from respirometry, was 81 mgO_2/L (46 $mgO_2/gVSS$).

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 3.4b). 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 O_2/g VSS.h. During the prolonged aerobic phase the total oxygen consumption was 290 mg O_2/L.

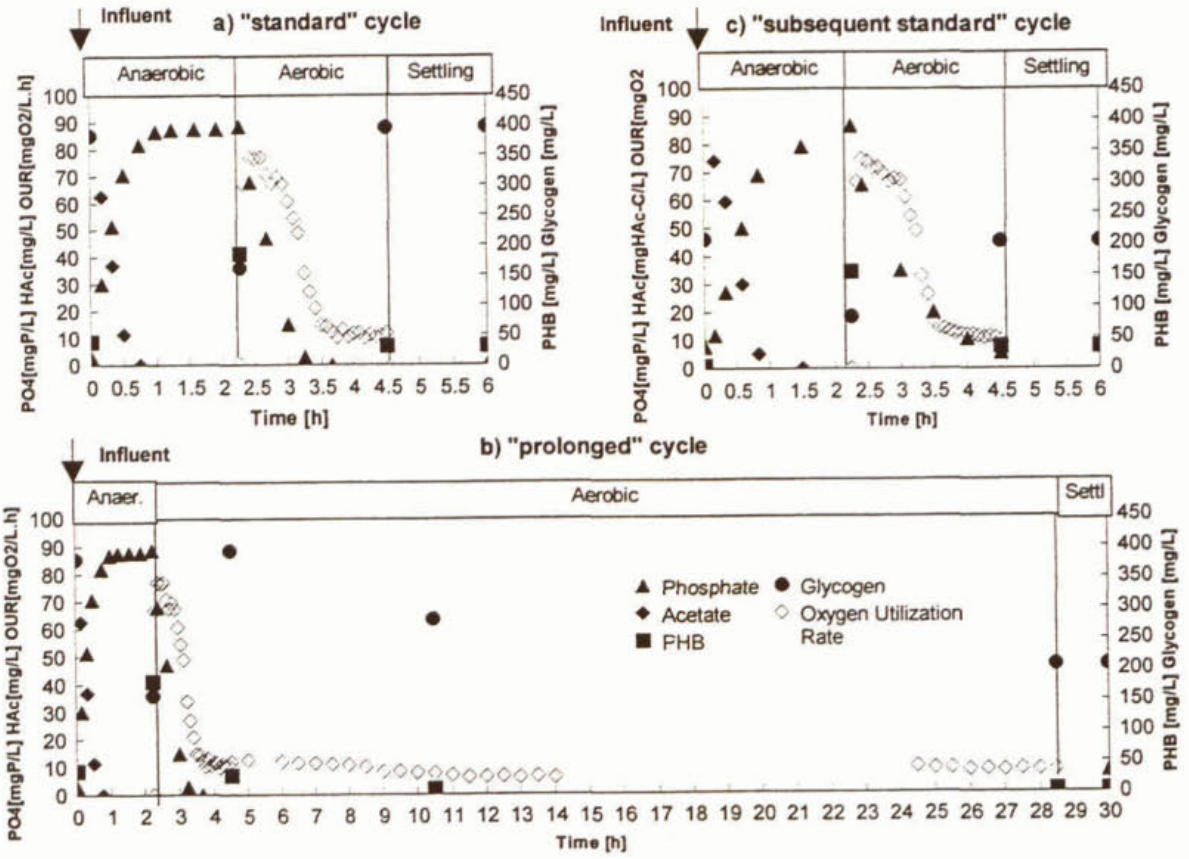


Figure 3.4. Concentration of relevant parameters in (a) standard, (b) prolonged and (c) subsequent standard SBR cycle of experiment E1.

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 3.4c) 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 (PO_4/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 O_2/L. The kinetic rates of selected parameters are summarized in table 3.1.

Table 3.1 Rates* of selected processes measured during anaerobic and aerobic phase of standard, prolonged and subsequent standard SBR cycle of experiment E1.

Parameter/Type of the cycle	Standard	Prolonged	Subsequent Standard
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	0.06

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

Experiment E2

Following an instant addition of acetate and phosphate at the beginning of the experiment E2 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 3.5a). 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 (biomass composition given in Brdjanovic et.al., 1998e).

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 3.5b). 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 (figure 4.5a). 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).

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).

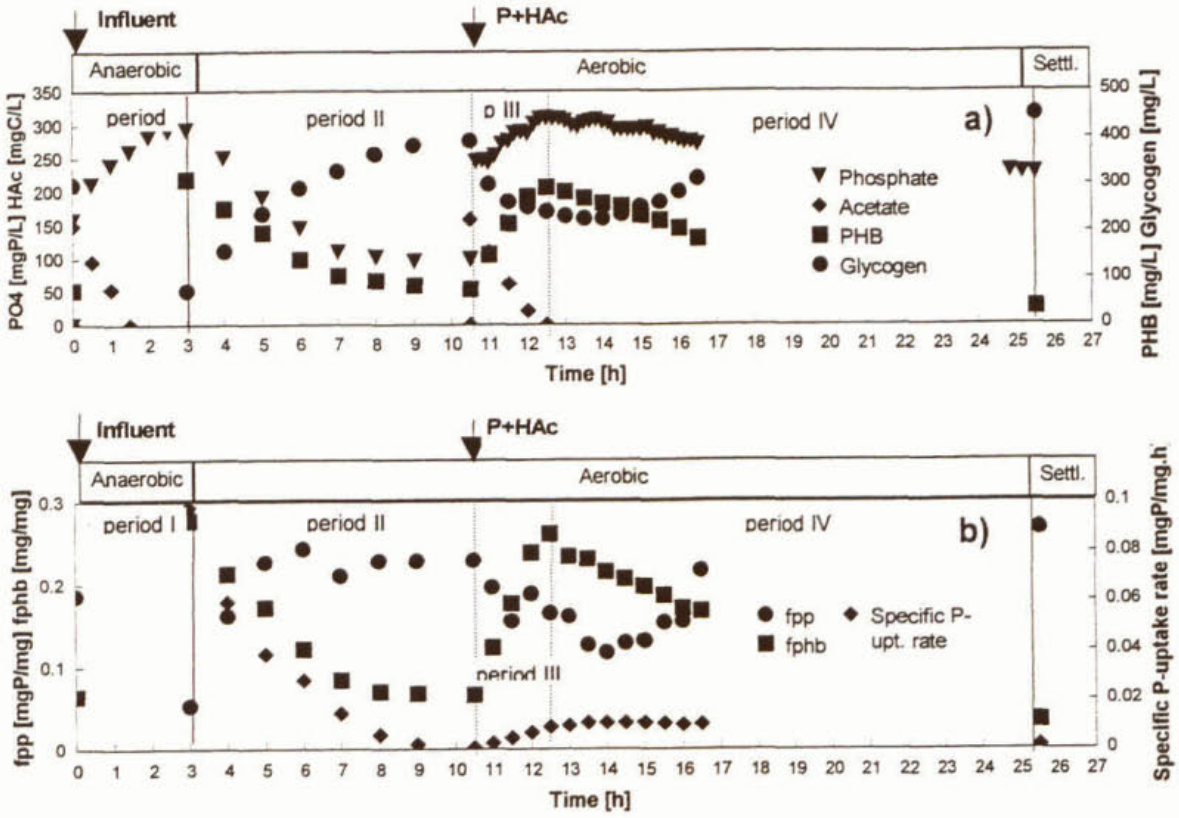


Figure 3.5 (a) conversion of relevant parameters of BPR during prolonged SBR cycle and, (b) dynamics of f_{pp} and f_{phb} fraction and specific phosphorus uptake rate during experiment E2.

Discussion

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 4.4 and tare in the range reported by Kuba et. al., (1993), Smolders et al., (1995a) and Brdjanovic et al., (1997b) 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., 1995b and Kuba et al., 1997c). 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 where 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 3.1). 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.

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 3.5b). 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 3.5a). 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.

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., 1995a and Murleitner et al., 1997) 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 mgO_2/gVSS.h observed in experiment E1 under starvation conditions agrees with the oxygen utilization rate for maintenance of 5.6 mg O_2/g VSS.h and 5.4 mg O_2/g VSS.h observed under similar conditions by Smolders et al., (1994c) and Brdjanovic et al., (1997b), respectively. This maintenance rate can adequately predict the relation between SRT and net sludge production (Smolders et al., 1995b). A comparison between the glycogen (4.66 mg COD/g VSS.h) and the oxygen utilization rates (4.74 mg O_2/g VSS.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 (additional experiment

not presented in this report) 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 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 (not presented) phosphate consumption was absent. The glycogen was still consumed by the rate assumably needed for maintenance.

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.

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.

Chapter 4

Modeling COD, N and P removal in a full-scale wwtp Haarlem Waarderpolder

INTRODUCTION

Biological phosphorus removal (BPR) is a complex process if compared to N and COD removal. Many different interferences with the other processes might occur. Mathematical simulations of full-scale BPR processes can help quantitatively evaluating these interactions. Several different models, such as Activated Sludge Model no.1 - ASM no.1 (Henze et al. 1987), ASM no.2 (Henze et al. 1994), ASM no.2d (Henze et al. in press), ASM no.3 (Gujer et al. in press), Delft bio-P Model (Murnleitner et al.1997, Van Veldhuizen, accepted) are suitable for application to full-scale wwtp. ASM no.1 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 ASM no.2, the situation is different. It has not been validated extensively due to the fact that the model became available only recently. Simultaneously to ASM no.2, the Delft BPR model was introduced. This model was validated in enriched BPR laboratory systems over a range of SRT values (Smolders et al. 1995b), or different anaerobic/aerobic times (Kuba et al., 1997c) and oxygen or nitrate as electron acceptor (Murnleitner et al., 1997), as well as during both start-up and steady state conditions (Smolders et al. 1995c). Recently, this combined model was for the first time applied on the full-scale wwtp Holten (BCFS® process) in the Netherlands (Van Veldhuizen et al., accepted). In this study, the model was used to check the performance of Phostrip®-like process at wwtp Haarlem Waarderpolder, also located in the Netherlands.

The purpose of this study was to evaluate: (a) how a complex model can be applied to a complex full-scale plant (conventional anaerobic-aerobic activated sludge system with side-stream BPR), (b) influent and sludge characterization procedures for bio-P modeling, (c) the use of batch tests to evaluate the model, and (d) different alternative BPR process schemes.

MATERIALS AND METHODS

Configuration of wwtp Haarlem Waarderpolder

The wwtp Haarlem Waarderpolder was built in 1969 and retrofitted in 1995 to its present state (figure 4.1). The plant was designed for removal of organic matter (COD), nitrogen (N) and phosphorus (P) from domestic and industrial wastewaters of Haarlem and five smaller nearby

communities, in total 160.000 P.E. (table 4.1). The COD and N removal is accomplished in a biological system which consists of four parallel activated sludge lines, while P removal takes place in a combined biological-chemical side-stream process where the side-stream sludge from all four activated sludge lines is treated (figure 4.2).

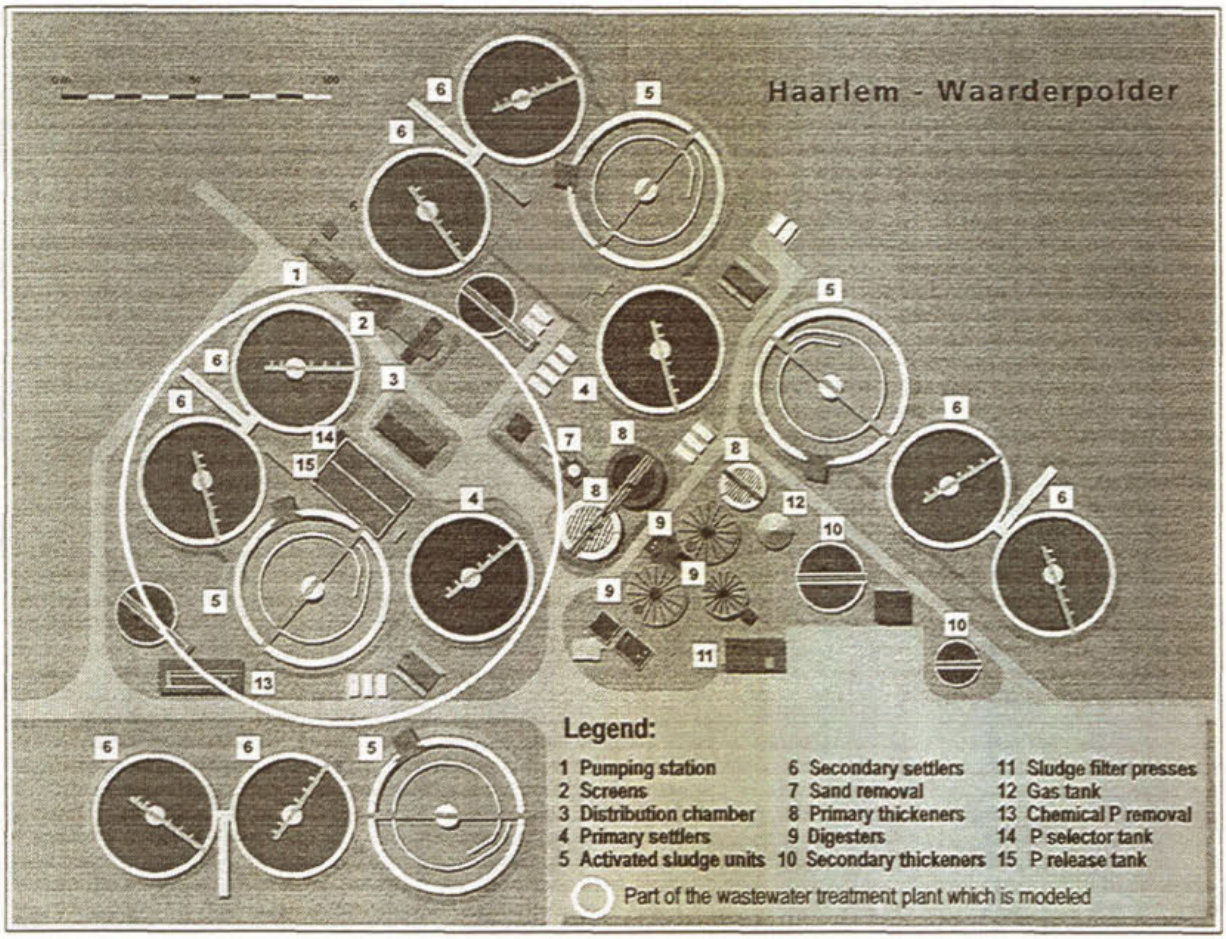


Figure 4.1. Layout of the wwtp Haarlem Waarderpolder.

Following conventional primary treatment, settled sewage is introduced in four parallel activated sludge units, each consisting of three concentric compartments, namely, a central compartment (non-bulking selector), an inner non-aerated ring (denitrification tank), and an outer, aerated ring (nitrification tank). A combination of diffused aeration, agitation by propellers and a high internal recirculation, makes the activated sludge in these units well mixed (except plug-flow-type central compartment). The activated sludge leaves the nitrification ring via a weir prior to splitting into two secondary settlers. After settling, the purified sewage is discharged into the nearby recipient.

A large fraction of the settled activated sludge (secondary sludge) is returned to the non-bulking selector and the denitrification ring to maintain the desired biomass content (currently 6 g MLSS/L). The remainder (side-stream sludge) is pumped to two compartmented agitated anaerobic tanks, called the P selector and the P release tank. They serve all four activated sludge units and have an identical structure. The source of volatile fatty acids (VFA) needed for P release by the phosphate accumulating organisms (PAO) in the P selector tank is the supernatant of the primary thickener, while in the P release tank, both concentrated acetic acid (HAc) and

Table 4.1. Designed and recorded data of the wwtp Haarlem Waarderpolder

Load	Unit	Designed 1994	Recorded 1996	Recorded 1997
Plant loading	P.E.	160000	109000	109100
Hydraulic loading				
❑ Average flow	m^3/h		1375	1333
❑ Dry weather flow	m^3/h	2175		
❑ Storm weather flow	m^3/h	7000		
Sewage strength				
❑ BOD (54 g BOD/P.E.d)	kg BOD/day	8640	3907	5082
❑ TKN (12 g N/P.E.d)	kg N/day	1920	1437	1436
❑ P_{total} (1.8 g P/P.E.d)	kg P/day	288	227	203
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

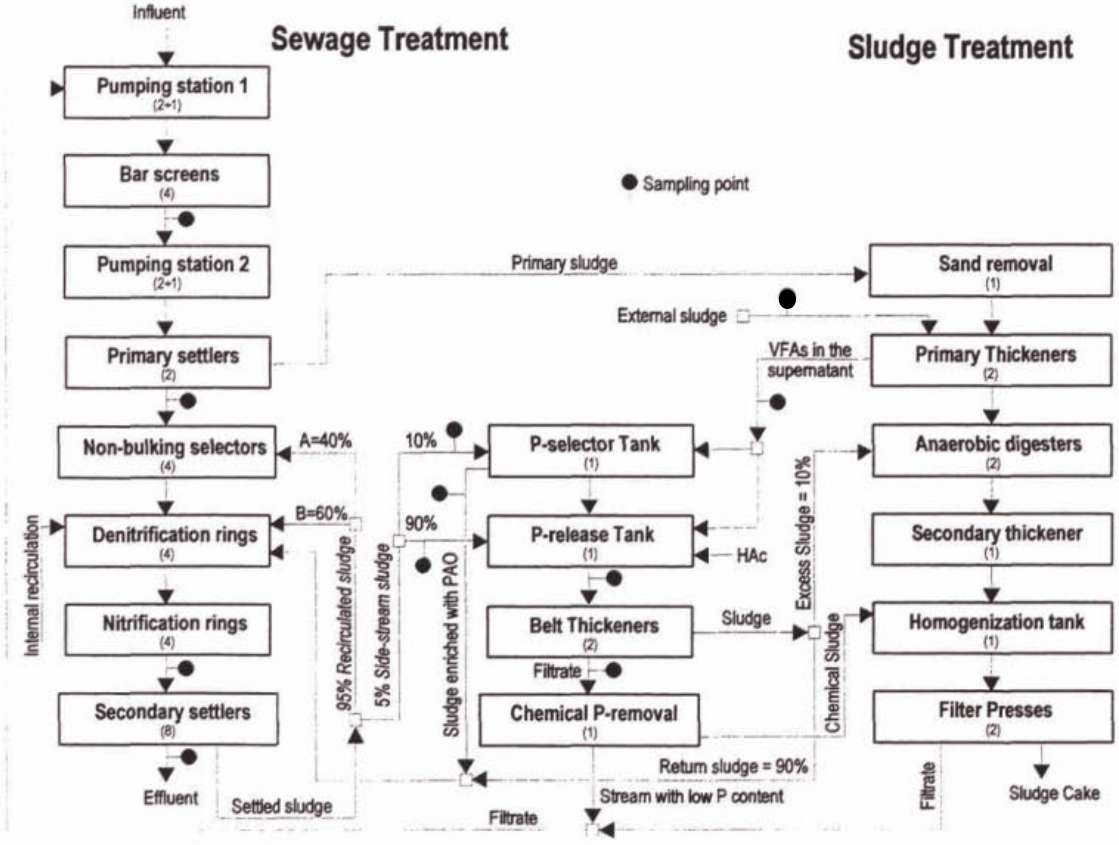


Figure 4.2. Simplified block process scheme of the wwtp Haarlem Waarderpolder (numbers in brackets indicate the number of process units).

supernatant of the primary thickener are added to the sludge. 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. 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 P release and a high P concentration in 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 most of the thickened sludge is returned to the denitrification ring, the remaining part (excess sludge) is wasted. The sludge wasting rate results in a total sludge retention time (SRT) in the system of 85 days. Phosphate-rich filtrate from belt thickeners is further treated by addition of lime to precipitate P from the filtrate.

Influent characterization

There are two influent flows to the section of the wwtp Haarlem Waarderpolder which needed to be characterized: (1) the settled sewage and, (2) the supernatant of the primary thickeners. The standard Dutch procedure (Roeleveld and Kruit, 1998) was used for characterization (see also appendix 2 and table 4.2).

Batch tests

Two double jacketed laboratory fermenters (each 1.5 L) with automated operation, control and monitoring were used in all batch tests (Brdjanovic et al. 1997b). The batch tests were performed at controlled temperature of 20 °C and pH of 7.0±0.1 in on-site lab using fresh return activated sludge. Following batch tests were performed (minimum three times) to determine: (a) anaerobic P-release rate, (b) aerobic P-uptake rate, (c) anoxic P-uptake rate, (d) fraction of denitrifying activity of PAOs, (e) nitrification rate, (f) denitrification rate and, (g) endogenous P-release rate. The description of the experimental procedure can be found elsewhere (Kuba et al., 1996b, Wachtmeister et al., 1997, Brdjanovic et al, 1997b and Brdjanovic et al., in press).

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 sewage temperature of around 13 °C). The on-site sampling locations are shown in figure 4.2. For influent characterization the following parameters were determined: COD_{total}, $COD_{filtered}$, $BOD_{5,total}$, PO_4, VFA (acetate, propionate, butyrate), NH_4, NO_3, pH, DO and temperature, while PHB (poly-hydroxy-butyrate), PHV (poly-hydrohy-valerate), glycogen, MLSS and MLVSS (mixed liquor volatile suspended solids) were determined for sludge characterization.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. (1997b), and in the Dutch guidelines for sewage and sludge characterization (Roeleveld and Kruit, 1998). The information obtained through the sampling program were combined with the data routinely collected (in weekly intervals) by the staff of the plant (see appendix 3). 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 the pumps at the plant.

Table 4.2. Influent characterization according to Dutch guidelines and combined ASM no.2 and Delft bio-P model

Influent characteristics required by the combined ASM2 and Delft BPR model			**Wastewater characteristics at WWTP Haarlem Waarderpolder on 3-10 April 1998 (T=12.6°C and pH 7.6)**		**Equations for determination of the influent characteristics (Roeleveld and Kruit, 1998)**	**Parameters needed to be measured for the influent characterization (Roeleveld and Kruit, 1998)**		
Symbol	Name	Unit	Settled sewage	Thick. overflow		Symbol	Name	Unit
	Soluble Components				**Soluble Components**			
S_{O2}	Oxygen (negative COD)	g COD/m³	2.1	0.05	$S_A = COD_{VFAs}$	$COD_{tot,inf}$	Total influent COD	g COD/m³
S_F	Readily biodegradable organics	g COD/m³	73	73	$S_I = 0.9 \cdot COD_{VFAs}$	$COD_{filt,inf}$	Influent COD filtered	g COD/m³
S_A	Volatile fatty acids	g COD/m³	32.4	49.9	$S_S = COD_{filt,inf} - S_I$	$COD_{filt,eff}$	Effluent COD filtered	g COD/m³
S_{NH}	Ammonium & ammonia nitrogen	g N/m³	46.5	55	$S_F = S_S - S_A$	COD_{VFAs}	COD from VFAs	g COD/m³
S_{NO3}	Nitrate & nitrite nitrogen	g N/m³	0.1	0	$S_{NH} = KjN - \Sigma(i^N \cdot X + i^N \cdot S)$	$BOD_{5,inf}$	influent BOD5	g BOD/m³
S_{PO4}	Inorganic soluble phosphorus	g P/m³	6.4	6	$S_{PO4} = P_{total} - \Sigma(i^N \cdot X + i^N \cdot S)$	$BOD_{5,eff}$	effluent BOD5	g BOD/m³
S_I	*Soluble inert organic matter*	g COD/m³	22	22		NH_4	Ammonia	g N/m³
S_{ALK}	Alkalinity	mol/m³	5	5	**Particulate Components**	PO_4	Phosphate	g P/m³
						k_{BOD}	BOD constant	1/d
	Particulate Components				$X_S = \alpha \cdot COD_{susp,inf}$	Y_{BOD}	Yield factor for BOD	
					$X_I = (1-\alpha) \cdot COD_{susp,inf}$			
					$X_H = 0$		Note:	
X_I	Particulate inert organic matter	g COD/m³	63	50	$X_{PHA} = 0$			
Xs	Slowly biodegradable substrate	g COD/m³	141	166	$X_{AUT} = 0.1$ to 1.0		In Dutch wastewater	
X_H	Active heterotrophic biomass	g COD/m³	0.01	0.01	$X_{PAO} = 0.1$ to 1.0		treatment practice the	
X_{PAO}	Phosphate acc. organisms	g COD/m³	0.01	0.01	$X_{PP} = 0$		majority of the above	
X_{PP}	Poly-phosphate	g P/m³	0.001	0.001			parameters are	
X_{PHA}	Poly-hydroxy-alkanoates	g COD/m³	0.001	0.001	**Other Components**		measured as a part of	
X_{GLY}	Glycogen	g COD/ m³	0.001	0.001			sampling programs	
X_{AUT}	Active autotrophic biomass	g COD/m³	0.01	0.01	$\alpha = [(BOD_{tot}/(1-Y_{H,BOD}) -$		routinely performed at	
X_{TSS}	Mixed liquor suspended solids	g MLSS/m³	153	162	$S_S]/COD_{susp,inf}$		their biological	
X_{MeOH}	Metal-hydroxides	g $Fe(OH)_4$/m³	0	0	$BOD_{tot} = BOD_5/(1-e^{-k \cdot 5})$		nutrient removal	
X_{MeP}	Metal-phosphate	g $FePO_4$/m³	0	0	$COD_{susp,inf} = COD_{tot,inf} - COD_{filt,inf}$		plants.	
Q	Flow	m³/day	5760	5520				

Modeling tools

The model used is given in appendix 4 (for more detailed description the reader is referred to Van Veldhuizen et al., accepted). Simulations were performed in SIMBA3.2+®. Simulations of plant operation were carried out (using average concentrations) 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 4.1). All plant simulations were performed using a 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 at 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 not possible to take into account different pH levels in the plant in one simulation.

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: (a) simulation of the treatment plant operation (both liquid phase and biomass) and model calibration based on measured data (current situation), (b) simulation of the batch tests using sludge properties as predicted in the previous step, (c) simulation of the performance of the treatment plant using feedback information from the batch test and their simulation, and (d) simulation of different alternative system configurations.

RESULTS

Set-up of the plant model

An overview of the results of influent characterization procedure is given in table 4.2. 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 present case, figure 4.3) made in SIMBA®. More information about this scheme is given in table 4.3.

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 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 return sludge to the measured level, 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.

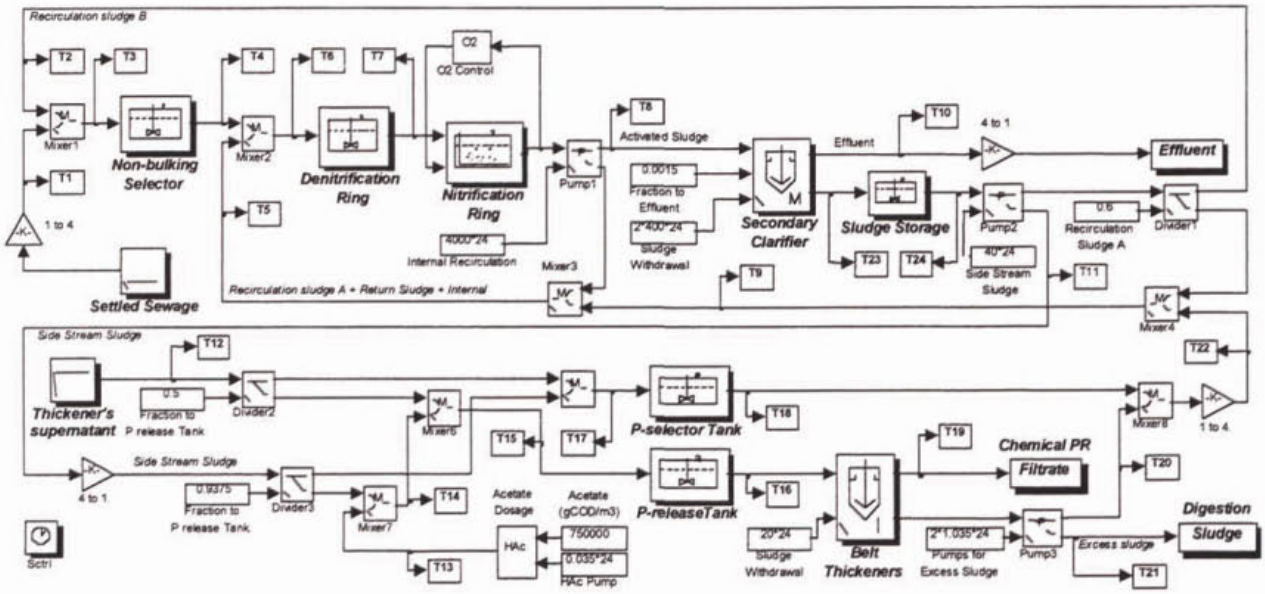


Figure 4.3. Hydraulic scheme of the wwtp Haarlem Waarderpolder.

Table 4.3. Major components of the model scheme for the wwtp Haarlem Waarderpolder.

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

Model calibration

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. Detailed sampling

revealed that the daily variation in concentrations was marginal. 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. (in press).

For the initial simulation of the plant performance the default set of parameters was used (Van Veldhuizen et al., submitted). The first step was to calibrate the SRT and the activated sludge concentration (MLVSS). The modeled biomass concentration in the activated sludge unit was adjusted to the observed level of around 4.5 g MLVSS/m^3 by varying the sludge wasting rate and taking into account the amount of biomass leaving the plant via effluent. The modeled SRT was 85 days. The estimated SRT from P-balance indicated an SRT of 80 days.

Following calibration of the SRT and activated sludge concentration, the ammonium and nitrate effluent concentration was calibrated. 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 mgO_2/L, reasonable results were achieved (2.4 $mgNH_4$-N/L and 5.3 mg NO_3-N/L versus 2.5± 0.5 $mgNH_4$-N/L and 4.6 ± 0.9 mg NO_3-N/L in reality as average for April '97), hence there was no need for further calibration for nitrification and denitrification. The aim was to predict the observed data within the uncertainty of the measurements themselves.

The P levels in the effluent of the wwtp and P release and selection tank were predicted well. The acetate concentration was however not correct in P selector and P release tanks, due to the likelihood (indicated by measurements and by financial administration) that the actual acetate dosage rate was higher than told by the plant operator. However, even with an increased acetate dosage rate predicted acetate concentration at the end of P release tank was lower than measured (data not shown). In addition, predicted acetate concentration was higher at the end than at the beginning in both tanks. Therefore, to further improve prediction of acetate levels in a side stream process, the maximal fermentation rate q_{fe} was decreased from 3 (default) to 1.0 gCOD/gCOD.d. Using the later value, the sum of the differences between predicted and measured acetate concentration at the end of the P release and P selection tank becomes minimal.

Although the batch sludge experiments were primarily 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 η^P_{NO3} was changed from 0.50 to 0.80. This gave a good fit of the ratio of phosphate removed in the aerobic ring to phosphate removed in the anoxic ring. It is striking that with this 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 unrealistically high (2 g COD/g COD, results not shown). Therefore, in the second loop the k_{gly} was changed from 1.09 to 0.15 g COD/g COD.d to fit the predicted data to the normal values of glycogen (~0.4 g COD/g COD). Change in k_{gly} influences f_{gly} which further affects f_{pp} and f_{pha} due to back coupling between these parameters in the bio-P model. By decreasing k_{gly}, f_{gly} is reduced, and consequently X_{PAO} increased. Based on the simulation results it was judged that no further calibration is needed. The list of parameters adjusted in the calibration procedure is given in table 4.4.

Table 4.4. Model parameters which value was changed in the calibration procedure.

Parameter	Symbol	Unit	Value 1)	2)	3)
Maximal fermentation rate	q_{fe}	gCOD/gCOD.d	3		1
Reduction factor under anoxic conditions	η^{P}_{NO3}		0.5		0.8
Glycogen formation rate	k_{gly}	gCOD/gCOD.d	1.09	0.45	0.15 & 0.45

1) model default (Murnleitner et al., 1997), 2) Van Veldhiuzen et al. (accepted), 3) this study

Model evaluation

The model evaluation was based on a check of its capability to describe (1) the liquid phase concentrations at several points in the plant using static influent data (separate sampling showed that there was no diurnal variation in influent concentration) and (2) the sludge properties as determined in batch tests. For this purpose batch experiments were simulated in SIMBA® (figure 4.4a-f).

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 4.4a and appendix 5). 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 g P/g COD, similar to the value observed at the wwtp in P release tank (0.27 g P/g COD), but lower than the default value of the model (0.36 g P/g COD). 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. 1997b) and that in both cases the same, external, acetate source was used. The default phosphate/acetate ratio was determined in laboratory tests using enriched bio-P culture (Smolders et al, 1994b) and was confirmed in tests with 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 the extremely long SRT or that in wwtp Haarlem Waarderpolder glycogen accumulating non-poly-P organisms (GAO, Cech and Hartman, 1993; Liu et al., 1994) have accumulated due to oversupply of acetate. GAOs are capable of anaerobic utilization of organic substrates which are converted and stored as PHA (poly-hydroxy-alkanoates), while the energy and reduction equivalents are provided by only glycogen degradation without involvement of poly-phosphate (poly-P). 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. in press). 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 assume there is significant presence of GAOs in the system, do not model their metabolism, retain the default PO4/HAc ratio, and leave this issue for further research. The last option was adopted in this work. This results in a correct description of the PAO population, but the HAc uptake under anaerobic conditions will be underestimated. The remaining HAc will be taken up by the heterotrophs to which group the GAO could be considered to belong.

Aerobic and anoxic phosphate uptake batch tests

The P uptake tests (figure 4.4b 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. By comparison of P uptake rate under aerobic and anoxic conditions (2.2 and 1.7 mg P/g VSS.h, respectively) the fraction of denitrifying activity of PAOs was estimated to be 80%.

Nitrification batch tests

Nitrification process performance in the batch test (figure 4.4d) 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 discrepance: either the amount of nitrifyers (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.

In both cases, firstly the full-scale plant was simulated, then the batch test with the "new" biomass obtained from a full-scale plant simulation. 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 mgNH_4-N/L and 1.3 mgNO_3-N/L when the b_{AUT} was increased, and 25 mgNH_4-N/L and 1.7 mgNO_3-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.

Denitrification batch tests

The model did not predict well observed concentrations in this test (figure 4.4e). Especially it is noted that in the denitrification test a very large release of phosphate and a rapid consumption of acetate was observed. 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 the TCA cycle instead of glycogen is delivering reduction equivalents (Kuba et al. 1996b). In general, simultaneous presence of acetate and nitrate at sewage plants does not occur often. Although denitrification in sludge batch test was not described satisfactorily, the nitrate concentrations in the treatment plant were predicted well.

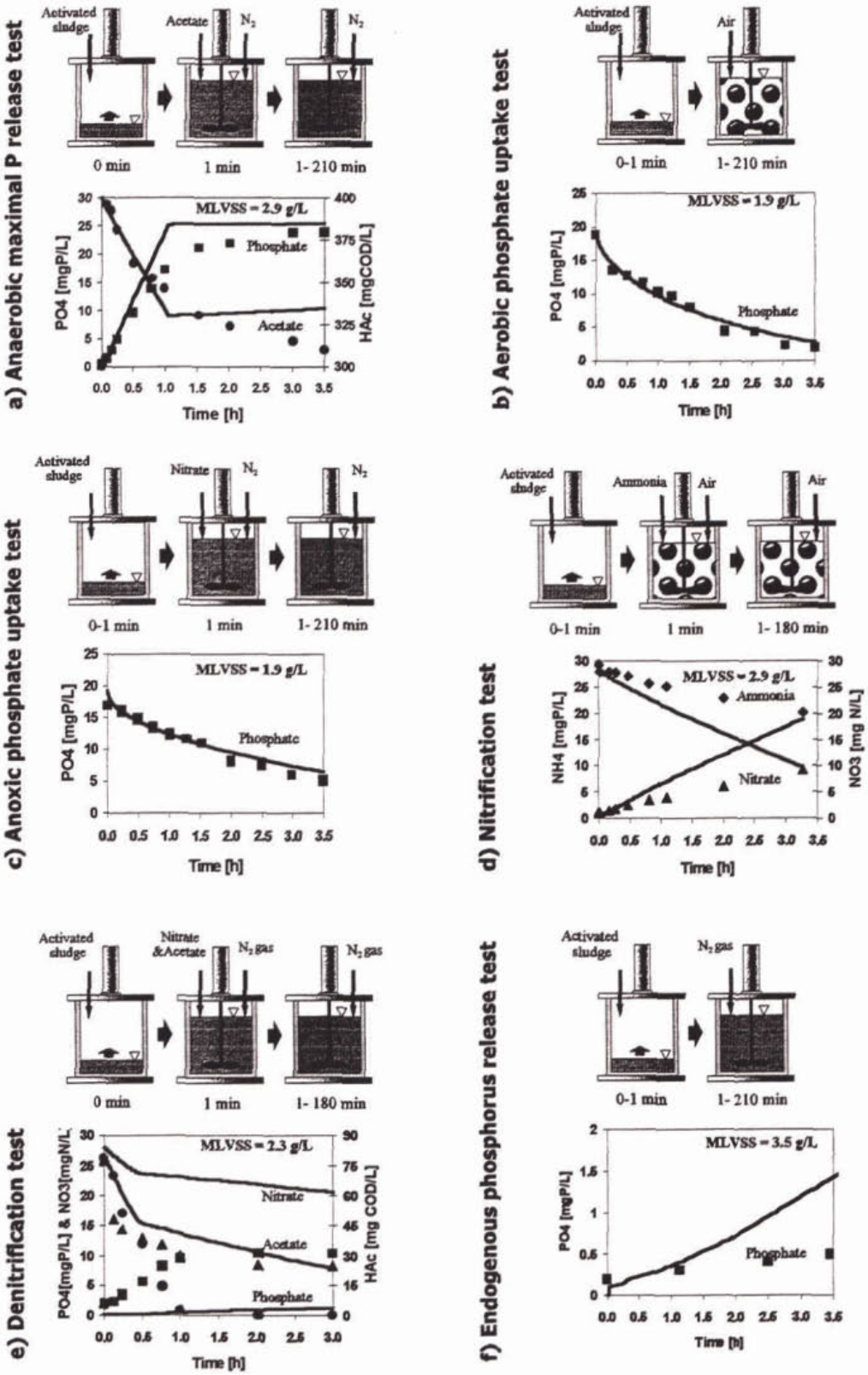


Figure 4.4. Concentration of phosphate (■) acetate (●), ammonia (▲), and nitrate (◆) in batch tests performed with settled activated sludge at temperature of 20°C and pH 7.0±0.1. The model prediction is represented with solid line. The experimental procedure is given schematically for each test.

Endogenous phosphorus release batch test

The results of this test (figure 4.4f) 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 ASM no. 2) leading to P release. We suggest that this decay of heterotrophs is overestimated. If endogenous respiration, as in ASM no.3, instead of decay would be used, probably the model and experiments would give the same results. Therefore it was decided not to change the parameters to fit this test.

It can be concluded that these batch tests delivered useful information on how the model performs. Some aspects like the maximal anaerobic P release, anoxic and aerobic P uptake were well described. Other aspects such as P release under denitrifying conditions and the endogenous P release pointed to places where the model could be improved. Finally, the nitrification process needed a better parameter estimation for correct fitting of full-scale and batch experiments. Since the default values however already gave good results in the full-scale model we decided to leave the values unchanged.

Alternative BPR process configurations

We used the model to evaluate different bio-P removal process schemes . Thereby we decided to keep the present physical configuration of the wwtp Harlem Waarderpolder model scheme (volume of tanks, influent flow rate etc.) the same. The model schemes of three alternative BPR process configurations, namely: (a) A/O, (b) modified UCT and (c) BCFS®, are described below (figure 4.5).

The calibrated model was used to evaluate BCFS® system, while in A/O and UCT systems k_{gly} was increased from 0.15 to 0.45 gCOD/gCOD.d to compensate for comparatively lower SRT (discussed later in detail). A lower SRT has a positive influence on the bio-P removal. Therefore, in those cases where the removal was not optimal the SRT was reduced to a value which would also under Dutch winter conditions resulted in a good nitrification (approximately 35 days or 15 days aerobic SRT). Selected simulation results are given in table 4.5 and complete set of results in appendix 6.

Alternatives 1a & 1b: A/O system configuration

The elements concerning side-stream BPR were removed. The rest of the plant which remained unchanged and was simulated as such (alternative 1a). Since alternative 1a presents, in essence, a non-optimized conventional configuration for COD and N removal, the predicted effluent quality strongly deteriorated in comparison with the present case. To achieve better process performance, including biological P removal, an anaerobic tank (larger than the already present non-bulking selector) is needed. The design criteria was that the acetate (from the thickener) entering this anaerobic tank is fully utilized. By increasing the volume of the non-bulking selector from 125 to 1500 m^3, a main-stream (A/O) BPR was obtained (alternative 1b) resulting in improved process efficiency. However, predicted effluent quality was still less good in comparison with the present case. The plant was simulated with SRTof 36 days; an increased SRT leads to even worse P-removal efficiency.

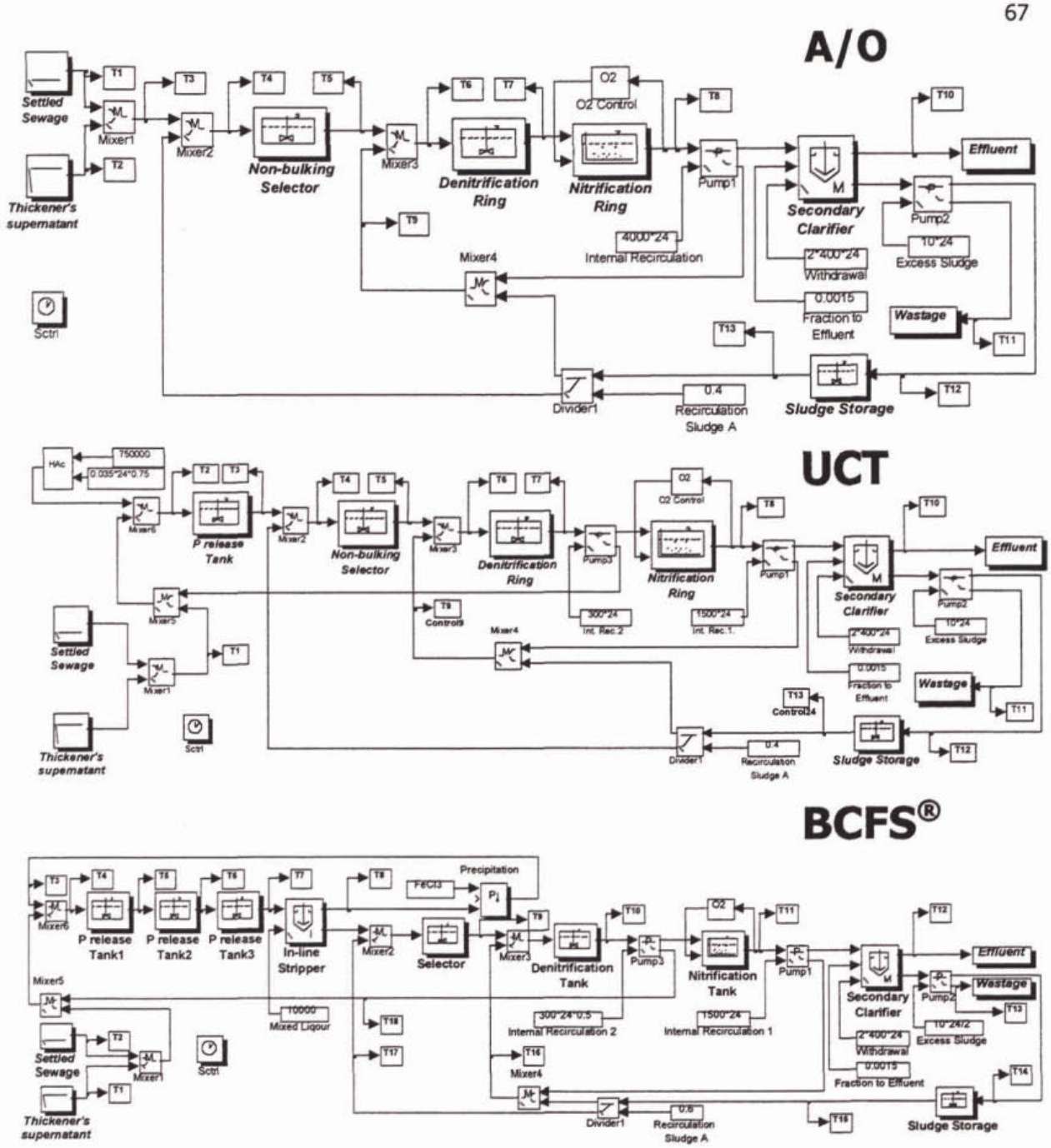


Figure 4.5 Hydraulic scheme of wwtp Haarlem Waarderpolder alternative configurations.

Alternatives 2a & 2b: Modified UCT system configuration

In alternative 2a an additional anaerobic P release tank (2000 m^3) was placed before the activated sludge unit in a modified UCT process configuration. Internal sludge recirculation - influent flow ratio between nitrification and denitrification rings was decreased from 1:13 (present case and alternative 1) to 1:5 and between denitrification ring and P release tank was 1:1. Operating sludge age was 37 days.

In this alternative, external addition of acetate to P release tank was not applied and the predicted effluent quality was similar to that from alternative 1b. This is likely because the nitrate concentrations in case 1b are already low. A good effluent quality was only achieved after external acetate dosing was introduced (alternative 2b). The amount of acetate needed to be added was sevenfold higher in comparison with optimized acetate addition in alternative 1b. The increased acetate need was predicted by the theoretical evaluation by Smolders et al. (1996), and shows the efficiency of a Phostrip® process with respect to HAc use.

Alternative 3a & 3b: BCFS® system configuration

Recently developed system configuration for chemical and biological P-removal with in-line 'P-stripping' and off-line precipitation (denoted as BCFS®-process, Van Loosdrecht et al., 1998) was applied. Side-stream BPR was removed, three anaerobic reactors in series (to simulate a plug-flow tank of 2000m^3) were introduced, followed by an in-line stripper (a baffled zone acting as clarifier). Phosphorus that can not be accumulated by the sludge (and removed via excess sludge) is removed by extracting a part (around 20% of the influent flow) of P-rich supernatant at in-line stripper. The amount of extracted flow equals the flow chemically treated in the original configuration (present case). After chemical treatment, P-free stream is returned to the beginning of the plant. Following in-line stripping, the reminder of the treatment was kept as in the present case; operating SRT was 90 days. The effluent quality was good without need for external acetate dosing (table 4.5).

DISCUSSION

Influent characterization

As far as the wwtp Haarlem Waarderpolder is concerned, the Dutch standard procedure for wastewater characterization (Roeleveld and Kruit, 1998) proved satisfactory for the model construction. This procedure was primarily developed for COD and N removal modeling; in this case it was used for COD, N and P modeling. The critical part of this procedure was the estimation of the coefficient k_{BOD}. The methodology for k_{BOD} determination is described elsewhere (Roeleveld and Kruit, 1998). 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 here. The impact of estimated k_{BOD} on the calculated SRT for wwtp Haarlem Waarderpolder is shown in figure 4.6. Even only a marginal change in this value (for example ±0.01) changes the calculated SRT by 5 days. It is a very important, but also very difficult task to have k_{BOD} determined at this accuracy. It is therefore expected that the k_{BOD} need to be adjusted in the model calibration for wwtp with long SRT due to the high sensitivity of the model to this parameter and to its relatively rough experimental estimation methodology (inaccuracy certainly higher then 0.01).

Table 4.5 Comparison of selected operational parameters of present and alternative process configurations of wwtp Haarlem Waarderpolder.

Parameter	Unit	Present Case Phostrip® process		Alternative 1: A/O process		Alternative 2: UCT process		Alternative 3: BCFS® process
		a	b	a	b	a	b	
Type of BPR process		Side stream	Side stream	Main stream	Main stream	Main stream	Main stream	Main stream
Acetate addition	m3/d	0.84 [1)]	0.36	none	none	none	2.5	none
SRT total	day	85	85	34	36	37	37	90
MLVSS aeration tank	mg/L	5927	5814	2595	2598	2564	3043	4574
Sludge Wasting	kg/d	2979	2927	3392	3348	3352	3964	2912
PO_4 effluent	mg P/L	0.3	0.8	7.5	8	2.5	0.7	0.9
NH_4 effluent	mg N/L	2.4	2.1	3.5	4.5	2.7	3.5	3.3
NO_3 effluent	mg N/L	5.3	5.9	7.8	2.6	9.3	5.6	5.8
Overall effluent quality		✓	✓	✗	✗	✗	✓	✓
Comments		Normal plant operation	Plant operation with reduced HAc		Non-bulking selector enlarged	Extra anaerobic tank added		Three anaerobic tanks in serial & in-line stripper

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 m^3/d (22 L/h) as 70% acetic acid

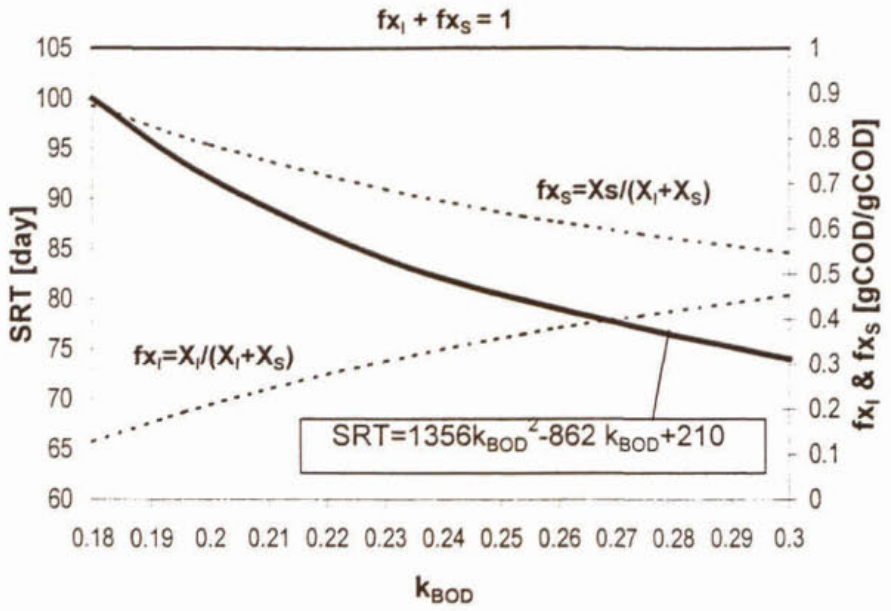


Figure 4.6. Influence of estimated k_{BOD} on calculated SRT and fraction of X_I and X_S of suspended COD.

Model calibration

The concentrations of the soluble components in the liquid phase in the wwtp were predicted well even without any calibration. This was true only to a lesser 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}).

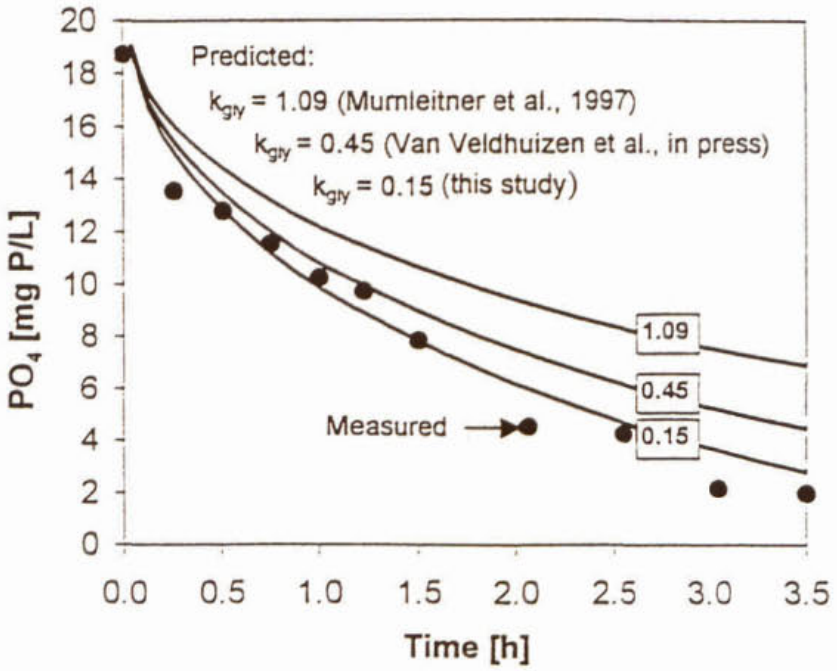


Figure 4.7. Influence of glycogen formation rate constant on aerobic phosphate uptake.

In the Delft bio-P model k_{gly} directly influences glycogen-active biomass ratio (f_{gly}). If the value of fgly 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. To check the influence of change in k_{gly} on predicted aerobic P uptake rate (and also anoxic P uptake rate) the 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 (figure 4.7), whereby the values for f_{gly} obtained for the high k_{gly} values are not realistic (Smolders et al., 1995b).

The sludge composition from above simulations was used to simulate aerobic and anoxic P uptake batch tests. Each batch test simulation was performed again with three different k_{gly} values. The results showed that this second variation in k_{gly} constant had no impact on predicted P uptake rates. However, a comparison (figure 4.7) 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 kgly either strongly depends on SRT or the kinetic expression for glycogen in the model is not fully correct. It is interesting to note that in the systems with longer SRT (table 4.6), the kgly needed to be lowered from 1.09 g COD/g COD.d at 20 days SRT (Murnleitner et al., 1997), to 0.45 at 50 days SRT (Van Veldhuizen et al., accepted) and 0.15 at 84 days SRT, to obtain a good prediction of aerobic and anoxic P uptake rate.

Table 4.6. An example of influence of variation in k_{gly} on fraction of storage materials in the biomass (source: simulation present case).

Parameter	Unit	k_{gly} [g COD/g COD.d]		
		0.15 This study SRT = 84 days	**0.45** Van Veldhuizen et al. (accepted) SRT = 50 days	**1.09** Murnleitner et al. (1997) 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

Operational aspects of BPR plants

According to project documentation and plant operational record (table 4.1), since its expansion in 1995, the wwtp Haarlem Waarderpolder has been under-loaded. Consequently, this resulted in a long SRT of 85 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 (simulated NO_3, NH_4 and P effluent concentrations were 3 mg N/L, 6.4 mg N/L and 0.4 mg P/L, respectively).

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 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 extra benefits in case the hypothesis of presence of significant amount of GAOs (see above) in this plant is true. If there would be less acetate available, the competition between PAOs and GAOs for substrate would intensify and, eventually, GAOs might be washed-out from the system. This will result in even further reduction in requirements for acetate in a side-stream process. Since this matter is highly speculative, more detailed research would be needed.

Due to low COD/P ratio relative poor P-removal was obtained in A/O and UCT process. In those systems insufficient amount of PAOs was produced to take up all phosphate. Larger anaerobic tank or shorter SRT would improve such situation. However, latter will certainly negatively affect nitrification process. It was shown that the processes with P-stripping are more efficient because part of P is chemically removed. For the strip processes little COD is needed for bio-P removal (10% of mainstream process, Smolders et al., 1996) leading to an efficient process, allowing a high SRT. This improves nitrification and reduces sludge production. Furthermore, a similarly good results were obtained by BCFS® process without need for HAc addition.

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 sewage 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 wether 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., in press).

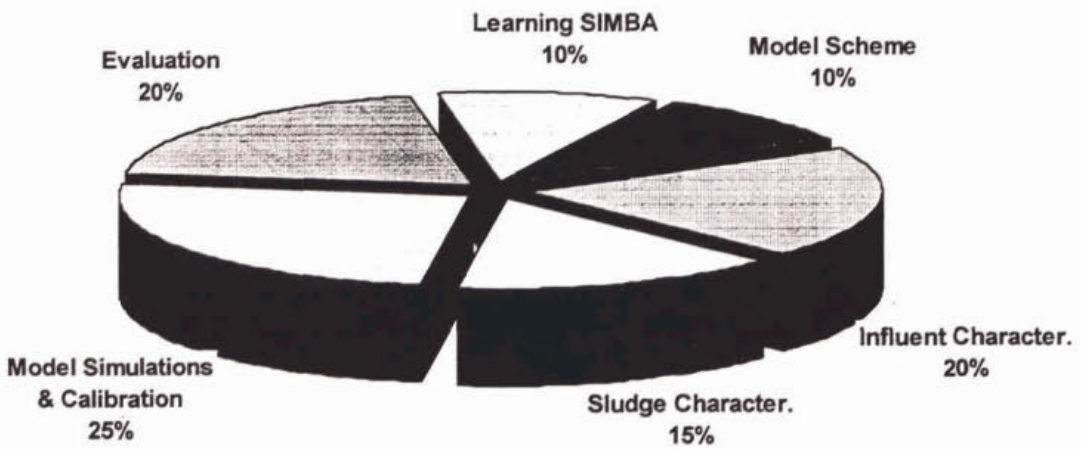


Figure 4.8. Time (in %) spent on various stages of modeling wwtp Haarlem Waarderpolder.

The time needed for the execution different phases of modeling the wwtp in Haarlem is given in figure 4.8. 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 4.8 can be used as indication only.

CONCLUSIONS

The combined ASM no.2 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. 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.

Chapter 5

Evaluation and outlook

ON THE STATUS OF MATHEMATICAL MODELING OF ACTIVATED SLUDGE SYSTEMS

An activated sludge model was used in this study to describe removal of organic matter, nitrogen and phosphorus in a full-scale 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 bio-P model) replaced the module for phosphorus removal of ASM no.2, and was combined with the equations for chemical oxygen demand (COD) and nitrogen (N) conversion of the ASM no.2.

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 wwtp. 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 bio-P model were determined using an enriched culture with PAOs grown on acetate in a lab-scale SBR.

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 standardised influent characterisation procedure (Roeleveld and Kruit, 1998) proved adequate for characterisation 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 ASM no.2 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 ASM no.2 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 bio-P 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 ASM no.2 by maintenance (as in Delft bio-P model) or endogenous respiration (as in ASM no.3).

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 know. Separate activated sludge batch tests using, in one case settled sewage (containing VFAs) and, in another acetate, could bring more information on this matter by comparing HAc concentrations at different pH.

The Delft bio-P 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 bio-P 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 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 bio-P 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.

ON THE STATUS OF THE ESSAYS

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. 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 report) 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 report 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. 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 report 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 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. submitted). 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), 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. More details on these bio-assays are given in appendix 1.

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 flourescent 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. 1999), 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. Obvious 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 then 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.

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 ASM no.2 and Delft bio-P 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 report 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 ASM no.4 may appear to describe COD, N and P removal for aerobic and anoxic conditions, possibly based on combination of ASM no.3 and a modified Delft bio-P 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 ASM no.1) prior to any further serious changes in the model take place.

Appendix 1

Methods for sludge characterization in BPR systems

Introduction

The ability of microorganisms to store reserve materials (like PHA, glycogen and poly-P) plays an important role in the process of BPR (Van Loosdrecht et al., 1997a). For the purposes of process evaluation and mathematical modeling of BPR it is needed to have a) an accurate measurement of the storage materials involved in the processes b) reliable value of the aerobic and anoxic ATP/$NADH_2$ ratio ($\delta_{aerobic}$ and δ_{anoxic}) and c) information of the proportion of bacterial populations in activated sludge important for BPR such as, phosphorus accumulating organisms (PAOs), denitrifying phosphorus accumulating organisms (DPAOs) and glycogen accumulating (non-poly-P) organisms (GAOs).

In this appendix several techniques and methods for sludge characterization which were recently developed and used in our ongoing research on BPR are described: (i) chemical measurement of the PHA content of the biomass, (ii) bioassay for glycogen determination in BPR systems, (iii) determination of the fraction of denitrifying PAOs in P-removing sludge, (iv) determination of the aerobic and anoxic ATP/$NADH_2$ ratio (δ value) in BPR systems, and (v) determination of the GAOs/PAOs ratio in activated sludge.

The first method (PHA analysis) is rather straightforward, tested, verified and routinely used in our laboratories. The other four methods (bioassays) consist of a calculation procedure based on the results of the batch tests. These methods are also tested and verified and already applied in several studies except the fifth method which is only proposed here.

Determination of the PHA content of the biomass

The hydroxyalkanoates are hydrolyzed in propanol/dichloroethane with hydrochloric acid. The monomer is esterified to n-propyl ester under these circumstances. The advantage of this method compared to sulfuric acid hydrolysis is that the ester remains stable under these reaction circumstances and will not be oxidized. After extraction with water a solution in dichloroethane remains. This solution is injected into a Gas Chromatograph (GC) and a complete separation of δ-hydroxyvaleric acid and γ-oxovaleric acid is obtained. Benzoic acid is used as internal standard.

Following reagents are used in this technique: Poly-β-hydroxybutyric acid (Sigma®); Benzoic acid, solution 20 mg/L in 1-propanol (internal standard solution); Hydrochloric acid (36%)/1-propanol [1:4]; Dichloroethane p.a.; Sodium sulphate anhydrous.

An activated sludge sample is centrifuged or filtered and the biomass is freeze dried. Lyophilized cell material is grinded and homogenized. Approximately 15 mg of sample is transferred to a glass tube; 50 µL of the internal standard solution is added; 1.5 mL of the hydrochloric acid /1-propanol mixture and 1.5 mL of dichloroethane is added; the tube is closed. The mixture is heated for 2 hours at 100°C and is occasionally stirred. After cooling-down 3 mL of water is added and the tube is well mixed using a Vortex® mixer. Then the tube is centrifuged for 5 min at 2000 rpm to obtain a complete separation of the two phases. 1 mL of the organic (bottom) layer is pipetted in an Eppendorf® cup, sodium sulphate is added and the sample is dried over night. After filtration (or centrifugation) the clear solution is transferred to a GC-vial.0.8 µL of solution is injected an a GC with a stabilwax (Restek®) column at 200°C with Flame Detection Ionisator (FDI) at 250°C.

Sigma polymer is used as a standard in the extraction procedure in amounts in between 0.5 and 2 mg. The other known compounds are not commercially available; so calibration is impossible. In order to obtain the ratio of response factors of δ-hydroxybutyric acid and the valeric acids the ratio of n-butyric acid and n-valeric acid is used. In other words the PHB calibration curve is used for PHV determination. The amount of PHA is calculated as a sum of PHB and PHV. The minimal measurable amount of PHB and PHV with satisfactory accuracy of±2% is 0.5 and 0.2% respectively (based on dry weight basis).

Bioassay for glycogen determination in BPR systems

The bioassay is based on the stoichiometric coupling between the anaerobic acetate uptake by the activated sludge and the related glycogen consumption (Smolders et al, 1994b, Brdjanovic et al., 1998b); for each C-mol of acetate taken up 0.5 C-mol of glycogen is consumed (1.125 mg glycogen/mg HAc-C, Smolders et al. 1994a). 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 an anaerobic batch test. However, the bioassay is only valid under the assumption that glycogen is exhausted due to acetate uptake. Here the result of the bioassay is compared with conventional method for glycogen determination by HPLC as glucose. The result obtained by this method presents the glycogen content of poly-P bacteria at the beginning of the batch test. An advantage of the method is that the result does not include glucose (originated from cell material), other carbohydrates or the glycogen content of bacteria other than poly-P bacteria.

An example of application of the bioassay for determination of the glycogen content of the sludge from an anaerobic-aerobic-settling sequencing batch reactor (SBR) operating in steady-state is given below (Brdjanovic et al., 1998b).

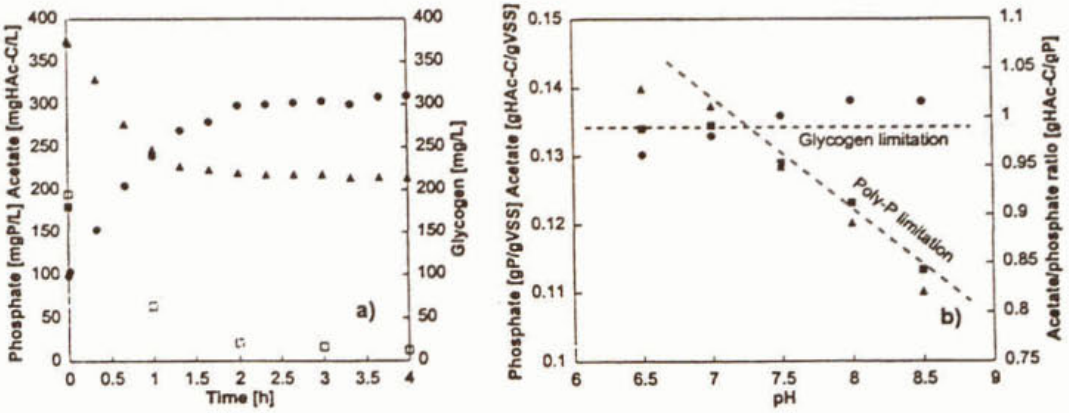


Figure.1. a) Acetate (▲), phosphate(●), glycogen (□), measured by conventional method) and glycogen (■, determined by bioassay) concentration during anaerobic batch test for glycogen determination using the sludge taken from the SBR at the aerobic phase, and b) specific phosphate release(●), specific acetate uptake (■), and acetate/phosphate ratio (▲) in anaerobic batch tests for determination of glycogen 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 (Brdjanovic et al., 1998b).

The sludge was taken from the SBR at the end of the aerobic phase and transferred to a double jacketed laboratory fermenter. The test was performed at controlled temperature 20°C and pH (7.0±0.1). Acetate (375 mgHAc-C/L as a concentration in the SBR) was added instantly at the beginning of the test under completely anaerobic conditions. During the test 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 test. The results of the test are presented in figure 1a.

An example of the calculation procedure to obtain the glycogen content of the biomass from the experimental observations is given in the table 1. In this procedure the terms "Glycogen (bioassay)" and "Glycogen (HPLC)" refer to the glycogen concentration determined using the bioassay and the conventional method (reference), respectively. Measured glycogen consumption of 264 mg glycogen/L or 156 mg glycogen/g active biomass ($G_{bioassay}$) 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 (as total glucose) was 6% higher than obtained by the bioassay, likely due to non-glycogen glucose, confirming the overestimating trend of conventional methods. In case of mixed culture this difference is larger due to the fact that sludge from full-scale sewage works is less enriched with bio-P bacteria. Therefore the concentration of glycogen relative to glucose becomes lower and larger deviations can be expected.

Table 1. Calculation procedure used in the bioassay for glycogen determination in BPR systems.

This example is based on the experimental data presented in figure 1a.	
Acetate conversion	
Acetate concentration at the start of the test ($_{Hacstart}$)	375 mgHAc-C/L
Acetate concentration at the end of the test ($_{Hacend}$)	140 mgHAc-C/L
Total HAc uptake ($HAc_{total}=HAc_{start}-HAc_{end}$)	235 mgHAc-C/L
Glycogen conversion	
Glycogen/HAc ratio (fixed stoichiometry)	1.125 mg/mgHAc-C
Glycogen concentration determined by the bioassay ($G_{bioassay}=1.125 \cdot {}_{Hactotal}$)	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)

The anaerobic acetate uptake may not only be halted by glycogen limitation, as required by the proposed test, but also poly-P limitation. In order to distinguish the possible shift from glycogen to poly-P limitation for anaerobic acetate-uptake as a function of pH a new method was developed by Brdjanovic et al., 1998b. The acetate/glycogen ratio is independent of pH, but the acetate/phosphate ratio decreases with increase in pH (Smolders et al., 1994b). In this method sludge 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 was instantly added into each reactor at the same moment and the anaerobic phosphate release and acetate

uptake were monitored hourly over 5 hours. The results of the method are illustrated in figure 1b. The glycogen was limiting at pH≤7.0, a transition zone was observed in the range 7.0<pH<8.0 and poly-P became limiting at pH≥8.0. Taking into account that the bioassay was originally tested at pH 7.0, glycogen was confirmed as the limiting factor.

Bioassay for determination of the fraction of denitrifying PAOs

It has been shown that besides oxygen nitrate can serve as an electron acceptor in BPR process (i.e. Kuba et al., 1993). Full-scale sewage works designed for combined nutrient removal consist of anaerobic (P-release) aerobic (nitrification and P-uptake) and anoxic (denitrification and P-uptake) zones. The use of denitrifying P-removing bacteria was demonstrated in studies in lab-scale systems (Kuba et al., 1993) as well as at full-scale treatment plants (Kuba et al., 1997a, and Van Loosdrecht et al., 1997b).

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. A direct comparison of P-uptake process under aerobic and anoxic conditions can straightforwardly lead to calculation of the contribution of denitrifying P-removal relative to total P-removal.

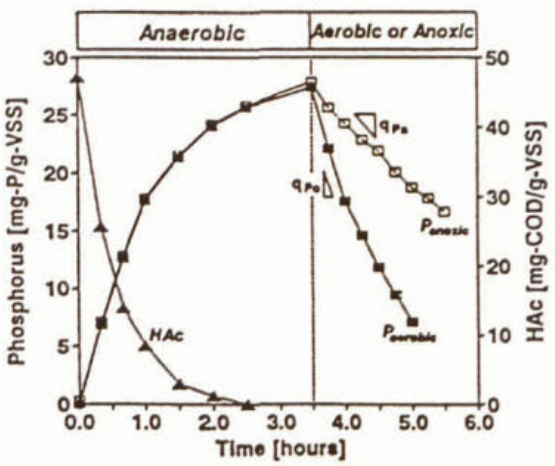


Figure 2. Phosphorus release and uptake under anaerobic-aerobic (■) and anaerobic-anoxic (□) conditions in activated sludge from a full-scale sewage works Acetic acid (▲) was added as an organic carbon source (Wachtmeister et al., 1997).

In the bioassay two batch tests are performed using the same sludge under aerobic and anoxic conditions. The sludge must be exposed firstly 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 the PHB does not limit the P-uptake process. After the acetate is taken up the sludge is split in two and one part is exposed to aerobic and another to anoxic conditions. In case that the surplus

acetate remains in solution after the anaerobic phase, the sludge should be washed and the phosphate should be manually added to the mixed liquor prior to splitting. Anoxic conditions are maintained by continuous addition of nitrate (instant addition of high amount of nitrate could cause undesirable build-up of nitrite in the system) and by the mixed liquor flushing by N2 gas. Oxygen and nitrate supply to the reactors should be in surplus. From the comparison of the P-uptake rates under aerobic (q_{Po}) and anoxic (q_{Pa}) conditions the relative proportion of denitrifying dephosphation activity (q_{Pa}/q_{Po}) in the phosphorus removing organisms can be calculated. An example of the result obtained by this procedure is given in figure 2 assuming that both organisms have identical q_P.

Method for determination of ATP/$NADH_2$ ratio (δ value)

Recently an integrated metabolic model for the aerobic and denitrifying biological phosphorus removal in activated sludge systems was developed (Murnleitner et al., 1997). This model is capable of describing the two known BPR processes, under aerobic and denitrifying conditions, with the same biochemical reactions and rate parameters, taking into account only the difference in electron acceptor (oxygen or nitrate). In this model the stoichiometry of the processes of the anaerobic and anoxic phases is identical except of the ATP/$NADH_2$ ratio (δ), [$\delta_{aerobic}$=1.8 mol/mol, Smolders et al., (1994b), and δ_{anoxic}=0.9 mol/mol, Murnleitner et al., (1997), for aerobic and denitrifying conditions in laboratory enriched sludge respectively]. Correct determination of the δ value is essential for the metabolic model. The experimental part of the methods for the determination of the δ value in practical activated sludge BPR systems is given below.

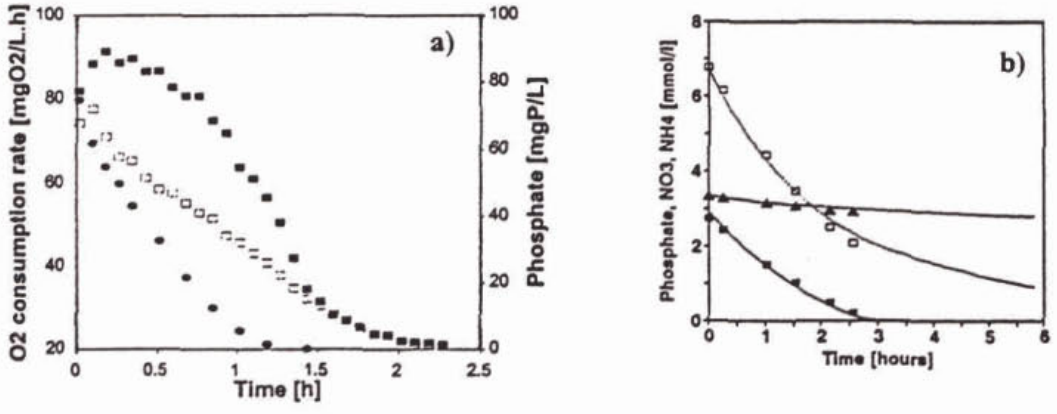


Figure 3. a) Oxygen utilization rate by the biomass in absence (□) and presence (■) of phosphate (●) during aerobic batch test (Smolders et al., 1994a), and b) Nitrate (□) ammonia (▲) and phosphate (■) concentration during batch test under denitrifying conditions (Kuba et al., 1996b).

In the oxidative phosphorylation ATP is produced from $NADH_2$. Under aerobic conditions the amount of ATP produced per electron pair, δ, so called phosphate/oxygen (P/O) ratio resembles the efficiency of oxidative phosphorylation. The P/O ratio can be established by studying the

effect of P-uptake and poly-P formation on the oxygen consumption in the batch test. The oxygen/phosphate yield was calculated from the oxygen consumption in the absence and presence of phosphate. Respirometry was used to establish the oxygen utilization rate (OUR) during the test. Based on the yield, the ATP/$NADH_2$ ratio ($\delta_{aerobic}$) was determined following the calculation method which is described in detail by Smolders et al. (1994a).

In a similar way, a value for ATP/$NADH_2$ ratio under anoxic conditions (δ_{anoxic}) can be determined by combining measured conversion rates of nitrate, biomass, PHB, and poly-P in batch tests with the enriched denitrifying dephosphatation sludge following the calculation procedure. Detailed description of the experimental procedure used for determination of $\delta_{aerobic}$ and δ_{anoxic} is space-demanding and reader is referred to Smolders et al. (1994a), Kuba et al. (1996b) and Brdjanovic et al. (1997b).

The method was applied in study of Smolders et al. (1994a), Kuba et al. (1996b), Brdjanovic et al. (1998b) and Murnleitner et al. (1997). An example of the results obtained from the experimental part of the method is given in figure 3.

Proposed method for determination of the GAOs/PAOs ratio in activated sludge

Beside PAOs there is another microbial population capable of anaerobic utilization of organic substrate in activated sludge processes: glycogen accumulating organisms (GAOs). 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 purposes 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 this ratio is proposed below.

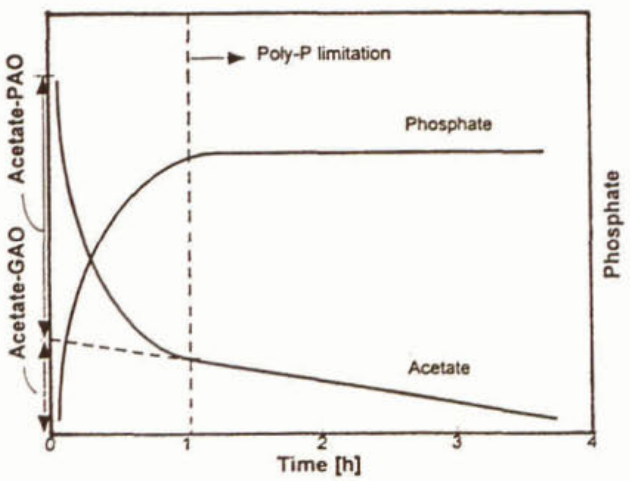


Figure 4. Determination of GAOs/PAOs ratio in BPR activated sludge based on acetate utilization during anaerobic batch test (fictive data resented).

The method is based on measurement of acetate consumption with an 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. 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, as illustrated in figure 4.

The method is based on the assumption that PAOs do not utilize acetate under anaerobic conditions when the internal poly-P storage is exhausted. Assumption was verified in a study of Brdjanovic et al. (1998c). A batch test was designed in which PAO-enriched sludge, after anaerobic release of poly-P in excess acetate at high pH (8-8.5, see also glycogen determination method), followed by aerobic incubation in a phosphate free medium to reestablish a high glycogen level, was subjected to acetate uptake under anaerobic conditions. The sludge could not take acetate up even though glycogen was not limiting. This result implies that PAOs can not utilize glycogen as the sole energy source for anaerobic acetate uptake, although GAOs should be able to do it. It appears that PAOs and GAOs have similar metabolic pathways but that they are regulated by different mechanisms.

This method is presumably most useful when applied on sludge consisting on large proportion of PAOs and GAOs. If the ratio of GAOs to PAOs is to low (say<10%) or high (>90%) the method will not be applicable. In addition to this the method can not be used to obtain the fraction of GAOs or PAOs in the biomass, but it is meant to be used to determine the approximate GAOs/PAOs ratio in the activated sludge systems.

Appendix 2

Influent characterization procedure according to Dutch guidelines

Characterization of the organic fractions of the influent

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

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

Soluble fraction of the COD consists of following components,

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

while the particulate fraction is represented by:

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

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 4.2). Therefore eq. 5.3 can be written as:

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

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

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

Readily biodegradable substrate S_S was introduced in the ASM no.1. In ASM no.2 it is replaced by:

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

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

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

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 ($COD_{filt,eff}$). The $COD_{filt,eff}$ 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 $BOD_{5,eff}$. The factor 1.5 is a conversion factor of BOD_5 to COD.

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

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

$$S_F = COD_{filt,inf} - 0.9 \cdot COD_{filt,eff} + 1.5\ BOD_{5,eff} - S_A \quad \ldots\ldots (5.9)$$

In ASM no.1, 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:

$$X_S = BCOD - S_S \quad (5.10)$$

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:

$$BOD_{tot} = BOD_t /(1 - e^{-k \cdot t}) \quad (5.11)$$

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, 1998). 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, 1998). The BCOD can be expressed as follows:

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

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

$$X_S = \{[BOD_t /(1 - e^{-k \cdot t})]/(1 - Y_{BOD})\} - S_S \quad (5.13)$$

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

$$X_I = COD_{part,inf} - X_S \quad (5.14)$$

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

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

Characterization of the N and P fractions in the influent

In ASM no.2 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_{NH4}) and nitrate and nitrite nitrogen (S_{NO3}).The phosphate compounds are represented also as soluble phosphorus (S_{PO4}). A small correction has to be made for an eventual poly-P fraction (X_{PP}) in the influent.

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

Control Point	Medium: sampling point	Unit	Liquid phase										Biomass							Others		
			mg COD /L	mg COD /L	mg COD /L	mg O_2/L	mg P/L	mg COD /L	mg COD /L	mg COD /L	mg N/L	mg N/L	g /L	g /L		mg COD /L	mg COD /L	mg COD /L	mg COD /L	grad C		mg O_2/L
		Parameter	COD_{total}	$COD_{filtered}$	$COD_{particulate}$	BOD_5	PO4	Acetate	Propionate	Butyrate	NH_4	NO_3	MLSS	MLVSS	MLVSS/MLSS	PHB	PHV	PHA	Glycogen	Temperature	pH	DO
	Raw sewage: after screens	8/4/97					6.7	45	9.1	2.5												
T1	Settled sewage: after the primary settler	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 the aerobic ring	8-9/4/97					0.5						6	4.7	0.76	55	10	65	190			1.6
T10	Effluent: after the secondary settler	3-4/4/97		31*		4*	0.9*				1.1*	2.3*										
		9-10/4/97					0.8				1.9	4										
	Primary sludge: outlet of primary settler	10/4/97					6.5															
	Schalkwijk sludge: outlet pipe	9/4/97					6.5	11.7														
T12	Supernatant: primary thickener	9/4/97	**360***	**144***	**216***	**157***	**6.0***	**50***			**55***	**0***										0.3
		10/4/97					6	37.4	9	3.7												
	Side stream sludge: P selector tank:	8/4/97																				
T17	section 1 (start)						5.7	33.2														
	section 2						7.1	32.1														
	section 3 (middle)						9.6	30.4					5	3.8	0.77	17	3	20	33		7.5	0.1
	section 4						10.5	29.5														
T18	section 5 (end)						11.1	29.2														
	Side stream sludge: P release tank:	8/4/97																				
T15	section 1 (start)						22.9	129														
	section 2						25.2	109														
	section 3 (middle)						26.6	107					1.1	0.9	0.78	77	17	94	156		6.5	0.1
	section 4						31.3	96.1														
T16	section 5 (end)						33.2	92.5														
T19	Filtrate: belt thickener	8/4/97					41.1															

Numbers printed in bold are used for influent characterization (table 5.5)
Numbers with * reffer to 24h composite samples, without *, to grab samples

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

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 *Plant Influent*	❑ Q	27580	m^3/d
	❑ pH	7.75	
	❑ COD_{tot}	495	$gCOD/m^3$
	❑ BOD_5	190	$gBOD/m^3$
	❑ Kj-N	56	gN/m^3
	❑ (NO_3+NO_2)-N	0.05	gN/m^3
	❑ P_{tot}	7.5	gP/m^3
	❑ TSS	220	$gTSS/m^3$
	❑ Load	109000	P.E.
❑ SETTLED SEWAGE *Biological Unit Influent*	❑ COD	265	$gCOD/m^3$
	❑ BOD	100	$gBOD/m^3$
	❑ Kj-N	48	gN/m^3
	❑ P_{otrho}	4.7	gP/m^3
	❑ P_{tot}	6.7	gP/m^3
	❑ TSS	100	$gTSS/m^3$
❑ ACTIVATED SLUDGE *Biological Unit Effluent*	❑ TSS	5200	$mgTSS/m^3$
	❑ SVI	60	mL/g
	❑ Inorganic matter	24	%TSS
❑ P-SELECTION TANK *Settled Sludge*	❑ TSS	860	$mgTSS/m^3$
	❑ P_{otrho}	13.3	gP/m^3
	❑ pH	7.5	
❑ P-RELEASE TANK *Settled Sludge*	❑ TSS	5540	$mgTSS/m^3$
	❑ P_{otrho}	41.2	gP/m^3
	❑ pH	6.8	
❑ EFFLUENT *Secondary Settlers Effluent*	❑ pH	8.05	
	❑ COD_{tot}	39	$gCOD/m^3$
	❑ COD_{filtr}	31	$gCOD/m^3$
	❑ BOD_5	4	$gBOD/m^3$
	❑ Kj-N	5.2	gN/m^3
	❑ NH_4-N	3.2	gN/m^3
	❑ (NO_3+NO_2)-N	8.3	gN/m^3
	❑ N_{tot}	13.5	gN/m^3
	❑ P_{tot}	2.3	gP/m^3
	❑ TSS	7	$gTSS/m^3$
	❑ Load	9.111	P.E.
❑ OVERALL TREATMENT EFFICIENCY	❑ COD_{tot}	92	%
	❑ BOD_5	98	%
	❑ Kj-N	91	%
	❑ N_{tot}	76	%
	❑ P_{tot}	69	%
	❑ TSS	97	%
	❑ Load	92	%
❑ PLANT LOADING	❑ COD_{tot}	13652	kgCOD/d
	❑ BOD_5	5240	kgBOD/d
	❑ Kj-N	1544	kgN/d
	❑ P_{tot}	207	kgP/d
	❑ TSS	5516	kgTSS/d

Appendix 4

Stoichiometry and kinetics of the combined ASM no.2 and Delft bio-P model

Stoichiometric matrix for dissolved and particulate components of ASM 2 & Delft BPRmodel

Process		S_{O2}	S_F	S_A	S_{NH4}	S_{NO3}	S_{PO4}	S_I	S_{ALK}	X_I	X_S	X_H	X_{PAO}	X_{PP}	X_{PHA}	X_{GLY}	X_{AUT}	X_{TSS}
1. Aerobic hydrolysis 2. Anoxic hydrolysis 3. Anaerobic hydrolysis			$1-f_{SI}$ $1-f_{SI}$ $1-f_{SI}$		$c_{1,n}$ $c_{1,n}$ $c_{1,n}$		$c_{1,p}$ $c_{1,p}$ $c_{1,p}$	f_{SI} f_{SI} f_{SI}	$c_{1,a}$ $c_{1,a}$ $c_{1,a}$		-1 -1 -1							$c_{1,i}$ $c_{1,i}$ $c_{1,i}$
HETEROTROPHIC ORGANISMS: X_H																		
4. Growth on S_F		$1-1/Y_H$	$-\frac{1}{Y_H}$		$c_{4,n}$		$c_{4,p}$		$c_{4,a}$			1						$c_{4,i}$
5. Growth on S_A		$1-1/Y_H$		$-1/Y_H$	$c_{5,n}$		$c_{5,p}$		$c_{5,a}$			1						$c_{5,i}$
6. Denitrification with S_F			$1/\tilde{Y}_H$		$c_{6,n}$	$-(1-Y_H)/2.86\ Y_H$	$c_{6,p}$		$c_{6,a}$			1						$c_{6,i}$
7. Denitrification with S_A				$-1/Y_H$	$c_{7,n}$	$-(1-Y_H)/2.86\ Y_H$	$c_{7,p}$		$c_{7,a}$			1						$c_{7,i}$
8. Fermentation			-1	1	$c_{8,n}$		$c_{8,p}$		$c_{8,a}$									
9. Lysis					$c_{9,n}$		$c_{9,p}$		$c_{9,a}$	f_{XIH}	$1-f_{XIH}$	-1						$c_{9,i}$
PHOSPHORUS ACCUMULATING ORGANISMS: X_{PAO}																		
Anaerobic	10. Storage of PHA			-1			Y_{PO4}		$c_{10,a}$					$-Y_{PO4}$	Y_{PHA}	$-Y_{GLY}$		$c_{10,i}$
	11. Maintenance						1		$c_{11,a}$					-1				$c_{11,i}$
Aerobic	12. Lysis of PHA	$1/Y_{PHA}^{O}-1$			$c_{12,n}$		$c_{12,p}$		$c_{12,a}$				$1/Y_{PHA}^{O}$		-1			$c_{12,i}$
	13. Storage of poly-P	$-1/Y_{PP}^{O}$			$c_{13,n}$		$c_{13,p}$		$c_{13,a}$				$-1/Y_{PP}^{O}$	1				$c_{13,i}$
	14. Glycogen formation	$1-1/Y_{GLY}^{O}$			$c_{14,n}$		$c_{14,p}$		$c_{14,a}$				$-1/Y_{GLY}^{O}$			1		$c_{14,i}$
	15. Maintenance	- 1			$c_{15,n}$		$c_{15,p}$		$c_{15,a}$				$-m_{PAO}^{O}/\ m_{O2}$					$c_{15,i}$
Anoxic	16. Lysis of PHA				$c_{16,n}$	$1-Y_{PHA}^{NO}/2.86\ Y_{PHA}^{NO}$	$c_{16,p}$		$c_{16,a}$				$1/Y_{PHA}^{NO}$		-1			$c_{16,i}$
	17. Storage of poly-P				$c_{17,n}$	$-1/2.86\ Y_{PP}^{NO}$	$c_{17,p}$		$c_{17,a}$				$-1/Y_{PP}^{NO}$	1				$c_{17,i}$
	18. Glycogen formation				$c_{18,n}$	$-(1-Y_{GLY}^{NO})/2.86\ Y_{GLY}^{NO}$	$c_{18,p}$		$c_{18,a}$				$-1/Y_{GLY}^{NO}$			1		$c_{18,i}$
	19. Maintenance				$c_{19,n}$	-1/2.86	$c_{19,p}$		$c_{19,a}$				$-m_{PAO}^{NO}/\ m_{NO}$					$c_{19,i}$
NITRIFYING ORGANISMS: X_{AUT}																		
20. Growth		$-(4.57-Y_A)/Y_A$			$c_{20,n}$	$1/Y_A$	$c_{20,p}$		$c_{20,a}$								1	$c_{20,i}$
21. Lysis					$c_{21,n}$		$c_{21,p}$		$c_{21,a}$	f_{XIA}	$1-f_{XIA}$						-1	$c_{21,i}$

Stoichiometric parameters of the combined model (20°C)

Symbol	Definition	Value	Unit	Reference
i^N_{SI}	N content of inert soluble COD (S_I)	0.01	gN/gCOD	Gujer et al, 1995
i^N_{SF}	N content of soluble COD (S_F)	0.03	gN/gCOD	"
i^N_{XI}	N content of inert particulate COD (X_I)	0.03	gN/gCOD	"
i^N_{XS}	N content of particulate COD (X_S)	0.04	gN/gCOD	"
i^N_{BM}	N content of biomass (X_H, X_{PAO} end X_{AUT})	0.07	gN/gCOD	"
i^P_{SI}	P content of inert soluble COD (S_I)	0	gP/gCOD	"
i^P_{SF}	P content of soluble COD (S_F)	0.01	gP/gCOD	"
i^P_{XI}	P content of inert particulate COD (X_I)	0.01	gP/gCOD	"
i^P_{XS}	P content of particulate COD (X_S)	0.01	gP/gCOD	"
i^P_{BM}	P content of biomass (X_H, X_{PAO} end X_{AUT})	0.02	gP/gCOD	"
i^{TSS}_{XI}	Ratio of TSS to X_I	0.75	gTSS/gCOD	"
i^{TSS}_{XS}	Ratio of TSS to X_S	0.75	gTSS/gCOD	"
i^{TSS}_{BM}	Ratio of TSS to biomass (X_H, X_{PAO} end	0.9	gTSS/gCOD	"
f_{SI}	Fraction of S_I from hydrolysis	0	gCOD/gCOD	"
Y_H	Yield van heterotrophic biomass (X_H)	0.63	gTSS/gCOD	"
f_{XIH}	Fraction of inert COD from lysis	0.1	gTSS/gCOD	"
Y_{PO4}	Yield coefficient (PO4/HAc)	0.36	gP/gCOD	Smolders et al, 1994b
Y_{PHA}	Yield coefficient (PHA/HAc)	1.5	gCOD/gCOD	"
Y_{GLY}	Yield coefficient (glycogen/HAc)	0.5	gCOD/gCOD	"
δ	Amount of ATP produced per NADH	1.8	mol/mol	Murnleitner et al,1997
m_{ATP}	ATP consumption for maintenance	0.019	mol/mol	"
Y_{PHA}^{O}	Yield coefficient (PHA/biomass)	1.44	gCOD/gCOD	"
Y_{PP}^{O}	Yield coefficient (PP/biomass)	4.52	gP/gCOD	"
Y_{GLY}^{O}	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

Definition of stoichiometric coefficients in the matrix

❑ Stoichiometric coefficients for S_{NH4}

$c_{1,n} = i^N_{XS} - i^N_{SI} * f_{SI} - (1-f_{SI}) * i^N_{SF}$
$c_{4,n} = i^N_{SF}/Y_H - i^N_{BM}$
$c_{5,n} = -i^N_{BM}$
$c_{6,n} = i^N_{SF}/Y_H - i^N_{BM}$
$c_{7,n} = -i^N_{BM}$
$c_{8,n} = i^N_{SF}$
$c_{9,n} = i^N_{BM} - i^N_{XI} * f_{XIH} - (1-f_{XIH}) * i^N_{XS}$
$c_{12,n} = -i^N_{BM}/Y^O_{PHA}$
$c_{13,n} = i^N_{BM}/Y^O_{PP}$
$c_{14,n} = i^N_{BM}/Y^O_{GLY}$
$c_{15,n} = i^N_{BM} * m^O_{PAO}/m_{O2}$
$c_{16,n} = -i^N_{BM}/Y^{NO}_{PHA}$
$c_{17,n} = i^N_{BM}/Y^{NO}_{PP}$
$c_{18,n} = i^N_{BM}/Y_{GLY}$
$c_{19,n} = i^N_{BM} * m^{NO}_{PAO}/m_{NO3}$
$c_{20,n} = -i^N_{BM} - 1/Y_A$
$c_{21,n} = i^N_{BM} - i^N_{XI} * f_{XIA} - i^N_{XS} * (1-f_{XIA})$

❑ Stoichiometric coefficients for S_{PO4}

$c_{1,p} = i^P_{XS} - i^P_{SI} * f_{SI} - i^P_{SF} * (1-f_{SI})$
$c_{4,p} = i^P_{SF}/Y_H - i^P_{BM}$
$c_{5,p} = -i^P_{BM}$
$c_{6,p} = i^P_{SF}/Y_H - i^P_{BM}$
$c_{7,p} = -i^P_{BM}$
$c_{8,p} = i^P_{SF}$
$c_{9,p} = i^P_{BM} - i^P_{XI} * f_{XIH} - (1-f_{XIH}) * i^P_{XS}$
$c_{12,p} = -i^P_{BM}/Y^O_{PHA}$
$c_{13,p} = i^P_{BM}/Y^O_{PP} - 1$
$c_{14,p} = i^P_{BM}/Y^O_{GLY}$
$c_{15,p} = i^P_{BM} * m^O_{PAO}/m_{O2}$
$c_{16,p} = -i^P_{BM}/Y^{NO}_{PHA}$
$c_{17,p} = i^P_{BM}/Y^{NO}_{PP} - 1$
$c_{18,p} = i^P_{BM}/Y^{NO}_{GLY}$
$c_{19,p} = i^P_{BM} * m^{NO}_{PAO}/m_{NO3}$
$c_{20,p} = -iP_{BM}$
$c_{21,p} = i^P_{BM} - i^P_{XI} * f_{XIA} - i^P_{XS} * (1-f_{XIA})$

❑ Stoichiometric coefficients for S_{ALK}

$c_{1,a} = c_{1,n}/14 - c_{1,p} * (1.5/31);$
$c_{4,a} = c_{4,n}/14 - c_{4,p} * (1.5/31);$
$c_{5,a} = c_{5,n}/14 - c_{5,p} * (1.5/31);$
$c_{6,a} = c_{6,n}/14 - c_{6,p} * (1.5/31) + (1-Y_H)/(14*2.86*Y_H);$
$c_{7,a} = c_{7,n}/14 - c_{7,p} * (1.5/31) + (1-Y_H)/(14*2.86*Y_H);$
$c_{8,a} = c_{8,n}/14 - c_{8,p} * (1.5/31) - 1/64;$
$c_{9,a} = c_{9,n}/14 - c_{9,p} * (1.5/31)$
$c_{10,a} = -Y_{PO4} * (1.5/31) + 1/64$
$c_{11,a} = -1.5/31$
$c_{12,a} = c_{12,n}/14 - c_{12,p} * (1.5/31)$
$c_{13,a} = c_{13,n}/14 - c_{13,p} * (1.5/31)$
$c_{14,a} = c_{14,n}/14 - c_{14,p} * (1.5/31)$
$c_{15,a} = c_{15,n}/14 - c_{15,p} * (1.5/31)$
$c_{16,a} = c_{16,n}/14 - c_{16,p} * (1.5/31) + (1-Y^{NO}_{PHA})/(14*2.86*Y^{NO}_{PHA})$
$c_{17,a} = c_{17,n}/14 - c_{17,p} * (1.5/31) + (1/Y^{NO}_{PP})/(14*2.86*Y^{NO}_{PP})$
$c_{18,a} = c_{18,n}/14 - c_{18,p} * (1.5/31) + (1-Y^{NO}_{GLY})/(14*2.86*Y^{NO}_{GLY})$
$c_{19,a} = c_{19,n}/14 - c_{19,p} * (1.5/31) + (1/2.86)/14$
$c_{20,a} = c_{20,n}/14 - c_{20,p} * (1.5/31) + (1/Y_A)/14$
$c_{21,a} = c_{21,n}/14 - c_{21,p} * (1.5/31)$
$c_{22,a} = 1.5/31$
$c_{23,a} = -1.5/31$

❑ Stoichiometric coefficients for X_{TSS}

$c_{1,t} = -i^{TSS}_{XS}$
$c_{4,t} = i^{TSS}_{BM}$
$c_{5,t} = i^{TSS}_{BM}$
$c_{6,t} = i^{TSS}_{BM}$
$c_{7,t} = i^{TSS}_{BM}$
$c_{9,t} = i^{TSS}_{XI} * f_{XIH} + i^{TSS}_{XS} * (1-f_{XIH}) - i^{TSS}_{BM}$
$c_{10,t} = -3.23 * Y_{PO4} + 0.6 * Y_{PHA} - 0.84 * Y_{GLY}$
$c_{11,t} = -3.23$
$c_{12,t} = i^{TSS}_{BM}/Y^O_{PHA} - 0.6$
$c_{13,t} = -i^{TSS}_{BM}/Y^O_{PP} + 3.23$
$c_{14,t} = -i^{TSS}_{BM}/Y^O_{GLY} + 0.84$
$c_{15,t} = -i^{TSS}_{BM} * m^O_{PAO}/m_{O2}$
$c_{17,t} = i^{TSS}_{BM}/Y^{NO}_{PHA} - 0.6$
$c_{17,t} = -i^{TSS}_{BM}/Y^{NO}_{PP} + 3.23$
$c_{18,t} = -i^{TSS}_{BM}/Y^{NO}_{GLY} + 0.84$
$c_{19,t} = -i^{TSS}_{BM} * m^{NO}_{PAO}/m_{NO3}$
$c_{20,t} = i^{TSS}_{BM}$
$c_{21,t} = i^{TSS}_{XI} * f_{XIA} + i^{TSS}_{XS} * (1-f_{XIA}) - i^{TSS}_{BM}$

Kinetic expressions for combined ASM no.2 and Delft bio-P model

Process	Rate equation
1. Aerobic hydrolysis	$K_h \cdot M_{LO2} \cdot \frac{f_S}{K_{LX}+f_S} \cdot X_H$
2. Anoxic hydrolysis	$K_h \cdot \eta_{LNO3} \cdot I_{LO2} \cdot M_{LNO3} \cdot \frac{f_S}{K_{LX}+f_S} \cdot X_H$
3. Anaerobic hydrolysis	$K_h \cdot \eta_{fe} \cdot I_{LO2} \cdot I_{LNO3} \cdot \frac{f_S}{K_{LX}+f_S} \cdot X_H$
HETEROTROPHIC ORGANISMS: X_H	
4. Growth on S_F	$\mu_H \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_F}{S_A+S_F} \cdot M_{HNH4} \cdot M_{HPO4} \cdot M_{HALK} \cdot X_H$
5. Growth on S_A	$\mu_H \cdot \frac{S_{O2}}{K_{HO2}+S_{O2}} \cdot \frac{S_A}{K_{HA}+S_A} \cdot \frac{S_A}{S_A+S_F} \cdot M_{HNH4} \cdot M_{HPO4} \cdot M_{HALK} \cdot X_H$
6. Denitrification with S_F	$\mu_H \cdot \eta_{HNO3} \cdot I_{HO2} \cdot \frac{S_F}{K_F+S_F} \cdot \frac{S_F}{S_F+S_A} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot M_{HNH4} \cdot M_{HPO4} \cdot X_H$
7. Denitrification with S_A	$\mu_H \cdot \eta_{HNO3} \cdot I_{HO2} \cdot \frac{S_A}{K_{HA}+S_A} \cdot \frac{S_A}{S_F+S_A} \cdot \frac{S_{NO3}}{K_{HNO3}+S_{NO3}} \cdot M_{HNH4} \cdot M_{HPO4} \cdot X_H$
8. Fermentation	$q_{fe} \cdot I_{HO2} \cdot I_{HNO3} \cdot \frac{S_F}{K_{fe}+S_F} \cdot \frac{S_{ALK}}{K_{HALK}+S_{ALK}} \cdot X_H$
9. Lysis	$b_H \cdot X_H$

$f_S = X_S/X_H$ $\quad f_{PHA} = X_{PHA}/X_{PAO}$ $\quad f_{PP} = X_{PP}/X_{PAO}$ $\quad f_{GLY} = X_{GLY}/X_{PAO}$

The terms M and I 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 I_{LO2} turns the reaction off in presence of oxygen in process 2 :

$$I_{LO2} = \frac{K_{LO2}}{S_{O2}+K_{LO2}}$$

Process	Rate equation
PHOSPHORUS ACCUMULATING ORGANISMS: X_{PAO}	
10. Anaerobic storage of PHA	$q_S^{max} \cdot \frac{S_A}{K_{PA}+S_A} \cdot I_{PO2} \cdot I_{PNO3} \cdot M_{PGLY} \cdot M_{PPP} \cdot X_{PAO}$
11. Anaerobic Maintenance	$m_{AN} \cdot I_{PO2} \cdot I_{PNO3} \cdot M_{PPP} \cdot X_{PAO}$
12. Aerobic lysis of PHA	$k_{PHA} \cdot f_{PHA}^{2/3} \cdot \frac{S_{O2}}{S_{O2}+K_{PO2}} \cdot M_{PNH4} \cdot M_{PALK} \cdot M_{PPO4} \cdot X_{PAO}$
13. Aerobic storage of poly-P	$k_{PP} \cdot \frac{1}{f_{PP}} \cdot \frac{S_{O2}}{K_{PO2} \cdot g_{PP}+S_{O2}} \cdot \frac{S_{PO4}}{K_{PP}+S_{PO4}} \cdot X_{PAO}$
14. Aerobic glycogen formation	$k_{GLY} \cdot \frac{1}{f_{GLY}} \cdot f_{PHA}^{2/3} \cdot \frac{S_{O2}}{S_{O2}+K_{PO2}} \cdot X_{PAO}$
15. Aerobic maintenance	$m_{O2} \cdot \frac{S_{O2}}{S_{O2}+K_{PO2}} \cdot M_{PPHA} \cdot X_{PAO}$
16. Anoxic lysis of PHA	$k_{PHA} \cdot \eta_{PNO3} \cdot f_{PHA}^{2/3} \cdot \frac{S_{NO3}}{S_{NO3}+K_{PNO3}} \cdot I_{PO2} \cdot M_{PNH4} \cdot M_{PALK} \cdot M_{PPO4} \cdot X_{PAO}$
17. Anoxic storage of poly-P	$k_{PP} \cdot \eta_{PNO3} \cdot \frac{1}{f_{PP}} \cdot \frac{S_{NO3}}{K_{PNO3} \cdot g_{PP}+S_{NO3}} \cdot \frac{S_{PO4}}{K_{PP}+S_{PO4}} \cdot I_{PO2} \cdot X_{PAO}$
18. Anoxic glycogen formation	$k_{GLY} \cdot \eta_{PNO3} \cdot \frac{1}{f_{GLY}} \cdot f_{PHA}^{2/3} \cdot \frac{S_{NO3}}{S_{NO3}+K_{PNO3}} \cdot I_{PO2} \cdot X_{PAO}$
19. Anoxic maintenance	$m_{NO3} \cdot I_{PO2} \cdot \frac{S_{NO3}}{S_{NO3}+K_{PNO3}} \cdot M_{PPHA} \cdot X_{PAO}$
NITRIFYING ORGANISMS: X_{AUT}	
20. Growth	$\mu_{AUT} \cdot \frac{S_{O2}}{K_{NO2}+S_{O2}} \cdot \frac{S_{NH4}}{S_{NH4}+K_{NNH4}} \cdot M_{NPO4} \cdot M_{NALK} \cdot X_{AUT}$
21. Lysis	$b_{AUT} \cdot X_{AUT}$

Kinetic parameters of the combined model (20°C)

Symbol	Definition	Value	Unit	Reference
K_h	Hydrolysis constant	3	1/d	Gujer et al, 1995
η^L_{NO3}	Anoxic hydrolysis reduction factor	0.6		"
η_{fe}	Anaerobic hydrolysis reduction factor	0.1		"
K^L_{O2}	Saturation/inhibition coefficient for oxygen	0.2	gO_2/m^3	"
K^L_{NO3}	Saturation/inhibition coefficient for nitrate	0.5	gN/m^3	"
K^L_X	Saturation coefficient for particulate COD	0.1	gCOD/gCOD	"
μ_H	Maximal growth rate on substrate	6	1/d	"
q_{fe}	Maximal fermentation rate	1	gCOD/gCOD.d	This study
η^H_{NO3}	Reduction factor for denitrification	0.8		Gujer et al, 1995
b_H	Lysis rate constant	0.4	1/d	"
K^H_{O2}	Saturation/inhibition coefficient for oxygen	0.2	gO_2/m^3	"
K_F	Saturation coefficient for growth on S_F	4	$gCOD/m^3$	"
K_{fe}	Saturation coefficient for fermentation on S_F	20	$gCOD/m^3$	"
K^H_A	saturation coefficient for acetate	4	$gCOD/m^3$	"
K^H_{NO3}	Saturation/inhibition coefficient for nitrate	0.5	gN/m^3	"
K^H_{NH4}	Saturation coefficient for S_{NH4} as nutrient	0.05	gN/m^3	"
K^H_P	Saturation coefficient for S_{PO4} as nutrient	0.01	gP/m^3	"
K^H_{ALK}	Saturation coefficient for alkalinity	0.1	$molHCO_3/m^3$	"
q^s_{max}	Acetate consumption rate	9.67	gCOD/gCOD.d	Smolders et al, 1994b
m_{AN}	Anaerobic maintenance coefficient	0.05	gP/gCOD.d	"
m_{NO3}	Anoxic maintenance coefficient	0.02	gN/gCOD.d	Murnleitner et al, 1997
m_{O2}	Aerobic maintenance coefficient	0.06	gCOD/gCOD.d	"
m^{NO}_{PAO}	Biomass consumption for anoxic maintenance	0.06	gCOD/gCOD.d	"
m^{O}_{PAO}	Biomass consumption for aerobic maintenance	0.07	gCOD/gCOD.d	"
K^P_A	Saturation coefficient for acetate	4	$gCOD/m^3$	Gujer et al, 1995
K^P_{NO3}	Saturation coefficient for nitrate	1.4	gN/m^3	Murnleitner et al, 1997
K^P_{O2}	Saturation coefficient for oxygen	0.2	$gCOD/m^3$	Gujer et al, 1995
K^P_{ALK}	Saturation coefficient for alkalinity	0.1	$molHCO_3/m^3$	"
K^P_{NH4}	Saturation coefficient for ammonium	0.05	gN/m^3	"
K^P_{PO4}	Saturation coefficient for phosphate	3.1	gP/m^3	Murnleitner et al, 1997
K^P_{PHA}	Saturation coefficient for PHA	0.01	$gCOD/m^3$	Gujer et al, 1995
K^P_{GLY}	Saturation coefficient for glycogen	0.01	$gCOD/m^3$	"
K^P_{PP}	Saturation coefficient for poly-P	0.01	$gCOD/m^3$	"
g_{PP}	Nitrate sensitivity factor for poly-P formation	0.1		Murnleitner et al, 1997
k_{PHA}	PHA decay rate	7.55	gCOD/gCOD.d	"
k_{PP}	Poly-P formation rate	0.11	gP/gCOD.d	Van Veldhuizen et al., 1999
k_{GLY}	Glycogen formation rate	0.15	gCOD/gCOD.d	This study
η^P_{NO3}	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^N_{O2}	Saturation/inhibition coefficient for oxygen	0.5	gO_2/m^3	"
K^N_{NH4}	Saturation coefficient for S_{NH4}	1	gN/m^3	"
K^N_{ALK}	Saturation coefficient for alkalinity	0.5	$molHCO_3/m^3$	"
K^N_P	Saturation coefficient for S_{PO4}	0.01	gP/m^3	"

Appendix 5

Results of the simulation of the batch tests performed with the activated sludge from wwtp Haarlem Waarderpolder using SIMBA® and combined ASM no.2 and Delft bio-P model

No.	Parameter	Unit		Test 1			Test 2			Test 3			Test 4			Test 5			Test 6	
				Start	*End*		*Start*	*End*		*Start*	*End*		*Start*	*End*		*Start*	*End*		*Start*	*End*
				T1	T2		T1	T2		T1	T2		T1	T2		T1	T2		T1	T2
1	S_{O_2}	g COD/m³	a) Anaerobic maximal phosphate release test	0.0	0.0	b) Aerobic phosphate uptake test	7.0	8.6	c) Anoxic phosphate uptake test	0.0	0.0	d) Nitrification test	7.0	8.2	e) Denitrification test	0.0	0.0	f) Endogenous phosphate release test	0.0	0.0
2	S_F	g COD/m³		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	S_A	g COD/m³		400	334		9	0		9.0	0.0		0	0		80	23		0	0
4	S_{NH_4}	g N/m³		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	S_{NO_3}	g N/m³		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	S_{PO_4}	g P/m³		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	S_I	g COD/m³		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	S_{ALK}	mol/m³		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	X_I	g COD/m³		3225	3227		2049	2051		2049	2044		3226	3215		2501	2502		3910	3653
10	X_S	g COD/m³		31	44		27	6		27	13		31	7		24	14		37	48
11	X_H	g COD/m³		405	384		245	259		245	253		406	409		315	333		492	435
12	X_{PAO}	g COD/m³		167	167		106	112		106	115		167	163		129	127		202	189
13	X_{PP}	g P/m³		25	0		0	16		0	12		30	30		23	22		36	32
14	X_{PHA}	g COD/m³		49	152		109	87		109	80		49	47		38	49		60	56
15	X_{GLY}	g COD/m³		83	48		26	32		26	29		83	86		64	62		100	93
16	X_{AUT}	g COD/m³		49	47		30	30		30	29		49	52		38	37		59	54
17	X_{MeOH}	g $Fe(OH)_3$/m³		0	0		0	0		0	0		0	0		0	0		0	0
18	X_{MeP}	g $FePO_4$/m³		0	0		0	0		0	0		0	0		0	0		0	0
19	COD_{part}	g COD/m³		4009	4069		2593	2575		2593	2564		4009	3979		3108	3123		4860	4527
20	VSS_{total}	g VSS/m³		2926	2970		1892	1880		1892	1872		2927	2905		2269	2280		3547	3305
21	$MLSS_{total}$	g MLSS/m³		4009	4069		2628	2575		2628	2564		4065	4034		3066	3081		4820	4590

Appendix 6

Results of the simulation of the wwtp Haarlem Waarderpolder using SIMBA® and combined ASM no.2 and Delft bio-P model

Simulation results present case 0a: Phostrip-like system configuration																									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	S_{O_2}	g COD/m³	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	0.0
2	S_F	g COD/m³	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	0.4
3	S_A	g COD/m³	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	125.0	38.2	45.4	38.8	38.2	38.2	38.2	38.7	0.0	0.0
4	S_{NH_4}	g N/m³	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	25.3	47.3	2.4	3.4
5	S_{NO_3}	g N/m³	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	0.5
6	S_{PO_4}	g P/m³	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	38.8	14.9	0.3	0.4
7	S_I	g COD/m³	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	S_{ALK}	mol/m³	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	8.7
9	X_I	g COD/m³	63	6452	3569	3569	5122	4951	4952	4952	6511	10	6452	50	0	6450	3890	3892	632	633	0	48659	48659	7364	6447	6452
10	X_S	g COD/m³	141	61	97	97	49	54	52	45	79	0	61	166	0	61	103	111	156	157	0	1384	1384	329	59	61
11	X_H	g COD/m³	0	812	445	447	657	634	636	638	816	1	812	0	0	811	487	470	74	68	0	5881	5881	883	831	812
12	X_{PAO}	g COD/m³	0	334	183	183	265	256	256	256	336	1	334	0	0	333	200	199	30	30	0	2490	2490	375	334	334
13	X_{PP}	g P/m³	0	60	33	31	47	45	45	46	56	0	60	0	0	60	36	0	5	0	0	0	0	0	59	60
14	X_{PHA}	g COD/m³	0	98	54	63	82	80	79	77	119	0	98	0	0	98	59	218	9	34	0	2724	2724	411	100	98
15	X_{GLY}	g COD/m³	0	165	91	88	130	125	125	126	160	0	165	0	0	165	99	46	15	7	0	577	577	87	164	165
16	X_{AUT}	g COD/m³	0	97	53	53	78	75	75	76	98	0	97	0	0	97	58	58	9	9	0	723	723	109	99	97
17	X_{MeOH}	g $Fe(OH)_3$/m³	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	X_{MeP}	g $FePO_4$/m³	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	m³/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	19200
20	COD_{part}	g COD/m³	204	8019	4492	4499	6383	6176	6175	6171	8120	12	8019	216	0	8017	4897	4994	925	937	0	62436	62436	9557	8034	8019
21	VSS_{total}	g VSS/m³	149	5853	3279	3284	4659	4508	4507	4504	5927	9	5853	158	0	5852	3574	3645	675	684	0	45574	45574	6976	5864	5853
22	$MLSS_{total}$	g MLSS/m³	196	7701	4314	4321	6130	5931	5931	5927	7798	12	7701	207	0	7700	4703	4797	889	900	0	59966	59966	9178	7716	7701

Simulation results present case 0b: Phostrip-like system configuration																								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	S_{O_2}	g COD/m^3	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	S_F	g COD/m^3	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	S_A	g COD/m^3	32.4	0.0	14.6	9.4	0.2	1.2	0.2	0.0	1.5	0.0	0.0	49.9	750000	75.0	65.0	4.3	45.4	26.4	4.3	4.3	4.3	23.3	0.0	0.0
4	S_{NH_4}	g N/m^3	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	S_{NO_3}	g N/m^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	S_{PO_4}	g P/m^3	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	S_I	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	S_{ALK}	mol/m^3	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	X_I	g COD/m^3	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	X_S	g COD/m^3	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	X_H	g COD/m^3	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	X_{PAO}	g COD/m^3	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	X_{PP}	g P/m^3	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	X_{PHA}	g COD/m^3	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	X_{GLY}	g COD/m^3	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	X_{AUT}	g COD/m^3	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	X_{MeOH}	$Fe(OH)_3$/m	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	X_{MeP}	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	107712	121008	121008	25008	11712	5808	960	4800	0.36	3600	6000	6000	2640	2640	5520	430	50	768	19200	19200
20	COD_{part}	g COD/m^3	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}	g VSS/m^3	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	$MLSS_{total}$	g MLSS/m^3	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 alternative 1a: A/O system configuration												SRT = 34 days		
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	S_{O_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	S_F	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	S_A	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	S_{NH_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	S_{NO_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	S_{PO_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	S_I	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	S_{ALK}	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	X_I	g COD/m^3	63	50	61	1651	1651	1947	1948	1948	2000	4	2653	2653	2658
10	X_S	g COD/m^3	141	166	145	87	86	48	45	40	41	0	54	54	51
11	X_H	g COD/m^3	0	0	0	530	535	644	646	648	664	1	882	882	866
12	X_{PAO}	g COD/m^3	0	0	0	0	0	0	0	0	0	0	0	0	0
13	X_{PP}	g P/m^3	0	0	0	0	0	0	0	0	0	0	0	0	0
14	X_{PHA}	g COD/m^3	0	0	0	0	0	0	0	0	0	0	0	0	0
15	X_{GLY}	g COD/m^3	0	0	0	0	0	0	0	0	0	0	0	0	0
16	X_{AUT}	g COD/m^3	0	0	0	54	54	66	66	66	68	0	90	90	89
17	X_{MeOH}	$Fe(OH)_3$/m	0	0	0	0	0	0	0	0	0	0	0	0	0
18	X_{MeP}	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 alternative 1b: A/O system configuration												SRT = 37 days		
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	S_{O_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	S_F	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	S_A	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	S_{NH_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	S_{NO_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	S_{PO_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	S_I	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	S_{ALK}	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	X_I	g COD/m^3	63	50	61	1551	1552	1828	1828	1829	1877	4	2490	2490	2494
10	X_S	g COD/m^3	141	166	145	82	84	43	41	36	36	0	48	48	42
11	X_H	g COD/m^3	0	0	0	401	402	486	487	488	501	1	665	665	655
12	X_{PAO}	g COD/m^3	0	0	0	177	177	212	213	213	219	0	290	290	289
13	X_{PP}	g P/m^3	0	0	0	88	81	103	104	105	107	0	142	142	144
14	X_{PHA}	g COD/m^3	0	0	0	6	34	14	13	11	11	0	15	15	11
15	X_{GLY}	g COD/m^3	0	0	0	25	16	27	28	28	29	0	38	38	41
16	X_{AUT}	g COD/m^3	0	0	0	50	50	61	61	61	63	0	83	83	82
17	X_{MeOH}	$Fe(OH)_3$/m	0	0	0	0	0	0	0	0	0	0	0	0	0
18	X_{MeP}	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 alternative 2a: modified UCT system configuration SRT = 37 days

Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	S_{O_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	S_F	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	S_A	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	S_{NH_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	S_{NO_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	S_{PO_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	S_I	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	S_{ALK}	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	X_I	g COD/m^3	63	61	944	945	1628	1628	1826	1827	1828	4	1944	2489	2489
10	X_S	g COD/m^3	141	145	94	94	70	69	47	43	33	0	34	45	45
11	X_H	g COD/m^3	0	0	245	247	429	431	488	489	492	1	521	670	670
12	X_{PAO}	g COD/m^3	0	0	106	106	187	187	211	212	213	0	226	290	290
13	X_{PP}	g P/m^3	0	0	52	44	89	89	104	105	105	0	112	144	144
14	X_{PHA}	g COD/m^3	0	0	7	42	28	28	17	14	10	0	10	14	14
15	X_{GLY}	g COD/m^3	0	0	14	3	21	21	28	29	30	0	32	41	41
16	X_{AUT}	g COD/m^3	0	0	32	31	55	55	63	63	64	0	68	87	87
17	X_{MeOH}	$Fe(OH)_3$/m	0	0	0	0	0	0	0	0	0	0	0	0	0
18	X_{MeP}	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 alternative 2b: modified UCT system configuration SRT = 37 days

Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
No.	Parameter	Unit													
1	S_{O_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	S_F	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	S_A	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	S_{NH_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	S_{NO_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	S_{PO_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	S_I	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	S_{ALK}	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	X_I	g COD/m^3	61	928	929	1601	1601	1795	1796	1797	1911	4	2447	2447	2450
10	X_S	g COD/m^3	145	100	102	80	80	57	55	42	44	0	57	57	52
11	X_H	g COD/m^3	0	230	224	401	401	459	460	466	493	1	634	634	624
12	X_{PAO}	g COD/m^3	0	274	274	483	483	547	549	551	585	1	750	750	746
13	X_{PP}	g P/m^3	0	68	47	109	109	132	136	138	146	0	187	187	188
14	X_{PHA}	g COD/m^3	0	56	145	139	140	119	113	102	107	0	139	139	132
15	X_{GLY}	g COD/m^3	0	67	37	107	107	133	134	139	149	0	189	189	195
16	X_{AUT}	g COD/m^3	0	30	30	53	53	61	61	62	65	0	84	84	83
17	X_{MeOH}	$Fe(OH)_3$/m	0	0	0	0	0	0	0	0	0	0	0	0	0
18	X_{MeP}	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 alternative 3: BCFS system configuration															SRT=85 days				
Sampling point			T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18
No.	Parameter	Unit																		
1	S_{O_2}	g COD/m^3	0.1	2.1	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	1.9	1.9	1.9	1.9	0.0	1.6	0.0	0.0
2	S_F	g COD/m^3	73.0	73.0	29.7	35.9	29.7	27.3	25.4	25.4	11.9	0.6	0.5	0.5	0.5	0.5	0.3	0.4	0.3	0.6
3	S_A	g COD/m^3	49.9	32.4	3.3	16.1	3.3	0.8	0.4	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	S_{NH_4}	g N/m^3	55.0	46.5	28.5	28.4	28.5	28.6	28.8	28.8	17.9	8.8	3.3	3.3	3.3	3.3	4.2	3.5	4.2	8.8
5	S_{NO_3}	g N/m^3	0.0	0.1	0.1	0.7	0.1	0.0	0.0	0.0	0.3	1.5	5.8	5.8	5.8	5.8	1.5	5.9	1.5	1.5
6	S_{PO_4}	g P/m^3	0.0	6.4	8.8	3.5	8.8	11.2	12.7	12.7	7.4	2.3	0.9	0.9	0.9	0.9	0.6	1.0	0.6	2.3
7	S_I	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	22.0	22.0	22.0	22.0	22.0
8	S_{ALK}	mol/m^3	5.0	5.0	6.4	6.4	6.4	6.4	6.3	6.3	7.2	7.9	8.0	8.0	8.0	8.0	8.4	8.0	8.4	7.9
9	X_I	g COD/m^3	50	63	1648	1648	1648	1648	1649	0	3164	3556	3556	7	4865	4865	4869	3786	4869	3556
10	X_S	g COD/m^3	166	141	89	90	89	90	91	0	78	52	40	0	55	55	50	42	50	52
11	X_H	g COD/m^3	0	0	239	238	239	237	235	0	458	522	526	1	719	719	707	558	707	522
12	X_{PAO}	g COD/m^3	0	0	153	152	153	153	153	0	296	335	335	1	459	459	457	357	457	335
13	X_{PP}	g P/m^3	0	0	52	58	52	50	48	0	107	126	127	0	174	174	175	136	175	126
14	X_{PHA}	g COD/m^3	0	0	36	13	36	44	50	0	43	28	24	0	32	32	28	24	28	28
15	X_{GLY}	g COD/m^3	0	0	22	30	22	19	18	0	53	66	68	0	92	92	95	72	95	66
16	X_{AUT}	g COD/m^3	0	0	33	33	33	33	33	0	64	72	73	0	101	101	99	78	99	72
17	X_{MeOH}	$Fe(OH)_3$/m	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	X_{MeP}	g $FePO_4$/m^3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Q	m^3/d	1200	6000	15800	15800	15800	15800	15800	1400	25848	69480	62280	7080	120	19080	19080	43632	11448	7200
20	COD_{part}	g COD/m^3	243	231	2006	1989	2006	2014	2019	28	3717	4126	4113	38	5615	5615	5609	4374	5609	4126
21	VSS_{total}	g VSS/m^3	177	169	1464	1452	1464	1470	1474	21	2713	3012	3002	28	4099	4099	4095	3193	4095	3012
22	$MLSS_{total}$	g MLSS/m^3	233	222	1927	1910	1927	1934	1939	27	3570	3963	3950	37	5393	5393	5388	4201	5388	3963

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