Biological Phosphorus Removal from Municipal Waste Water - Interactions in the Anoxic Zone and Consequences on Process Operations

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Biological Phosphorus Removal from Municipal Waste water - Interactions in the Anoxic Zone and Consequences on Process Operations-.

Ph.D. Thesis

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ABSTRACT

Activated sludge processes designed for biological phosphorus removal (BPR) generally include biological nitrogen removal. This implies that a number of the different processes for nutrient removal (N and P) take place simultaneously at the same location of the plant. With respect to BPR there is still a lack in understanding the interactions in the anoxic zones of such systems. This is of significance, since appropriate BPR performance will rely on the understanding of the factors influencing the behaviour of the phosphate accumulating organisms (PAO).
As an original contribution to increase and improve the knowledge on the BPR process, the principal objective of this study was to identify and investigate cause and effect relationships of BPR in the anoxic zone and to evaluate their consequences on plant-wide operation and performance.

The research of this thesis involved experimental phases as well as model evaluations. Process behaviour and performance were monitored in batch and pilot plant experiments at different imposed conditions. Monitoring included measurements of NO_X-N, NH_4-N, PO_4-P, COD, acetate, SS, VSS and intracellular stored poly-β-hydroxy-alkanoate (PHA) concentrations.

- The batch set-up consisted of up to 4 reactors operated in parallel with activated sludge obtained from the pilot plant. Corresponding to the specific type of investigation, different sequences of anaerobic, anoxic and aerobic phases were applied. The set-up ensured defined conditions, allowing direct comparison of different scenarios and an improved assessment of the different factors influencing BPR.
- The pilot plant consisted of a 2,6 m^3, alternating BioDeniPho™ type plant, fed with municipal waste water from an adjacent treatment plant. The plant was operated during the whole period of the study and nutrient concentrations were monitored continuously at four different locations.

The experimental work focussed on the anoxic condition and the governing phenomena for BPR.
The results and conclusions were implemented in the evaluation of suitable operational and control strategies with emphasis on avoiding nitrate accumulation in the system, as this is known to interfere with BPR performance. A strategy to control denitrification by external COD addition to the anoxic phase, was tested in the pilot plant and modified with respect to BPR performance.
Model evaluation addressed the modifications to be performed for an improved description of the biological nutrient removal process, based on the experimental findings. As a starting point, the combination of ASM2 (Activated Sludge Model No 2) and the TU Delft model was employed.

In the following a short description of the main chapters in this thesis is given.

Chapter 2 addresses the background information concerning the most important aspects with regard to microbiology, process engineering and modelling of biological waste water treatment. Focus is put on the process of biological phosphate removal, presenting the general principle and the current understanding of the microbial metabolism. Present knowledge of the factors influencing the P-removal performance is addressed. Attention is drawn to the response of a BPR system

under anoxic cultivation conditions, with and without the presence of organic substrate. The second part of this chapter addresses the main existing designs of activated sludge system for enhanced nutrient removal. Information on typical process characteristics, such as the large variety of time constants and the variation of incoming waste water are given. Common aspects of operation and control of activated sludge system are presented.

Chapter 3 presents the objective of this thesis.

Chapter 4 deals with different experimental investigations of series of batch tests, addressing cause and effect relationships under anoxic and aerobic conditions:

- Results are presented that clearly demonstrate the dependency of aerobic and anoxic P-uptake rates on the level of internally stored PHB. Activated sludge obtained from the pilot plant was submitted to a sequence of anaerobic/anoxic or anaerobic/aerobic phases. The obtained P-uptake rates as a function of the PHB content in the cells are summarised and compared to literature values (Petersen *et al.*, 1998). Furthermore the achievable net P-uptake is investigated, when submitting the activated sludge to different anaerobic COD loads. A decrease in the BPR performance has been noticed, once the COD load exceeded the corresponding load of the pilot plant. The observed response is discussed based on the current understanding of the underlying mechanisms and its behaviour compared to similar observations reported in literature.

- Results are presented that strongly support the hypothesis that PAO from activated sludge systems consist of two groups: a) denitrifying PAO (DNPAO) capable of using oxygen and nitrate and b) non-denitrifying PAO (O2-PAO) only able to use oxygen. Activated sludge obtained from the pilot plant was submitted to a sequence of anaerobic/anoxic/aerobic, anaerobic/aerobic or anaerobic/anoxic conditions. Several methods for the determination of the two fractions of PAO are performed and compared.
  This section extends previously reported results (Kerrn-Jespersen and Henze, 1993) in that the pH was controlled to around pH 7 to assure that phosphate precipitation was minimal, and in the measurement of PHB and PHV. Simulations implementing existing models for the growth of O2-PAO and DNPAO are used to confirm the experimental results and to gain a better understanding of some of the observations.
  The limitation and restrictions in the use of the presented methods are pointed out and discussed.

- Results are presented, addressing the effect of nitrite on anoxic phosphate uptake. Sludge obtained from the pilot plant was exposed to nitrite or mixtures of nitrite and nitrate at various concentration levels. The course of phosphate, nitrite and nitrate and the internal storage component PHA was compared with batches exposed to nitrate only. Nitrite at low concentration levels (up to about 4 to 5 mg NO_2-N/l) was not detrimental to anoxic P-uptake and hence, can serve also as electron acceptor for P-uptake. Exposure to higher concentration levels induced a complete stop of the anoxic P-uptake, and damaged severely the aerobic P-uptake. The critical nitrite concentration, above which inhibition of phosphate uptake occurred, was in the range of 5 to 8 mg NO_2-N/L. The detrimental effect of nitrite was found to last for at least several hours after the nitrite exposure

- The continuous introduction of an organic substrate to the denitrifying reactor of a biological phosphorus removal (BPR) process was examined. Acetate was used as BPR promoting, model organic substrate. Several observations were made regarding the influence of substrate

availability on PHA storage/utilisation and phosphate uptake/release. At low acetate addition rates the P-uptake and PHB utilisation rates were reduced compared to when no acetate was available. At higher acetate addition rates a net P-release occurred and PHB was accumulated. For certain intermediate acetate addition rates the PHB level increased while a net P-uptake occurred. Whether the introduction of BPR promoting organic substrates to the denitrifying reactor was detrimental to overall P-removal appeared to depend on the interaction between aerobic P-uptake, being a function of PHB level, and the aerobic residence time.

In chapter 5 investigations are presented, dealing with the response of a BioDeniPho pilot plant to the continuous introduction of a BPR promoting organic substrate to the denitrifying zone.

The study addresses the effect of potential leakage of easily biodegradable COD from the anaerobic to the anoxic zone, as well as the use of a model based control routine for the external carbon source addition in order to control nitrate in the system. In addition to the control performance, focus was put on the arising phosphate dynamics and the limits induced by the goal of satisfactory phosphate removal.

The pilot plant experiments were performed over several cycles while monitoring the course of NO_X-N, NH_4-N, PO_4-P, PHB and PHV, COD and Acetate. The experimental period covered a time interval of approximately 2 months. The results are discussed in conjunction with the calculated P-uptake, PHB utilisation and denitrification rates.

No negative impact on BPR was noticed, at external acetate addition rates that were of the same order of magnitude as the detected flow (leakage) of COD from the anaerobic zone. The control routine applied proved to be suitable for nitrate control. A simple modification assured that phosphate accumulation in the plant due to the acetate addition was avoided, i.e. no increase in the phosphate concentration of the effluent. Anoxic activity of the PAO was maintained during the experimental period and checked by batch tests. Furthermore, the possibility of BPR stabilisation through external carbon source addition to the anoxic zone is discussed.

BPR deterioration was detected during some experiments and seemed to be due to sudden increases of the COD load in the inlet. In order to account for these scenarios too, control strategies could consist of a combination of the external carbon source addition with ,e.g., aeration time length control or equalisation of the inlet.

Chapter 6 investigates modelling aspects of the BPR process. Using a priori knowledge and experimental results, areas of model deficiency are indicated with respect to BPR. A revised/extended model is proposed. The model evaluation focused on the qualitative ability of the model to predict the phosphorus uptake as a function of the initial PHA level. Revised rate expressions were implemented for poly-phosphate storage and PHA utilisation of the phosphate accumulating organisms (PAO). Furthermore, the process of anoxic acetate uptake and storage as PHA was added to the model. Both aspects are essential, as they have been observed to occur in praxis. Simulations were evaluated and validated with data from an alternating type pilot plant, covering a time period of several cycles. The revised model exhibited an improved prediction quality with regard to the nutrient and internal PHA concentration. It was capable to capture PHA limited P-uptake as well as the effect of acetate flow into the anoxic phase on BPR dynamics. For the investigations only a few parameter had to be adjusted and the proposed extensions lead to 5 additional parameters compared to the original model.

In a second step, the revised model was extended to two groups of PAO, according to the electron acceptor used. The simulation study assessed the ability of DNPAO, capable of using both nitrate and oxygen, to compete successfully in BPR systems to purely aerobic PAO (O2PAO). It is proposed that the proliferation of DNPAO is relying to a certain extent on external impacts, such as the influent composition (presence of DNPAO). However, growth depending only on internal cell storage materials (PHA) represents a severe restriction of the model.

Dansk resume'

Aktiverede slam processer til biologisk fosfor fjernelse (BPR) omfatter sædvanligvis også kvælstof fjernelse. Denne kombination betyder at et antal processer til fjernelse af næringsstoffer (N og P) finder sted samtidigt og på det samme sted i et spildevandsanlæg. For biologisk fosfor fjernelse er der stadig en manglende forståelse af interaktioner i de anoxiske zoner i spildevandsanlæg. Denne mangel på viden er væsentlig eftersom hensigtsmæssig biologisk fosfor fjernelse hviler på forståelse af de faktorer der påvirker opførslen af de fosfat akkumulerende organismer (PAO).
Det væsentligste formål med og originale bidrag fra dette ph.d. projekt var at finde og undersøge årsagssammenhængene for biologisk fosforfjernelse i den anoxiske zone og at evaluere deres effekt på operation og opførslen af det totale anlæg.

Det forskningsmæssige arbejdet har omfattet eksperimenter og evaluering af modeller. Processens opførsel blev fulgt i batch og pilotanlægs eksperimenter ved forskellige betingelser. De forskellige procesvariables koncentrationer, der blev målt omfattede NO_X-N, NH_4-N, PO_4-P, COD, acetat, SS, VSS og intracellulært lagret poly-β-hydroxy-alkanoate (PHA).

- Batch eksperimenterne blev gennemført i op til fire parallelt opererede reaktorer med aktiveret slam fra pilotanlægget. Forskellige sekvenser af anaerobe, anoxiske og aerobe faser blev gennemført. Dette udstyr sikrede veldefinerede betingelser, der tillod en direkte sammenligning af forskellige scenarier. Disse forhold tillod en forbedret evaluering af de forskellige faktorer, der påvirker biologisk fosforfjernelse.

- Pilotanlægget bestod af et 2,6 m^3, alternerende BioDeniPho™ anlæg, der blev forsynet med spildevand fra et nærliggende spildevands anlæg. Pilotanlægget var i drift gennem hele ph.d. studiet, hvor næringsstoffernes koncentrationer blev målt kontinuerligt på fire steder i anlægget.

Det eksperimentelle arbejde fokuserede på anoxiske betingelser og de dominerende fænomener for biologisk fosfor fjernelse. Resultaterne og konklusionerne blev implementeret i evalueringen af hensigtsmæssige operations- og reguleringsstrategier med henblik på at undgå nitrat akkumulation i systemet, hvilket vides at kunne interferere med med den biologiske fosfor fjernelse.
En strategi med henblik på regulering af denitrifikation ved tilsætning af en ekstern kulstofkilde til den anoxiske fase blev testet og modificeret på anlægget med henblik på biologisk fosfor fjernelse. De modifikationer, der blev udført på modellen med henblik på at opnå en forbedret beskrivelse af den biologiske næringsstofs fjernelse, blev evalueret udfra de eksperimentelle resultater. Udgangspunktet for model evalueringen var at gennemføre en forbedret Model evaluering. Til en begyndelse anvendes en kombination af TU Delft og (Activated Sludge Model No 2) ASM2 modellerne.

Nedenfor gives en kort beskrivelse af afhandlingens hovedkapitler.

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Kapitel 2 indeholder baggrundsinformation om de vigtigste aspekter for mikrobologi, proces teknologi og model dannelse for biologisk spildevands rensning. Her er der fokusseret på biologisk fosfor fjernelse, hvor de generalle principper og den nuværende forståelse af kulturens mikrobielle stofskifte. Ligeledes præsenteres den nuværende viden om de faktorer der påvirker fosfor fjernelse. Specielt rettes opmærksomheden mod opførslen af en biologisk fosforfjernelses proces ved anoxiske betingelser med og uden tilstedeværelse af organisk substrat. Den anden del af dette kapitel beskriver de væsentligste eksisterende aktiverede slam processer til forøget fjernelse af næringsstof. Der informers om typiske process karakteristika, som f.eks. det store spænd af tidskonstanter og variationen i spildevandsforsyningen. Endelig præsenteres fælles aspekter vedrørende operation og regulering af aktiverede slam processer.

Kapitel 3 præsenterer formålene med denne afhandling.

Kapitel 4 behandler en række eksperimentelle batch reaktor undersøgelser med henblik på afklaring af årsagssammenhænge ved anoxiske og aerobe betingelser:

- Der præsenteres resultater der klart demonstrerer afhængigheden af aerob og anoxisk fosforoptagelseshastigheder som funktion af indholdet af internt lagret PHB. Aktiveret slam fra pilot anlægget udsattes for en sekvens af anaerobe/anoxiske eller anaerobe/aerobe faser. De opnåede fosfor optagelses hastigheder i afhængighhed af kulturens PHB indhold sammenlignes med litteratur værdier (Petersen *et al.*, 1998). Desuden undersøges det opnåelige netto fosfor optag, når den aktiverede slam udsættes for forskellige anaerobe COD belastninger. Der observedes et fald i den biologiske fosfor fjernelse når COD belasningen overskred pilotanlæggets korresponderende belastning. Observationerne diskuteres udfra den nuværende forståelse af de underliggende mekanismer og den biologiske fosforfjernelses opførsel sammenlignet med lignende observationer i litteraturen.

- Der præsenteres resultater der stærkt underbygger hypotesen om at PAO fra aktiveret slam består af to grupper: a) denitrifierende PAO (DNPAO) der er istand til at bruge oxygen og nitrat og b) ikke denitrifierende PAO (O2-PAO) der kun kan bruge oxygen. Her blev aktiveret slam fra pilotanlægget udsat for en sekvens af anaerob/anoxisk/aerob, anaerob/aerob eller anaerob/anoxiske betingelser. Der benyttedes flere metoder til bestemmelse af de to PAO fraktioner, ligsom disse blev sammenlignet.

  Denne section udvider tidligere rapporterede resultater (Kerrn-Jespersen and Henze, 1993) idet pH blev reguleret omkring 7 for at sikre at fosfat bundfældningen var minimal samt ved at PHB og PHV blev målt. Der anvendes simuleringer med eksisterende modeller til vækst af O2-PAO og DNPAO til at konfirmere de eksperimentelle resultater og for at opnå en bedre forståelse af nogle af observationerne.
  De præsenterede metoders begrænsninger og restiktioner påpeges og diskuteres.

- Der præsenteres resultater vedrørende nitrits effekt på anoxisk fosfat optag. Slam fra pilotanlægget blev udsat for nitrit eller blandinger af nitrit og nitrat ved forskellige koncentrations nivauer. Forløbet af fosfat, nitrit, nitrat og den internt lagrede PHA blev sammenlignet med forløbet i batche der kun blev udsat for nitrat. Lave koncentrationer af nitrit (op til 4 - 5 mg NO_2-N/l) var ikke ødelæggende for anoxisk fosfat optagelse, og kan derfor tjene som elektron akceptor for fosfat optagelse. Højere koncentrations niveauer inducerede et komplet stop for anoxisk fosfat optagelse, samt reducerede det aerobe fosfat optag betydeligt. Den kritiske nitrit

koncentration over hvilken der skete inhibering af fosfat optagelse var i området fra 5 til 8 mg NO_2-N/L. Nitrits inhiberende effekt varede adskillige timer efter kulturen var udsat for nitrit.

- Kontinuerlig tilførsel af organisk substrat til den denitifierende reaktor i en biologisk fosfor fjernelses process blev undersøgt. Acetat blev anvendt som et model stof for organisk substrat til fremme af biologisk fosfor fjernelse. Der gøres adskillige observationer vedrørende substratets effekt på PHA lagring/forbrug og fosfat optag/frigørelse. Ved lave acetat tilsætningshastigheder var fosfatoptagelse og PHB forbrug reduceret sammenlignet med forholdene uden acetat tilsætning. Ved højere acetat tilsætningshastigheder skete der en netto fosfat frigørelse og PHB blev akkumuleret. Ved visse mellemliggende acetat tilsætningshastigheder forøgedes PHB niveauet mens der skete en netto fosfat optagelse. Hvorvidt introduktion af organiske substrater til fremme af biologisk fosfor fjernelse til den denitrifierende reaktor var ødelæggende for den totale fosfat fjernelse afhang tilsyneladende af interaktionen mellem aerobt fosfor optag, der afhænger af PHB niveauet, og den aerobe opholdstid.

I kapitel 5 præsenteres undersøgelser af opførslen af et BioDeniPho pilot anlæg med kontinuerlig introduktion af et organisk substrat til fremme af biologisk fosfor fjernelse til den denitrifierende zone. Studiet vedrører effekten af en potentiel lækage af let nedbrydelig COD fra den anaerobe til den anoxiske zone, samt brugen af en model baseret reguleringsrutine til styring af tilsætningen af den eksterne kulstof kilde for at regulere nitrat nivauet i processen. Udover reguleringens opførsel blev der fokuseret på den resulterende fosfat dynamik og de begrænsninger der blev induceret af formålet vedrørende tilfredsstillende fosfat fjernelse.

Eksperimenterne på pilot anlægget blev gennemført over adskillige cyklus perioder med måling af NO_X-N, NH_4-N, PO_4-P, PHB og PHV, COD og Acetat. Eksperimenterne blev gennemført over en, periode på ca. 2 måneder. Resultaterne diskuteres sammen med de beregnede fosfatoptags, PHb forbrugs og denitrifikations hastigheder.

Ved eksterne acetat tilsætningshastigheder, der var af same størrelsesorden som den detekterede COD strømning (lækage) fra den anaerobe zone blev der ikke observeret nogen negativ effekt på bilogisk fosforfjernelse. Den anvendte regulerings rutine var tilstrækkelig til regulering af nitrat niveauet. En simpel modifikation sikrede at man kunne undgå fosfat akkumulation, dvs. der skete ingen forøgelse af effluentens fosfat koncentration, som følge af acetat tilsætningen. Den anoxiske aktivitet af PAO blev vedligeholdt under den eksperimentelle periode og blev overvåget ved batch forsøg. Desuden diskuteres muligheden for stabilisering af den biologiske fosfor fjernelse ved tilsætning af ekstern kulstof kilde til den anoxiske zone. Der blev detekteret forringelse af den biologiske fosfor fjernelse under nogle eksperimenter, dette synes at skyldes pludselige forøgelse af COD belastningen i det indkommende spildevand. Med henblik på også at kunne tage hensyn til disse scenarier kunne regulerings strategier bestå af tilsætning af ekstern kulstof kilde kombineret med f.eks. styring af beluftningstiden eller udjævning af variationen i indkommende spildevand vha. et ekstra basin.

I kapitel 6 undersøges modellerings aspekter for biologisk fosfor fjernelse. Udfra a priori viden og eksperimentelle resultater indikeres forskellige model defekter for biologisk fosfor fjernelse. Der foreslås en revideret/udvidet model. Model evalueringen fokuserede på modellens kvalitative evne til at prediktere fosfor optaget som funktion af det initielle PHA niveau. Der blev implementeret

revideredе hastighedsudtryk for fosfat akkumlerende organismers (PAO) lagring af polyfosfat og PHA forbrug. Desuden blev anoxisk acetat optagelse og lagring tilføjet til modellen. Begge aspekter er essentielle, eftersom de er observeret i praksis. Simuleringer blev evalueret og valideret med data fra et pilot anlæg af den alternerende type, over adskillige cyklus perioder.Den revideredе model udviste en forbedret prediktions kvalitet for næringssalte og interne PHA koncentrationer. Modellen var istand til at beskrive både PHA begrænset fosfat optag og effekten af acetat tilstrømning til den anoxiske fase på biologisk fosforfjernelses dynamik. For disse undersøgelser var det tilstrækkeligt kun at justere få parametre ligesom de foreslåede udvidelser tilførte 5 yderligere parametre sammenlignet med den originale model.

I en anden fase blev den revideredе model udvidet til to grupper fosfat akkumulerende organismer i henhold til den anvendte elektron akceptor. I et simuleringsstudie undersøgtes evnen af DNPAO, der kan benytte både nitrat og oxygen, til at konkurrere med rent aerob PAO (O2PAO) ved biologisk fosfor fjernelses processer. Det foreslås at vækst af DNPAO i et vist omfang er baseret på eksterne påvirkninger, som f.eks. sammensætningen af indkommende spildevand (tilstedeværelse af DNPAO). Imidlertid sætter vækst der kun afhænger af interne lagrings materialer (PHA) en alvorlig begrænsning ved modellen.

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1 INTRODUCTION

Decreasing surface water quality often reveals itself in the phenomenon of eutrophication, i.e. the extensive growth of algae and aquatic plants. In addition to imposing detrimental effects on aquatic life, it represents a significant problem for areas relying on water supply from surface water. Furthermore, being known to appear in lakes, eutrophication has also been observed in coastal areas in the recent years.

During the approach to protect the surface water quality, nitrogen and phosphorus have been identified as the limiting nutrients for algae growth. Limiting the discharge of phosphorus, however, has been recognised as the more effective method of these two to prevent eutrophication. For a successful implementation of such a strategy, the sources contributing to the overall phosphorus load must be well identified. In some areas the contribution from diffuse (non-point) sources, such as urban or agricultural runoff, may be sufficiently high that the benefit from point source control is decreased significantly. In most cases, however, phosphorus removal during waste water treatment can be regarded as an appropriate means to induce P-limitation in the surface waters, as the major contribution can be attributed to municipal and industrial waste water (approximately 70%).

The increasing attention to the eutrophication related problems resulted in the implementation of the EU Directive 91/271/EEC in 1991, stating new effluent requirements for urban wastewater. These include, for example, 2 mg P/L for small (10,000 to 100,000 population equivalent) and 1 mg P/L for large waste water treatment plants (> 100,000 p.e.). Discharge into sensitive waters is more restricted and the allowed effluent concentrations are expected to be lowered further in the near future.

The common methods to remove phosphorus from waste water rely on chemical precipitation (with iron, aluminium or calcium salts) and increasingly on biological phosphorus removal (BPR). The latter is achieved by enhancing the presence of a group of bacteria, which accumulate phosphate in excess to their metabolic requirements for growth. These bacteria incorporate up to 5 times more phosphate compared to 'normal' biomass. By withdrawing the excess sludge, phosphorus is removed from the water system. BPR is often favoured over chemical precipitation due to several features:

- Lower sludge production, being positive for downstream sludge treatment.
- No chemicals required, implying no additional discharge of chloride & sulphide salts in the w
- Increasing fertiliser value of the sludge, as accumulation of salts and heavy metals from impurities of the added chemical is avoided.
- Cost advantages in most cases.

However, sometimes BPR alone is insufficient and must be supported for example by co-precipitation. On one side, this is due to the fact that the fraction of biologically removed P depends on the amount and quality of organic substrate in the waste water. On the other side, despite being well established in practice today, there exists still a certain lack in understanding of the BPR process and thus also in the development of optimised operation and control strategies.

2 BACKGROUND OF BIOLOGICAL WASTEWATER TREATMENT

ABSTRACT

Biological treatment of municipal wastewater addresses the removal of organic compounds, nitrogen and phosphorus. This involves an increasing number of complex microbial interactions, inducing a more advanced design and operation of biological waste water treatment systems. Understanding the processes at a microbial level as well as the typical characteristics of an activated sludge system with regard to process operation is essential for further development of waste water treatment systems.

This chapter is intended to supply background information concerning the most important aspects with regard to microbiology, process engineering and modelling. Focus is on the biological phosphate removal process, presenting the general principle and the current understanding of the microbial metabolism. Present knowledge about the factors, influencing the P-removal performance, is addressed. Attention is drawn to the response of a BPR system under anoxic cultivation conditions, with and without the presence of organic substrate.

Due to combined biological nitrogen and phosphorus removal, the operational complexity of the activated sludge processes is increased considerably. Hence, new developments in operational and control strategies are required in order to preserve acceptable effluent water quality. The second part of this chapter supplies a general overview on existing design, operation and control issues. In addition to the various designs of activated sludge system for enhanced nutrient removal, information on typical process characteristics, such as the large variety of time constants and the variation of incoming waste water are given. Common aspects of operation and control of activated sludge system are presented

2.1 Aspects of Biological Nutrient Removal

In waste water treatment terminology normally only nitrogen and phosphorus are referred to as nutrients, because these elements are considered to be the limiting nutrients for the growth of algae and bacteria in eutrophic surface waters. Besides the removal of suspended solids, the objective of biological waste water treatment is the removal of carbon, nitrogen and phosphorus compounds from the waste water. Hence, although not regarded as nutrient in a strict sense, carbon sources are included in this section - also due to their importance for biological waste water treatment (all micro-organisms require carbon sources for the new cell synthesis). This section is intended to give an overview to important aspects concerning the removal of the three groups of pollutants. Evidently, main focus is put on the mechanism of biological phosphorus removal.

2.1.1 Removal of Organic Compounds

Organic substrates in waste water- characterisation

Waste water represents a complex mixture of organic substances, present in various forms. Lumped parameters as COD (chemical oxygen demand) are used for measuring their quantity, since the measurement of the concentration of all the individual compounds is not possible. As the organic substrates differ also widely in their accessibility to activated sludge micro-organisms, a classification of the organics seems to be advisable. The classical division of carbon compounds on the basis of COD is shown in Figure 2.2-1.

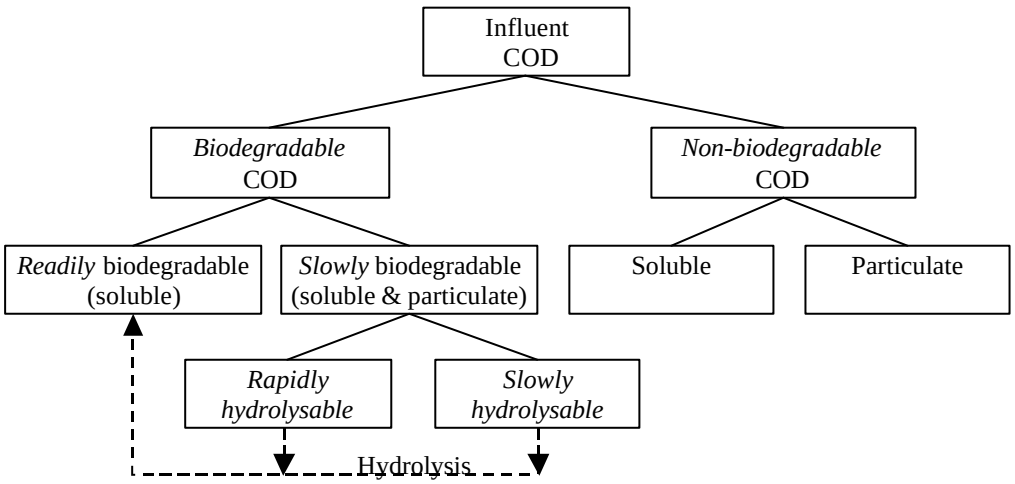

Figure 2.1-1. Division of the influent COD in municipal waste water according to Henze *et al.*, 1997.

Non-biodegradable organic substances are present in *soluble* and *particulate* form. The term *non-biodegradable* is relative and dependent on the cultivation conditions. Allowing sufficient solid retention time, the activated sludge might adapt, i.e. induction of specific enzymes and enrichment of species containing the appropriate enzyme system occur. Under these conditions also compounds commonly considered non-biodegradable can be biologically degraded.

Traditionally, the *soluble non-biodegradable* fraction refers to the soluble part of the COD being inert and passing through the activated sludge system to the final effluent, without undergoing conversion reactions.

The *non-biodegradable particles* are also inert, but can accumulate in the system. They are captured to some extent in the flocs of the activated sludge., causing a certain change in the

composition of the sludge in the system. By the withdrawal of the excess sludge, a part of the *non-biodegradable particles* is removed from the system.

Biodegradable substances are also present in soluble and particulate forms. The terms readily and slowly biodegradable refer to whether or not the molecule of the organic compound can be transported directly through the cell membrane. Hence, slowly biodegradable substances are particulate substrates and larger dissolved molecules, which have to be hydrolysed (enzymatic breakdown) prior to their utilisation by the micro-organisms. The products of hydrolysis are considered to be readily biodegradable substrates, as indicated in Figure 2.1-1. Overall, three groups of biodegradable substances in waste water can be distinguished (Henze *et al*., 1997):

a) Readily biodegradable substrates

This fraction involves organic compounds, consisting of small and simple molecules, which can be directly metabolised inside the cell. Hence they are utilised at high rates under all cultivation conditions. Table 2.1-1 displays an example for the composition of readily biodegradable sub-fraction in raw waste water:

Table 2.1-1. Readily biodegradable COD in raw waste water according to Henze *et al*., 1997.,

Acetic acid	25 COD mg/L	Alcohol (ethanol, methanol)	5 COD mg/L
Higher volatile fatty acids	10 COD mg/L	Lower amino acids	10 COD mg/L
		Monocarbohydrates	10 COD mg/L

b) Rapidly hydrolysable – slowly biodegradable substrates

Dissolved and colloid solids are the dominant forms of the rapidly hydrolysable organic compounds. However, some suspended solids may also hydrolyse rapidly. The whole fraction can account for 15-25% of the total COD in raw municipal waste water (Wanner, 1994).

c) Slowly hydrolysable - slowly biodegradable substrates

This fraction mainly involves suspended solids being only slowly hydrolysed. This substrate fraction is utilised at rather low rates.

In general, hydrolysable substrates are referred to as slowly biodegradable substrates, as they are not immediately available for internal cell metabolism, because of their high molecular weight and complex molecules. Before being transported across the cell membrane, they have to be split by extracellular enzymes. They represent almost 75% of the utilisable organic substrates in municipal waste water.

Processes like hydrolysis and fermentation (conversion of readily biodegradable substrates to volatile fatty acids, preferably acetic acid) have a major impact on the composition of the waste water. Hydrolysis takes place under all cultivation conditions, but at different rates (Henze *et al.*, 1995), whereas fermentation is believed to be limited to anaerobic conditions. Depending on the conditions in the sewer system, significant changes in the fractions of biodegradable substrates can occur during waste water transport in the sewers.

Removal of organic substrates by activated sludge

Depending on the composition of the organic substances, their removal will have to be a combination of physico-chemical and biochemical processes and reactions. In the following only a schematic overview is given, as a detailed description would be beyond the scope of this section. Upon contact of the activated sludge with the waste water, instantaneous removal of carbon substances from the

liquid phase occurs. Several processes happen simultaneously (Wanner, 1994) and are referred together to as biosorption (Eikelboom *et al.*, 1982):

a) Enmeshment of particles into the structure of activated sludge flocs.
b) Entrapment and adsorption of colloidal matters.
c) Sorption of soluble high molecular organic compounds.
d) Uptake of single organic compounds with small molecules.

After the biosorption, most of the sorbed organic molecules are present in a form, which is not suitable for intracellular metabolism, as they cannot permeate the cell membrane. The majority of these common high-molecular-weight compounds are organic polymers like lipids, polysaccharides and proteins. Before enzymatic transport through the cell membrane these polymers have to be broken down to smaller structures, or directly to monomers by hydrolysis.

During this process the *polysaccharides* are converted to glucose and fructose in a two step process serving both as energy and carbon source. *Lipids*, being organic polymers formed of glycerol and long chain fatty acids, are split into glycerol, which is further metabolised in glycolysis, and into long-chain fatty acids. The latter ones are subsequently shortened during β-oxidation, before entering the internal metabolism The main role of *proteins* in the metabolism of organotrophic micro-organisms is the supply of building blocks for the synthesis of new biomass. The aspect of providing energy is not as important compared to carbohydrates and lipids.

After the extracellular hydrolysis, the fragments of polymers and single molecules are transferred to the cells, where they are metabolised in the cell's internal enzymatic apparatus. The intracellular metabolism is divided into the catabolism, generating energy for the cell's energy requirements, and the anabolism, leading to synthesis of new cell material. Both processes are taking place simultaneously. A schematic example (overview) of the aerobic catabolism is shown in Figure 2.1-2.

The intracellular metabolism is dependent on the cultivation conditions (anaerobic, anoxic or aerobic) and the type of organisms. A large group of micro-organisms is capable of aerobic C-removal. But also anaerobic C-removal is known and applied in waste water treatment, though to higher extent to industrial waste water. For a detailed description of the biochemical and microbial mechanisms the reader is referred to the corresponding literature (e.g., Schlegel, 1992).

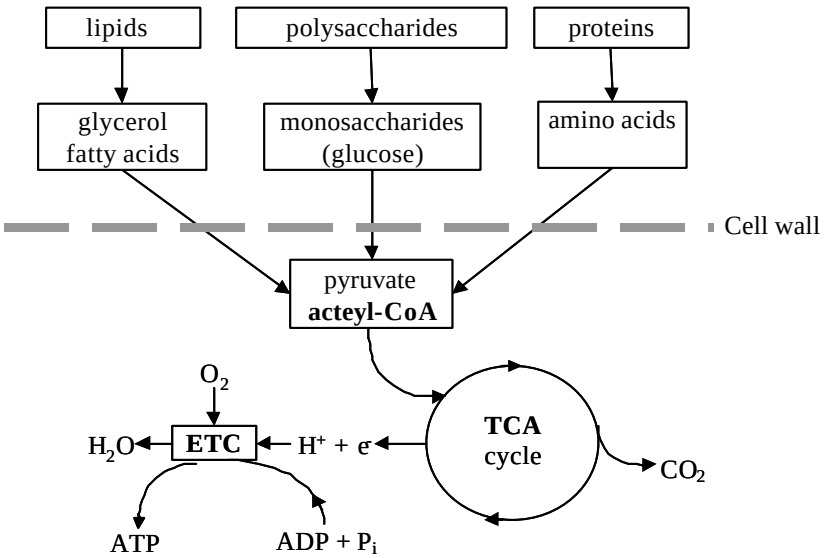

Figure 2.1-2. Schematic diagram of an aerobic catabolism (from Wanner, 1994)

2.1.2 Nitrogen Removal

Elimination of nitrogen from waste waters has become one of the most important nutrient removal process in waste water treatment. Nitrogen compounds present in municipal waste water are basically divided into two classes: *inorganic* and *organic nitrogen*. The sum of both is referred to as *total nitrogen* and can be determined analytically, e.g. *total Kjeldahl nitrogen* (TKN) analysis.
The *inorganic nitrogen* consists mainly of the reduced (ammonia) and oxidised (nitrite and nitrate) nitrogen forms. Due to reducing conditions in most sewer systems, the amount of oxidised nitrogen in the inlet of a treatment plant is normally insignificant. Although ammonia nitrogen can be present in aquatic systems as dissolved gaseous ammonia (NH_3) or as ionised ammonia (NH_4^+), see eq 2.1, approximately 95 % of the reduced nitrogen in municipal waste water is present as NH_4^+. This is induced by the typical temperature (8-20°C) and pH (7-8.5) of municipal wastewater.

$$NH_3 + H_2O = NH_4^+ + OH^- \qquad \text{eq 2.1}$$

The *organically bound nitrogen* in municipal waste water consists mainly of compounds containing amino groups (Wanner, 1994). The biodegradable part of the organic nitrogen undergoes conversion in hydrolytic reactions, converting further the amino groups to ammonia, which is released from the cells into the bulk liquid. During these processes, also referred to as *ammonification*, large organic molecules are depolymerised by extracellular enzymes and amino acids are formed. These amino acids are transported into the cells, where further degradation to ammonia and different types of organic compounds takes place (as described in. section 2.1.1). As a consequence ionised ammonia (NH_4^+) represents the main starting point for most biological N-removal techniques/processes.

The first investigations of the biological conversion processes date back to the end of the 19th century (ammonia oxidation - Winogradsky, 1890, reduction of nitrate/nitrite - Breal, 1892). Apart from the sequence of nitrification and denitrification, being considered as the conventional method of N-removal, a range of new microbial processes have been reported in literature recently. In the following a short overview of certain mechanisms for nitrogen removal will be given. Some of the important possible microbial nitrogen conversions are schematically shown in Figure 2.1-3. For a survey of the detailed biochemistry involved in these processes, the reader is referred to Jetten *et al.*, (1997a)

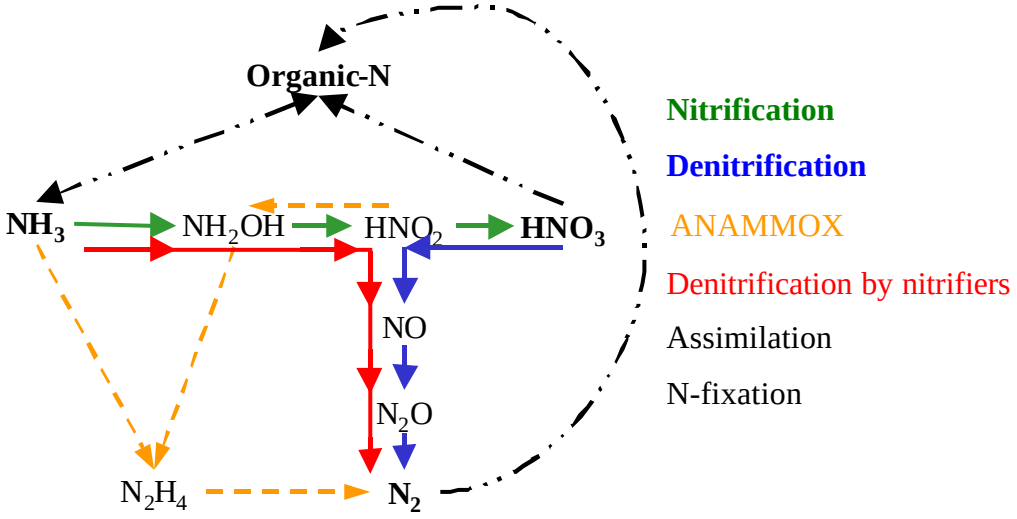

Figure 2.1-3: Schemes of microbial nitrogen conversions.

Autotrophic Nitrification

During autotrophic nitrification ammonia is oxidised in at least two steps via nitrite to nitrate.

Step A $NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_20 + 2\ H^+$ eq 2.2

Step B $NO_2^- + 0.5\ O_2 \rightarrow NO_3$ eq 2.3

These two steps have been generally attributed to two different types of micro-organisms: step A to *Nitrosomonas spe* and step B to *Nitrobacter spe*. Recent microbiological studies employing gene probes for the analysis of the microbial community confirm the predominant role of *Nitrosomonas spe* as ammonia oxidiser (Wagner *et al.*, 1996). Nitrobacter *spe*, however, were often not found in nitrifying sludge. The contradictory results of natural population studies, based on traditional isolation methods, are today explained by the 'incorrect' enrichment conditions applied. While non-limiting conditions, with regard to oxygen and substrate are usually applied during the isolation procedures, the actual system exhibits mostly limiting conditions (Van Loosdrecht and Jetten, 1998). As a consequence the competitive advantages within the microbial community might change and bacteria originally present in only a small fraction will be found in abundance.

Sensors such as gene probes, on the other hand, enable to study the microbial community in-situ, i.e. under the conditions present in the actual system, and therefore tend to give a more precise picture (Wagner *et al*., 1993a). Interesting to note is that up to date, no isolate/ single organism capable of direct oxidation of ammonia to nitrate has been identified (Van Loosdrecht and Jetten, 1998).

Nitrifying organisms in general exhibit a low specific growth rate (1/day, Henze *et al*., 1987). Therefore process operation and /or layout must deal with this characteristic feature in order to prevent a wash out of the nitrifying bacteria. This is achieved by fixation of the bacteria (biofilm reactors) or by controlling the sludge age in activated sludge systems (Henze *et al*., 1997). Due to their low specific growth rate the amount of autotrophic organisms is always significantly smaller than the amount of heterotrophs in common activated sludge systems (Henze *et al*., 1997).

Furthermore, nitrification adds to the overall oxygen demand of an activated sludge unit, which should be accounted for by the design of the aeration system. Accepting the stoichiometry in equations eq 2.2 and eq 2.3 (Schlegel, 1992), the oxygen requirement for the first reaction is 3.43 gO_2/gN and 1.14 gO_2/gN for the oxidation of nitrite. Under normal conditions the oxidation rate of nitrite is higher than the one of ammonia, thus no accumulation of nitrite is expected (s. section 4.3).

Denitrification

Denitrification is one of the most important biochemical processes, reducing nitrogenous oxides, principally nitrate and nitrite, to nitrogen gas, N_2 (Figure 2.1-3). Hence, it is substantial in order to maintain the nitrogen balance in terrestrial ecology. It is a process that only takes place under oxygen limited conditions (< 10 mM O_2, Ye *et al*., 1994), as already low concentrations of dissolved oxygen can inhibit important enzymes involved.

Furthermore, denitrification requires electron donors such as organic substances or reduced compounds such as sulphide or hydrogen. The type of the carbon source, however, exhibits a strong influence on the denitrification rate, with the tendency of higher rates for easily biodegradable substances (Henze, 1991, Henze *et al.*, 1997, Tam *et al*., 1992, Gerber *et al.*, 1986). If the external substrates are exhausted, slow endogenous anoxic respiration with activated hydrogen derived from cellular materials will be also possible (Henze *et al*., 1993). However, as the rate of hydrolysis under anoxic conditions is low, the availability of hydrolysed particulate substrates for denitrification is rather limited (Wanner, 1994).

Due to the use organic carbon sources, denitrifying species are classified as heterotrophic organisms. There exists a large variety of organisms capable of nitrate reduction: e.g. *Pseudomonas denitrificans*, *Pseudomonas aeruginosa*, *Paracoccus denitrificans*, *Thiobacillus denitrificans* and *Bacillus lichenformis* are known as denitrifiers (Schlegel, 1992).

Denitrification occurs in a sequence of several reactions, being catalysed by different enzymes. These enzymes are located in the cytoplasm membrane and the periplasmatic (Ye et al., 1994). Substrates for the denitrification pathway, such as nitrate, nitrite, and N_2O, are required for the full expression of enzymatic activities for denitrification, as they activate the transcription of the genes involved in nitrite reduction (Ye et al., 1994). Most denitrifying organisms do have the entire pathway, but some strains lack the ability of one or more steps (Tiedje, 1988).
Formation of the intermediates during the conversion of nitrate (Figure 2.1-3) can easily occur under electron donor limitation. Furthermore, low dissolved oxygen concentration can increase the risk of intermediate accumulation, as it might inhibit the various enzymes involved in the metabolism differently. The release of the intermediates into the environment is of some concern, as for example N_2O is involved in the stratospheric reactions, which results in the depletion of ozone. Similarly, the accumulation of nitrite has to be prevented, due to its toxicity. Nitrite concentration might build up in the system during denitrification because of a lower reduction rate compared to the rate of nitrate reduction (Wilderer *et al*., 1987, see also section 4.3).
A distinct feature of denitrification is the fact that it is coupled to the electron transport chain. Consequently the denitrifiers are able to gain large amounts of energy without oxygen being present. This is accomplished by the membrane bound ATPase enzyme regenerating large amounts of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic orthophosphate. As a result denitrifiers exhibit a relatively high specific growth rate (6/d, Henze *et al.*, 1987).

Non- conventional Nitrification-denitrification mechanisms

Anoxic Ammonium Oxidation (ANAMMOX)

Mulder *et al.* (1995) observed ammonium removal in an anaerobic (anoxic) fluidised denitrifying bed, while treating the effluent from a methanogenic reactor. It is suggested that the responsible organisms catalyse two peculiar conversions: anaerobic oxidation of ammonium to nitrogen gas and anaerobic oxidation of nitrite to nitrate (Van de Graaf *et al.*, 1996,1997):

$$NH_4^+ + 1.3\ NO_2^- + 0.042\ CO_2 \Rightarrow 0{,}042\ \text{biomass} + N_2 + 0.22\ NO_3^- + 0.08\ OH^- + 1.87\ H_2O \qquad \text{eq. 2.4}$$

In theory ammonium can be oxidised by serving as an electron donor in a denitrification reaction, but the oxidation of ammonium with nitrite to nitrogen gas represents a new biochemical pathway and is still under investigation.
Due to the extremely low specific growth rate 0.1-0.05/day the responsible organisms can only be enriched in specific selective conditions (Jetten and van Loosdrecht, 1998). Nevertheless, due to rather high conversion capacity, the process seems suitable for commercial exploitation.

Denitrification by autotrophic nitrifiers

This term refers to the conversions of ammonium to nitrite, which is further reduced to N_2 by autotrophic nitrifiers (Figure 2.1-3). In general, it is possible that autotrophic nitrifying bacteria produce N_2O, NO, N_2 gas, thus perform a kind of denitrification without using organic substrates. These types of ammonium conversions have been the subject of several microbiological papers (e.g. Bock *et al.*, 1995; Poth, 1986). Nevertheless, it seems that they do not play a significant role in treatment of municipal waste water, as their rates are more than one order of magnitude lower compared to the ones of conventional nitrification/denitrification (Van Loosdrecht and Jetten, 1998).

Heterotrophic nitrification- aerobic denitrification

Heterotrophic nitrification refers to the ability of heterotrophic organisms to oxidise ammonium (Robertson and Kuenen, 1990). Aerobic denitrification, i.e. the ability of micro-organisms to denitrify while they sense oxygen, has been illustrated by several microbial studies (e.g. Robertson *et al.*, 1995, Patureau *et al.*, 1998). Both conversion possibilities imply the simultaneous use of oxygen and nitrate as electron acceptor, thus leading to an increased specific growth rate for the corresponding organisms (Patureau *et al.*, 1994).
Sometimes aerobic denitrification is used in a different context: it is referred to as denitrification in an aerobic reactor. In this case diffusion limitations into the flocs or biofilm provide anaerobic or anoxic conditions where conventional denitrification can occur. As a consequence the term of aerobic denitrification includes in this case all conceivable processes for the conversion of ammonium to elementary nitrogen under aerobic operating conditions (e.g. Hippen *et al.*, 1998).

Conclusion on nitrogen removal from municipal waste water

Despite the variety of nitrogen conversion processes, the conventional nitrogen removal mechanism is still considered as the most important one for the treatment of municipal waste water in traditional activated sludge plants. However, for specific conditions (waste water characteristics or new plant/reactor design), the contribution of other nitrogen conversion processes gains importance for the overall nitrogen removal. Hippen *et al.* (1998), for example, describe the significance of aerobic denitrification during the treatment of leachate from landfills. New suggestions for process layouts for nitrogen removal, mainly based on the ANAMMOX mechanism, can also be found in literature (Jetten *et al.*, 1997a and Strous *et al.*, 1997).

2.1.3 Phosphorus Removal

Biological phosphorus removal from waste water is based on two microbial mechanisms. As phosphorus is one key element in the synthesis of new biomass, a part of the phosphate present in the waste water is removed due to the stoichiometric coupling to the microbial growth. The second mechanism of phosphate removal is characterised by the uptake of phosphate in excess to the need for growth and its storage as intracellular polyphosphate (poly-P). This capability is the key aspect of enhanced biological phosphate removal (EBPR) process, referred to only as *BPR*.

The introduction of the inlet waste water into the anaerobic zone (Barnard, 1975) together with the circulation of activated sludge through the anaerobic and aerobic or anoxic zones are considered as the distinct features of the BPR process layout. This aspect allows an enrichment of **p**olyphosphate **a**ccumulating **o**rganisms (PAO) in the system. PAO are characterised by the capability to take up carbon sources anaerobically and to store them as intracellular organic polymers in order to use them in the proceeding aerobic/anoxic phases. This ability represents an advantage over the majority of other micro-organisms in the system, who are not capable of anaerobic substrate storage. Hence an enrichment of PAO can be achieved in the system, despite a lower specific growth rate compared to 'normal' heterotrophic bacteria (Nakuamura and Dazai, 1986; Smolders *et al.*, 1994a).

The dynamics of the key compounds involved in BPR when submitting the sludge to an anaerobic-aerobic sequence in a batch reactor, are illustrated schematically in Figure 2.1-4.

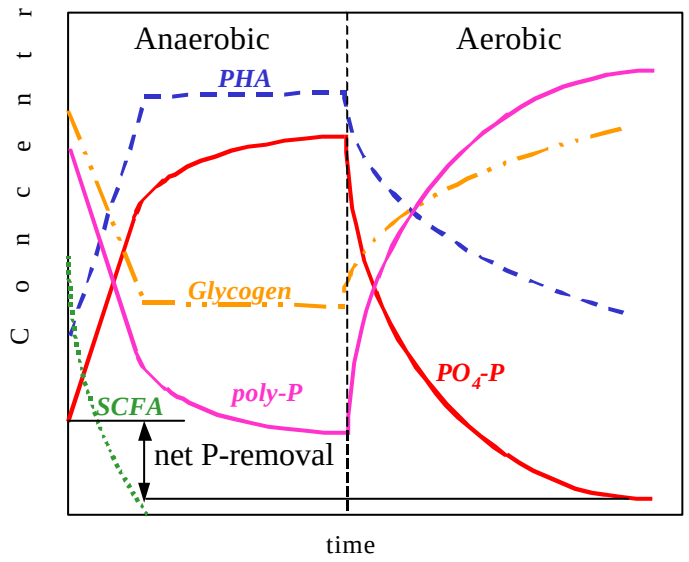

Figure 2.1-4: Dynamics of key compounds in BPR during an anaerobic-aerobic sequence

In the *anaerobic phase* the PAO take up carbon sources, mainly short chain fatty acids (SCFAs), and store them in the form of polyhydroxyalkanoates (PHA). The energy needed for this process is derived by the degradation of intracellular poly-P, resulting in a subsequent release of orthophosphate (PO_4^{-3}) into the mixed liquor. Glycogen, as a third storage compound, is degraded during this phase to supply required reducing power as well as part of the energy.

In the *aerobic phase* the PAO use the stored PHA as a carbon and energy source for aerobic growth, for the uptake of PO_4-P to recover the poly-P level and for the recovery of the glycogen pool. A net uptake of phosphate is achieved, as the PAO are capable of accumulating more phosphate during the aerobic phase than previously released under anaerobic conditions. The actual phosphate removal is accomplished by withdrawing excess sludge, with high phosphorus content, from the system.

Since the first report on biological phosphorus removal from waste water a considerable amount of research was dedicated towards its prerequisites and mechanism. Literature reviews have been presented by Barnard (1983), Wentzel *et al.* (1989), Torien *et al.* (1990), Jenkins and Tandoi (1991) and most recently by Mino *et al.* (1998). In recent years the discussion has focused on the different metabolic pathways leading to phosphorus accumulation. Basically two models for the metabolic mechanism have evolved, differing in the incorporation/acceptance of glycogen as a third storage compound. Although the role of glycogen is generally accepted today (Mino *et al.*, 1998), a more detailed understanding of both models is useful. Hence, both will be presented in the following sections.

2.1.3.1 Anaerobic conditions

During anaerobic conditions acetate, representing a carbon source, which readily promotes BPR, is transported into the cell and activated to acetyl-CoA. Acetyl-CoA is further converted to PHA, consisting mainly of polyhydroxybutyrate (PHB). The energy required for these processes is provided by ATP hydrolysis, leading to a release of cations (usually K^+, Mg^{2+} or Ca^{2+}) and the anion $H_2PO_4^-$. The regeneration of ATP from ADP is accomplished by transferring energy–rich phosphoryl groups from the poly-P pool. Since PHA is a reduced polymer, its synthesis requires reducing power. The proposed models vary mainly in the source of reduction equivalents as pointed out by Wentzel *et al.* (1991), who summarised the two main biochemical models : the Comeau-Wentzel model and the Mino model, schematically depicted in Figure 2.1-5.

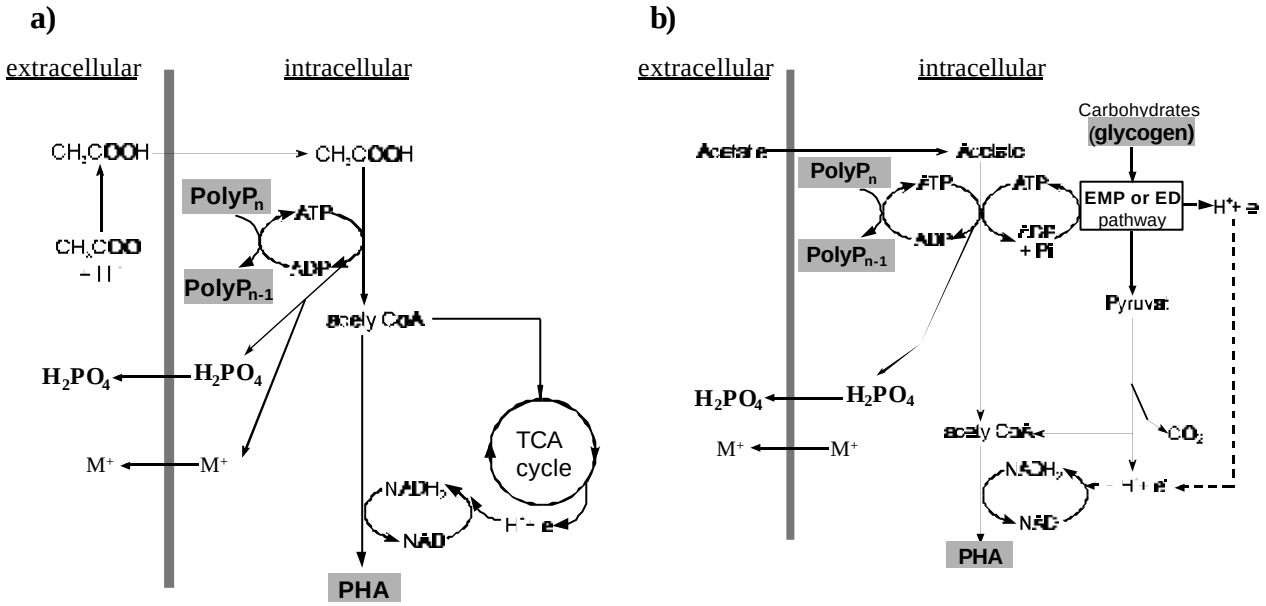

Figure 2.1-5. Models for anaerobic metabolism using acetate as a substrate.
a) Comeau/ Wentzel model b) Mino model (slightly simplified)

In order to obtain a better overview, the sources for the reducing power in the different models, are shown isolated in Figure 2.1-6.

In the *Comeau-Wentzel model* partial oxidation of acetyl-CoA through the TCA cycle is assumed to produce the required reducing power (Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Matsuo, 1985). Of the accumulated acetate, 11% enter the TCA cycle while the remaining acetate is converted into PHA. Per mol of acetyl-CoA four mols of $NADH_2$ are produced (Figure 2.1-6a).

In the *Mino model* degradation of intracellularly stored glycogen to acetyl-CoA is considered as the source for reducing equivalents as well as for part of the energy. (Mino *et al.*, 1987; Arun *et al.*, 1988). The Embden-Meyerhoff-Parnas (EMP) pathway was suggested as a source of reduction equivalents (Figure 2.1-6b). In the EMP pathway the degradation of 1 mol of glycogen produces 4 mols of $NADH_2$. Wentzel *et al.* (1991) suggested an adaptation of the Mino model, proposing the Entner-Doudoroff (ED) pathway for glycogen degradation. (Figure 2.1-6c).

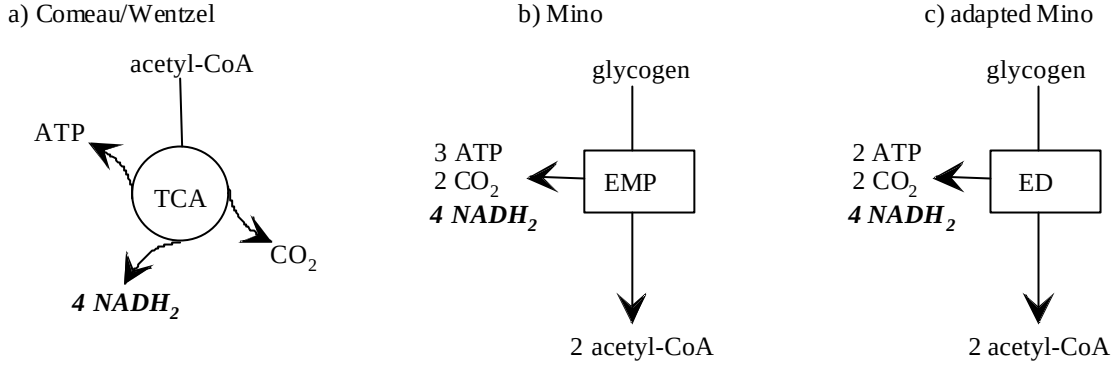

Figure 2.1-6. Sources of reduction equivalents ($NADH_2$) under anaerobic conditions according to: a) Comeau/Wentzel model, b) Mino model (Embden-Meyerhoff-Parnas pathway), c) adapted Mino model (Embden-Doudoroff pathway).

As mentioned before the adapted Mino model is favoured today, since several recent experimental results exhibited strong support for this model :

- Indications, that the acetate taken up anaerobically is not oxidised to CO_2 and thus not metabolised through the TCA cycle were obtained by Bordace and Chicsa (1989). They applied radioactively labelled acetate as a carbon source and found only a very small portion of the labelled carbon in the CO_2 generated under anaerobic conditions.
- By evaluating energy balances, Smolders (1995) demonstrated that there must be an additional mechanism to supply energy (ATP) for the formation of acetyl-CoA besides poly-P degradation. During experiments carried out under low pH conditions the P-release was found to be less than the amount theoretically required for the formation of acetyl-CoA.
- Several studies applying 13C NMR (^{13}C labelled acetate traced with solid state carbon NMR) demonstrate the role of glycogen within the anaerobic and aerobic metabolism of BPR sludge (Satoh *et al.*, 1992; Maurer *et al.*, 1997; Pereira *et al.*, 1996). It was shown that the major source of reduction equivalents was derived from glycogen.

Despite the strong support of the adapted Mino model due to the experimental evidences, the possibility of partial functioning of the TCA cycle under anaerobic conditions cannot be ruled out. In general the TCA cycle is linked to respiration and assumed to be operable only under aerobic and anoxic conditions. However, Pereira *et al.* (1996) suggested, based on in vivo 13^C-NMR and 31^P-NMR experiments, that the TCA cycle still contributes to the production of reduction equivalents under anaerobic conditions. Similar Maurer *et al.* (1997) proposed that PHA is formed from metabolised acetate together with degraded glycogen. In the same study, they supported the suggestion of Wentzel *et al.* (1991) that glycogen is degraded via the Entner-Doudoroff pathway instead of the Embden-Meyerhoff-Parnas pathway .

As a conclusion, a combination of both models shown in Figure 2.1-5 seems to be currently the most adequate approach.

2.1.3.2 Aerobic and anoxic conditions

Aerobic metabolism

Under aerobic conditions the PAO utilise the stored PHA as a carbon and energy source for growth (synthesis of new biomass) as well as for the recovery of the glycogen and poly-P pools. A schematic presentation of the metabolism is shown in Figure 2.1-7. In catabolic reactions PHA is broken down to acetyl-CoA, entering the TCA cycle and associated glyoxylate cycles. The reduction equivalents ($NADH_2$) generated in these cycles are subsequently oxidised via the electron transfer chain (ETC). ATP is produced simultaneously by the oxidative phosphorylation.

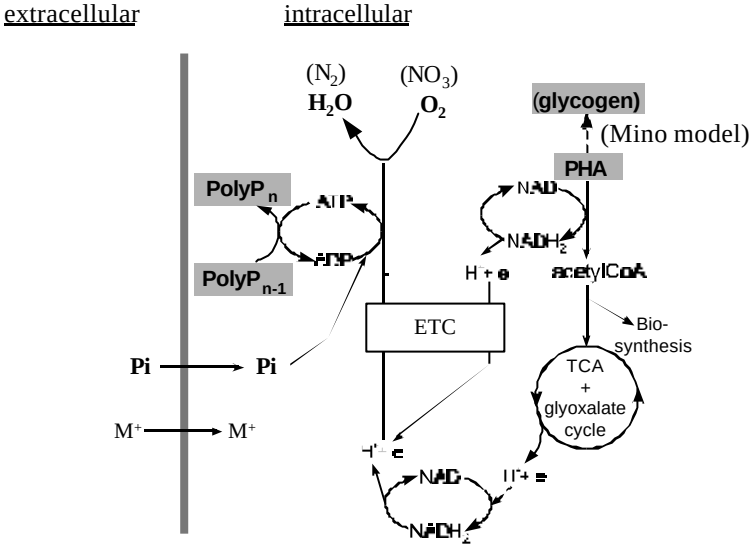

Figure 2.1-7. Aerobic/anoxic metabolic mechanism for BPR

Anoxic conditions: Denitrification by PAO

Initially it was suggested that phosphorus accumulation can only be achieved using oxygen as an electron acceptor. It was postulated that during the anoxic phase of a combined N & P removal process the PAO are more or less inactive or reacting as under anaerobic conditions. However, anoxic P uptake by PAO, using internally stored organics and nitrate instead of oxygen as an electron acceptor, has been observed in the past by several research groups (e.g. Hascoet and Florentz, 1985; Vlekke *et al.*, 1988; Kuba *et al.*, 1993). A review on denitrifying phosphorus uptake has been presented by Barker and Dold (1996).

Today it is well accepted that at least a fraction of PAO are able to use the respiratory mechanism under anoxic conditions, i.e. to perform the same metabolism under anoxic conditions as under aerobic conditions (Kuba *et al*., 1993, 1996b). Though offering the possibility for good P-removal performance, the anoxic P-uptake reveals a lower energy efficiency. Operating two SBRs (anaerobic-anoxic and anaerobic-aerobic) Vlekke *et al.* (1988) found nitrate to be not as efficient as oxygen, but suitable as a sole electron acceptor for BPR. They determined the ratio of $P_{taken\text{-}up}/PHA_{consumed}$ for the anoxic system to be 32% less than for the aerobic one. Also based on laboratory SBR operation, Kuba *et al.*, (1994) estimated the energy production efficiency with nitrate to be 40% lower than that with oxygen (expressed as mol ATP/mol NADH). The overall P-removal performance was good for both systems, but the P-uptake rates were lower in the anoxic SBR than in the aerobic one (Kuba *et*

al., 1994). Due to the lower energy production efficiency a 20 % lower cell yield value was reported for the anaerobic-anoxic process.

Research so far indicates that only a fraction of the PAO has this ability to utilise nitrate as electron acceptor (e.g. Kerrn-Jespersen and Henze, 1993; Bortone *et al.*, 1994). A division of the PAO with respect to the electron acceptor used was suggested by Kerrn-Jespersen and Henze (1993). The fraction able to use nitrate as well as oxygen can be termed denitrifying PAO (DNPAO), whereas the PAO only able to use oxygen are further referred to as O2PAO. An approach to characterise the microbial population in terms of phosphorus removal activity was presented by Wachtmeister *et al.* (1997), using the ratio of anoxic vs. aerobic P-uptake rate to determine the relative fraction of DNPAO. In the same study they also pointed out the possibility that there is only one population of PAO, which can acquire, depending on the intensity of the exposure to aerobic and anoxic conditions, different levels of denitrification activity. Hence the measured ratio $q_{P,anox}$ / $q_{P,aerobic}$ would represent the level of induced denitrification capacity. Although the assumption of two groups of PAO seems to find wider acceptance, there exists no definite proof yet. Also a combination of both hypotheses remains possible.

Up to now it remained unclear whether the DNPAO reduce nitrate only to nitrite, or if they are also able to use nitrite as an electron acceptor for P-uptake. The scarce available data seems to indicate that DNPAO are only capable of nitrate reduction. Comeau *et al*. (1987) reported that anoxic phosphate uptake did not occur with nitrite as electron acceptor. Kuba *et al.* (1996b) attributed a reduction in phosphate uptake activities, observed during an experiment involving enriched cultures in a sequential batch reactor, to nitrite accumulation. Lotter *et al.* (1986) carried out microbiological studies with isolates from different systems (AE, AN-AE, Bardenpho) and found that a majority of isolates capable of denitrification reduced nitrate only to nitrite. Overall, not sufficient data is available for a conclusive statement and further investigation is needed with respect to the role of nitrite in the denitrification by DNPAO.

Anoxic phosphorus uptake has also been reported from operation of full scale waste water treatment plants. Ostegaard *et al.* (1997), for example, performed a one year study on a full scale UCT system. They found PHA to play a mayor role as a carbon source for denitrification, with a corresponding phosphate uptake in the anoxic zone. They identified at least 30% of the COD consumed in the anoxic zone of the system as PHA and estimated the anoxic P-uptake to be 30% of the total one. Kuba *et al.* (1997) tested the activated sludge of two full scale UCT-type WWTPs with regard to the occurrence of DNPAO. Much lower DNPAO activity was found in one of the sludges, which was attributed to the transfer of nitrate to the anaerobic zones, lower fatty acids concentrations and lower amounts of nitrate recycled to the anoxic zone for one of the plants. This study illustrates that there exist a high degree of variation within the anoxic P-uptake activity and consequently also in the amount of DNPAO present in different systems.

So far only few factors have been identified, that influence the anoxic P-uptake ability of the PAO. The aeration time was found to be of crucial importance for the DNPAO and a minimisation of the aeration time is recommended to increase anoxic P-removal activity (Kuba *et al.*, 1996c). They recommend furthermore to avoid the carry over of biodegradable COD sources from the anaerobic to the subsequent phases as well as nitrate recirculation to the anaerobic zone. Hence, post-denitrification systems conflict with optimised anoxic P-removal, because large quantities of PHA are aerobically oxidised. Considerations like the above resulted in suggestions for a process

configuration, which consists of a two sludge system, performing nitrification with fixed film cultures and denitrification as well as P-removal with activated sludge (Kuba *et al.*, 1996b; Sorm *et al.*, 1996; Bortone *et al.*, 1994).

Simultaneous presence of BPR promoting substrates and electron acceptors (oxygen/nitrate)

In nutrient removal systems there exists strong competition for readily biodegradable substrates between the different microbial groups (oxic chemoorganotrophic, anoxic chemoorganotrophic (denitrifying) bacteria and PAO). The presence or absence of these substrates is a decisive factor influencing the microbial composition of activated sludge (e.g. Mudrack and Kunst, 1986).

Typical scenarios, which induce the simultaneous presence of carbon sources and an electron acceptor, are the introduction of nitrate into the anaerobic zone via the return sludge and the flow of organic substrate from the anaerobic zone downstream to the anoxic or aerobic one. Ongoing hydrolysis can also contribute further to the presence of readily biodegradable carbon sources.

In all cases of e^--acceptor entrainement into the anaerobic zone , the strong competition for substrate causes a decline in the availability of organic substrate for the PAO metabolism, resulting in a decreasing P-removal performance (Wentzel *et al.*, 1988, Smolders *et al.*, 1994a, Kuba *et al.*, 1994, Brdjanovic *et al.*, 1998). Furthermore the presence of nitrate in the anaerobic zone is believed to inhibit the hydrolysis of slowly biodegradable substrates. Consequently there will be a lack of fermentation products available for PAO, inducing a negative effect on BPR. Hence, in order to maintain BPR performance, the occurrence of such situations should be prevented.

Concerning the metabolism of PAO during the simultaneous presence of carbon sources and an electron acceptor, little detailed research has appeared in literature. Generally it is assumed that carbon sources available under these conditions will be primarily used for PHA formation (Mino *et al.*, 1998, Filipe and Daigger, 1997, Kuba *et al.*, 1994). However, there exists no fundamental reason against a direct usage/growth on acetate omitting the storage of PHA.

Assuming oxygen to be the electron acceptor present, the TCA cycle can be expected to be fully operative. Hence, theoretically, all PAO have two possible sources of reducing equivalents: the TCA cycle and the glycogen degradation. As the electron transport chain (ETC) is also fully operative during aerobic growth, excess reducing equivalents, generated in the TCA cycle, can be used to produce ATP. Thus several possible ways to fulfil their energy requirements (sources of ATP) are available for PAO : poly-P and glycogen degradation, the TCA cycle and oxidative phosphorylation. Which mechanism (pathways) will be used, is probably governed by the levels of ATP, Acetyl-CoA and reducing equivalents, as well as the levels of internally stored poly-P, glycogen and PHA. Depending on the cell needs and induced driving forces, the mechanisms applied, i.e. the sources, might vary. For example, it might be possible that situations occur, in which poly-P degradation is not applied during acetate uptake and PHA storage as sufficient energy is provided via the TCA cycle.

Accepting the existence of two groups of PAO the situation becomes more complicated in the simultaneous presence of carbon sources and nitrate. O2PAO metabolism can be assumed to be similar as under anaerobic conditions (s. section 2.1.3.1) as they are not able to use nitrate. The DNPAO on the other side might have all the possibilities, as described above for oxygen. The measurable result in terms of phosphate and PHA dynamics would be an overlay of the different microbial actions. P-uptake and PHA oxidation are suggested to occur simultaneously with P-release

and PHA storage when SCFA are available under anoxic conditions (Barker and Dold 1996, Gerber *et al.*, 1986, 1987). Hence, the net result of anoxic P-uptake or P-release seems also to depend on the relative number and activity of obligate aerobic P-removing bacteria (O2PAO), of nitrate reducing P-removing bacteria (DNPAO) and of non P-removing heterotroph bacteria (denitrifiers).

The overview given illustrates that some details of BPR metabolism have yet not been fully understood. The fact that the simultaneous presence of carbon sources and nitrate as an electron acceptor occurs quite often during operation of WWTPs, illustrates the importance of further investigations addressing these scenarios.

Practical importance of denitrification by PAO

In practice the denitrifying capability of PAO is expected to be beneficial to the overall nutrient removal for several reasons. Due to the ability of the PAO to take up and store phosphate, using nitrate as electron acceptor, the same organic substrates are effectively utilised for both P and N removal. This is of significance since organic substrate availability is often a limiting factor in nutrient removal processes. Hence, usage of anoxic BPR can achieve phosphorus removal and denitrification at the same time and save significant amounts of COD (Wanner *et al.*, 1992; Kuba *et al.*, 1996b). The appropriate choice of process layout and operation may lead to an improved process performance by maximising the organic substrate utilisation. Other advantages associated with denitrifying PAO activity may include a reduction in aeration energy and a reduced sludge production.

Mathematical modelling of the nutrient removal processes is used for an increasing number of applications With respect to BPR, process behaviour of phosphate and nitrogenous compounds can only be predicted by introducing the denitrifying ability of PAO into the model.

2.1.3.3 Responsible organisms

In general PAO are characterised by their capability to store poly-P and by their ability to take up and store carbon sources under anaerobic conditions. Conventionally it has been assumed that a single dominant group of micro-organisms would be enriched in BPR sludge with high phosphate removal capacity. Former investigations focussed on bacteria of the type *Acinetobacter sp.*, which were shown not to be responsible for BPR in recent studies (e.g. Wagner *et al.*, 1993b, 1994; Kampfer *et al.*, 1996). Despite a certain degree of research, so far no pure cultures were isolated, that exhibited all characteristics a BPR sludge should posses (Jenkins and Tandoi, 1991; Mino *et al.*, 1998).

Based on recent studies (Wagner *et al.*, 1994; Bond *et al.*, 1995; Kampfer *et al.*, 1996) it is likely that the PAO consist of different bacterial groups and are not dominated by a single bacterium. This is supported by the work of Liu (1995), who found at least three morphological distinguishable micro-organisms, dominating the microbial community of a sludge with high BPR performance. These recent studies are in line with former observations (e.g. Fuhs and Chen 1975; Buchan 1983; Streichan *et al.*, 1990; Matsuo 1994), which indicated that the BPR sludge (community) can change with time and can well be different from place to place.

Further studies and research applying new molecular techniques for microbial identification of BPR sludge are considered as the appropriate tools in order to identify the organisms responsible for BPR.

A proliferation of other organisms, also capable of anaerobic substrate uptake, has first been observed by Cech and Hartman, 1990. As their metabolism involves intracellular glycogen and PHA storage, but no poly-P accumulation (Satoh *et al.,* 1994), they are commonly referred to as glycogen accumulating organisms (GAO). Often a deterioration of BPR performance has been observed along with the enrichment of GAO, directing the research towards investigations concerning the reason for such a proliferation of GAO (e.g. Satoh *et al.,* 1994; Cech *et al.*, 1993, 1994; Liu *et al.,* 1994, 1996a). So far the presence of glucose in the feed (Cech and Hartman 1990, 1993) long SRT and HRT (Fukase *et al.,* 1985; Matsuo, 1994) seem to play an important role for the enrichment of GAO.
In practice the BPR process treating municipal waste water is relatively stable in terms of phosphate removal. Deterioration of BPR due to GAO enrichment has so far been mostly reported from laboratory scale processes. For a more detailed review concerning the microbiology of BPR systems, including the expected characteristics of PAO, the reader is referred to Mino *et al*. (1998).

2.1.3.4 Mathematical models for BPR.

With ongoing research concerning BPR, a variety of models have been developed, reflecting the status of the understanding of the biochemical aspects at that time. Wentzel *et al.* (1988, 1989 a, b, 1992) proposed a first comprehensive mathematical model for BPR, which was based on the Comeau/Wentzel model (Comeau *et al*., 1986; Wentzel *et al*., 1986). This model was taken as a basis and restructured by the IAWQ task group on mathematical modelling (Henze *et al.*, 1995), presenting the Activated sludge model No. 2 (ASM2). The ASM2 deals with the several processes of waste water treatment (C, N and P removal), including also simultaneous precipitation of phosphorus with ferric hydroxide. The capability of PAO to denitrify, was first not considered, but later introduced in the new version, the ASM2d (Henze *et al.*, 1998). In both models glycogen is not introduced as a variable, but incorporated in the organic substrate pool of PHA. Mino *et al.*, (1995) proposed an extension to the ASM2 to include glycogen as a component and the process of denitrifying phosphorus removal.
Smolders *et al.,* (1994a, 1995) proposed a metabolic model solely for anaerobic-aerobic BPR with acetate as a substrate, explicitly taking into account the internal processes in the cell. Considerations of the fate of slowly biodegradable organic substrate were not included. This model was further extended to capture anoxic BPR processes (Kuba *et al.,* 1996a; Murnleitner *et al.,* 1997). A combination of this model with the C and N removing part of the ASM2 was presented by Brdjanovic (1998) in order to simulate biological waste water treatment of full-scale treatment plants.
Barker and Dold (1997 a, b) also proposed a model for BPR with 19 components and 36 processes, including phosphate uptake under aerobic as well as anoxic conditions.
Filipe and Daigger (1998) refined the metabolic model of Smolders (1995) by proposing a different mechanism for acetate transport into the cell. They assumed passive diffusion instead of active transport as suggested by Smolders and illustrated a better agreement with experimental data of Wentzel (1989 a, b) .
Pramanik *et al.*(1999) presented a flux-based stoichiometric model for the BPR metabolism with the intention to test and improve the assumptions made in the kinetic models. Using linear optimisation for solving the vast numbers of reactions (163 reversible and 166 irreversible) the model provides

some information on the pathways of energy and reducing equivalents production (ATP, NADH and NADPH).

From the review of proposed metabolic pathways for BPR and associated mathematical models it can be seen that some details of BPR have not been fully understood and are still under investigation. Mathematical models differ in their scope and the amount of details included in the model. The obtained models today are able to describe rather well the behaviour of laboratory scale BPR processes, enriched with PAO and fed with acetate as a main substrate. For the purpose of applying these models to practical solutions, kinetic information of PAO is still needed and should be further investigated. Also the aspect of using a substrate other than acetate will be a point of interest in the future.

2.1.3.5 Further factors influencing BPR

Types of carbon sources

The amount of phosphorus that can be removed by BPR is directly related to the amount of low molecular organics, preferably acetate, taken up by the PAOs under anaerobic conditions. Those organic substrates are derived either from the raw wastewater or from fermentation of higher molecular organics under anaerobic conditions (Lötter and Pitmann, 1992; Randall et al., 1994; Skalsky and Daigger, 1995). However, it should be kept in mind that other low molecular organic substances than acetate, including propionate, lactate, pyruvate, malate and succinate, can also be metabolised by PAO (Satoh *et al.*, 1996). Whereas acetate mainly leads to the accumulation of polyhydroxybutyrate (PHB), the other mentioned low molecular organics induce also the accumulation of other PHA (e.g., polyhydroxyvalerate, PHV) though to a lower extent (Satoh *et al.*, 1992).

The current model of the BPR metabolism (section 2.1.3.1) originates from acetate as a carbon source. Investigations concerning the pathways for organic substrates other than acetate have been carried out by some research groups. An overall concept is presented by Mino *et al.* (1996). An overview of the approaches used and of a conceptual model for the anaerobic uptake of organic substrates and their conversion to PHA by PAO is presented by Mino *et al.* (1998).

Precipitation

The addition of calcium or metal ions, such as ferric iron or aluminium is known as a means to achieve chemical phosphorus removal as a alternative to BPR (Henze *et al.*, 1997). Hence, it is evident that the possible presence of these substances in the waste water reaching a biological treatment plant will also induce chemical phosphate precipitation, simultaneous to the biological one. This has to be taken into consideration when BPR is the objective of the investigation. Generally, the concentration of metal ions (iron and aluminium) is negligible in municipal waste water, unless it is added upstream of the treatment plant (preventing odour problems or industry discharge). Calcium on the other side is often present in the waste water in varying amounts. Phosphate precipitation by calcium is highly pH dependent and will increase significantly at higher pH levels. To avoid a mixing of different P-removal mechanisms, which are not easy to differentiate, the pH must be controlled at least below 7.5 (Maurer and Boller, 1998).

Influence of pH

Biological processes in general are dependent on the pH value, exhibiting an optimal pH range for each biological process. With regard to biological wastewater treatment a pH in the range of 6.5 up to 9 is considered to be adequate (Henze *et al.*, 1997).

Concerning BPR, the pH can significantly effect the energy budget of anaerobic substrate uptake by PAO. The ratio of phosphorus released to carbon taken up reflects the energy provided by poly-P degradation for this anaerobic substrate uptake. The values reported in literature for this ratio exhibit quite a variation (Mino *et al.*, 1998). Smolders *et al.* (1994 a, b) linked this variation to the pH of the system, showing that a higher pH results in a higher ratio of P-release/HAc taken up, with a variation of 0,25-0,75 P-mol/C-mol in a range of pH=5.5 to 8.5. They suggested that the transport of acetate into the cell is thermodynamically influenced by the pH. As the pH of the waste water influences the electrical potential difference across the cell membrane, the energy requirement for the transport of acetate into the cell increases with increasing pH, i.e. more 'work' is necessary to take up a negatively charged ion, like acetate, against the negative electric potential of the cells. The experimental results were confirmed by Liu *et al.* (1996b).

In addition to the influence discussed above the variation of the pH can also influence the overall P-removal, as higher pH for example can lead to increasing chemical precipitation. Consequently, controlling the pH to a fixed value or at least monitoring the pH is indispensable if aspects of BPR are investigated.

Temperature

The influence of the temperature on the conversion rates of PAO seems to be within the same order of magnitude as for other heterotrophic organisms (Schreiner 1994; Meinhold 1994; Brdjanovic *et al.*, 1997; Baetens *et al.*, 1999). In contrast to the significant effect on the rates under anaerobic conditions, being dependent on the temperature according to the Arrhenius type equation, no influence on stoichiometry was detected. An optimum temperature interval of 20 to 30 °C was determined for the anaerobic processes.

Counterbalancing ions

As discussed in section 2.1.3.2, the phosphate taken up and stored as polyphosphates in the cells is counterbalanced with ions such as Ca^{2+}, Mg^{2+} and K^{+}. Investigations by Brdjanovic *et al.* (1996) showed that for example potassium limitation has a negative effect on BPR. However, a severe shortage or limitation of potassium during the treatment of municipal wastewater is unlikely to occur due to its high potassium content. Treatment of industrial wastewater, which contains high amounts of phosphate but no potassium, can cause problems.

2.2 Activated Sludge Systems for Enhanced Nutrient Removal

The term activated sludge evolved in the beginning of this century from investigations, addressing the aeration of waste water and introducing a recycle of the suspension formed during the aeration period. This suspension consisted of active biomass responsible for the improvement of treatment efficiency and was termed *activated sludge*. The recycle of sludge remained a characteristic feature of the activated sludge process, allowing elevated biomass concentration in the aeration tanks.

The initial objective of the activated sludge process in waste water treatment was to remove carbonaceous pollution and suspended solids. Since then a vast variety of process configuration were developed (Alleman and Prakasam, 1983; Wanner, 1994). Design has been dependent, amongst others, on the waste water characteristics, the effluent standards and the state of the knowledge about cause and effect relationships within the biological processes.

Nitrogen removal was introduced in the treatment plant layout at the beginning of the 1970s. First the nitrification process was included in the treatment line and some years later denitrification was added as a process step. In the subsequent years biological phosphorus removal was increasingly incorporated in the biological waste water treatment.

The affect of waste water characteristics and the optimisation of process configurations have been the main aspects influencing the further development of biological treatment systems. Wastewater characteristics are evidently influenced by the types of pollution sources, but also by the processes within the sewer system. Both cannot be modified easily. Primary sedimentation or acid fermentation of primary sludge (Wentzel and Ekama, 1997) are known processes, which can be applied on the site of the treatment plant in order to change waste water characteristics, being more favourable to desired microbial reactions. Optimisation of the process configuration aims at creating environmental conditions, most suitable for the optimal activity of bacteria, performing the corresponding treatment step (e.g. avoiding oxygen entrainement in zones dedicated for denitrification; preventing wash-out of nitrifying bacteria). Furthermore, the plant layout has to account also for the negative impacts of the dynamics in the incoming load on the performance of the biological treatment. Construction of equalisation basins and/or oversizing of the plant, for example, were the consequences.

As a results various process configurations exist today with manifold reactors, including aerobic zones for carbon oxidation and nitrification, anoxic zones for denitrification and anaerobic zones necessary for BPR. Some configurations incorporate multiple series of reactors, with various recycle streams and phase schedules, others are based on sequentially operated reactors with different multiple phases.

2.2.1 Principle/ Basic Process Configurations for BPR

Despite a lack in the fundamental understanding from microbiological and biochemical points of view, the BPR process has been incorporated in the operation of existing treatment plants and can be considered today as well established in practice. With increasing understanding and operational experiences, the design of the BPR process configurations evolved. The common prerequisite for all BPR configurations is the alternating cycling of the sludge through anaerobic and aerobic/anoxic conditions and the introduction of the influent to the anaerobic zone. The process alternatives are differentiated according to the location of the anaerobic zone. Mainstream configurations incorporate

the anaerobic zone in the water line, whereas in sidestream processes the anaerobic phase is present in the sludge line. Common continuous flow, mainstream processes for BPR are illustrated in Figure 2.2-1.

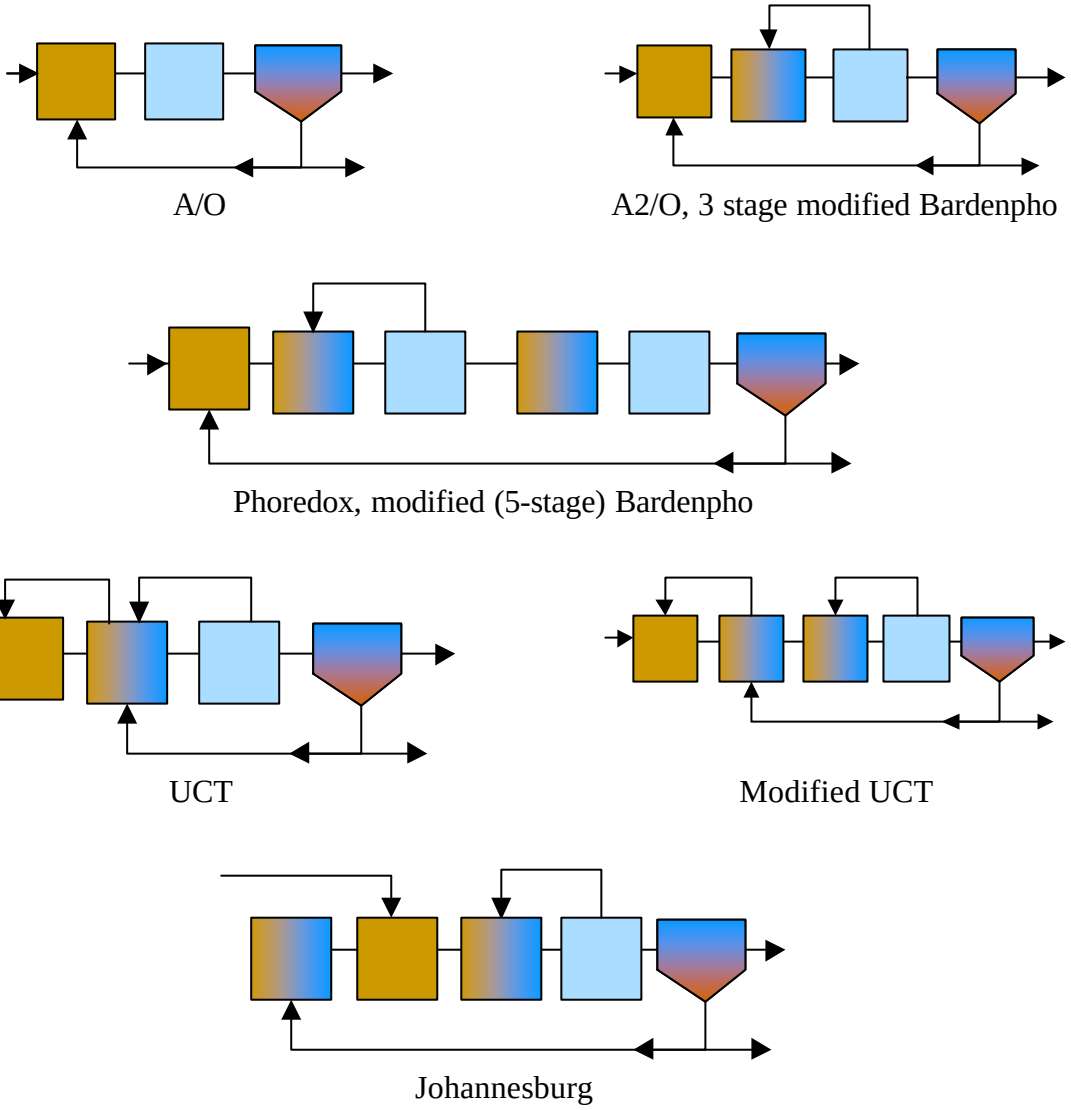

Figure 2.2-1 Mainstream processes for BPR. anaerobic : ; anoxic: ; aerobic:

The simplest configuration for BPR consists only of a sequence of an anaerobic and an aerobic reactor with the return sludge being recycled to the anaerobic reactor. This *A/O process* usually does not incorporate nitrification, due to the low sludge residence time applied. Once nitrification is to be included, the design becomes more complex, as nitrate recirculation to the anaerobic zone has to be avoided. Hence, the layout has to account for appropriate denitrification. In the *A2/O process* this is accomplished by a pre-denitrification step, i.e. recycling nitrate from the aeration zone to the anoxic one. The high recycle ratio between the reactors, needed to assure low nitrate concentration in the effluent represents a major disadvantage. Further modification by inserting an additional post-denitrification step resulted in the *Phoredox* process. Both process types exhibit the disadvantage of introducing a certain amount of nitrate to the anaerobic zone with the return sludge. In the *UCT* system this effect is minimised, by returning the sludge to the anoxic reactor for further denitrification and by adding a supplementary recycle between anoxic and anaerobic zones. Optimisation of this configuration lead to the division of the denitrifying reactor in two reactors (*modified UCT*).

The *Johannesburg* process addresses the problem of nitrate in the return sludge in a different way. Here the sludge is returned to a non-aerated reactor at the head of the treatment line for endogenous denitrification.

The process configurations shown in Figure 2.2-2 still represent mainstream processes, but exhibit some specific characteristics, which are shortly described in the following.

The *BioDeniPho* system represents an alternating process type, in which the nutrient removal process is performed sequentially in each of the two tanks by switching the flow path and the aeration pattern according to a cyclic strategy. Within each phase the flow from the anaerobic reactor is always directed to the anoxic reactor to provide organic matter for the nitrate reduction. A more detailed description of this process is provided in Appendix 8.4.

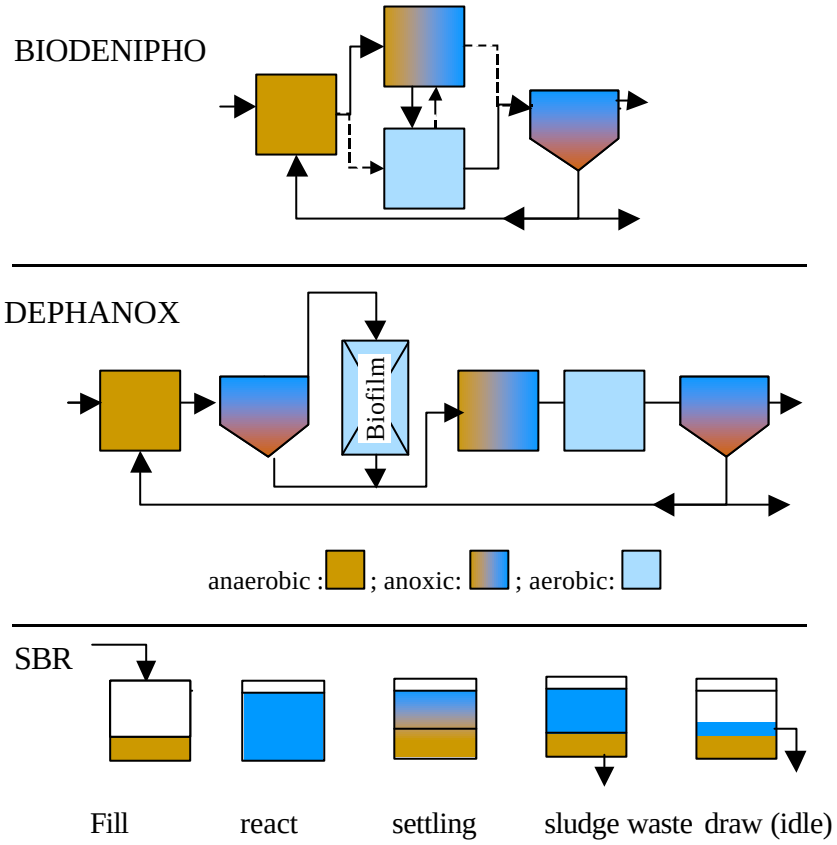

Figure 2.2-2. Mainstream processes for BPR: sequential operation mode (BioDeniPho™, sequencing batch reactor) and with a biofilm reactor for nitrification (DEPHANOX)

The particular characteristic of the *DEPHANOX* process is the combination of nitrification in a biofilm reactor with an activated sludge system for BPR and denitrification, thus representing a two sludge system. After the anaerobic reactor the wastewater is separated from the sludge and sent to the biofilm reactor for nitrification. In the subsequent reactor the two streams are mixed again, providing favourable conditions for anoxic phosphorus uptake. The final aerobic reactor is supposed to ensure satisfactory P-removal from the system (Bortone *et al*., 1994).

Apart from the continuous systems, presented above, BPR can also be performed in a sequencing batch reactor (*SBR*). In the *SBR* process a sequence of phases with different environmental conditions are applied on a time scale to the reactor. A variety of phase combinations is possible, enabling the achievement of similar process layouts as in continuous flow activated sludge systems.

The *Phostrip*™ process, shown in Figure 2.2-3, represents a sidestream process for BPR. Here the anaerobic phase is present in the sludge line. Phosphate release from the sludge is induced in the

anaerobic zone by appropriate means, e.g. addition of acetate, and subsequently precipitated chemically after separation. Hence, phosphate is not removed with the biological excess sludge, but with the 'chemical sludge' from the precipitation unit.

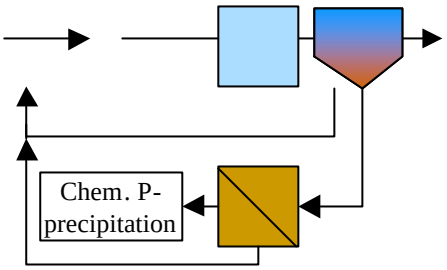

Figure 2.2-3 Side-stream process: Phostrip

As shown a large variety of process layouts exist, which all exhibit reasonable performance. Design rules were mostly deduced from operational experience, but more and more the increasing knowledge about the biological process and availability of mathematical models play an important role within the layout of waste water treatment plants. The choice of the appropriate system for BPR and nitrogen removal depends mainly on the Nutrient/COD ratio in the influent and the amount of readily biodegradable COD available, but it is also governed by patent regulation and by country specific preferences.

2.2.2 Process Characteristics of Activated Sludge Systems

A characteristic of activated sludge processes for waste water treatment is the wide range of time constants, which is due to the vast variety of physical, chemical or biochemical processes involved. An overview over the essential time constants, encountered in activated sludge systems, is presented in Figure 2.2-4. Changes in dissolved oxygen concentration occur with time constants in the order of seconds. Variations in nutrient concentrations due to microbial actions or caused by redirecting internal flow patterns, are processes which occur in the order of minutes. Internal sludge redistribution by control of the return sludge flow rate may take hours before significant changes are noticeable. Finally, changes in sludge inventory occur in the order of days, while significant changes in sludge biomass composition may require weeks or more.

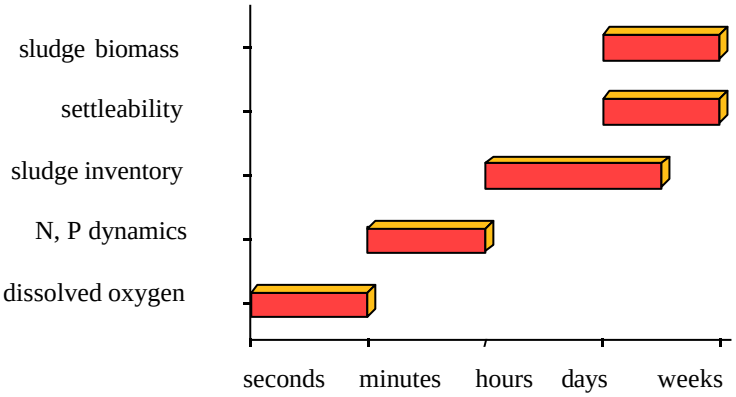

Figure 2.2-4 Essential time constants of activated sludge nutrient removal processes

The high degree of interaction between the different biological processes and the wide range of time constants illustrate the complex and complicated task of operation and control of activated sludge

systems. Its complexity is further increased due to the process reactivity being an intricate and slowly changing function of ambient temperature and waste water composition. This is even intensified, as the influent flow, its composition and the temperature are continuously subject to variation on an hourly, daily, weekly and seasonal time scale. Hence, the main challenge in control of activated sludge processes is the disturbance attenuation in this complex non-linear, multivariable system. The principal objective is to maintain sufficient nutrient removal, which meets the required effluent standards at lowest possible costs, i.e. creating environmental conditions for efficient pollutants removal. This implies also, due to being open systems, maintaining of a proper micro-organism consortium through selective pressure by adequate operation and design. For a comprehensive overview of the problems involved the reader is referred to the corresponding literature (e.g. Marsili-Libelli, 1989 and Olsson *et al.*, 1989, 1992).

The development of control strategies is closely related to the development of the understanding of the processes involved as well as to the improvement of fast, robust and inexpensive measurements systems (e.g. Thornberg *et al.*, 1993). Despite increasing efforts and recent developments, still only a small number of variables relevant to waste water and sludge components can be assessed by appropriate (real-time) sensors. Hence, indirect measurements are often evaluated and used for control purposes, e.g. oxidation reduction potential (ORP) (Sekine *et al., 1985*; Menardiere *et al.*, 1991; Wouters-Wasiak *et al.*, 1994; Paul *et al.*, 1998), pH (Al-Ghusian *et al.*, 1994) and oxygen utilisation rate (OUR) (Surmacs-Gorska *et al.*, 1995; Larose *et al.*, 1997; Klapwijk *et al.*, 1998).

In recent years, with increasing process knowledge and computational power and improvement in data collection (including on-line sensors), more advanced control strategies evolved (e.g. Lukasse, 1999). Often rule-based control is applied (e.g. Thornberg *et al.*,1992; Nielsen *et al.*, 1995), but more and more the model based control approach, involving models with different levels of complexity can be found (e.g. Isaacs and Thornberg, 1997). In order to facilitate the overall multivariable problem, a decomposition by decoupling the control of fast and slow processes, see Figure 2.2-4, is often applied (e.g. Hiraoka and Tsumara, 1989). An overall control strategy, aiming at a minimal plant size while ensuring efficient and robust performance, will have to consist of a hierarchical structure, incorporating several levels with various grades of sophistication. The lowest level is represented, for example, by dissolved oxygen (DO) control in the aerated reactors or biomass control, maintaining a certain MLSS concentration. These controller just serve to create an environment for efficient substrate and nutrient removal, hence their setpoint is being dictated by the higher level control routines. Global optimisation strategies designed to maintain optimal sludge activities and properties, are at the highest level and may involve long term control decisions.

The control handles available to achieve the desired goals are dependent on the type of nutrient removal processes involved in the activated sludge system. The control handles available for a pure *nitrification process* involve the waste flow rate, the aerobic hydraulic residence time and the air flow rate. The waste flow rate seems unsuitable for active control of the process, as the response time of the sludge inventory to changes is quite long. Moreover due to affecting the sludge residence time, and hence the concentration of autotrophs, severe constraints to the freedom of manipulation are imposed by settler design and the request to avoid the risk of settler malfunctioning. Common active control handles for the nitrification process are the aerobic hydraulic residence time and the airflow rate, controlling the dissolved oxygen (DO) concentration around a fixed setpoint, preventing DO limitation or inducing controlled DO limitation (e.g. Sekine *et al.*, 1985; Isaacs,1996).

The *denitrification process* introduces as new control handles, the internal recirculation flow rate, the size of the anoxic zone, which can be changed by switching off the individual aerators and the external carbon source addition, which increases the denitrification rate (Sekoulov *et al.*, 1990; Tam *et al.*, 1992; Isaacs *et al.*, 1995).

Alternating processes simplify to some extent the problem of automatic control. They do not require the large internal recirculation flow rate with its inherent pumping costs and continuous O_2 transport to the anoxic phase. Moreover, the switch between aerobic and anoxic conditions (flowpath and aeration switching times) can be performed in minutes independent of hydraulic hold up times. Furthermore, the internal dynamics of the process allow easy estimation of reaction rates from measurement signals. This offers an ideal setting for (recursive) identification of process dynamics (e.g. Cartensen *et al.*, 1995).

The different control handles and control goals with their corresponding response time are illustrated in Figure 2.2-5.

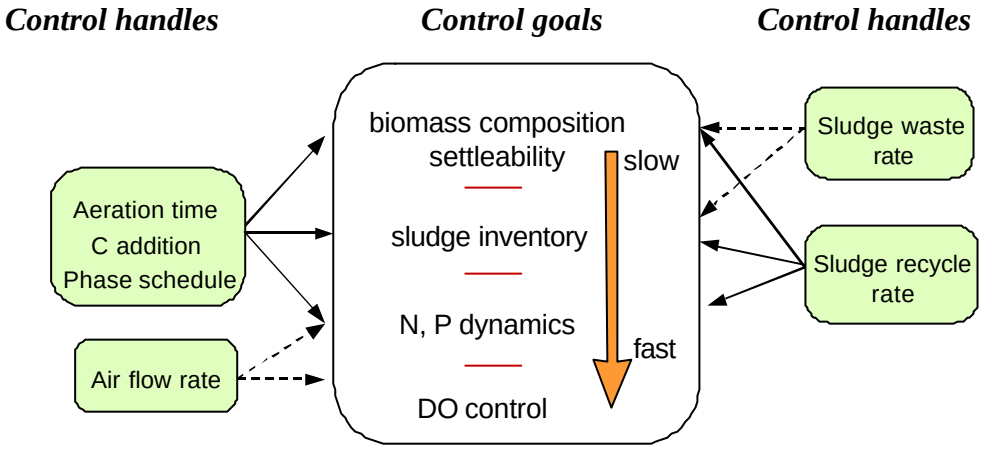

Figure 2.2-5 . Control goals and handles, incl. the response time, in process control of AS processes

The aeration intensity, sludge recycle rate and sludge wasting rate are considered, amongst others,. as traditional control signals employed for activated sludge processes. These do not offer the control authority and operational flexibility needed to maintain effluent standards (see review by Olsson, 1985, 1992). Hence new ways to effectively influence process dynamics were investigated during the last years. These include control handles such as manipulation of dissolved oxygen levels, external carbon addition rates, recirculation rates and, for periodic processes, the timing of switching of flow paths and aeration (Isaacs, 1996, Isaacs, 1997; Isaacs and Thornberg, 1997). The majority of these investigations dealt with C and N removal processes. This is partly due to the fact that the process knowledge is more sufficient for N-removal and consequently also the models needed for advanced control are less complex and better validated than for BPR. Unlike biological N removal, the interactions between waste water components and activated sludge micro-organisms affecting biological P removal are still not very well understood today. Although implemented in many full scale plants throughout the world, relatively little is known concerning optimised plant operation with regard to maximisation of P removal and avoidance of disturbance related losses in performance. In case of periods with poor BPR performance, for example, often chemical precipitation is applied (in parallel or in serial) in order to meet the effluent standard regulations with regard to phosphorus. Hence, a first step towards advanced control of the BPR processes is the identification of the essential causal relationships, which can then be translated to implementable

control handles. A key to improving biological P removal is the understanding of the factors limiting the rate and the extent of P-uptake in the aerobic and anoxic reactors. The ability of PAO to denitrify opens up the possibility of using the same waste water organic substrates to carry out both tasks of N and P removal. It remains to be investigated which factors affect the ability of PAO to denitrify, and how this ability can be implemented in operational strategies, i.e. in what way can it be used to improve the process performance.

In summary, the introduction of phosphorus removal in addition to biological nitrogen removal increases considerably the operational complexity of activated sludge processes. Consequently, maintaining acceptable effluent water quality at reasonable costs requires new developments in operational and control strategies.

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3 OBJECTIVES AND APPROACH

The introduction of biological nitrogen and phosphorus removal increases considerably the operational complexity of activated sludge processes. Appropriate operational and control strategies to maintain acceptable effluent water quality, rely on the understanding of the important underlying mechanisms. Despite intensive research on the BPR mechanism over the past 20 years and this process being well established in practice today, there are still lacks in the understanding of BPR. In particular, little is known about the factors influencing the behaviour of the phosphate accumulating organisms (PAO) under denitrifying conditions. As an original contribution to increase and improve the knowledge on the BPR process, the principal objective of this study is to:

- Investigate and identify the interactions in the anoxic zone of a combined nitrogen and phosphorus removal process and evaluate their consequences on plant-wide operation and performance.

To achieve this goal, the work is subdivided into different steps, involving batch and pilot plant experiments as well as model evaluation. Based on current knowledge and the literature review (*Chapter 2*), important aspects to focus upon have been identified for each step.

Major Steps

A) Understanding of biological phosphorus removal (BPR) through experimental work with focus on anoxic conditions and its governing phenomena.

Cause and effect relationships are examined both in laboratory and pilot plant scale.

This step includes investigations on:

- The dependency of anoxic and aerobic P-uptake on the PHA content and the factors that influence the rate and the extent of phosphate storage *(Chapter 4.1).*
- The hypothesis of two fractions of PAO (DNPAO and O2PAO) and possible ways to assess their activity with simple batch experiments *(Chapter 4.2).*
- The effect of nitrite, as an intermediate in nitrification and denitrification, on the PAO activity *(Chapter 4.3).*
- The impact of an easily degradable substrate present in the anoxic zone on BPR.

(Chapter 4.4 and Chapter 5).

B) Suitable Operational and control strategies

Information currently available about potential control strategies for systems involving N and P removal is scarce. Evaluation of the microbiological reactions under anoxic conditions is expected to lead to the identification of potential control handles. Emphasis will be put on operational strategies to avoid nitrate accumulation in the system, as this is known to interfere with BPR performance. The investigations will include implementation and testing on pilot plant scale of the operational strategy as a function of the process conditions *(Chapter 5)*

C) Model evaluation and modification

Based on the knowledge of the cause and effect relationships gained from previous steps, an existing mathematical model is analysed. As a starting point, the combination of ASM2 (Activated Sludge Model No 2) and the TU Delft model is employed. Refinement and modification are performed and tested for an improved description of the biological nutrient removal process, especially regarding:

- The dependency of the phosphate uptake rates on the PHA content.
- The anoxic acetate uptake of PAO.

(Chapter 6)

Thesis outline

Chapter 2, *Background of biological wastewater treatment*, presents general background information as well as detailed information on biological phosphorus removal. Based on current knowledge and the literature review presented, the important aspects to focus upon in this work have been identified. These aspects are summarised and listed above as the major steps of this work

There is a particular need to improve the understanding of the P-uptake before an amelioration of the operation and control of the BNR system can be undertaken. As a consequence *Chapter 4* addresses the investigations concerning major cause and effect relationships for biological P-uptake. It is structured such that each section addresses an individual aspect of BPR, listed above under major step A). All sections have been, or will be, published separately. Consequently they can be read independently, however with the inherent drawback of some unavoidable repetitions. A summarising conclusion, addressing the important findings of all sections including their significance for the subsequent work of this study, is presented at the end of the chapter (section 4.5).
Process behaviour and performance were examined predominantly in batch experiments in chapter 4. Sludge obtained from a BioDeniPho type pilot plant was submitted to different imposed conditions, using liquid phase and internal storage compounds (PHA) measurements for detailed analysis.

The rate of P-uptake is often the crucial step in achieving satisfactory BPR. Identification of the important parameters affecting the P-uptake rates is hence significant. Section 4.1 addresses and demonstrates the high dependency of aerobic and anoxic P-uptake rates on the level of intracellularly stored PHA. Consequently, this dependency is taken into account when discussing/analysing BPR behaviour during the subsequent work. In addition it is emphasised that any modelling approach

should incorporate this aspect and that strategies for improved and stabilised BPR should include actions to keep the PHA level sufficiently high.
It is known that BPR performance is relying on the dynamics of the COD load in the incoming waste water. In that context, section 4.1 addresses another interesting aspect: the tendency of BPR to deteriorate at sudden increases of the COD content in the influent. Evaluation of the PHA utilisation rate and the P-uptake rate are used to discuss possible underlying reasons. This aspect is of significant importance for any kind of additional discharge of high concentrated streams to the system (discharge of industrial waste water, external addition of hydrolysate etc.).

In general during this study, phosphorus uptake rates under anoxic conditions were found to be 50 to 60 % of the aerobic ones, which is within the range reported in literature. This difference is not explainable only based on the type of electron acceptors. Section 4.2, hence, picks up the theory of two fractions of PAO presenting experimental investigations that strongly substantiate this theory: the denitrifying part (DNPAO) able to use nitrate and oxygen as electron acceptors, and the second group (O2-PAO) only capable of oxygen utilisation. Changes of phosphate and PHA pattern in a sequence of anaerobic-anoxic-aerobic phases are used to discuss the existence of these two groups of PAO. Furthermore, there is an interest in being able to detect shifts in the PAO population or/and anoxic activity. Possible ways, including their drawbacks, to assess the two fractions of PAO by applying simple batch tests are presented and discussed. The most appropriate method is analysed in detail and found to be suitable for detecting changes in the population distribution or anoxic BPR activity, that might take place due to changes in operational strategies.

Another issue where little information is available concerns the effect of nitrite on BPR. BPR could be affected by nitrite, as the accumulation of nitrite, appearing as an intermediate in nitrification and denitrification, is known to cause severe problems in biological processes in general. Section 4.3 addresses this topic by analysing batch experiments designed to cover a range of nitrite concentrations and their effect on P-uptake during anoxic and aerobic conditions. It is demonstrated that above certain critical nitrite concentration, both anoxic and aerobic P-uptake are damaged. This is of relevance for treatment scenarios that might favour nitrite accumulation, as for example SBRs or the treatment of waste water highly loaded with ammonium. However, accumulation of nitrite up the critical level were not observed during pilot plant operation and are not to be expected in continuous systems treating municipal waste water.

Section 4.4 addresses the aspect of simultaneous presence of organic substrate and nitrate in BPR systems. These circumstances are not unusual, in particular due to the inherent requirement of organic substrate for denitrification. Concerning the effect on BPR, main focus in research so far was put on nitrate introduced to the anaerobic zone via the recirculation of return sludge. However, BPR promoting organic substrates, i.e. VFA, and nitrate can well be simultaneously present also in the anoxic reactors. Organic substrate is made available at a slow rate, either due to conversion reactions (hydrolysis, fermentation) within the anoxic reactor or due to incoming readily degradable substrates not taken up in the anaerobic zone. Hence, it is of interest to examine what occurs with respect to BPR dynamics, e.g. PHA storage/utilisation and phosphate uptake/release, when organic substrates are continuously added to the anoxic zone. In section 4.4 this subject is addressed via a series of batch experiments, including a continuous addition of an external carbon source to the anoxic phase.

The responses of the system are evaluated for a large interval of concentrations added and cover the interaction in the anoxic phase as well as their consequences for the subsequent aerobic phase.
Based on the results essential tendencies are outlined with respect to the leakage of easily biodegradable substrate from the anaerobic zone to the anoxic reactor. Moreover, first indications and trends are presented concerning the feasibility of controlled addition of an external organic substrate to the anoxic reactor as a means to improve N removal in BPR systems.

The conclusions drawn in Chapter 4 give first insight to the consequences on operation and performance related to the interactions in the anoxic zone. In addition they are used as a fundamental base for the subsequent work concerning detailed investigation of the behaviour of a continuous system (pilot plant scale) and for required model modifications for simulating BPR.

In *Chapter 5* the investigations are extended from batch experiments to a continuous system at pilot plant scale level (BioDeniPho® process). Scenarios are analysed, that deal with the response of the pilot plant to the continuous introduction of a BPR promoting organic substrate to the denitrifying zone. The study addresses the effect of potential leakage of easy biodegradable COD from the anaerobic to the anoxic zone, as well as the use of a model based control routine for the external carbon source addition in order to control nitrate in a BPR system. Aim of the control strategy is to improve N-removal, by increasing the denitrification rate. Thereby it is expected to considerably decrease the risk of nitrate accumulation leading to a reduction in BPR performance due to nitrate introduction to the anaerobic zone. The experiments are discussed in conjunction with the calculated P-release, P-uptake, PHA utilisation and denitrification rates of the corresponding environmental zones (anaerobic, anoxic, aerobic).
Several important questions are addressed: a) can the conclusions drawn from batch experiments be confirmed for a continuous system; b) are there distinct differences in the response of the two systems; c) when does BPR deteriorate due to the external addition of organic substrate to the anoxic zone; d) can the model based approach chosen be adapted to the criteria of satisfactory BPR; e) how is the anoxic activity of PAO affected?

Models for simulations of activated sludge systems are used for an increasing number of applications, nowadays. However, models incorporating BPR are still under discussion. Main reason for this situation is the evolving understanding of the underlying mechanism combined with the need to reduce the complexity of the model.
Chapter 6 deals with model evaluation addressing the modifications to be performed for an improved description of the biological nutrient removal process, based on the experimental findings of this thesis. As a starting point, the combination of the part of ASM2 related to COD and N removal and the TU Delft model for BPR is employed. Refinement addresses two distinct aspects : the dependency of the P-uptake rates on the intracellular PHA content and ability of PAO for anoxic acetate uptake. Both issues have been identified in the previous chapters, as being important for a correct description of the BPR process. Question addressed during model extension included: a) does a refinement of the model improve the prediction quality to a noticeable extent; b) can it be accomplished with a minimal increase in amount of parameters?
In a second step the revised model has been extended to two groups of PAO, differing in their ability to use either only oxygen (O2-PAO) or oxygen and nitrate (DNPAO) as electron acceptor. Focus was put on external disturbances, that might have a potential impact on the proliferation of the DNPAO.

Investigation concerning the ‘two-group’ model are carried out as pure simulation studies, as its applicability is severely restricted due to a lack of measurements for differentiating the distribution of the internal storage pools between the two groups of PAO. However, it offers a research-tool or approach to improve the understanding BPR. Modification of the ‘one-group’ model, on the other side, is destined for practical applications.

Chapter 7 contains the overall conclusions of this thesis and in addition the recommendations for further research concerning BPR. As often in research, the work addressing a certain set of objectives tends to open up new set of questions. The topics for future research outlined in this section originate to an extent from this study as well as from discussion with researcher working within the same field.

3.1 Publications and Contributions

Meinhold, J. and Isaacs, S.; 1997. Biological Phosphorus removal from wastewater: Use of oxygen and nitrate as electron acceptor. Oral presentation and poster presentation at the *Danish Biotechnology Conference III* in Vejle, 1997

Meinhold J., Filipe C.D.M., Daigger G.T. and Isaacs S.; (1999a) Charaterization of the Denitrifying Fraction of Phosphate Accumulating Organisms in Biological Phosphate Removal. *Wat. Sci. Tech*., **39** (1), 31 – 42.

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Filipe C. D. M., Meinhold J., Daigger Glen T., Jørgensen S.-B. and Grady C.P.L.Jr. (2001). The Effects of Equalization on the Performance of Biological Phosphorus Removal Systems. *Wat. Envir.* ***Res.***, May/June 2001, 276 – 285. (parts presented at the WEFTEC 99).

Jensen J.L., Meinhold J., Krühne U. and Jørgensen S.-B.. Model Predictive Control Design for an Alternating Nutrient Removal Process. INRA Conference on New Advances in Biological Nitrogen and Phosphorus Removal for Municipal or Industrial Wastewaters, pp 231-239, Narbonne, France October 1998.

Articles prepared for submission

To be submitted to Water Research :

Meinhold J., Larose C.A. and Jørgensen S.-B. Anoxic and Aerobic Phosphate Uptake rates as a function of the initial PHA content.
Based on section 4.1.

Meinhold J., Larose C.A. and Jørgensen S.-B. Performance and behaviour of Biological Phosphorus Removal during External Carbon Source Addition for Denitrification.
Based on section 5 (5.2).

Meinhold J., Knoche R., Larose C.A. and Jørgensen S.-B. Adaptation of a control algorithm for improved nitrogen removal to constraints imposed by biological phosphorus removal performance.
Based on section 5 (5.3)

To be submitted to Biotechnology and Bioengineering :

Meinhold J., Larose C.A. and Jørgensen S.-B. Anoxic acetate uptake and the dependency of phosphorus dynamics on PHA content – A revised model structure for biological phosphorus removal.
Based on section 6 (6.2).

4 PHOSPHORUS UPTAKE UNDER ANOXIC AND AEROBIC CONDITIONS -CAUSE AND EFFECT RELATIONSHIPS –

Introduction

The performance of biological phosphorus removal (BPR) depends on a complex and still not fully understood mechanism (section 2). Although the process has been extensively investigated, most research in this field has been directed towards the anaerobic phase, as this was assumed to be the most essential step in the overall process (Matsuo *et al.*, 1992). Designing an anaerobic reactor, which will give a maximum poly-hydroxy-butyrate (PHB) storage capacity and a maximum potential for P-uptake, relies on the knowledge of the stoichiometry and kinetics of this phase. Investigations of ruling phenomena and interactions in the P-uptake phases are scarce. Smolders *et al.* (1994b) used respirometric measurements to calibrate a metabolic model for the aerobic phase, and Kuba *et al.*, (1996) studied the anoxic uptake behaviour in a SBR, presenting a metabolic model for denitrifying PAO, based on the model for the aerobic phase presented by Smolders *et al.* (1994b). But the majority of these studies have been performed on enriched cultures under non-limiting conditions, using synthetic wastewater as a substrate, thus not necessarily reflecting the conditions encountered in full scale plants.

Most activated sludge processes designed for biological phosphorus removal (BPR) include denitrifying zones for N-removal. Consequently different microbial activities for nutrient removal (N and P) take place simultaneously at the same location of the plant; i.e. phosphate is known to be taken up in the aerobic and anoxic stages during which also nitrification and denitrification, respectively, are taking place. Therefore it is most likely that interactions between the different processes occur and that the processes will affect each other. The understanding of these interactions and essential phenomena has to be improved, in order to optimise process design and operation and to suggest appropriate control strategies. With regard to control and operation of BPR processes the anoxic and aerobic phases are at least as important as the anaerobic phase. Although the amount of PHB stored in the anaerobic phase is decisive for the P-uptake potential, the interactions and essential phenomena in the uptake phases together with the aerobic and anoxic retention time, determine the actual amount of phosphorus that can be taken up.

In this chapter the results from a series of investigations are presented, aiming at different aspects with regard to the anoxic and aerobic P-uptake behaviour. The experiments were carried out as batch tests to ensure more defined conditions than encountered in the pilot plant, thus improving the assessment of the different influencing factors.

4.1 Anoxic and Aerobic P-uptake Rates as a Function of the initial PHA Content

ABSTRACT

Results of experimental investigations are presented that clearly demonstrate the dependency of aerobic and anoxic P-uptake rates on the level of internally stored PHB. Batch experiments were performed in which activated sludge obtained from a pilot scale BiodeniphoTM was submitted to a sequence of anaerobic/anoxic or anaerobic/aerobic, conditions while monitoring the course of NOx-N, NH4-N, PO4-P, PHB and PHV. The obtained P-uptake rates as a function of the PHB content in the cells are summarised and compared to literature values (Petersen *et al.*, 1998).

Furthermore the achievable net P-uptake is investigated, when submitting the activated sludge to different COD loads during the anaerobic phase. A decrease in the BPR performance has been noticed, once the COD load exceeded the normal, corresponding load of the pilot plant. The observed response is discussed based on the current understanding of the underlying mechanisms and its behaviour compared to similar observations reported in literature.

4.1.1 Introduction

The interaction between the different intracellular components, with poly-hydroxy-alkanoates (PHA) as key substances, is characteristic of the biological phosphorus removal process. Hence, an understanding of the effects of the intracellularly stored PHA on BPR is essential for the practical application of the process. The studies of Smolders *et al.* (1994b) on the aerobic metabolism did not include the evaluation of the dependency of the aerobic P-uptake rate on the PHA level, since the work was based on non-limiting conditions with respect to PHA. In a recent study of the aerobic phosphate uptake Petersen *et al.*, (1998) pointed out that the aerobic P-uptake rate is highly dependent on the PHB concentration. In this section the relationship between the internal PHB level and the observed P-uptake rate is investigated for anoxic as well as aerobic conditions. Furthermore the questions of achievable net uptake of phosphorus and evolution of the denitrification rate for different amounts of initially added COD are addressed.

4.1.2 P-uptake Rates as a Function of the initial PHA level

Several sets of batch experiments with up to 4 reactors in parallel were carried out, using activated sludge from a BioDeniPho pilot plant and applying the experimental batch set-up and the analytical methods described in section 8.2. During the anaerobic phase the four reactors of each set received different amounts of acetate.The sets differed in the type of P-uptake period, i.e. applying aerobic or anoxic conditions, in order to asses the effect of the initial PHA level on the P-uptake rates under both environmental conditions. After the acetate induced P-release ended, potassium phosphate (KH_2PO_4) was added to the reactors to bring the phosphate concentration to the same level in all four reactors of each set. Subsequently aeration was started or nitrate added in order to establish aerobic or anoxic conditions, respectively. The pH was continuously monitored and adjusted to 7.0 ± 0.1 and the temperature remained between 18 and 19 °C.

Figure 4.1-1 illustrates the results of two representative set of experiments. The concentration patterns show the typical behaviour, applying either aerobic or anoxic conditions.

Under aerobic conditions a higher phosphorus uptake, along with higher PHA utilisation, is observed compared to anoxic conditions. Anoxic phosphorus uptake slows down considerably after some time, despite PHA concentrations being at higher level than at the start of the experiment. Possible explanations for this reduced activity under anoxic conditions will be discussed in section 5.2. The contribution of phosphate accumulating organisms (PAO) to denitrification can clearly be seen in Figure 4.1-1. As all reactors exhibit the same biomass composition and extracellular COD sources are severely limited in the anoxic phase, the increase in denitrification can be attributed to the activity of the PAO, which increases according to the amount of PHA stored in the anaerobic phase.

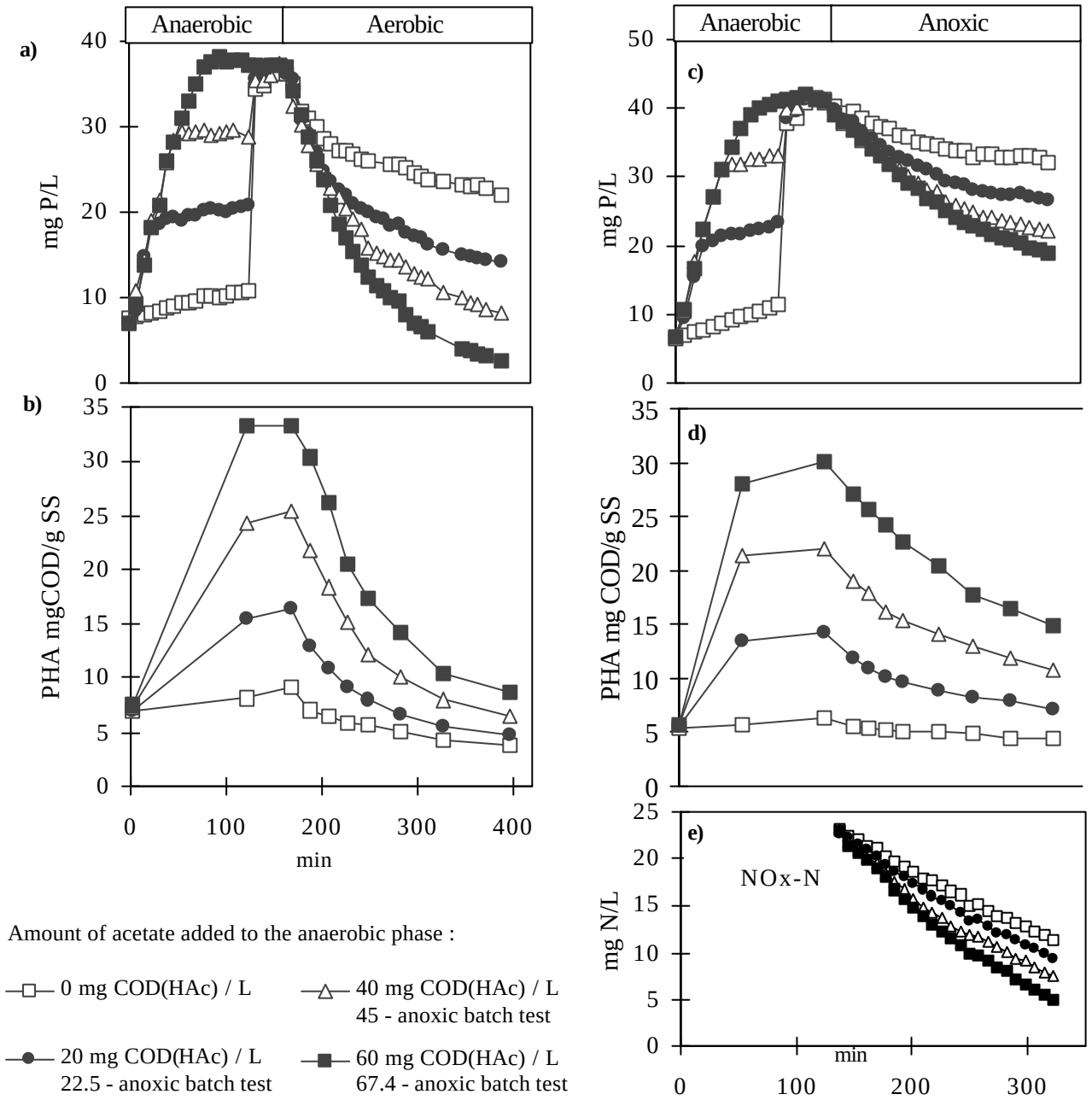

Figure 4.1-1. Set of batch experiments with different amount of acetate initially added:
a) to b) measured concentration in anaerobic- aerobic batch tests.
c) to e) anaerobic-anoxic batch tests.

The corresponding rates of P-uptake, denitrification and PHA utilisation, calculated from a regression from initial portions of the experimental data, are shown in Figure 4.1-2. For both conditions applied, the initial P-uptake rate and the PHA utilisation rate increase with increasing level of internally stored PHA. It is noteworthy, that while the aerobic P-uptake rate is about twice as high as the anoxic one, the PHA utilisation under aerobic conditions is about 3 times the value of the anoxic one. A possible explanation could be the presence of an organic substrate due to hydrolysis of slowly biodegradable substances and subsequent fermentation under anoxic conditions. Fermentation, being the process producing VFA or easily biodegradable substrate, could induce PHA storage in parallel to the PHA utilisation and thereby reduce the measured PHA utilisation rate. Similar would account for phosphate: the measured data would reflect the sum/overlay of P-release

and P-uptake. The P-uptake, being significantly higher, would mask the release, similar to the observation and suggestions of Gerber *et al.* (1987). In the aerobic reactor, the processes producing easily biodegradable substrate might not play a role as important compared to anoxic conditions. Consequently the aerobic data reflects mainly the P-uptake and PHA utilisation processes. In ASM2 (Henze *et al.*, 1995) hydrolysis takes places under all process conditions (anaerobicc, anoxic, aerobic), though at different rates, while fermentation is limited to anaerobic conditions. Accepting these assumptions the explanation given above might not hold, but little is known up to now concerning hydrolysis and fermentation under anoxic or aerobic conditions. Furthermore the conditions closed to or within the sludge might be different compared to the bulk liquid, e.g. there might be anaerobic conditions in the center of the floc, with fermentation as a consequence, while the liquid is anoxic.

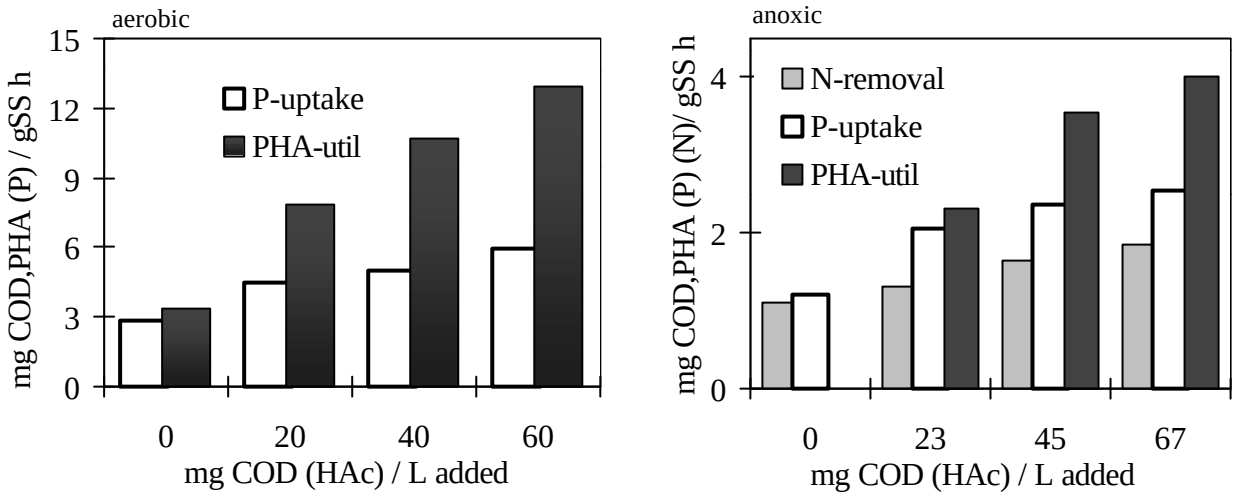

Figure 4.1-2. Rates for P-uptake, PHA utilisation and denitrification for the aerobic and anoxic uptake phases of the batch tests.

The contribution of PAO to denitrification in the presented batch test is quite significant. Comparing the reactors with the two highest additions of acetate to the one receiving 23 mg COD(HAc)/L, the denitrification rate increased by around 30% and 46 % respectively. Background denitrification, i.e. NO3-N removal by denitrifiers using extracellular COD sources, will be higher in the pilot plant operated with real municipal wastewater, since the presence of extracellular COD sources during anoxic conditions is not as severely limited as in the batch tests. Consequently the relative contribution of the PAO to denitrification using internal stored PHA, though still significant, will be less in full scale or pilot plant operation.

The *initial P-uptake rates as a function of the initial PHB level* from a series of batch experiments are presented in Figure 4.1-3a. For compariative purposes the figure is supplemented with data reported by Petersen *et al.* (1998) (white Δ) for aerobic conditions. The grey data points show the values obtained in this study for aerobic conditions whereas the black ones represent the anoxic data.

With regard to aerobic conditions the increase of the P-uptake rates with increasing PHB level is obvious. Despite different conditions, i.e. different amount of PAO, the data is quite in line with those from Petersen *et al.* (1998). They estimated the initial concentration of PAO to 600mg-COD/L via the maximum observed P-release rate during anaerobic conditions, the VSS concentration, the observed ratio of P/HAc and the rate constant for PHA storage according to the ASM2 default value (Henze *et al.*, 1995). Performing the same estimation for the batch tests of this study, the amount of

PAO varied between 420 and 580 mg-COD/L (grey circles and squares in Figure 4.1-3a). In order to eliminate deviations due to different amount of PAO in the system, the aerobic P-uptake rates are plotted against the PHB content in the PAO in Figure 4.1-3b. At lower PHB concentration levels the values from both investigations are quite in line with each other. For PHB concentrations above 15 $mgCOD_{PHB}/gVSS$ there seems to be a deviation, although it is hard to compare, as there is a lack of data from Petersen *et al.* (1998) for the higher concentration region. They determined the maximum P-uptake rate for their data to 14 mg P/g VSS h, which is not supported by the data obtained in this study.

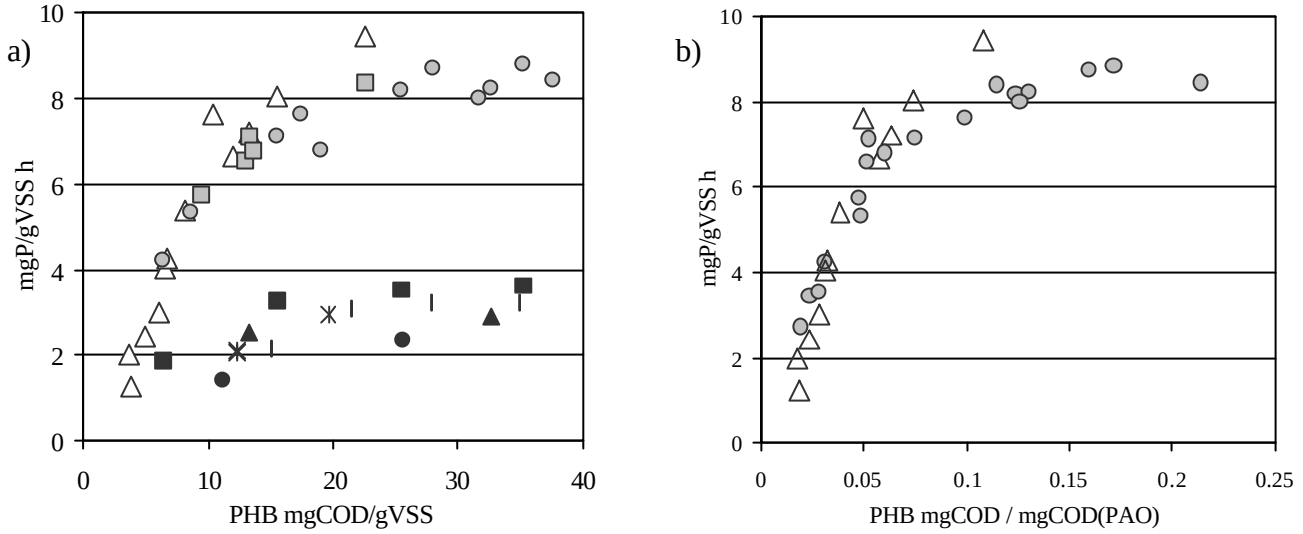

Figure 4.1-3. Observed initial P-uptake rate as a function of the initial PHB level from batch tests.
Results of this study are compared to the results from Petersen *et al*.(1998).
white Δ - aerobic P-uptake rates (Petersen *et al.*, 1998);
grey○ □- aerobic P-uptake rates this study; black symbols- anoxic P-uptake rates

Besides clearly revealing the dependency of P-uptake rates on the PHB level, no further, definite conclusion can be drawn. This is partly due to an insufficient amount of data and, moreover, due to possible differences in the poly-P concentration between the different experiments, which also affects the uptake rate to a certain degree (Brdjanovic *et al.*, 1998). Comparing the P-uptake rates of two reactors at a specific PHB level results in different values. As the operation of the reactors differed only in the amount of acetate initially added, this observation underlines that the P-uptake rate is not completely described by the PHB level alone and hints towards a dependency of the P-uptake rate on the poly-P concentration.

The P-uptake rates calculated for the anoxic conditions, are more scattered than for aerobic conditions (Figure 4.1-3a). This behaviour is most probably due to the varying activity of anoxic P-uptake in the different experiments and, similar to the aerobic case, influenced by the different amounts of PAO in the system. Looking at each batch experiment separately (represented by the different black symbols) it can be clearly stated, that the anoxic P-uptake rate is also highly dependent on the PHB level.

Fraction of PHA not available for biodegradation.

Several investigations report that a certain amount of PHA is not or only partly accessible for biodegradation. Brdjanovic *et al.* (1998), investigating the impact of excessive aeration on BPR in a SBR using biomass highly enriched with PAO, stated that 2.1 mg COD_{PHB} /g VSS of PHB remained in the biomass. Petersen *et al.* (1998) and Temmink *et al.* (1996) estimated the amount of PHB not

available for biodegradation to 2.6 mg COD/g VSS or 11.7 mg COD_{PHB}/g COD_{PAO}, after aerating the biomass from an activated sludge pilot plant for more than 12 hours.
In the present study the PHA (sum of PHB and PHV) concentration approaches a value of 1.5 mg COD_{PHA}/g VSS or 9.6 mg COD_{PHA}/g COD_{PAO}, which is somewhat lower than the values stated above, despite applying a shorter aeration period than in the investigations mentioned above. All values, however, remain in the same order of magnitude. The assumption that this residual amount of PHA is less accessible for biodegradation arises from the observation that even after long aeration the PHA pool will not be depleted down to zero. This observation could also be possible due to the existence of micro-organisms other than PAO, being able to store PHA. Glycogen accumulating organisms (GAO) use glycogen and PHA as internal storage components, but do not exhibit poly-P formation (Cech and Hartman, 1990, 1993; Mino *et al.*, 1994; Satoh *et al.*, 1994). It is assumed that the presence of glucose, sugars and other complex carbon sources in the inlet is one essential factor supporting the growth of GAO (Liu *et al.*, 1996, Satoh Y. *et al.*, 1994). But several aspects suggest that these organisms are not present or play an insignificant role in the activated sludge investigated :

a) the stoichiometric parameters calculated from batch tests for the anaerobic phase are well in line with the current model understanding;
b) glucose and sugars are not a major carbon source in the pilot plant operation;
c) during situations without 'COD shock load', the dynamic of the internal PHA storage is found to follow well the phosphorus dynamics according to the current understanding of the BPR process.
d) The influent P/C (g P/gC_{HAc}) ratio of the Biodenipho pilot plant (P/C ≈ from 12/100 to 20/100) is high enough to favour the growth of PAO, out competing GAO (Liu et al., 1997).

4.1.3 Achievable net Uptake of Phosphorus for different amount of initially added COD

In order to be able to compare the phosphorus removal capacity of the reactors receiving different amounts of acetate in the anaerobic phase, the ratio of the phosphorus taken up within the first hours to the amount of P released due to acetate uptake was chosen as a criterion. In Figure 4.1-4 the values obtained for several batch tests (indicated by different symbols) are displayed. Values above 100% reflect net P-removal, whereas values below 100% indicate that no net removal has been achieved. For both uptake conditions, aerobic and anoxic, the removal capacity decreases with increasing initial COD addition, as more P is released in the anaerobic phase than taken up in the subsequent aerobic or anoxic phase. Looking at the each batch test seperately (different symbols) this tendency becomes even more evident.
Although the P-uptake rate is at a higher level for the reactor receiving a higher amount of acetate, this can not compensate completely for the higher P-release. This response, however, can not be regarded as typical for BPR at higher COD levels, as it is known that plants operating at higher COD levels achieve satisfactory phosphorus removal. The investigations made are characterised by special conditions, representing a sudden increase in the COD load compared to the load level observed in the pilot plant just before the experiments.

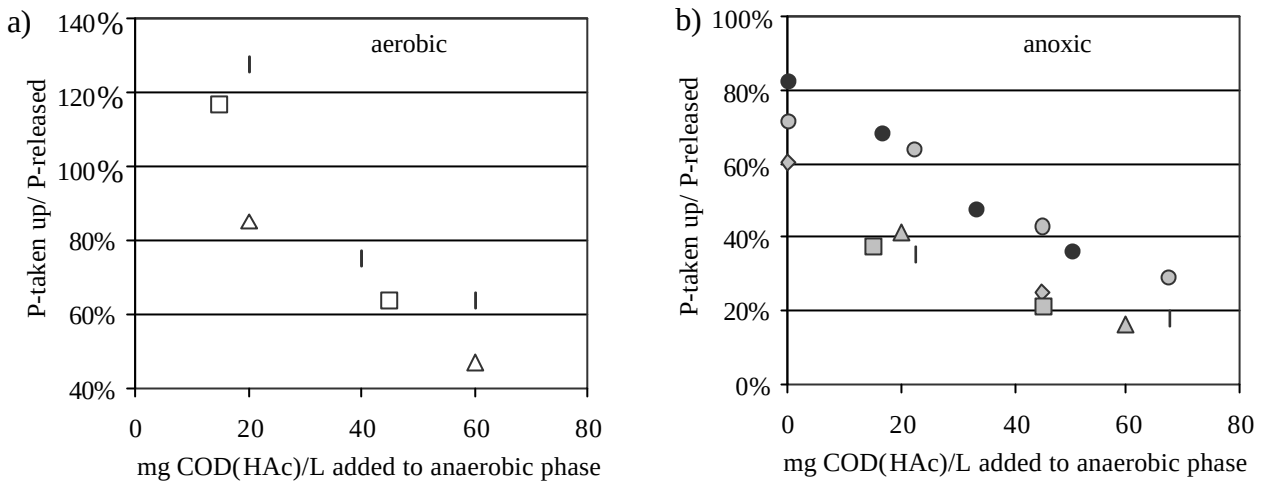

Figure 4.1-4. Ratio of PO_4-P taken up within the 1st hour to PO_4-P released due to HAc uptake.

a) Results from anaerobic-aerobic batch tests
b) Results from anaerobic-anoxic batch tests

Values > 100% reflect net P-removal; values < 100% indicate that no net removal has been achieved.
The different symbols indicate values obtained for different batch tests.

In some reactors with higher COD addition, the P-uptake ceases or slows down considerably without obtaining net-P-elimination although the PHA level is still higher than at the beginning of the experiments. Similar observations were made by Brdjanovic *et al.* (1998), who attributed this behaviour to a high level of poly-P in the cells, being close to the maximum poly-P content of the cells reported by Smolders *et al.* (1996) and Wentzel *et al.* (1989) and thus limiting the phosphorus uptake. In the present study the observed behaviour cannot be attributed to a possible influence of the poly-P pool , i.e. reaching a maximum level, as the decrease of the P-uptake occurs before the amount of phosphorus previously released has been taken up. Consequently the poly-P pool should be at a lower level than at the start of the experiment.

The stoichiometric parameters of the anaerobic acetate uptake, P-release and PHA storage calculated for these batch tests are close to the values observed in other investigations (e.g. Table 4.1-1).

Table 4.1-1. Stoichiometric coefficients of the anaerobic phase

Y_{PO4} (P_{rel}/HAc_{up}) gP/gCOD	Y_{PHA} [1)] ($PHA_{stor.}/HAc_{up}$) gCOD/gCOD	Y_{PHB} ($PHB_{stor.}/HAc_{up}$) gCOD/gCOD	source
0.5 –0;6	1.2 – 1.3	0.9 – 1.1	this study
0.35		1.5	Murnleitner *et al.*, 1997
0.43		1.5	Smolders *et al.*, 1995
0.52		0.89	Barker and Dold, 1997a, 1997b
0.5			Wentzel *et al.*, 1992
0.43		1.18	Kuba *et al.*, 1997
0.37 - 0.55			Kuba et al., (1993) AN-ANOX SBR
0.41 – 0.49			Kuba et al., (1993) AN AE SBR

1) PHA representing the sum of PHB and PHV.

Consequently the amount of PHA stored during the anaerobic phase should in most cases satisfy the requirements for complete removal /uptake of the phosphate previously released. But the response in

the batch tests reveals that this is not the case. Calculating the ratio of the PHA utilisation rate and the P-uptake rate reveals an increase in the amount of PHA used with respect to phosphate taken up (Figure 4.1-5). In general it is possible that more energy is required to take up phosphate, presumed that certain conditions, as for example pH or temperature will change. The pH is known to influence the energy requirement for the substrate transport over the cell membrane (Smolders *et al.*, 1994a). However, as both, pH and temperature, were kept constant during the batch tests in this study, there seems to be no reason for an increase in the energy requirement for phosphorus uptake. Possible limitation of the P-uptake due to the lack of Mg^{2+} and K^{+}ions (Brdjanovic *et al.*, 1996), needed as counter ions (s. section 2.1.3), did not occur (experimental data not shown). Hence, the increased PHA utilisation seems to be due to another PHA consuming process. The refill of the glycogen pool and cell growth are two processes known to be associated with PHA consumption (s. section 2.1.3). Both processes are not covered by the measurement set-up. The differences in the yields for growth of new biomass, production of glycogen and phosphorus uptake are known to result in an increase in the ratios mentioned. An additional possibility could be that the PAO, cultivated/living under permanent starving conditions, will use a sudden increase in carbon availability to increase their growth. An increase of the carbon flux from the PHA pool towards growth would reduce the amount of PHA available for P-uptake and thus lead to a reduced P-uptake. A behaviour like this could well be an explanation for the observed responses in the batch tests and the calculated utilisation rates, as the measured PHA utilisation rates represent the overall utilisation due to all processes related to PHA consumption rates and not only the ones referring to P-uptake.

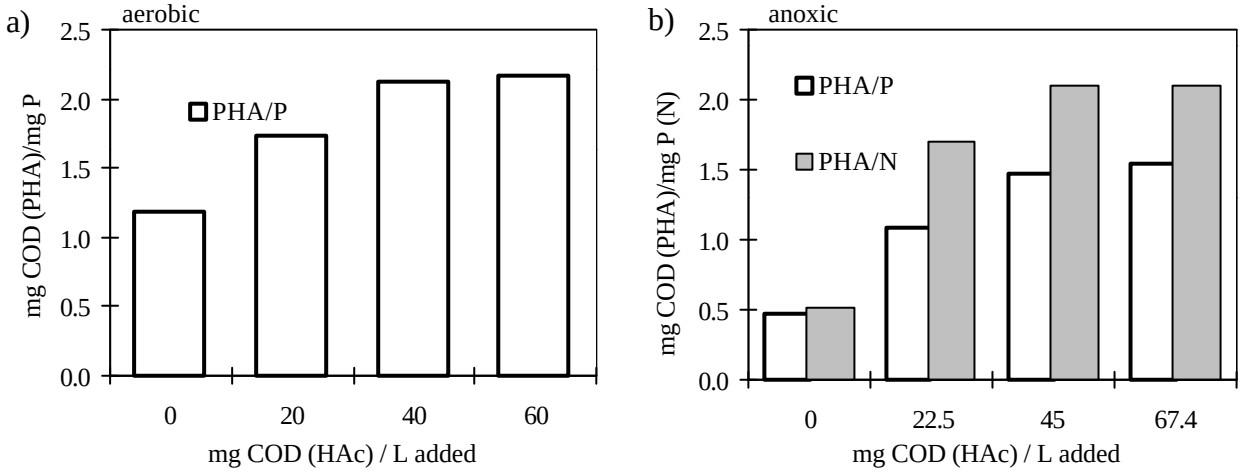

Figure 4.1-5. PHA utilisation per amount of P taken up and/or nitrate reduced during batch tests

a) PHA/P ratios for the aerobic phase of anaerobic-aerobic batch tests.
b) PHA/P and PHA/N ratios for the anoxic phase of anaerobic–anoxic batch tests.

Looking at Figure 4.1-5, a higher PHA/P ratio for aerobic conditions is observed compared to anoxic ones. From the current understanding (Kuba *et al.*, 1996, Murnleitner *et al.*, 1997) the amount of PHA utilised per P taken up will be larger under anoxic conditions compared to aerobic ones. This is not reflected by Figure 4.1-5, probably because the measured PHA utilisation rates under anoxic conditions reflect the result of the overlay of anoxic PHA storage and utilisation, as discussed in section 4.1.2. Despite this, Figure 4.1-5 clearly reveals for both conditions the important tendency of an increasing PHA utilisation per amount of phosphate taken up at increasing amount of initially added acetate.

Overall the evaluation of these experiments indicate that temporary decrease of the removal capacity will occur upon sudden increases of the COD load. Though not performed in this study, submitting the sludge to several cycles of a higher but constant acetate load in the anaerobic phase, similar to a SBR operation, should result in a recovery of the removal capacity within a few cycles, similar as describes by Brdjanovic *et al.* (1998).

4.1.4 Summary and Discussion

The results from batch experiments clearly illustrated that the aerobic as well as the anoxic P-uptake rates are highly dependent on the PHA level in the cells (Figure 4.1-3). In this study a saturation effect with regard to PHA started to become important at levels of around 0.15 mg COD_{PHB}/mg COD_{PAO}.

Accordingly, the denitrification improves at higher internally stored PHA levels, due to the increased activity of PAO under anoxic conditions. Contribution of PAO to overall denitrification was quite significant during the batch experiment and about 50% could be attributed to PAO. In full scale or pilot plant operation the relative contribution of the PAO to denitrification, though still significant, will be less, as denitrification by 'normal' denitrifiers (non PAO) will be higher due to an increased availability of extracellular COD sources during anoxic conditions.

The PHA content not or less available for biodegradation was estimated in this study in the order of 0.01 g COD_{PHA}/g COD_{PAO}, which is in the same order of magnitude as reported in literature (Temmink *et al.*, 1996, Petersen *et al.*, 1998).

The batch tests were performed in such a way, that in some reactors the activated sludge was exposed to a sudden increase in the anaerobic COD load compared to the load experienced in the pilot plant, which leads to a significant decrease in the phosphate removal capacity. Evaluation of the PHA utilisation rate and the P-uptake rate indicates, that the yield of PHA to biomass might increase for the PAO upon sudden increase of the COD load, i.e. more carbon is directed to growth, resulting in less PHA available for P-uptake. The phosphate responses of the batch tests resemble the behaviour of BPR processes after prolonged starvation due to extensive aeration or dilution (rain events) or after low loading during weekends, described in literature (Krühne and Jørgensen, 1999; Temmink *et al.*, 1995; Carucci *et al.*, 1999). In case of dilution due to rain events the plant receives low concentrated sewage and high hydraulic loading, whereas 'weekend effect' is mainly characterised by a low COD loading. In both situations, the deterioration of BPR occurs after the actual event, i.e. when the COD load increases again back to its original level (Krühne and Jørgensen, (1999)), which is comparable to the sudden increase in the COD load as applied in this study.

Essential factors, leading to a disturbance of the operational stability and efficiency of BPR processes in such cases, seem to be :

a) partial depletion of the internal PHA stores, due to excessive aeration (Temmink *et al.*, 1996; Brdjanovic *et al.*, 1998).
b) limited P-uptake due to poly-P content reaching its maximum level (Brdjanovic *et al.*, 1998).
c) low loading resulting in nitrate accumulation and high nitrate input to the anaerobic tank (Pitman *et al.*, 1983; Wolf and Telgmann, 1991).

The results of this study underline the importance of maintaining the PHA content above a minimum level for satisfactory BPR performance. Increased stabilisation of the process can be reached by

assuring a high PHA content in the cells, inducing high P-uptake rates. Furthermore the results point out the possibility of an additional factor leading to deterioration of BPR efficiency and performance: the increase of the carbon flow towards growth upon an increase in COD load, resulting in less PHA available for P-uptake.

For plant operation these observations / results indicate that it is advisable to keep the PHA pool at a continuously high level and to avoid sudden increases in the COD load in order to maintain BPR efficiency.

Several strategies to avoid the negative effects on BPR and to compensate the essential factors mentioned above are recommendable. Control of the (adjustable) aeration time helps to avoid unnecessary oxidation of the PHA pool. The use of preceding equalisation tanks (Filipe *et al.* 2001) can reduce the fluctuation of the COD load, thus counteract the BPR deterioration due to a sudden COD increase in the influent. In a similar way the external addition of COD sources can be applied, to prevent a drop of the COD in the influent during low loading situations (Teichfischer, 1995; Krühne and Jørgensen, 1999). Furthermore, when adding external BPR promoting substrate to stabilise the process, e.g. use of pre-fermenters or hydrolysate (Thornberg *et al.*, 1995), a sudden increase in the COD load should be avoided, i.e. the addition should be performed continuously with a slowly rising rate, instead of allowing a large step upward in the COD load. Though not investigated, the return sludge rate might also offer the possibility to counteract a certain COD load increase. When increasing the return sludge flowrate , the same COD load is distributed to more sludge within the same time interval. This strategy is of course limited by constraints from plant operation (sludge blanket height, hydraulic load and sludge sedimentation characteristics, etc.) and its impact on the other compartments of the plant have to be evaluated carefully.

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4.2 Division of the PAO into 2 Groups and the Consequences on BPR

ABSTRACT

Results of experimental investigations are presented that strongly support the hypothesis that PAO in activated sludge systems consists of two groups: a) denitrifying PAO (DNPAO) capable of using oxygen and nitrate and b) non-denitrifying PAO (O2-PAO) only able to use oxygen. Batch experiments were performed in which activated sludge obtained from a pilot scale Biodenipho^{TM} was submitted to a sequence of anaerobic/anoxic/aerobic, anaerobic/aerobic or anaerobic/anoxic conditions while monitoring the course of NOx-N, NH4-N, PO4-P, PHB and PHV. Several methods for the determination of the two fractions of PAO were performed and compared.

This study extends on previously reported results (Kerrn-Jespersen and Henze, 1993) in that the pH was controlled to around pH 7 to assure that phosphate precipitation was minimal, and in the measurement of PHB and PHV. Simulations implementing existing models for the growth of O2-PAO and DNPAO are used to confirm the experimental results and to gain a better understanding of some of the observations.

The limitation/ restrictions in the use of the presented methods are pointed out and discussed

This section is based on the article :

> Meinhold J.,. Filipe C. D. M, Daigger G.T. and Isaacs S. (1999). Characterization of the denitrifying fraction of phosphate accumulating organisms in biological phosphate removal., *Wat. Sci. Tech.*, **39** (1), 31-42.

Simulation results of this section were supplied by C.D.M. Filipe.

Supplementary investigations with regard to restrictions/limitations in the use of the methods and a final discussion of this subject are presented at the end of this section.

4.2.1 **Characterisation of the Denitrifying Fraction of Phosphate Accumulating Organisms in Biological Phosphate Removal**

4.2.1.1 Introduction

Biological phosphorus removal (BPR) in activated sludge systems is accomplished by heterotrophic micro-organisms collectively referred to as phosphate accumulating organisms (PAO). They can store large amounts of phosphorus in the form of polyphosphate granules. Growth of PAO is stimulated by continuously recirculating the biomass between anaerobic and aerobic environments. During the anaerobic period the PAO store organic substrate, preferably volatile fatty acids (VFA) in the form of polyhydroxy-alkanoates (PHA; predominantly poly-β-hydroxy-butyrate (PHB) or poly-β-hydroxy-valerate (PHV)). The energy necessary to drive substrate uptake is provided by cleaving the internally stored polyphosphate, resulting in the release of phosphorus to the liquid phase. Simultaneously, a decrease in the glycogen content of cells occurs to maintain the redox balance in the cell (Smolders *et al*., 1994a; Arun *et al*., 1989; Mino *et al*., 1987). In the presence of an electron acceptor (oxygen or nitrate), PAO use the stored PHA for growth, replenishment of the glycogen utilised anaerobically, and excess uptake of phosphorus, regenerating the intracellular polyphosphate pool (Smolders *et al*., 1994b; Kuba *et al*., 1996c). Net phosphorus removal from the system is achieved by withdrawing phosphate-rich waste sludge.

Although it was initially thought that PAO could not grow and accumulate phosphorus under anoxic conditions, it has been demonstrated experimentally that PAO can do so (Kerrn-Jespersen and Henze, 1993; Vlekke *et al*., 1988; Kuba *et al*., 1993). Anoxic phosphorus uptake has been observed in bench-scale systems (Kuba *et al*., 1993, 1996a, 1996c) and in full-scale wastewater treatment plants (Kuba *et al*., 1997). The use of denitrifying phosphorus accumulating organisms (DNPAO) in BPR systems can be advantageous because the same organic substrate is efficiently used both for nitrogen and phosphorus removal. This is significant since organic substrate availability is often a limiting factor in nutrient removal processes. Other advantages associated with DNPAO activity include a reduction in aeration energy and sludge production (Kuba *et al*., 1996a).

The objectives of this study were to: provide additional evidence of anoxic phosphorus uptake in a pilot scale system; investigate the dependency of the anoxic phosphorus uptake rate on the PHA content of PAO; develop methods that allow to quantify the fraction of the PAO population able to use nitrate as a terminal electron acceptor; and use existing metabolic models for the growth of O2-PAO and DNPAO to gain a better understanding of some of the experimental observations.

4.2.1.2 Material and methods

Batch experiments:

The experimental set-up is illustrated in Figure 4.2-1 and consisted of four 5 litre Plexiglas cylindrical batch reactors. During the course of an experiment nitrogen gas was sparged above the liquid surface to exclude atmospheric oxygen and maintain anaerobic/anoxic conditions. Aerobic periods were initiated by sparging compressed air through a diffuser at the bottom of the reactors.

Chemical addition was performed by pipette or, for continuous addition, with a calibrated peristaltic pump. The pH was manually controlled to 7.0±0.1 through additions of 1.0 M HCl or 0.5 M NaOH. The temperature of the bulk liquid remained at 18 °C ± 1. Automatic measurement of nitrate plus

nitrite (NO_x-N), phosphate (PO_4-P) and ammonia (NH_4-N) or nitrite (NO_2-N), was performed by flow injection analysis (FIA) using a modified version of the FIA system described elsewhere (Pedersen *et al*., 1990; Meinhold *et al*., (1999).

The protocol common to each batch experiment was as follows. Activated sludge was obtained from a Biodenipho^{TM} pilot plant treating municipal wastewater (Isaacs and Temmink, 1996). The sludge

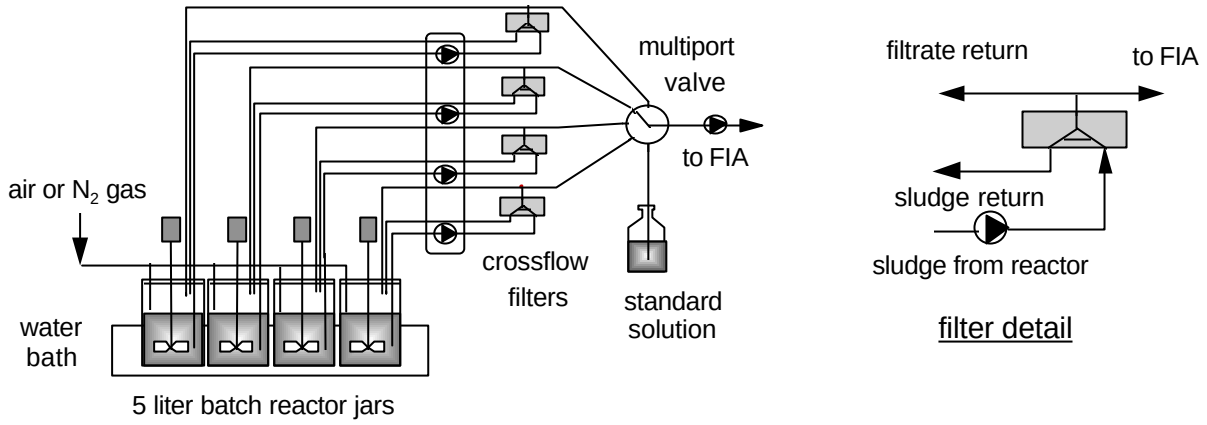

Figure 4.2-1. Schematic diagram of the experimental batch set-up

was obtained on the day each experiment was performed, and therefore sludge characteristics varied somewhat from experiment to experiment. Before taking the sludge, the pilot plant reactor was first isolated without aeration until nitrate was totally consumed. Four liters of sludge were then transferred to each of the four batch reactors, which immediately thereafter were stirred and placed under nitrogen gas. For some experiments the aqueous phosphate concentration level was raised by adding potassium phosphate. Each experiment was initiated with an anaerobic PHA-uptake/ phosphate-release step by adding sodium acetate (HAc) and maintaining the reactors anaerobic until the phosphate release associated with acetate uptake was complete in all reactors. Subsequently either anoxic or aerobic conditions were initiated.

PHB and PHV were measured from samples collected manually. The procedure for sample collection consisted of withdrawing 30 ml of mixed liquid from each reactor followed by immediate centrifugation (5 min. at 4000 rpm) and immediate freezing of the sludge pellet. The pellets were then freeze dried before further analysis.

Analytical methods

Ammonia nitrogen (NH4-N), nitrate plus nitrite nitrogen (NOx-N) and ortho-phosphate (PO4-P) were analysed with FIA (Pedersen *et al*. 1990). PHB was measured as described in Smolders *et al.* (1994a) with minor modifications. MLSS and MLVSS were determined according to APHA Standard Methods (1985).

Simulation studies:

The studies were initiated by simulating the growth of PAO and DNPAO in two sequencing batch reactors, as described in Filipe and Daigger (1997). The first SBR was used to generate DNPAO biomass. Each cycle consisted of an initial anaerobic period (2.0 hours), where the feed (containing 400 mg-COD/l of acetate and 15 mg-P/l) was added in the first 2 minutes, followed by an anoxic period (1.75 hours) where nitrate was added at a flux equal to 6.382 mmol-N/h for the first hour. The final period was aerobic, with a duration of 1.75 hours. This system was simulated for an SRT of 7

days until steady-state conditions were achieved. The second SBR was simulated in the same fashion, but with no nitrate added, resulting in an initial anaerobic period of 3.75 hours and an aerobic period of 1.75 hours. This system was used to generate PAO only capable of using oxygen as the terminal electron acceptor (O2-PAO)

Three types of batch tests were simulated: **Anaerobic/anoxic/aerobic batch tests.** The duration of each period was set to 2 hours. At the beginning of the anoxic period nitrate was added to a final concentration of 7 mmol-N/l, so that nitrate was never limiting. **Anaerobic/anoxic batch tests**. The anaerobic period also had a duration of 2 hours, and the anoxic period with a duration of 4 hours was started with the addition of 7 mmol-N/l of nitrate. **Anaerobic/aerobic batch tests.** The conditions were the same as in the anaerobic/anoxic batches, but aerobic conditions were created instead of adding nitrate.

The behaviour in batch test for different initial proportions of DNPAO biomass (0, 25, 50, 75, and 100%) were simulated. Each batch was simulated for 3 different initial acetate concentrations (3.125, 6.25, and 12.5 mmol-C/l). The initial phosphorus concentration in all batches was set to 1.5 mmol-P/l).

The DNPAO model was the one developed by Kuba *et al*. (1996c). These authors summarised the kinetic expressions found by Smolders *et al*. (1995a, 1995b, 1995c) for the growth of PAO under aerobic conditions. The stoichiometry used for the aerobic processes was as described by Smolders *et al*. (1994b, 1995a). A set of switches were used to turn processes on and off depending on the terminal electron acceptor being used (Filipe and Daigger, 1997). All simulations were done using Aquasim (Reichert, 1994)

4.2.1.3 Results and discussion

The interaction between nitrate, organic substrates and phosphate can readily be observed in the alternating type BIODENIPHO^{TM} process (Einfeldt, 1992) due to its semi-batch manner of operation. Figure 4.2-2 shows nitrate and phosphate measurements collected over about one process cycle in one of two anoxic/aerobic reactors of a pilot scale plant at two different conditions. The right plot shows data collected during "normal" process conditions with municipal wastewater as feed.

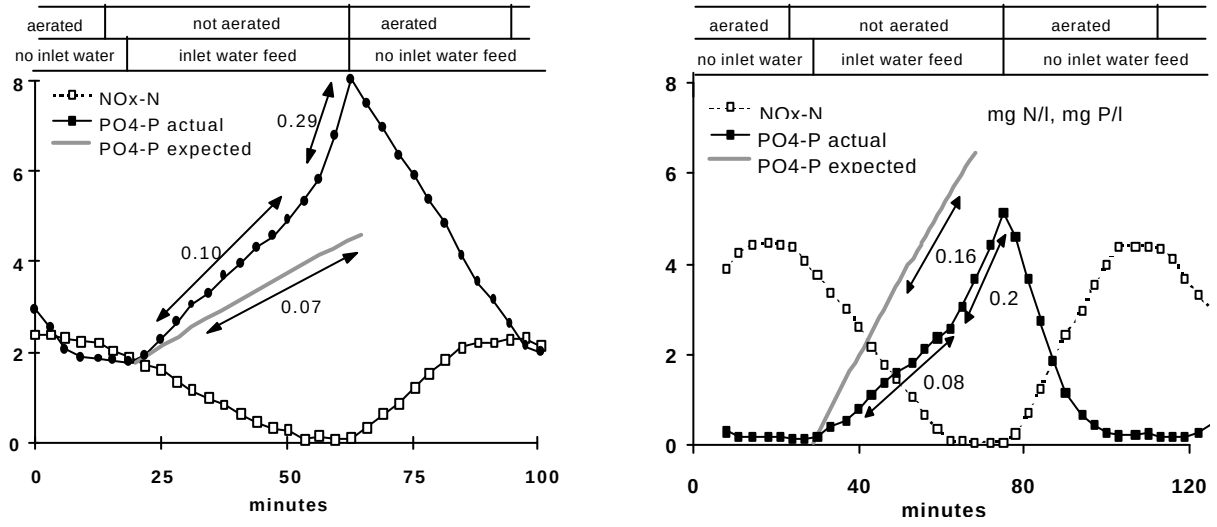

Figure 4.2-2. Nitrate and phosphate measurements in one of two anoxic/aerobic reactors of a BIODENIPHO^{TM} pilot plant. The numbers by the double arrow segments are rates of phosphate increase in mg P(l·min)$^{-1}$.

The left plot presents data immediately after step addition of acetate to the feed. The straight line, obtained from a mass balance, is the course that phosphate would take with no reaction. For the right hand plot a comparison of the slope of this curve with the rate of increase of the actual phosphate measurements indicates that P-uptake occurs while nitrate is present (denitrification by PAO), and that some phosphate is released after nitrate has been consumed. Phosphate release after the anoxic reactor has become anaerobic is attributed to maintenance and the take-up of organic substrate made available at a slow rate, either due to conversion reactions (hydrolysis, fermentation) or due to incoming readily degradable substrates not taken up in the anaerobic zone. The effect of a substrate source being also available in the anoxic phase can been seen in the left hand plot, where the slope of the phosphate measurements is always higher than the calculated one. Nevertheless anoxic P-uptake still occurs, as the slope of the phosphate increase is considerable lower as long as nitrate is present. The data presented in Figure 4.2-2 clearly show that anoxic phosphorus uptake took place in the pilot plant.

Experimental evidence suggests that two different populations of PAO exist in BPR systems (Kerrn-Jespersen and Henze, 1993; Bortone *et al.*, 1996). In previous research it was observed that P-uptake resumed under aerobic conditions even though it ceased during a previous anoxic period. This provided the basis for dividing the PAO into two groups (Kerrn-Jespersen and Henze (1993)).

In this research batch experiments with 3 reactors in parallel were conducted, submitting the sludge to a sequence of anaerobic/anoxic/aerobic, anaerobic/aerobic or anaerobic/anoxic conditions. Carefully defined conditions were provided, however, with a higher sampling rate, controlled pH and supplemental PHB and PHV measurements. The results of one representative experiment are shown in Figure 4.2-3.

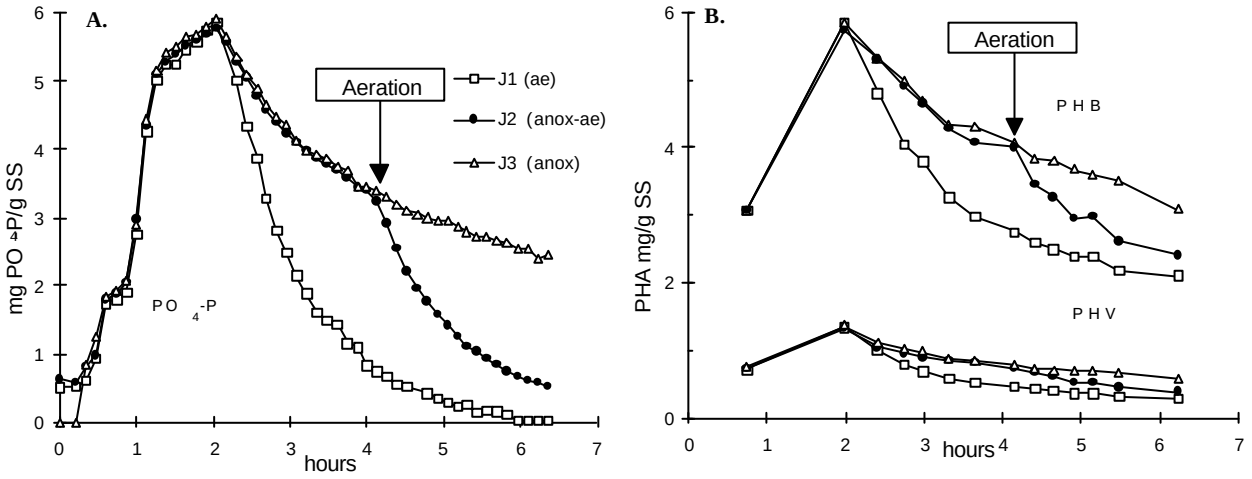

Figure 4.2-3. Concentration profiles for the three different batch tests.

A.) orthophosphate concentrations; B.) PHA expressed as PHB and PHV.

All the reactors received the same amount of acetate as well as 5 mg/L of PO_4-P (as KH_2PO_4). After the acetate induced P-release ended, aeration was started in reactor J1, while nitrate was added to the other two reactors. Reactor J1, being aerated, exhibits rapid phosphorus uptake achieving a net phosphorus removal from the liquid. P-uptake gradually slows down in the anoxic reactors (J2 and J3), and no net uptake is achieved. Phosphorus uptake rapidly increases in J2 when aeration is provided. Only minor P-uptake occurs in J3 during the last 2.5 hours of the experiment, which remained anoxic, compared to the pattern of J2.

PHB and PHV were also measured during this experiment and the results appear in the right hand plot of Figure 4.2-3. The dynamics of both internal storage compounds are consistent with the dynamics of soluble phosphate. During the anaerobic period there is an increase in PHA as phosphate is released and acetate is taken up. During the anoxic and aerobic phases both PHA utilisation rate and phosphate uptake rate are highest initially, with a gradual reduction as the experiment proceeds. Similar to the phosphate pattern, PHA utilisation exhibits a relatively strong increase in reactor J2 upon the start of aeration.

There are several possible reasons for the significant decrease in phosphorus uptake towards the end of the anoxic phase.

The first could be the accumulation of nitrite in the system, which is known to inhibit severely several biological processes. This seems very unlikely, however, because phosphorus uptake immediately increased after aeration was started in reactor J2 and it seems reasonable to assume that nitrite would also affect the biomass under aerobic conditions. In addition, experiments were performed (not shown) to evaluate the effect of nitrite on BPR. The results showed that nitrite can serve as an electron acceptor up to a certain critical concentration, which was ten times higher than the nitrite concentrations observed in the pilot plant and during the batch experiments, (Meinhold *et al.*,1999).

The second reason could be limitations of either K^+ or Mg^{2+}. This cannot explain the observations because P-uptake occurred normally under aerobic conditions.

The most likely explanation for the behaviour presented in Figure 4.2-3 is the existence of two populations of PAO - one that can use only oxygen as the terminal electron acceptor and the other that can use either oxygen or nitrate. Anoxic phosphate uptake is due to DNPAO, using nitrate as e^- acceptor, and this uptake slows down as their intracellular storage material (PHA) becomes limiting. The other group, the O2-PAO, still have sufficient intracellular storage, as they are not able to use nitrate. Increased phosphate uptake at the start of aeration is due to the activity of O2-PAO.

Simulations performed assuming the existence of the two populations reflect the same qualitative behaviour as observed in the experiments (Figure 4.2-4). The simulations for the anaerobic/anoxic/aerobic batch test show the same type of profiles as observed experimentally. Figure 4.2-4.B. reveals that the phosphorus uptake rate under anoxic conditions is significantly lower than under aerobic conditions, which is due to the fact that only 50% of the population is able to use nitrate in this simulation.

Of interest is also that upon subsequent aeration the contribution of DNPAO to the total rate of phosphorus uptake is much lower than the one of the purely aerobic PAO. This occurs because DNPAO will consume significant amounts of PHB under anoxic conditions, reducing their ability to remove phosphorus under aerobic conditions.

The same behaviour was observed when two different batch tests (anaerobic/anoxic and anaerobic/aerobic) were simulated (Figure 4.2-4 C and D). Under anoxic conditions a lower rate of phosphorus uptake was observed, due to a lower percentage of the biomass being able to accomplish it. But under aerobic conditions, the contribution of each population to phosphorus uptake is basically the same.

Note how steep the phosphorus uptake rate is under aerobic conditions (Figure 4.2-4.B and D), whereas the anoxic uptake rate shows a smoother pattern. This observation will become important when the proportion of DNPAO in the pilot plant is measured .

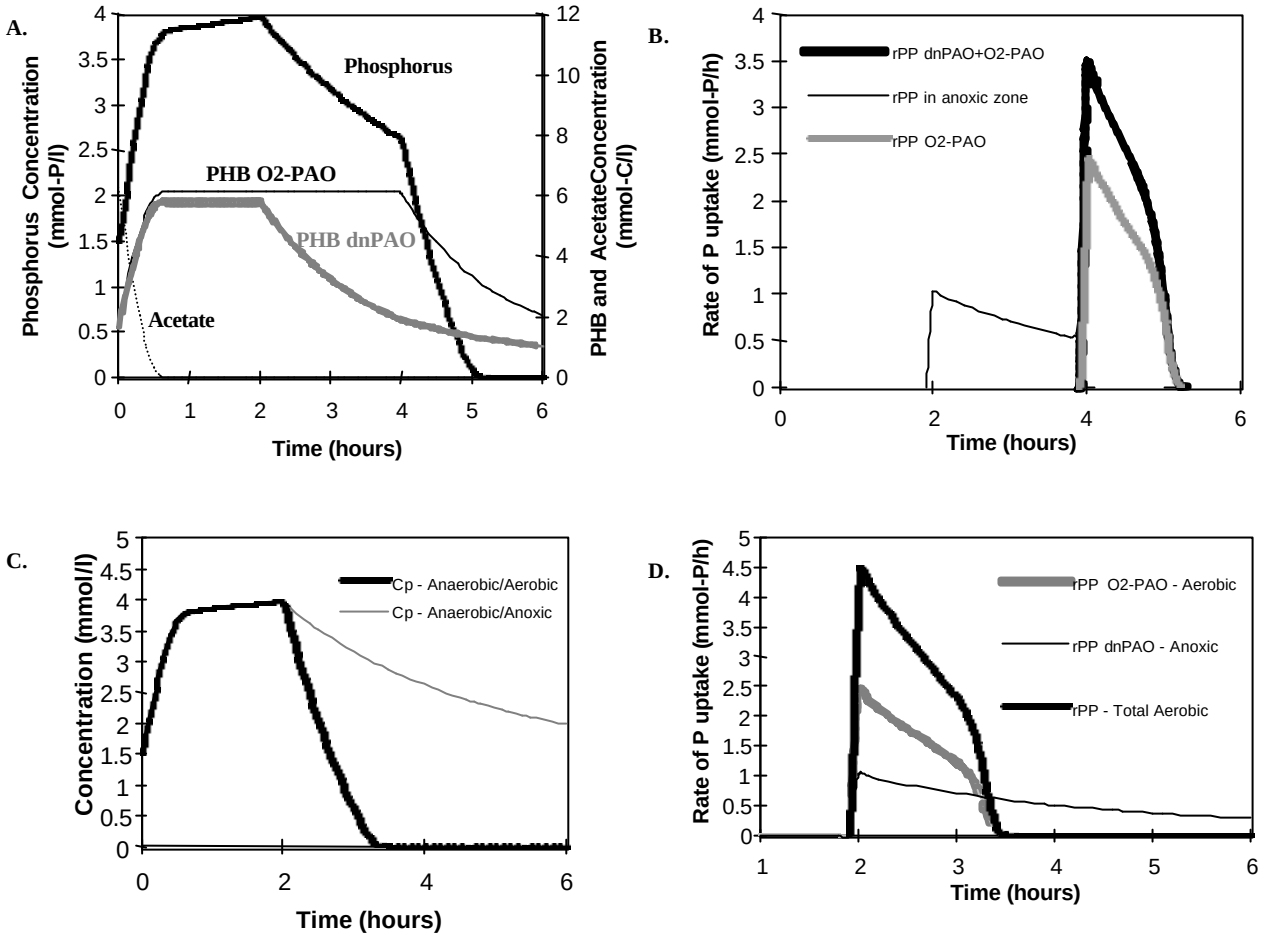

Figure 4.2-4. A.) Simulated profiles for an anaerobic/anoxic/aerobic batch test with an initial acetate concentration of 6.25-mmol-C/l and for 50% DNPAO;
B.) Phosphorus uptake rates for test presented in plot A;
C.) Phosphorus profiles for an anaerobic/anoxic and a anaerobic/aerobic batch test with an initial acetate concentration of 6.25 mmol-C/l and for 50% DNPAO;
D.) Phosphorus uptake rates for tests presented in plot C.

Figure 4.2-5 presents PHB profiles for the simulations presented in Figure 4.2-4.C. The PHB content of O2-PAO in the anoxic batch test does not change. However, when appropriate terminal electron acceptors are present, the PHB profiles for all organisms are essentially the same. This is due to the fact that the model assumes the same specific PHB oxidation rate for the two populations under aerobic and anoxic conditions, as long as the PHB content at the end of the anaerobic phase is the same. The experimental results (Figure 4.2-3) showed that the PHB consumption rate under anoxic conditions was lower than under aerobic conditions. This is consistent with the two population hypothesis, as illustrated in Figure 4.2-5.B where composite PHB curves are presented. This Figure clearly shows that the existence of a population that cannot use nitrate will cause a significant decrease in the observed rate of PHB utilisation under anoxic conditions.

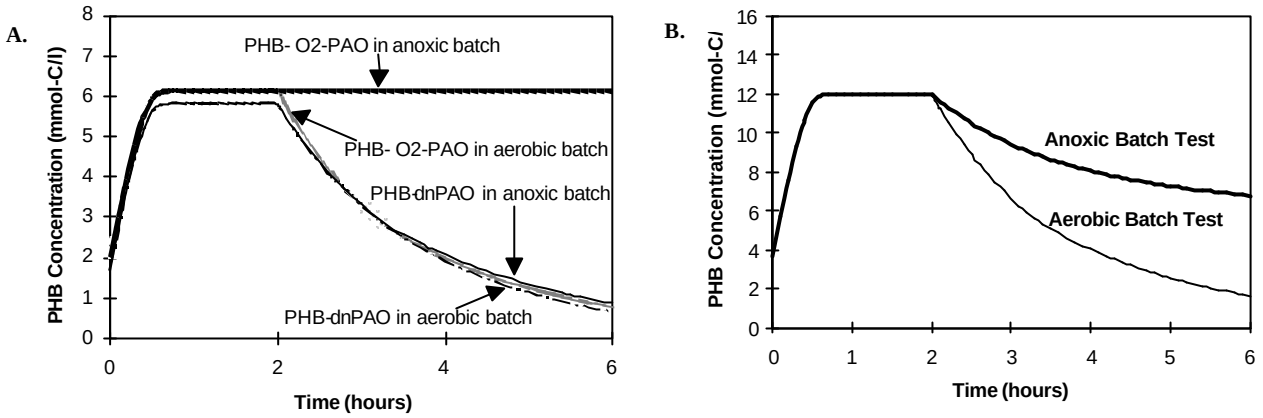

Figure 4.2-5. A.) PHB concentrations in the two populations from the simulated batch tests in Figure 4.2-4.C.; B.) Observed PHB concentrations.

4.2.1.4 Characterisation methods of the denitrifying fraction of the PAO

Two different ways to determine the DNPAO fraction were investigated. As these procedures can be applied to data from either one (anaerobic/anoxic/aerobic) or two reactors (one being aerated the other running anoxically) there are four possible methods for determining the denitrifying PAO fraction. The results from the derivation of expressions to calculate the PAO fractions are presented below.

The following assumptions were used in the derivations:

- the specific poly-P formation rate for DNPAO under anoxic conditions is reduced compared to their aerobic rate ($q_{PP,\ anox,DNPAO} = \eta_{NO3} * q_{PP,\ aerob,DNPAO}$, ($\eta_{NO3} = 0.8$));
- O2-PAO do not store phosphate under anoxic conditions;
- no nitrate is present in the anaerobic phase; i.e. no disturbance of the acetate uptake by PAO;
- DNPAO and O2-PAO, at t=0, have stored the same amounts of intracellular storage material and they have the same kinetics according to these polymeric substances;
- DNPAO and O2-PAO have the same stoichiometry for acetate uptake under anaerobic conditions.

1. Fractionation of PAO based on initial P-uptake rate:

Based on the assumptions listed above, the fraction of DNPAO can be determined based on the initial P-uptake rates, which are proportional to the amounts of DNPAO and non- DNPAO.

Comparing the two uptake rates, and taking into account that the anoxic P-uptake rate has to be divided by $\eta_{NO3} = 0.8$ in order to 'transform' or relate the rate to aerobic conditions, the following two equations for the fraction of DNPAO and O2-PAO can be obtained:

$$X_{PAO} = 100\,\% = X_{DNPAO} + X_{non-DNPAO} \qquad \text{and} \qquad \frac{q_{anoxic}}{\eta_{NO3}} \bullet \frac{1}{q_{ae}} = \frac{X_{DNPAO}}{X_{PAO}} \qquad \text{(eq. 4.2-1).}$$

The profiles obtained from a single anaerobic/anoxic/aerobic batch test can also be used. Again the ratio of the P-uptake rates is proportional to the ratio of DNPAO and O2-PAO. But, the initial P-uptake rate after starting aeration can not be used as such for the determination. Since the DNPAO are still able to take up phosphate in the aeration phase, the initial rate is not only due to O2-PAO. To obtain the aerobic P-uptake rate of the O2-PAO, one must correct the original rate, calculated from

measurements, by extrapolating the (preceding) anoxic curve and subtracting this extrapolated anoxic P-uptake rate (divided by η_{NO3}) from the original aerobic P-uptake rate:

$$\frac{q_{anoxic}}{\eta_{NO3}} \bullet \frac{1}{q_{ae,corr}} = \frac{X_{DNPAO}}{X_{non-DNPAO}} \quad \text{with } q_{ae,corr}(t) = q_{ae}(t) - \frac{q_{anox}(t)}{\eta_{NO3}} \qquad \text{(eq. 4.2-2).}$$

2. Fractionation of PAO based on total P-uptake:

This method is conducted by comparing the quantity of phosphate taken up within a time period equal to values where the anoxic and aerobic uptake rates have decreased to an equal fraction of their initial uptake rate. The amount of phosphorus taken up in this time period is then proportional to the ratio of DNPAO and O2-PAO :

$$\frac{q_{anox}(t_0)}{q_{anox}(t_1)} = \frac{q_{ae}(t_0)}{q_{ae}(t_2)} \Rightarrow \frac{X_{DNPAO}}{X_{PAO}} = \frac{\Delta P_{anox}(t_{0/1})}{\Delta P_{ae}(t_{ae,0/2})} \qquad \text{(eq. 4.2-3).}$$

Using the profiles from an anaerobic/anoxic/aerobic batch test, this procedure follows the same pattern as for two reactors. But again the aerobic P-uptake rate has to be corrected in the same way as above :

$$\frac{q_{anox}(t_0)}{q_{anox}(t_1)} = \frac{q_{ae,corr}(t_{ae,0})}{q_{ae,corr}(t_2)} \Rightarrow \frac{X_{DNPAO}}{X_{non-DNPAO}} = \frac{\Delta P_{anox}(t_{0/1})}{\Delta P_{ae,corr}(t_{ae,0/2})} \text{ (eq. 4.2-4).}$$

According to the procedures described above, the fraction of denitrifying PAO (DNPAO) was determined for several experiments similar to the one presented in Figure 4.2-3. For the first method the average P-uptake rates for the half hour of the experiment were used. For the second method the time interval was chosen during which the rates drop to half their initial value. The results obtained with the data presented in Figure 4.2-3, which are representative of the various experiments, are shown in table 2 . All 4 methods produce almost equal results, that is about 56 % DNPAO and, consequently, 44 % O2-PAO.

Table 4.2-1. Fraction of DNPAO determined for experiment shown in Figure 4.2-3.

Method based on :	2 reactors		1 reactor (An-anoxic-aerobic)	
	P-uptake rate	P taken up	P-uptake rate	P taken up
DNPAO	55 %	54 %	58 %	56 %
O2-PAO	45 %	46 %	42 %	44 %

The method using just one reactor with an anaerobic-anoxic-aerobic sequence exhibits a higher possibility for errors occurring due to extrapolation of anoxic P-uptake. The extrapolation contributes to uncertainty of the estimate, so the procedures using two reactors seem to be more suitable for further use.

To assure good results by any of the procedures, several prerequisites have to be fulfilled. First, it is absolutely necessary to ensure good quality in the phosphorus measurements. The data used for the procedures must be collected during the initial part of the phosphate uptake curves, i.e. outside of severe PHA limitation. Thus, the initial rates should be used. Consequently the uptake rates for the different approaches were calculated for the first hour (average). Experiments exhibiting low

phosphate uptake rates due to prolonged periods of low inlet organic matter concentrations were found not to be suitable for the procedures discussed above.

Another problem identified was that the ratio of uptake rates seems not to be constant over time. Figure 4.2-6 shows the measured DNPAO fraction based on the P-uptake rates in two reactors (one anoxic and one aerobic) with time. A higher fraction of DNPAO is calculated with time. The same phenomenon was observed during the simulations. As seen in Figure 4.2-4, the aerobic P-uptake rate changes much more abruptly throughout the test than the anoxic P-uptake rate, despite the fact that the phosphorus profile in the aerobic test seems to be fairly smooth. This leads to overestimation of the fraction of DNPAO. The simulations also demonstrated that, if an average value of the uptake rates was taken only from the initial part of the test, the standard deviation associated with the average was fairly low and tended to increase significantly as the period of time used was increased. As long as an appropriate time interval was used for the estimation of the rates, the ratio of the rates observed between the anoxic and aerobic batch tests was linearly dependent on the fraction of DNPAO in the system during the simulations. Consequently this can be seen as a validation of the first fractionation method by the simulation studies. It is, therefore, of maximum importance to fix a time interval over which the uptake rates are averaged in order to remove this source of variability on the determination of the respective fractions of the two organisms. Consequently, the results of the estimation procedures should not be understood as a precise measurement of the two fractions. However, by using the same fixed time interval, changes in the population distribution can be detected. So, the procedures are a valid relative measure of changes in the population distribution of the system studied.

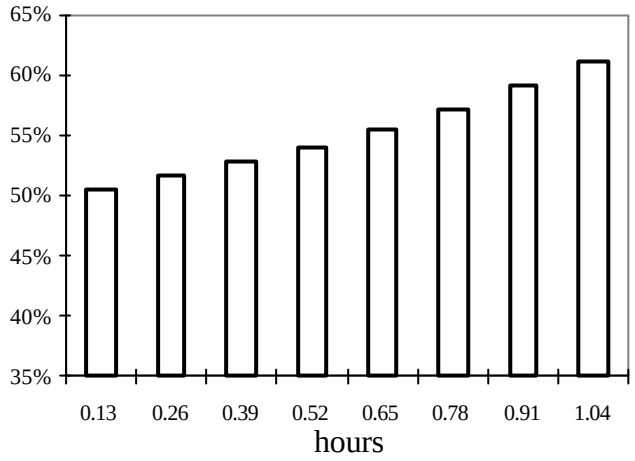

Figure 4.2-6 Determined DNPAO fraction from the P-uptake rates of a anoxic and a aerobic reactor.

These results also illustrate that PAO fractions reported in the literature may be flawed. In most cases the fractions are simply determined by the ratio of P-uptake rates without any detailed specification of time (Wachtmeister *et al.*, 1997). These values are not comparable unless the determination was done in the same time interval.

The use of the η -value is not without discussion. In the ASM1 model, introduced by the IAWQ task group (Henze *et al.*, 1987), it is implemented reflecting the observations that have been made on anoxic growth, as nitrate is in general less efficient than oxygen as an electron acceptor. Other research groups (Kuba *et al.*, 1993, 1996c) do not make use of the η -value, as they observed practically the same efficiency of anoxic P-uptake compared to the aerobic one. For this study, the value of $\eta = 0.8$ was used, as suggested for normal denitrifiers in ASM1. Possible deviation in this parameter will only have an impact on the estimation of the absolute fractions of the populations. It will not hinder the observation in changes of the relative fractions of the 2 populations, i.e. the procedures remain suitable for detecting relative changes within the system studied.

4.2.1.5 Conclusions

1. Anoxic P-uptake occurred in a Biodenipho^{TM} pilot plant, as well as in batch tests including an anoxic period.
2. The results obtained in batch tests, as well as through simulation, point strongly to the existence of two populations of PAO. Some have the ability to use nitrate and oxygen as the terminal electron acceptors, while the remainder only use oxygen
3. The selection of an appropriate time interval for the estimation of P-uptake rates is a key factor that must be taken into account. A fixed value should be used to avoid introducing unnecessary variability in the estimation of PAO fractions.
4. The methods proposed will find best use in detecting changes in the population distribution that might take place due to changes in operational strategies.

4.2.2 Limitation in the Use of the Methods

Accepting the existence of two groups, the assumption (s. section 4.2.1.4), that DNPAO and O2-PAO, at t=0, have stored the same amounts of intracellular storage seems questionable. Due to the ability of the DNPAO to use nitrate as an electron acceptor, they will exhibit a different utilisation of their internal storage pools than the O2-PAO. As measurements of PHA (and glycogen and poly-P) only represent the sum of the corresponding storage pool of the two groups, no information is obtained about their distribution within these two groups. Due to the dependency of the P-uptake rates on the PHB level (s. section 4.1), this induces the risk of applying a fractionation method to a region where severe limitation is occurring for one group and thus deflecting the results. Experiments, as shown in Figure 4.2-7, involving two distinct level of measured PHA content in the cells, were conducted to address this problems.

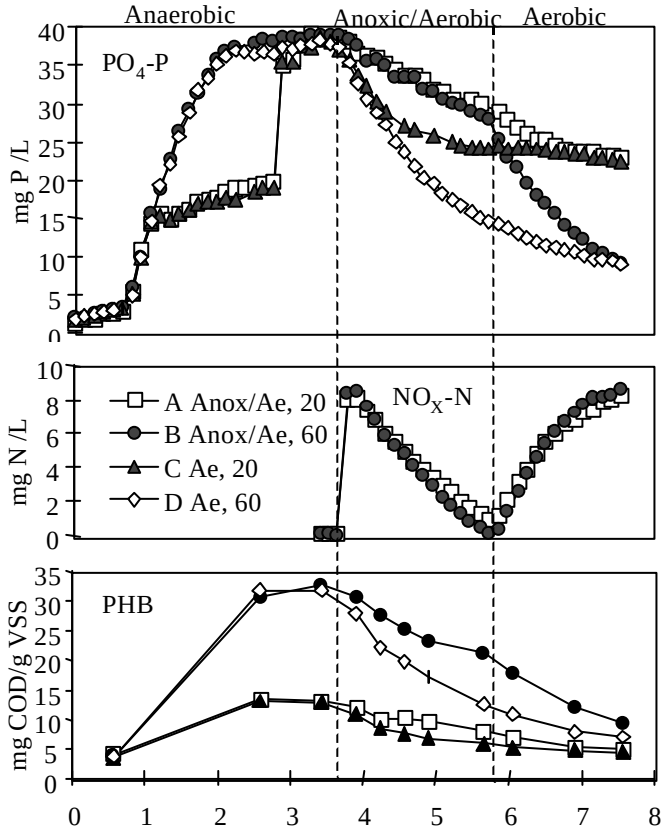

Figure 4.2-7. Batch test at different PHB levels. The numbers next to the symbols: amount of acetate added.

Anaerobic–aerobic conditions were applied to two of the four reactors. The remaining two reactors were conducted with a anaerobic-anoxic-aerobic sequence. 20 and 60 mg COD_{HAc}/L, respectively, were added initially in the anaerobic phase. After the acetate induced P-release has stopped, K_2HPO_4 was added to bring the ortho-phosphate concentration up to the same level in all reactors. Subsequently the corresponding P-uptake phase was initiated.
The fractionation procedures were only applied to two reactors (one aerated, one anoxic), as the use of one reactor (anoxic-aerobic sequence) introduces an additional uncertainty due to the extrapolation needed (s. section 4.2.1.4). The results obtained are listed in Table 4.2-2.

Table 4.2-2. Fraction of DNPAO determined for experiment shown in Figure 4.2-3

Method based on:	P-uptake		P-up rate 1st hr		**initial P-up rate**[1]	
	20 mg COD	60 mg COD	20 mg COD	60 mg COD	20 mg COD	60 mg COD
DNPAO	96.4%	67.4%	60.2%	50.1%	**47.3%**	**45.2%**
non - DNPAO	3.6%	32.6%	39.8%	49.9%	**52.7%**	**54.8%**

[1] Rates were determined from the first two measurement points (7.5 min).

The method based on total P-uptake (s. 4.2.1.4), utilises data from a time interval of well more than one hour, thus it is highly probable that part of data originates from a region where severe PHB limitation is occurring. This is reflected in the results obtained, showing a large deviation for the two levels of initially added acetate and unreasonable results for the reactors receiving 20 mg COD/L. Using the method based on the average of the P-uptake rates within the first hour, involves the same problem. The results show less deviation than the ones obtained before, as the time interval of the data used is considerably smaller than the one used for the method based on total P-uptake. But limitation of the P-uptake rates, at least in the reactor receiving 20 mg COD_{HAc} /L, still seems to be very likely. By choosing a shorter interval to determine the initial P-uptake rates, the probability of severe PHB limitation is reduced. Values obtained, from the first measurement points in the corresponding uptake phases (last two columns in table Table 4.2-2) suggest less influence of PHB on the P-uptake rates, i.e. the values obtained for both reactors are much closer to each other, exhibiting an acceptable deviation.
It seems that the situation where all methods exhibit the same results (Table 4.2-1) represents rather an exceptional case. Evaluating several experiments at different PHB levels, only methods based on initial P-uptake rates (first measurement points), involving two batch reactors, covered a wider range of batch tests with reasonable results. Choosing a narrow time interval for determining the P-uptake rates, will be the most appropriate way to avoid occurence of severe PHA limitation or at least to reduce this effect as much as possible. Hence, the observations underline the importance of using a short, but constant, time interval, involving the first measurement points in the uptake phase, and suggest to utilise a higher level of COD addition ($\geq$ 30mg COD_{HAc}/L) to avoid severe PHB influence on the P-uptake rates of the two groups.

4.2.3 Summary

Batch results obtained, supported by simulations, strongly substantiate the theory of two group of PAO. The denitrifying part (DNPAO) exhibits the ability to use nitrate and oxygen as electron

acceptors, whereas the second group (O2-PAO) use only oxygen. No definite techniques are yet available to access the microbial groups responsible for BPR, but new microbial techniques such as FISH analysis might be useful in the future to specify exactly the microbial distribution.

Possible severe PHB limitation of the P-uptake rates for one or both groups, induces problems with the fractionation procedures presented. The method based on the ratio of the initial anoxic and aerobic P-uptake rates exhibited the most reliable results, as choosing the very first data points for the calculation and providing sufficient acetate in the anaerobic phase, reduces the influence of PHB on the determined P-uptake rates. As the influence of the poly-P content of the cells was not accounted for, the method will only give a rough estimate of the distribution. Interpretation of the results as an anoxic BPR activity seems to be more appropriate.

Provided severe PHB limitation is reduced to a minimum, the method proposed will find best use in detecting changes in the population distribution or anoxic BPR activity, that might take place due to changes in operational strategies. Furthermore it can be useful for the determination of the reduction factor for the anoxic P-uptake during calibration of a model consisting of one group of PAO (Brdjanovic, 1998).

The difficulties encountered due to the PHB dependency of the P-uptake rates, can actually be used to gain additional information about the system studied. Applying the method at two different COD loads (one equal to plant load, the other excessive) can give first insight / information about a possible severe PHB limitation of the P-uptake of one group in the system.

In any case, the selection of an appropriate time interval for the estimation of P-uptake rates is a key factor that must be taken into account. A fixed value should be used to avoid introducing unnecessary variability in the estimation of PAO fractions. For comparison purposes the batch tests should always be performed under the same conditions.

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4.3 Effect of Nitrite on Phosphate Uptake in Biological Phosphorus Removal

ABSTRACT

Results from a series of batch experiments are presented, in which activated sludge obtained from an alternating type biological phosphorus removal process was exposed to nitrite or mixtures of nitrite and nitrate at various concentration levels. By comparing the course of phosphate, nitrite and nitrate (measured on-line) and the internal storage components PHB and PHV (measured manually for some experiments) with batches exposed to nitrate only, the effect of nitrite on anoxic phosphate uptake was investigated. How nitrite exposure affects subsequent aerobic phosphate uptake was also examined in one experiment. The experiments show that nitrite at low concentration levels (up to about 4 to 5 mg NO_2-N/l) is not detrimental to anoxic phosphate uptake and can serve as electron acceptor for anoxic phosphate uptake. Exposure to higher concentration levels (roughly 8 mg NO_2-N/l and greater) inhibits anoxic phosphate uptake completely, and aerobic phosphate uptake severely. The critical nitrite concentration, above which nitrite inhibition of phosphate uptake occurs, is in the range of 5 to 8 mg NO_2-N/l for the experiments performed in this study, but appears to be dependent on sludge conditions. The inhibiting effect of nitrite was found to last for at least several hours after the nitrite exposure.

This chapter is based on the article :

Meinhold J., Arnold E. and Isaacs S. (1999). Effect of nitrite on anoxic phosphate uptake in biological phosphorus removal activated sludge. Wat. Res., 33 (8), 1871-1883.

4.3.1 Introduction

In activated sludge systems, biological phosphorus removal (BPR) occurs due to the ability of a particular group of micro-organisms to take up and store excessive amounts of phosphate. These micro-organisms, known collectively as phosphate accumulating organisms (PAO), store the phosphate internally as polyphosphate polymers. The breakdown and release of the polyphosphate provides energy for the take up and storage of certain simple organic substrates under anaerobic conditions. The simple organics are stored internally as polyhydroxyalkanoates (PHA), predominantly polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV), and serve as substrates for growth under conditions allowing respiratory metabolism. Parallel to growth, the PAO regenerate their polyphosphate stores, thus removing the released as well as new phosphate from the mixed liquor. The phosphate is then removed from the process as polyphosphate stored in the waste activated sludge.

The original consensus concerning PAO metabolism based on early studies was that these micro-organisms lacked the ability to denitrify and, hence, could only grow and accumulate phosphate under aerobic conditions. More recent investigations have made it clear, however, that at least a fraction of the PAO can accumulate phosphate under anoxic conditions (Barker and Dold, 1996; Comeau *et al.,* 1986; Kerrn-Jespersen and Henze, 1993; Gerber *et al*., 1987; Hascoet and Florentz, 1985; Jørgensen and Pauli, 1995; Kuba *et al.*, 1993; Vlekke *et al.,* 1988). This is of significance since BPR activated sludge processes generally include biological nitrogen removal, meaning that nitrate is invariably present during the phosphate release - phosphate uptake cycle. The use of nitrate rather than oxygen for PAO metabolism is advantageous for several reasons. The supply of organic substrates in wastewater, needed for both biological phosphorus and nitrogen removal, is normally limited. Hence, improved nutrient removal is expected if the same organics can be used for both purposes. This “double use” of wastewater organics will also result in a reduced sludge production, and the use of nitrate rather than oxygen as electron acceptor for at least a portion of the phosphate uptake will reduce aeration demand (Copp and Dold, 1998). Consequently, phosphate uptake under denitrifying conditions in BPR processes is a subject of interest currently being studied (Kuba *et al.*, 1994; Meinhold *et al.*, 1998, 1999; Sorm *et al.*, 1996).

In the biological removal of nitrogen, nitrite appears as an intermediate in the two major steps involved, nitrification and denitrification. During nitrification, ammonia is converted to nitrite which is further oxidised to nitrate. During denitrification, nitrate is reduced to nitrogen gas in a sequence of four reactions: $NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$. Although most denitrifiers are capable of carrying out the entire pathway, there exist some strains which lack the ability to perform one or more steps (Tiedje, 1988; Jørgensen and Pauli, 1995).

Generally, the rate of nitrite reduction during denitrification in activated sludge systems is assumed to be sufficiently high to preclude the accumulation of nitrite as an intermediate. Blaszczyk *et al.* (1980) and Wilderer *et al.* (1987) found, however, that using glucose as carbon source promoted nitrite accumulation. Wilderer *et al.* (1987) explained this in terms of the fermentative conditions allowing an enrichment of the biocommunity for bacteria which reduce nitrate to nitrite only. Nitrite accumulation can also come about due to repression of nitrite reductase (Wilderer *et al.*, 1987) or other factors influencing denitrification activity such as the presence of oxygen, substrate concentration (electron donor), temperature and pH in the bulk solution (Halling-Sørensen and

Jørgensen, 1993). Nitrite accumulation was also observed in activated sludge systems by Requa and Schroeder (1973), and Kone and Behrens (1981) concluded that nitrite accumulation would be triggered by discontinuous operation such as batch reactors. In a sequencing batch reactor Wilderer *et al.* (1987) observed nitrite accumulation in excess of 10 mg NO_2-N/l with either acetate or glucose as carbon source.

As the accumulation of nitrite is known to cause severe problems in biological processes in general, BPR could also be affected. Until now relatively little has appeared in the literature concerning the affect of nitrite on BPR. Comeau *et al.* (1987) reported that anoxic phosphate uptake did not occur with nitrite as electron acceptor, but only one rather high concentration (10 mg NO_2-N/l) was examined in his study. During one experiment involving enriched cultures in a sequential batch reactor, Kuba *et al.* (1996) attributed a reduction in phosphate uptake activities to nitrite accumulation but the quoted concentration range was rather broad (approx. 5 to 10 mg NO_2-N/l).

This paper reports results of batch experiments designed to examine the effect of nitrite on anoxic phosphate uptake. All experiments employed activated sludge obtained from a pilot scale BPR plant with a real wastewater feed. To provide a sufficiently high measurement frequency and accuracy, nitrite (NO_2-N), nitrate plus nitrite (NO_x-N) and phosphate (PO_4-P) were measured automatically on-line using flow injection analysis. To determine the influence of nitrite on the dynamics of organic storage products, PHB and PHV were also measured manually during a number of experiments.

4.3.2 Material and Methods

Batch experiments

The experimental set-up is illustrated in Figure 4.3-1and consisted of four 5 litre Plexiglas cylindrical batch reactors. Each reactor was equipped with a motor driven stirrer. The reactors were covered with a Plexiglas lid but were not airtight. During the course of an experiment nitrogen gas was sparged to just above the liquid surface to exclude atmospheric oxygen and maintain anaerobic/anoxic conditions. Chemical addition was performed by pipette or, for continuous

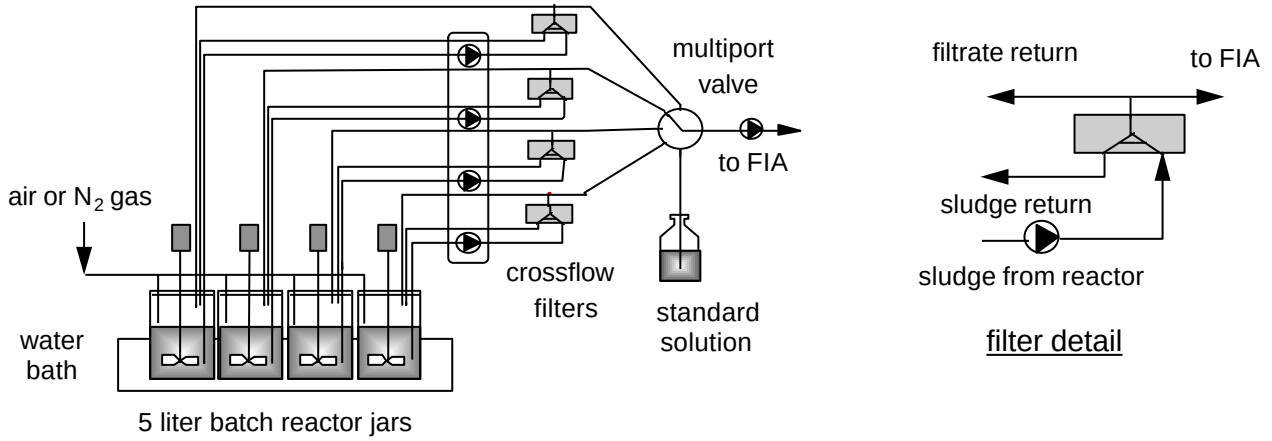

Figure 4.3-1: Schematic diagram of the experimental batch set-up

addition, with a calibrated peristaltic pump. The pH was manually controlled to 7.0±0.1 through additions of 1.0 M HCl or 0.5 M NaOH throughout the course of the experiments. A pH of 7 was chosen to minimise chemical precipitation of phosphate based on an experimental study indicating

that phosphate precipitation in this experimental set-up is minor if pH does not rise much above 7 (Pedersen, 1996).

Automatic measurement of nitrite (NO_2-N), nitrate plus nitrite (NO_x-N) and phosphate (PO_4-P) was performed by flow injection analysis (FIA) using a modified version of the FIA system described elsewhere (Pedersen *et al.*, 1990; Isaacs and Søeberg, 1998). The ammonia analyser was converted to a nitrite analyser for this investigation. Mixed liquor from each reactor was continuously pumped through a crossflow filter unit (pump: 4 channel Watson Marlow 505S; peristaltic tubing: 6.4 × 1.6 Maprene; transport tubing: 5 × 1.5 PVC; filter membrane: DOW Denmark ETNA20A; filter area: 36 cm^2; all tubing sizes are bore diameter × wall thickness in mm.) and back to the reactor. The filtrate from each filter unit was selected for analysis in turn by means of a multiposition valve. When not selected for injection, the filtrate was returned to the reactor from where it originated by means of 1.6 × 0.8 PFTE tubes. These tubes also served as sample storage buffers since the filtrate flowrates normally were slightly less than the pumping rate to the FIA system. The FIA system measured all three species NO_2-N, NO_x-N and PO_4-P in a given sample in parallel every 1.5 minutes. The four reactors were measured periodically in turn along with a standard solution (2 mg NO_2-N/l, 8 mg NO_3-N/l and 10 mg PO_4-P/l), giving a measurement frequency for each reactor of 7.5 minutes (6 minutes when only 3 reactors were employed).

Protocol common to each batch experiment was as follows: Activated sludge was obtained from an anoxic/aerobic reactor of a BIODENIPHO™ pilot plant (Isaacs and Temmink, 1996). The sludge was obtained on the day each experiment was performed, and so sludge characteristics varied somewhat from experiment to experiment. Before taking the sludge the pilot plant reactor was first isolated without aeration until nitrate was totally consumed. Four litres of sludge were then transferred to each of the four batch reactors. Immediately thereafter the reactors were covered and the flow of nitrogen gas and the stirrers were started. 5 mg N/l ammonium chloride was added initially to each reactor to avoid ammonia limitation during the course of the experiment. For some experiments the aqueous phosphate concentration level was raised by adding an aqueous solution of potassium phosphate. Each experiment was initiated with an anaerobic PHA-uptake/phosphate-release step by adding an amount of sodium acetate (HAc) to each reactor and maintaining the reactors anaerobic until the phosphate release associated with acetate uptake was complete in all reactors. After the anaerobic period an anoxic period was initiated by adding either nitrate (sodium nitrate in water) or nitrite (sodium nitrite in water) either instantaneously or continuously. The flowrate vs. pump speed setting of the peristaltic pump used for the continuous nitrite addition was calibrated prior to each experiment, and the pump speed settings were recorded during the course of an experiment to allow calculation of the nitrite addition rate. Additionally, as a control, the nitrite solution was weighed at the start and end of the continuous addition.

In experiments performed later in the investigation PHB and PHV were measured from samples collected manually. The procedure for sample collection consisted of withdrawing 30 ml of mixed liquor from each reactor followed by immediate centrifugation (3 min. at 4000 rpm) and immediate freezing of the sludge pellet. The pellets were then freeze dried before further analysis.

Analytical methods

Nitrite nitrogen (NO_2-N), nitrate plus nitrite nitrogen (NO_x-N) and ortho-phosphate (PO_4-P) were analysed with FIA as described above. PHB was measured as described in Smolders *et al.* (1994) with minor modifications. MLSS was determined according to APHA Standard Methods (1985).

4.3.3 Results

In the following, all experiments are referred to as Exp. # and the batch reactors are referred to as #A, #B, #C and #D, where # represents the experiment number in chronological order of performance. The numbering only includes those experiments which provide the most relevant results and which are described below in detail. In all experiments, the observed rise in the measured nitrate or nitrite concentration after an instantaneous addition was generally less than the actual concentration added. The reason for this is that step changes in concentrations could not be precisely tracked. Each reactor was measured only every 6 to 7.5 minutes and, consequently, some denitrification usually occurred before the concentration increase could be registered.

Exp. 1 shown in Figure 4.3-2 was designed to study anoxic phosphate uptake in the presence of nitrite at two concentration levels compared to anoxic phosphate uptake in the presence of nitrate only. MLSS was measured to be 4.1 g/l.

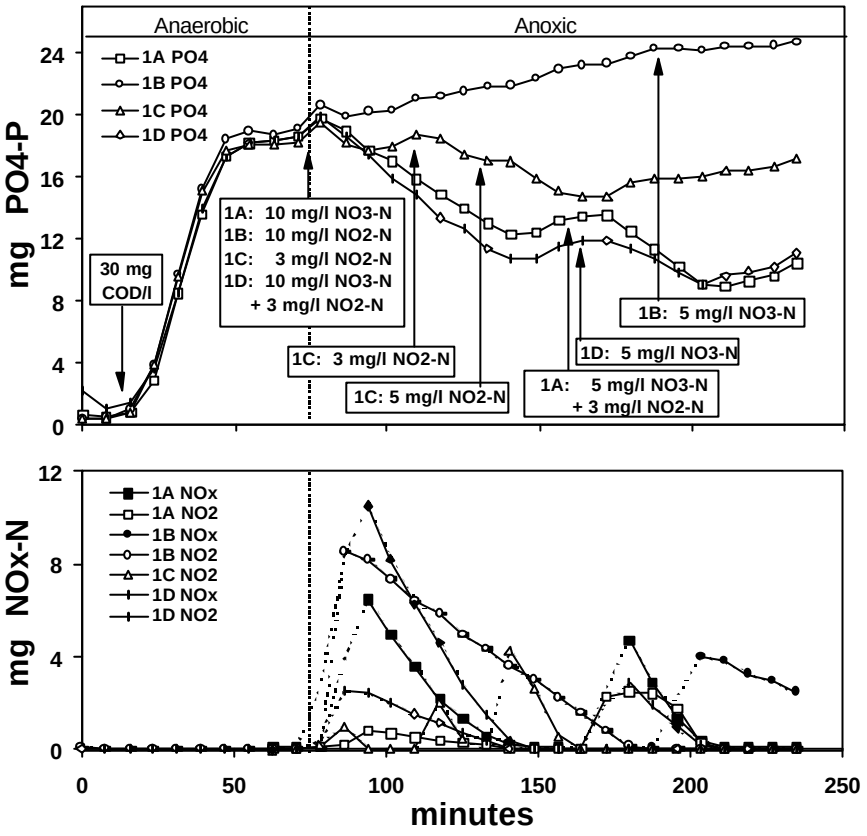

Figure 4.3-2 Exp.1. Anoxic P-uptake in the presence of nitrite, nitrate and a mixture of nitrite/nitrate.
PO_4-P, NO_2-N and NO_X-N measurement in the four reactors, operated in parallel and receiving either only nitrate or only nitrite or a mixture of both.

Reactor **1A** received only nitrate initially (10 mg NO_3-N/l) and this reactor exhibited the typical behaviour when nitrate serves as electron acceptor. Phosphate uptake occurred as long as nitrate was present, and as soon as nitrate was totally consumed a slow release of phosphate took place. This phosphate release, further referred to as secondary release, is presumably mainly associated with the storage of organic substrates arising from the hydrolysis of slowly biodegradable organic substrates, but endogenous effects might also contribute to this P-release, although to a lesser extent. Upon adding oxidised nitrate later in the experiment (5 mg NO_3-N/l + 3 mg NO_2-N/l) phosphate uptake resumed. Nitrite up to concentrations of about 0.9 mg NO_2-N/l appeared as an intermediate product

after the initial nitrate addition in reactor 1A. This, however, was the only instance during the course of this study where nitrite concentrations in excess of 0.2 mg NO_2-N were recorded after an addition of only nitrate.

In reactor **1B** phosphate uptake was inhibited completely after a nitrite addition of 10 mg NO_2-N/l. The rate of nitrite consumption was also much reduced (0.10 mg N $(l \cdot min)^{-1}$) compared to nitrate consumption in reactor 1A (0.19 mg N $(l \cdot min)^{-1}$). Nitrate (5 mg NO_3-N/l) was added to reactor 1B after nitrite was totally consumed but this did not lead to a phosphate uptake. This nitrate was also consumed at a low rate compared to reactor 1A. This indicates that the detrimental effect of nitrite is not just momentary but rather lasts for at least a period of time after nitrite no longer is present (compare to Exp. 6 below).

In reactors **1C** and **1D** phosphate uptake did not appear to be negatively influenced by nitrite at the lower concentration of 3 mg NO_2-N/l. Reactor 1C received three separate additions of nitrite (3, 3 and 5 mg NO_2-N/l), and phosphate uptake occurred immediately after each addition. Nitrite was consumed too quickly to allow for a good estimation of denitrification and phosphate uptake rates. However, the rate of phosphate uptake after each nitrite addition appears to be similar to the phosphate uptake rate in reactor 1A. The waviness in the phosphate curve is an artefact of the repeated switching between anoxic and anaerobic conditions. In reactor 1D the initial addition of 10 mg NO_3-N/l was supplemented with 3 mg NO_2-N/l and this again did not negatively influence the phosphate uptake. A comparison of the phosphate uptake rate in reactors 1A and 1D suggests that phosphate uptake occurs more quickly in the presence of both nitrate plus nitrite instead of nitrate alone. However, this behaviour was not observed in the other experiments of this study.

Exp. 2 shown in Figure 4.3-3 was one of several experiments performed to determine the nitrite concentration at which the inhibition of anoxic phosphate uptake begins to occur. MLSS was measured to be 3.5 g/l.

Reactor **2A** served as control reactor receiving 10 mg NO_3-N/l initially and an additional 10 mg NO_3-N/l after the first nitrate addition was consumed. Reactor **2B** received four sequential additions of 4 mg NO_2-N/l. As with reactor 1C, nitrite was consumed too quickly in reactor 2B to allow a good estimation of the rates of denitrification and phosphate, and the waviness in the phosphate curve is due to repeated switching between anoxic and anaerobic conditions. A comparison with reactor 2A clearly shows, however, that phosphate uptake occurred with instantaneous additions of 4 mg NO_2-N/l at least as quickly as with nitrate as electron acceptor. Nitrite was also consumed in reactor 2B at a much higher rate than nitrate in reactor 2A.

Reactors **2C** and **2D** received initial instantaneous nitrite additions of respectively 6 and 8 mg NO_2-N/l, and anoxic phosphate uptake was inhibited in both reactors. The nitrite consumption rates in these two reactors were also much lower than in reactor 2B, and slightly lower than the nitrate consumption rate in reactor 2A. Neither a phosphate uptake nor an increase in the denitrification rate occurred in either reactor when nitrite was added at lower concentrations (4 to 5 mg NO_2-N/l) later in the experiment after the initial nitrite addition was totally consumed.

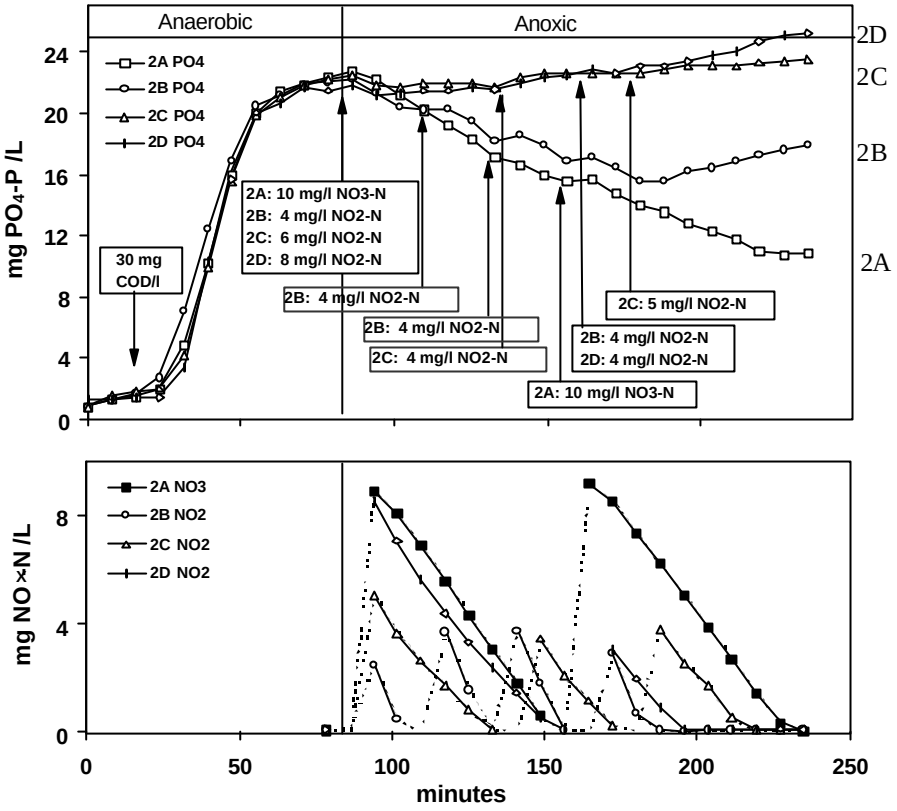

Figure 4.3-3 Exp.2, determining nitrite concentration level, at which inhibition occurs.

PO_4-P, NO_2-N and NO_X-N measurement in the four reactors, operated in parallel.
The control reactor (2A) received only nitrate; the other three reactors received different levels of nitrite during the anoxic phase.

Exp. 3 shown in Figure 4.3-4 was intended to narrow down the critical nitrite concentration at which inhibition of anoxic phosphate uptake begins. The MLSS was 3.4 g/l. Reactor **3A** served as control receiving 12 mg NO_3-N/l initially while reactors **3B, 3C and 3D** received nitrite additions of respectively 4, 5 and 6 mg NO_2-N/l. These same nitrite additions were repeated after the initial nitrite was consumed.

Based on the results of Exp. 2, nitrite inhibition had been expected to occur within the concentration range applied here. However, phosphate uptake occurred in all three reactors at rates which appear to be at least as high as with nitrate in reactor 3A. The rates of nitrite consumption during the first two additions in reactors 3B, 3C and 3D were similar, and higher than the nitrate consumption rate in reactor 3A. The third addition to reactors 3B, 3C and 3D was made with higher nitrite concentrations (8, 10 and 7 mg NO_2-N/l, respectively) and phosphate uptake was inhibited in all three reactors. The nitrite consumption rates in all three reactors were also affected, with a reduction to approximately one third of the rate occurring with the first two additions and approximately one half of the nitrate consumption rate in reactor 3A

In two other similarly performed experiments (not shown) phosphate uptake was found to be inhibited at 8 and 10 mg NO_2-N/l but not at 4 and 6 mg NO_2-N/l, and at 6 and 8 mg NO_2-N/l but not at 5 mg NO_2-N/l. Apparently, the critical nitrite concentration is a function of sludge conditions. For the activated sludge employed in this study, it lies between 5 and 8 mg NO_2-N/l.

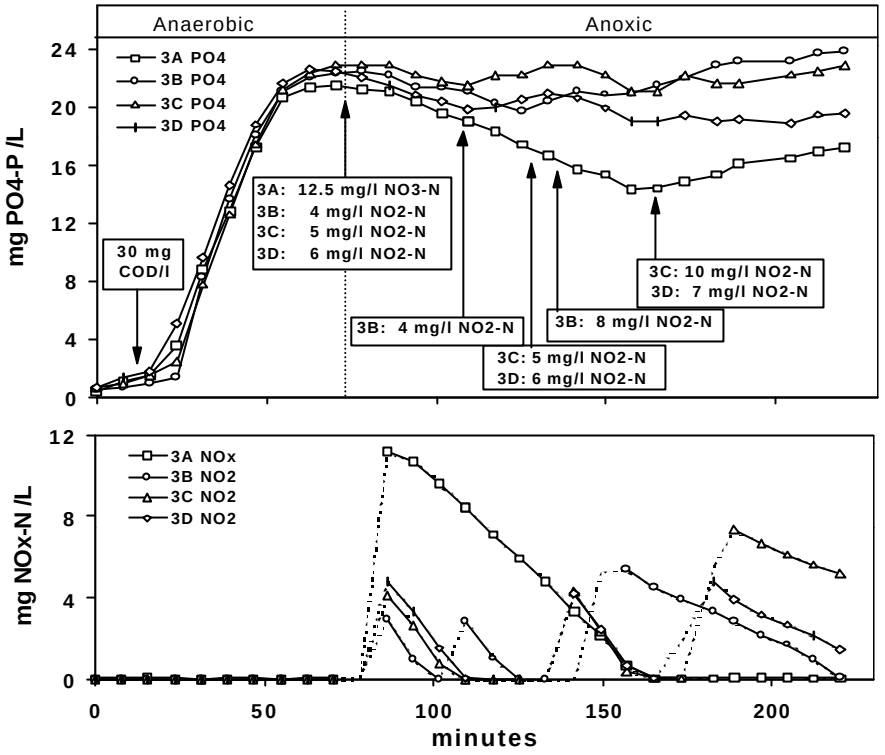

Figure 4.3-4 Exp.3, determining critical nitrite concentration, at which inhibition occurs.

PO_4-P, NO_2-N and NO_X-N measurement in the four reactors, operated in parallel.

Reactor A – control reactor receiving only NO_3-N, Reactors B, C, D receiving only NO2-N at increasing concentration

Exp. 4 shown in Figure 4.3-5 (MLSS=3.4 g/l) was one of several experiments by which nitrite was added continuously to one of the reactors. This was done in order to better assess phosphate uptake in the continuous presence of low concentration levels of nitrite. Only three reactors were employed.

During the initial portion of the experiment, reactors **4A** and **4B** differed from each other only in that the 12.5 mg NO_3-N/l added to reactor 4B was supplemented with 4 mg NO_2-N/l. The phosphate uptake rates in both reactors were similar. The denitrification rate in reactor 4B (nitrate plus nitrite consumption rate) was about 10% higher than the denitrification rate in reactor 4A. After total consumption of nitrate in reactor 4A (t=210 min.) the normal slow release of phosphate occurred. The addition of 4 mg NO_2-N/l to reactor 4B after the consumption of the first addition of nitrate and nitrite resulted in a continued phosphate uptake and denitrification with little change in the respective rates.

Nitrite was added continuously to reactor **4C** after an initial instantaneous addition of 3 mg NO_2-N/l. The nitrite addition rate was manually adjusted during the course of the experiment in an attempt to maintain a nitrite concentration between 2 and 4 mg NO_2-N/l. Phosphate uptake occurred in this reactor but at about a 20% lower rate than in reactors 4A and 4B. The nitrite consumption rate in reactor 4C, calculated from the known nitrite addition rate and the nitrite change in the nitrite measurements, was about 30% to 45% higher than the denitrification rates in the other two reactors.

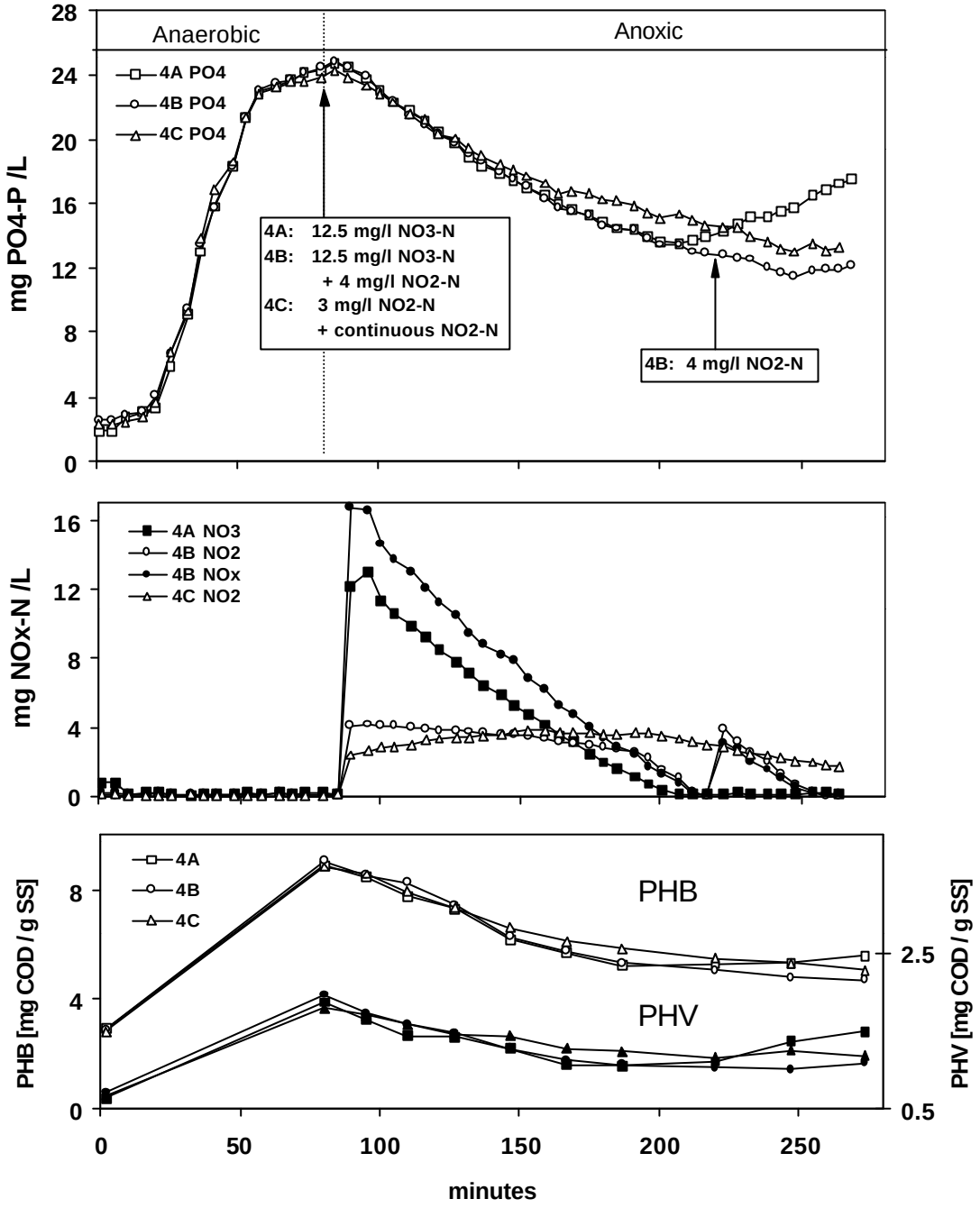

Figure 4.3-5 Exp. 4, P- uptake in the continuous presence of low concentration of nitrite.

PO_4-P, NO_2-N, NO_X-N and PHA measurement in the three reactors, operated in parallel. Reactor 4A – control reactor receiving only NO_3-N, reactor 4B received a mixture of nitrate/nitrite; reactor C received continuously nitrite, attempting to keep the NO_2-N concentration between 2 and 4 mgN/L.

PHB and PHV were also measured during this experiment and the results appear in the lower plot of Figure 4.3-5. The dynamics of both internal storage compounds are consistent with the dynamics of soluble phosphate. During the anaerobic period there is an increase in PHA as phosphate is released and acetate is taken up. During the anoxic phase both the PHA utilisation rate and the anoxic phosphate uptake rate are highest initially, with a gradual reduction as long as nitrate or nitrite is present. For reactor 4A, phosphate was released and PHA accumulated again after the reactor became anaerobic at about 220 minutes. As with the anoxic phosphate uptake rate, the rate of PHA

utilisation in the presence of nitrite only (reactor 4C) is lower compared to when nitrate was present. Contrary to the results of Exp. 1, the trajectories of both PHA and phosphate in reactors 4A and 4B provide no indication that anoxic phosphate uptake occurs at a higher rate when nitrite is present (at a low concentration) in addition to nitrate.

In **Exp. 5** shown in Figure 4.3-6 (MLSS=3.4 g/l), reactors **5A**, **5B** and **5C** were intended to investigate the relative utilisation of nitrate and nitrite by BPR activated sludge. The three reactors received the same total concentration of oxidised nitrogen but with varying proportions of nitrate and nitrite: 10 mg NO_3-N/l (reactor 5A), 8 mg NO_3-N/l + 2 mg NO_2-N/l (reactor 5B) and 6 mg NO_3-N/l + 4 mg NO_2-N/l (reactor 5C). During the anoxic period following this first addition, all three reactors exhibited phosphate uptake at about the same rate. The NO_x-N consumption rate during this period, however, is higher with increasing proportion of nitrite. After nitrate and nitrite were totally consumed the same additions were performed again. This second addition was not performed soon enough for reactors 5A and 5B and so a short anaerobic period caused a momentary pause in the phosphate uptake between the two additions. During the anoxic period after this second addition all three reactors exhibited a similar NO_x-N consumption rate.

Nitrite was added continuously to reactor 5D after an initial instantaneous addition of 3 mg NO_2-N/l. The nitrite utilisation rate was underestimated which is why nitrite decreased to zero after the first few measurements. The nitrite addition rate was then increased and occasionally adjusted, so that the nitrite concentration remained between 1.7 and 2.7 mg NO_2-N/l. As in Exp. 4, the anoxic phosphate uptake rate was slightly lower in reactor 5D with continuous nitrite addition compared to the other reactors receiving higher levels of nitrate. A mass balance was again employed to calculated the nitrite consumption rate in reactor 5D.

As with Exp. 4, the initial nitrite consumption rate in this reactor was about 30% higher than the initial NO_x consumption rate in the other reactors and was generally higher throughout the experiment.

PHB and PHV measurements were also performed in Exp. 5. As with Exp. 4, the PHA dynamics are consistent with the phosphate dynamics. Reactors 5A, 5B and 5C exhibited about the same PHA utilisation rates, which were slightly higher than the PHA utilisation rate of reactor 5D. Once again, a comparison of reactors 5A, 5B and 5C indicates that anoxic phosphate uptake does not occur at a higher rate when nitrite in addition to nitrate is present at a low concentration.

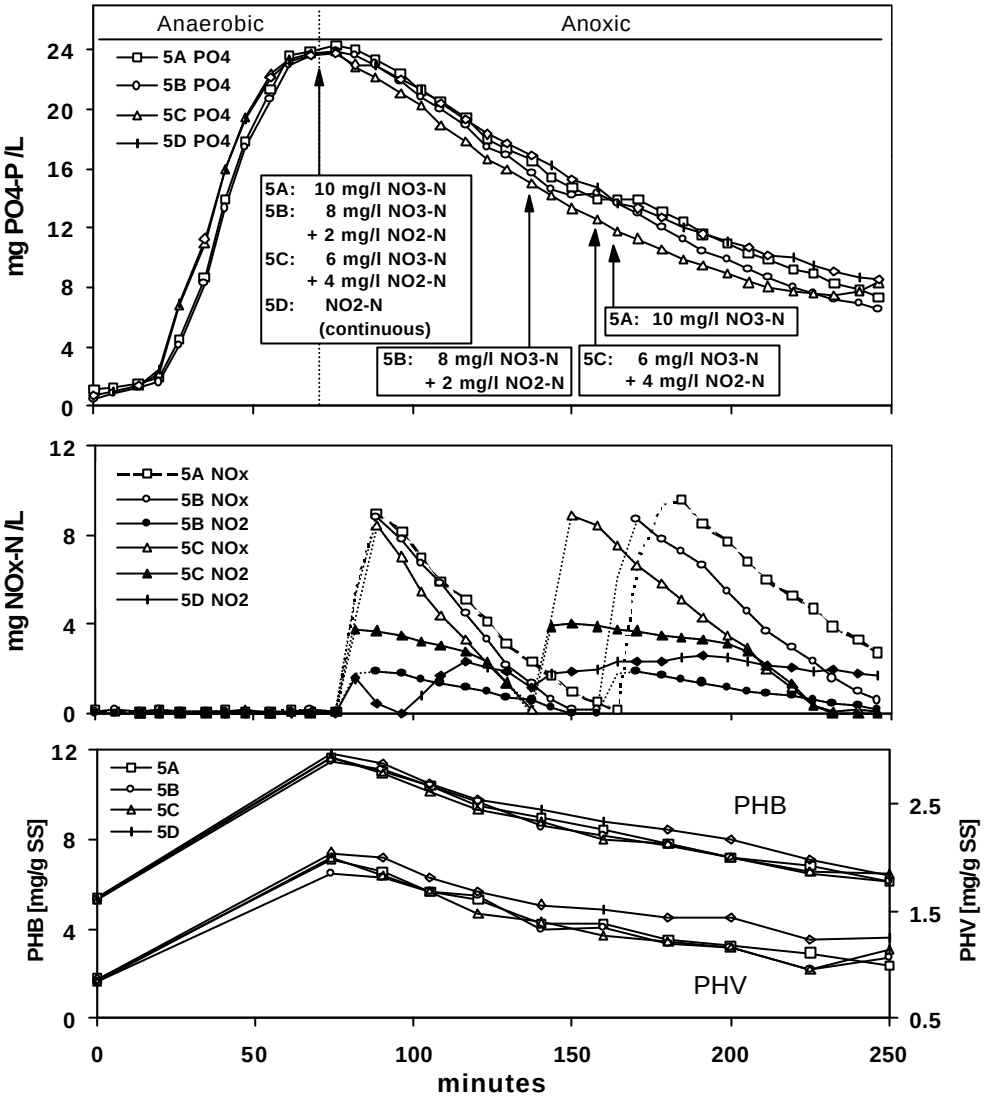

Figure 4.3-6 Exp. 5, investigating the relative utilisation of nitrate and nitrite.

PO_4-P, NO_2-N, NO_X-N and PHA measurement in the four reactors, operated in parallel.
Three reactors received the same total concentration of oxidised nitrogen but with varying proportions of nitrate and nitrite: 10 mg NO_3-N/L (reactor 5A), 8 mg NO_3-N/l + 2 mg NO_2-N/L (reactor 5B) and 6 mg NO_3-N/L + 4 mg NO_2-N/L (reactor 5C).

Exp. 6 shown in Figure 4.3-7 (MLSS=3.8 g/l) was performed to supplement the results of reactors 1A and 1B of Exp. 1. Here, PHB and PHV were measured, and the experiment was performed over a longer time period to see whether anoxic phosphate uptake would resume within several hours after the exposure to inhibitive levels of nitrite. The sludge in reactor **6A** was continuously exposed to nitrate during the anoxic period by repeated additions of nitrate. Nitrite (10 mg NO_2-N) was added initially to reactor **6B**. After total consumption of this nitrite the sludge was continuously exposed to nitrate for the remainder of the experiment.

As with Exp. 1, phosphate uptake was inhibited in the reactor exposed to 10 mg NO_2-N/l. The initial slight drop in phosphate is due to a procedural error, by which only a low, non-inhibitory amount of nitrite was initially added. This caused some phosphate uptake to occur until the error was noted and the proper amount of nitrite was added.

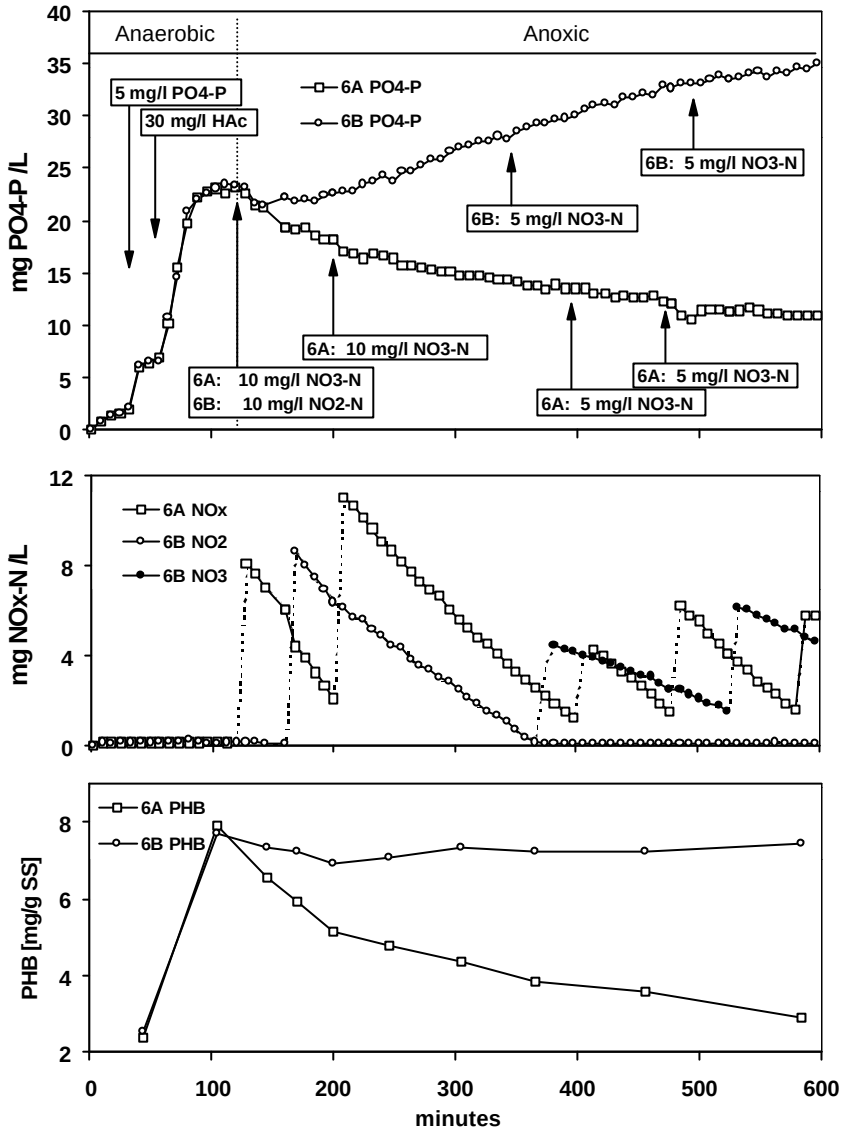

Figure 4.3-7. Exp. 6, persistent BPR inhibition after temporary exposure a critical level of nitrite.

PO_4-P, NO_2-N, NO_X-N and PHA measurement in the two reactors, operated in parallel.
Reactor 6A (control reactor) was continuously exposed to nitrate during the anoxic period
Reactor 6B received initially 10 mg NO_2-N and later on nitrate for the rest of the experiment.

No significant PHB consumption occurred during or after the exposure to the high nitrite concentration. The sludge in reactor 6B was exposed to nitrate for a period of about 4 hours after nitrite was totally consumed, and there appeared to be no recovery of the ability to utilise internally stored PHB to take up phosphate within this time frame. Reactor 6B also exhibited a much lower denitrification rate after nitrite exposure, where rate of nitrate consumption between 370 and 540 minutes in reactor 6B was about 50% of the nitrate consumption rate during the same time period in reactor 6A. Clearly anoxic P-uptake does not resume within 4 hours after exposure to high nitrite concentration.

Experiment 7 shown in Figure 4.3-8 was performed in order to see whether the inhibiting effect of nitrite exposure also affected aerobic phosphate uptake. After the anaerobic phosphate release period reactors **7A** received 10 mg NO_3-N/l and **7B** and **7C** 10 and 15 mg NO_2-N/l respectively, while reactor **7D** remained anaerobic. At about 200 minutes into the experiment after all nitrate and nitrite was consumed all reactors were aerated.

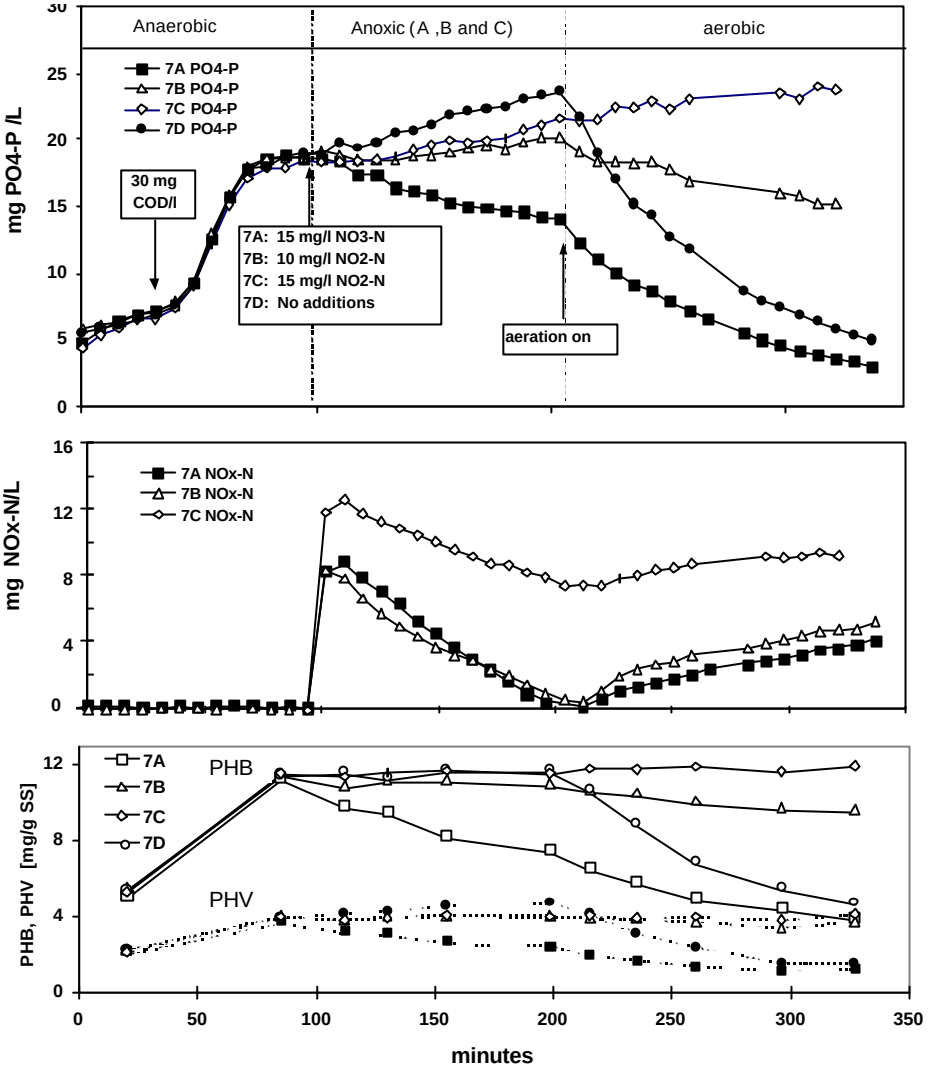

Figure 4.3-8 Exp.7, inhibitive effect of nitrite on aerobic P-uptake in a AN-ANOXIC-AE batch test.
PO_4-P, NO_2-N, NO_X-N and PHA measurement in the four reactors, operated in parallel.
Reactor 7A: 15 mg/l NO_3-N; reactor 7B: 10 mg/l NO_2-N;
reactor 7C: 15 mg/l NO_2-N; reactor 7D: No additions

Phosphate and PHA in reactor 7A exhibited the typical response supporting the hypothesis that only a fraction of the PAO can utilise nitrate as electron acceptor. The behaviour exhibited by reactor 7A can be explained by the existence of two populations of PAO (Kerrn-Jespersen and Henze, 1993; Meinhold *et al*,1999): one that can use only oxygen as the terminal electron acceptor and the other that can use either oxygen or nitrate. Anoxic phosphate uptake is due to DNPAO, using nitrate as e^--acceptor. It slows down as their intracellular storage material (PHA) becomes limiting. The other group, the non-DNPAO, still have sufficient intracellular storage, as they are not able to use nitrate. Increased phosphate uptake upon the start of aeration is then due to the activity of non-DNPAO.

In reactor 7B and 7C the anoxic phosphate uptake was severely inhibited. The observed P-release, however, is not as high as in reactor 7D (no NO_X-N), which exhibited secondary phosphate release. This suggests that the activity of the PAO was not completely inhibited. This is also in line with the observation, that for reactor 7B both reactions (P-uptake and PHB-utilisation) were able to proceed to some extent under aerobic conditions, but at a much reduced rate compared to the reactors 7A and 7D. The reactor, receiving 15 mg NO_2-N/L, showed an increase of phosphate in the bulk liquid throughout the course of the experiment, indicating a higher degree of inhibition. The PHA

concentration, however, showed no concomitant increase but stayed rather constant during the whole experiment. This suggests that there was still some metabolic activity.

4.3.4 Discussion

Since the batch experiments were performed on different dates using activated sludge from a pilot scale plant fed with real wastewater, the characteristics of the sludge varied somewhat from experiment to experiment. Some of the relevant factors subject to variation include MLSS, the fractions of active denitrifying PAO, non-denitrifying PAO and denitrifiers without phosphate accumulating activity, the initial PHA content of the PAO, the initial organic substrate pool and the rate of hydrolysis of slowly biodegradable organic substrates. With the exception of MLSS and PHA none of these quantities were measured. The total range over which the MLSS varied was 3.4 g/l to 4.2 g/l but for the majority of the experiments the MLSS remained within the range of 3.4 g/l to 3.6 g/l. Due to this variability in sludge characteristics, several aspects of sludge behaviour, e.g. the uninhibited rates of anoxic phosphate uptake and denitrification, varied among the experiments.

The results of all experiments performed indicate that nitrite at low concentrations (up to 4 or 5 mg NO_2-N/l) is not detrimental to anoxic biological phosphate uptake and, with respect to PHA utilisation and phosphate uptake, can serve as electron acceptor in a similar manner as nitrate. At higher concentrations (above 8 mg NO_2-N/l) nitrite interferes with PAO metabolism, so that PHA utilisation and anoxic phosphate uptake cease. The critical nitrite concentration at which nitrite inhibition occurs appears to be dependent on the condition of the activated sludge and, based on this study, lies somewhere in the range of 6 to 8 mg NO_2-N/l. It should be noted that these experiments were performed with activated sludge which is normally not exposed to nitrite. The situation might be different for activated sludge systems with high ammonia loading or high nitrate concentrations, both favouring the accumulation of nitrite, if adaptation to nitrite can occur.

The relationship between initial phosphate uptake rate (mg P/min l) and initial nitrite concentration, on a per volume basis, can be seen in the left portion of Figure 4.3-9. Each point represents the initial rate of change in phosphate concentration, calculated by a linear regression using up to the first 6 measurements following the nitrite addition. Included in the figure are all batches of all experiments performed with instantaneous nitrite addition and for which the linear regression could be made with at least three measurements. Positive and negative values signify phosphate release and uptake, respectively. At first glance the data appear to be spread around a line crossing the abscissa between 6 and 8 mg NO_2-N/l. However, the large spread in the data is largely due to the variability in sludge characteristics among the experiments, and the actual behaviour might instead be described by an "S"-shaped curve, with a relatively constant phosphate uptake under 6 mg NO_2-N/l, a relatively constant phosphate release above 8 mg NO_2-N/l and a sharp increase (i.e. a sharp drop off of phosphate uptake rate) in-between 6 and 8 mg NO_2-N/l. Except for a few outliers in the region of higher concentrations, this type of behaviour is more apparent in the right hand plot in Figure 4.3-9., where the data from the left plot is presented on a per suspended solids basis.

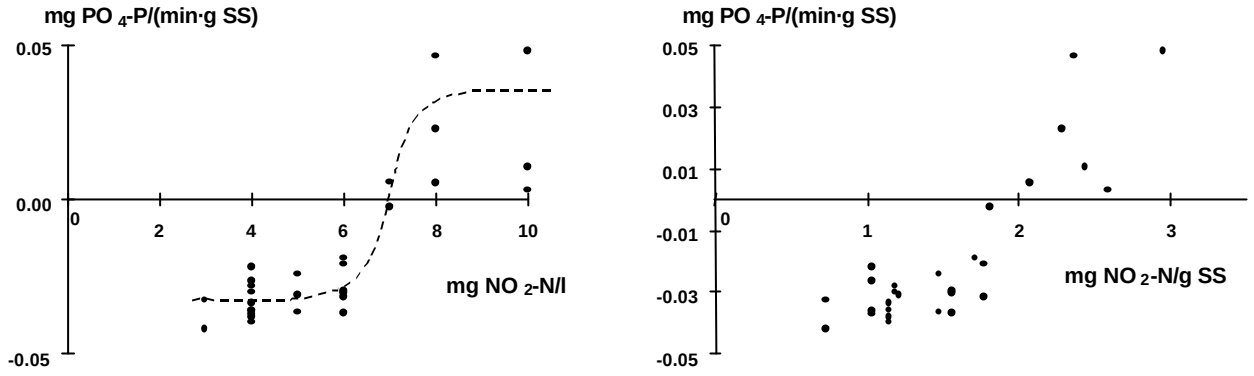

Figure 4.3-9 Relationship between initial phosphate uptake rate and NO_2-N concentration added.

The points represent the initial rate of change of phosphate after nitrite addition plotted against the initial nitrite concentration for all experiments performed (incl. those not shown in Figures 2 through 8).

Left hand plot: volumetric basis; right hand plot: MLSS basis.

For experiments for which the internal storage products PHB and PHV were measured, these two components were found to follow well the dynamics of soluble phosphate. The rates of PHB and PHV consumption decreased with decreasing phosphate uptake. PHB and PHV stores increased as phosphate was released under anaerobic conditions after oxidised nitrogen was totally consumed. Furthermore, PHB and PHV were not consumed when phosphate uptake was inhibited by nitrite. In case of nitrite inhibition the PHA dynamics, however, did not show an increase according to the P-release observed. (Exp. 6, Figure 4.3-7 and Exp. 7, Figure 4.3-8). This indicates that the observed increase in phosphate is probably not due to take up of organic substrate and storage as PHA, i.e. it is not equal to the metabolism known from the anaerobic phase. Whether this P-release is due to lysis or death of PAO or maybe due to changes in the pH in the sludge floc can not be stated from the measurements made. Lysis/death could explain the P-release and long term deterioration of BPR: exposure to nitrite causes lysis/death of a large portion (depending on amount of nitrite added) of the PAO, resulting in the release of phosphate and organics into the bulk liquid. The fraction of PAO still active might be able to take up these released organics and store them as PHA. This could balance out the overall PHA content, resulting in the constant concentration observed (Exp.6 and Exp.7). The activity of the still functioning PAO could also explain the slight phosphate uptake observed in reactor 7C during the aerobic phase. However, based on the measurements, no precise conclusion can be drawn with regard to which part of the metabolism and how exactly it is inhibited.

The relative rates of nitrate and nitrite reduction occurring in the activated sludge obtained from the pilot plant can be observed with the help of Figure 4.3-10, which shows the measured NO_x-N and NO_2-N concentrations of reactor 5C. Also shown is NO_3-N, which was calculated by subtracting the measured values for NO_2-N from those for NO_x-N. The numbers shown on the plot are volumetric utilisation rates in mg N $(l \cdot min)^{-1}$ for various measurement segments, calculated with a linear regression. A comparison of the rates for the two subsequent additions shows that these rates decrease with time, which can be explained by a decrease in the available organic substrate. This should cause the rates to be non-linear, but over the time period of one NO_X-N addition this non-linear effect is minor and the rates can be estimated by linear regression. The calculation of the rates for the two segments was performed to illustrate the decrease in NO_X-N utilisation rate.

Since nitrate is first reduced to nitrite which is then reduced further, the decreasing trend in the nitrite measurements while nitrate is present indicates that the nitrite utilisation rate is higher than the

nitrate utilisation rate. The nitrite utilisation rate is equal to the slope of the NO_x-N curve, which for both measurement segments is about 15% higher than the nitrate utilisation rate even though presumably only a certain portion of the heterotrophic bacteria is able to reduce nitrite.

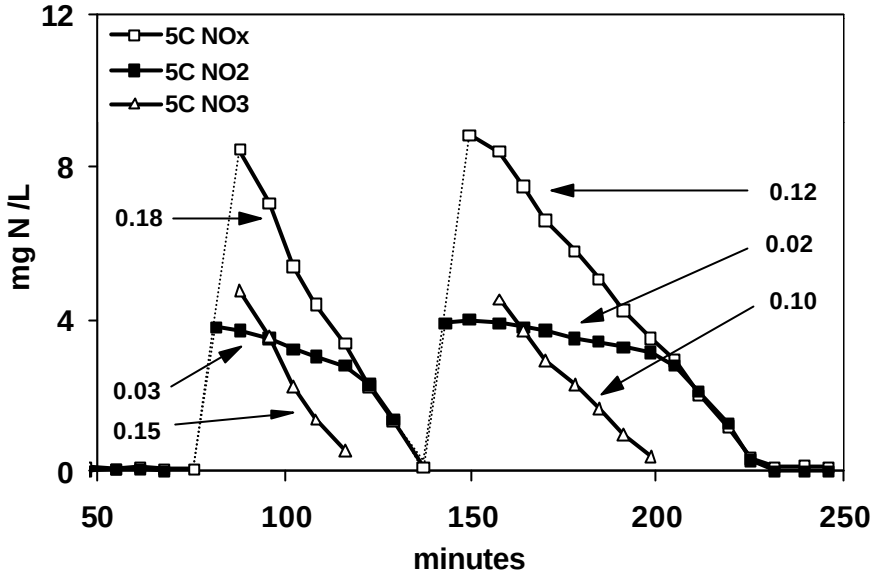

Figure 4.3-10. Nitrate and nitrite in reactor 5C - relative rates of NO_3-N and NO_2-N reduction.
The numbers shown on the plot are volumetric utilisation rates in mg N $(l \cdot min)^{-1}$ for various measurement segments.

The relative nitrite and nitrate utilisation rates are presumably dependent on the condition of the sludge, and a slightly lower rate for nitrite initially may explain the small nitrite accumulation observed in Exp. 1. In all other experiments performed, however, the nitrite utilisation rate appeared to be the higher one of the two. This observation is of relevance because it means that nitrite would not be expected to accumulate to inhibiting levels.

Several experiments indicate that the inhibiting action of nitrite is not only momentary, occurring only while the nitrite is present, but lasts for at least several hours after the time of exposure. This factor is significant because it means that even momentary exposure, as may for example occur with a periodic sequencing batch reactor, needs to be avoided. Exposure to nitrite is damaging not only anoxic phosphate uptake but aerobic P-uptake as well. Depending on the degree of inhibition (amount of nitrite added) some aerobic phosphate uptake could be observed (e.g. reactor 7B after nitrite exposure). The phosphate uptake rate, however, was minor compared to the rates in reactors 7A and 7D. Reactor 7C exhibited complete P-uptake inhibition. Accepting the suggestion of two fractions of PAO, Exp. 7 illustrates that nitrite has affected both, denitrifying and non-denitrifying, PAO fractions to a severe extent.

To summarise, the higher rate of nitrite compared to nitrate reduction means that only little or no accumulation of nitrite is expected in the alternating or recirculating processes under normal circumstances. At low concentration levels, nitrite will serve as electron acceptor and promote phosphate uptake similar to nitrate with no adverse effects. The uptake of phosphate with nitrite shows that the denitrifying fraction of PAO is capable of the entire pathway of nitrate reduction to nitrogen gas (the fact that at least some PAO can perform the first stage of nitrate to nitrite is demonstrated in the enriched cultures by Jørgensen and Pauli, 1995). On the other hand, problems with phosphate removal will occur in processes where nitrite may accumulate, even momentarily, to concentrations in excess of around 5 mg NO_2-N/l. This might be the case, for example, with

industrial wastes or with sequencing batch reactor processes where the biological reactor is loaded with high levels of ammonia.

4.3.5 Conclusions

A series of batch experiments have shown that nitrite at low concentrations (up till roughly 4 mg NO_2-N/l) does not adversely affect anoxic phosphate uptake in activated sludge obtained from an alternating type biological phosphorus removal process. In this concentration range phosphate uptake occurs in the absence of both oxygen and nitrate using nitrite as electron acceptor. Employing nitrite, nitrate and mixtures of both resulted in the same performance with regard to anoxic phosphate uptake rates.

On the other hand, after exposure to higher nitrite concentrations ($\geq$ 8 mg NO_2-N/l) anoxic phosphate uptake is totally inhibited. The inhibition is not only momentary, occurring only as long as nitrite is present, but lasts for at least several hours after the nitrite exposure. Aerobic phosphate uptake is also inhibited severely and ceases after exposure to slightly higher levels of nitrite.

The critical nitrite concentration, above which severe nitrite inhibition of phosphate uptake occurs, has been found to vary among the experiments and, hence, appears to be dependent on sludge conditions. For the experiments performed in this study, the critical nitrite concentration lies in the range of 5 to 8 mg NO_2-N/l.

The aim of this study was to use BPR activated sludge being acclimatised to municipal wastewater, with its complex composition, and to investigate the response of the sludge to different nitrite concentration levels. The results obtained give good insights in the usage of nitrite as an electron acceptor for BPR and also illustrate its limitations. The investigation of the biochemical mechanism for the usage of nitrite as well as for the inhibition cases is desirable, but will require more defined experimental conditions such as known substrate and biomass composition and a higher number of measured variables.

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4.4 Continuous Addition of Acetate to the Anoxic Phase in BPR Batch Experiments

ABSTRACT

The continuous introduction of a biological phosphorus removal (BPR) promoting organic substrate to the denitrifying reactor of a BPR process is examined through a series of batch experiments using acetate as model organic substrate. Several observations are made regarding the influence of substrate availability on PHA storage/utilisation and phosphate uptake/release. Under anoxic conditions PHB is utilised and phosphate is taken up, indicating that at least a fraction of the PAO can denitrify. The rates of anoxic P-uptake, PHB utilisation and denitrification are found to increase with increasing initial PHB level. At low acetate addition rates the P-uptake and PHB utilisation rates are reduced compared to when no acetate is available. At higher acetate addition rates a net P-release occurs and PHB is accumulated. For certain intermediate acetate addition rates the PHB level can increase while a net P-uptake occurs. Whether the introduction of BPR promoting organic substrates to the denitrifying reactor is detrimental to overall P-removal appears to be dependent on the interaction between aerobic P-uptake, which is a function of PHB level, and the aerobic residence time.

This section is based on the article :

> Meinhold J., Pedersen H.; Arnold E., Isaacs S. and Henze M. (1998). Effect of continuous addition of an organic substrate to the anoxic phase on biological phosphorus removal. *Wat. Sci. Tech.*, **38** (1), 97-107.

Supplementary investigations and final discussion of this subject are presented at the end of this section.

4.4.1 Effect of Continuous Addition of an Organic Substrate to the Anoxic Phase on Biological Phosphorus Removal

4.4.1.1 Introduction

Activated sludge processes designed for biological phosphorus removal (BPR) generally include biological nitrogen removal. This means that nitrate is invariably present during the phosphate-release / phosphate-uptake cycle. Of interest, consequently, is an understanding of the effects of nitrate on BPR performance. Nitrate is usually considered to be inhibitive to BPR activity, since nitrate introduced to the anaerobic zone via return sludge can be denitrified in this zone, thereby reducing the supply of organic substrates available for uptake and later utilisation by the phosphate accumulating organisms (PAO) responsible for BPR activity.

Generally accepted today, however, based on numerous investigations (see e.g. the review by Barker and Dold, 1996), is that at least a fraction of the PAO can denitrify. Gerber *et al.* (1986, 1987) postulated that phosphorus uptake and release occur simultaneously under anoxic conditions due to the activity of a denitrifying and a non-denitrifying fraction of PAO. The more predominant reaction supposedly masks the less predominant one, i.e. at low acetate concentrations under anoxic conditions, P-release associated with acetate uptake by PAO may be hidden by simultaneous anoxic P-uptake. They also stated that the overall denitrification rate reflects the sum of the denitrification rate due to normal (non-PAO) denitrifiers and due to the denitrifying fraction of PAO. Using SBR's, Kuba *et al.* (1993) demonstrated the use of nitrate as sole electron acceptor for BPR. Kerrn-Jespersen and Henze (1993) and Gerber *et al.* (1986, 1987) have observed anoxic phosphorus uptake and concomitant denitrification in full scale activated sludge plants.

Nitrate, therefore, may be beneficial to BPR systems due to denitrifying PAO. If the PAO take up and store phosphate using nitrate as electron acceptor then the same waste water organic substrates are effectively utilised for both P and N removal. This is of significance since organic substrate availability is often a limiting factor in nutrient removal processes. Other advantages associated with denitrifying PAO activity include a reduction in aeration energy and a reduced sludge production.

The interaction between nitrate and organic substrates can readily be observed in the alternating type BIODENIPHO^{TM} process (Einfeldt, 1992) due to its semi-batch manner of operation. Figure 4.4-1 shows nitrate and phosphate measurements collected over a little more than one process cycle in one of two anoxic/aerobic reactors of a BIODENIPHO pilot scale plant. The curve without symbols is the course that phosphate would take were no reactions involving phosphate to occur in the reactor, calculated from the dilution rate and incoming phosphate concentration. A comparison of the slope of this curve with the rate of increase of the actual phosphate measurements (the numbers next to the arrowed line segments in the figure) indicates that P-uptake occurs while nitrate is present (denitrification by PAO), and that some phosphate is released after nitrate has been consumed. This release has been observed to be much greater during periods of high waste water strength.

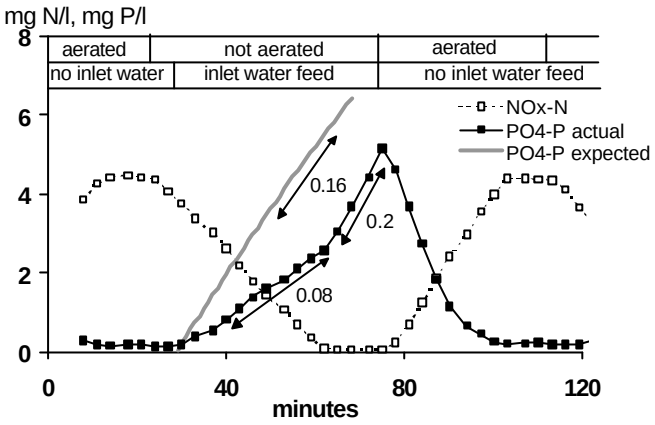

Figure 4.4-1 Nitrate and phosphate measurements in one of two anoxic/aerobic reactors of a BIODENIPHO™ pilot plant. The numbers by the double arrow segments are rates of phosphate increase in mg $P(l \cdot min)^{-1}$.

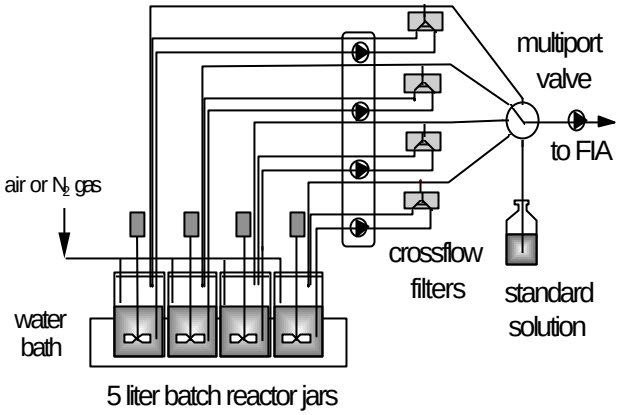

Figure 4.4-2 Experimental batch set-up.

Phosphate release after the anoxic reactor has become anaerobic is attributed to the take-up of organic substrate made available at a slow rate, either due to conversion reactions (hydrolysis, fermentation) within the reactor or due to incoming readily degradable substrates not taken up in the anaerobic zone. At least in the latter case it can be assumed that this substrate source is also available while the reactor is anoxic. Moreover, the controlled addition of an external organic substrate to the anoxic reactor has been examined as a means to improve N removal (Isaacs *et al.*, 1994). Hence, it is of interest to examine what occurs with respect to BPR dynamics, e.g. PHA storage/utilisation and phosphate uptake/release, when organic substrates are continuously added to the anoxic zone. This paper presents results from several batch experiments which were performed to address this question.

4.4.1.2 Methods

Batch experiments

The set-up consists of four 5 litre plexiglass cylindrical reactors, each equipped with a motor driven stirrer (Figure 4.4-2). Atmospheric oxygen is excluded during non-aerobic periods by supplying nitrogen gas at a low flowrate to just above the liquid surface. During aerobic periods air is sparged through the liquid using an aquarium diffuser for each reactor. Chemical addition is performed by pipette or, for continuous addition, with a calibrated peristaltic pump. Automatic measurement of NH_4-N, NO_x-N and PO_4-P is performed as follows: Mixed liquor from each reactor is continuously pumped through a crossflow filter and back to the reactor with a 4 channel peristaltic pump, and the filtrate from each reactor is sent in turn by means of a multiport valve to be analysed for all three species by flow injection analysis (FIA). Filtrate not taken for analysis is returned to the reactor by means of a tube which also serves as a sample liquid buffer since the pumping rate to the FIA system does not always equal the filtrate flowrate. All three species are measured in each reactor plus in a standard solution every 7.5 minutes (see also Isaacs and Temmink, 1996).

Activated sludge for each batch experiment was obtained from one of two anoxic/aerobic reactors of a BIODENIPHO™ pilot plant. Before taking the sludge the pilot plant reactor was first isolated without aeration to totally remove nitrate. Four litres of sludge were then transferred to each of the four batch reactors, which immediately thereafter were stirred and placed under nitrogen gas. Each experiment was initiated with an anaerobic PHB-uptake/phosphate-release step by adding an amount of sodium acetate (HAc) to each reactor and maintaining the reactors anaerobic until the phosphate release associated with acetate uptake was complete in all reactors. If the sludge contained negligible ammonia, 5 mg N/l ammonium chloride was also added initially to avoid ammonia limitation during the course of the experiment. After the anaerobic period an

anoxic period was initiated by adding sodium nitrate. At the same time the continuous addition of HAc was started. Additional sodium nitrate was later added to a reactor if the nitrate in the reactor was about to be completely consumed. In case an aerobic period was to follow an anoxic period (Exp. 5), this was done by stopping the flow of nitrogen gas as well as the continuous HAc addition and starting the flow of compressed air. An experimental series (not shown) indicated that phosphate disappearance due to precipitation in the batch set-up is minor if pH does not rise much above 7. The pH for all experiments was, therefore, manually monitored and maintained at 7.0±0.2 with additions of 1.0 M HCl or 0.5 M NaOH.

Analytical methods

Ammonia nitrogen (NH_4-N), nitrate plus nitrite nitrogen (NO_x-N) and ortho-phosphate (PO_4-P) were analysed with FIA (Pedersen *et al.* 1990). PHB was measured as described in Smolders *et al.* (1994) with minor modifications. MLSS and MLVSS were determined according to APHA Standard Methods (1985).

4.4.1.3 Results

In all figures presented, continuous curves are portrayed for the nitrate measurements by appropriate vertical adjustment of the data between times of nitrate addition. The curves shown thus correspond to the situation in which all nitrate added during the experiment was injected at the beginning of the anoxic period. The continuous addition rates of acetate given in the text and figures are in units of mg COD $(l \cdot min)^{-1}$.

Several experiments were first performed to examine the effect of initial PHB level on anoxic P-uptake. Exp. 1 shown in Figure 4.4-3 exhibits the typical behaviour observed. During the anaerobic phase the four reactors received different amounts of acetate (0, 22.5, 45 and 67.5 mg COD/l). After the acetate induced P-release ended, potassium phosphate (KH_2PO_4) was added to the reactors to bring the phosphate concentration to the same level in all four reactors. Nitrate was then added to all four reactors. Table 4.4-1 summarises the rates of anoxic P-uptake, denitrification and PHB utilisation calculated from a regression from portions of the experimental data. The initial P-uptake rate and the denitrification rate increase with increasing level of internally stored PHB. There appears to be a saturation level above which further increases in the initial P-uptake rate are negligible. The PHB/N ratio indicates that another organic source, e.g. hydrolysis of slowly biodegradables, contributes to denitrification. This contribution is greater when denitrification by the PAO occurs to a lesser extent due to a lower PHB availability.

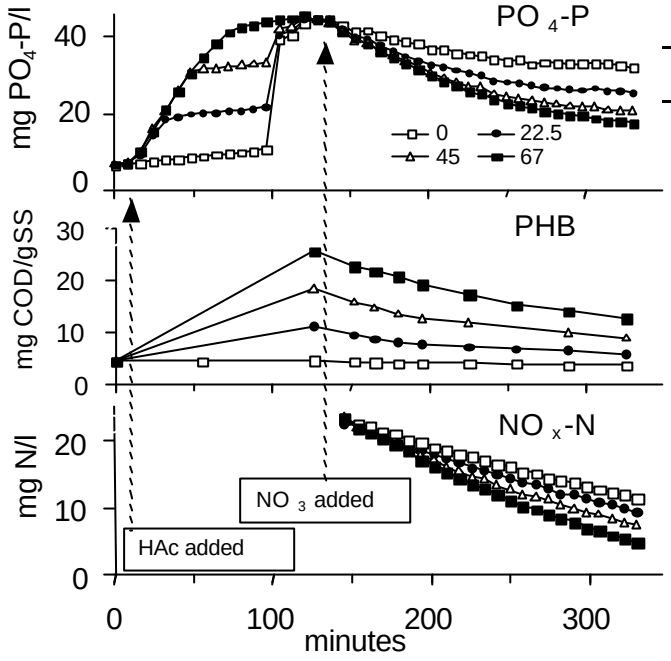

Figure 4.4-3 Exp. 1, effect of different amounts of PHB anaerobically stored on initial anoxic P-uptake rates. Legend values are the amounts of HAc added (mg COD/l).

Table 4.4-1. Rates for Exp.1

HAc added mg COD/L		0	22.5	45	67.5
P-uptake b)	mg P / gSS h[a)]	1.2	2.1	2.4	2.6
N-removal b)	mg N / gSS h[a)]	1.1	1.31	1.63	1.82
PHB util. rate b)	mg COD / gSS h[a)]	0.29	1.79	3.0	3.36
c)		0.11	0.30	0.61	1.07
PHB/ N b)	mg COD / mg N	0.27	1.36	1.84	1.85
c)		0.12	0.3	0.56	0.87

a) SS =3.71g/L b) 1st hour anoxic c) 200-320 min

The effect of continuous anoxic acetate addition was studied at two initial PHB levels with Exp. 2 shown in Figure 4.4-4. During the anaerobic phase two reactors each received 15 and 30 mg COD/l HAc. After the acetate induced P-release ended, potassium phosphate was added to the two reactors receiving 15 mg COD/l to bring phosphate in all reactors to the same level. Nitrate was then added to all four reactors, and the continuous addition of acetate was started at a rate of 0.2 mg COD $(l \cdot min)^{-1}$ to one reactor for each initial acetate addition level. Anoxic acetate addition had the effect of decreasing the P-uptake and PHB utilisation rates and increasing the denitrification rate.

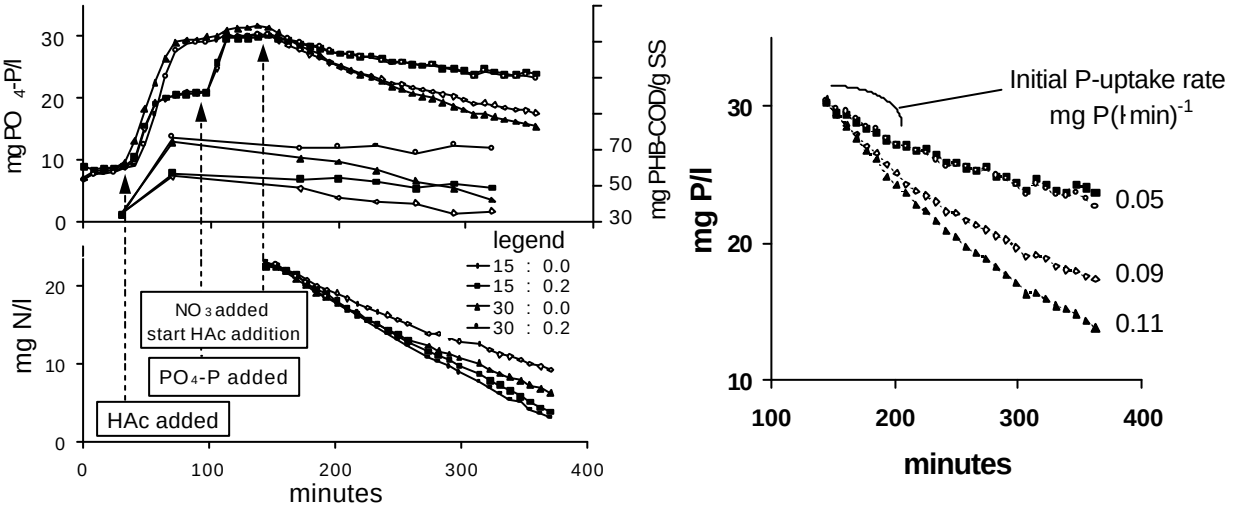

Figure 4.4-4. Exp. 2. Effect of continuous anoxic acetate addition at two initial PHB levels.
The legend shows the initial anaerobic acetate addition (mg COD/l) and the anoxic acetate addition rate in mg COD$(l \cdot min)^{-1}$. Right plot: detail of the phosphate curves. The numbers shown are the initial P-uptake rates.

As with Exp. 1, a higher initial PHB level resulted in higher denitrification, P-uptake and PHB utilisation rates for the reactors with no anoxic acetate addition. With anoxic acetate addition,

however, these rates were not significantly influenced by the initial PHB level. Of interest is the fact that with anoxic addition the PHB levels remained almost constant while a net P-uptake still occurred.

Several experiments were performed with acetate added continuously at various addition rates and two sets of results are shown in Figure 4.4-5. The left hand plots (Exp. 3) and right hand plots (Exp. 4) differ in the range of acetate addition rates employed, and in the initial amount of acetate added anaerobically.

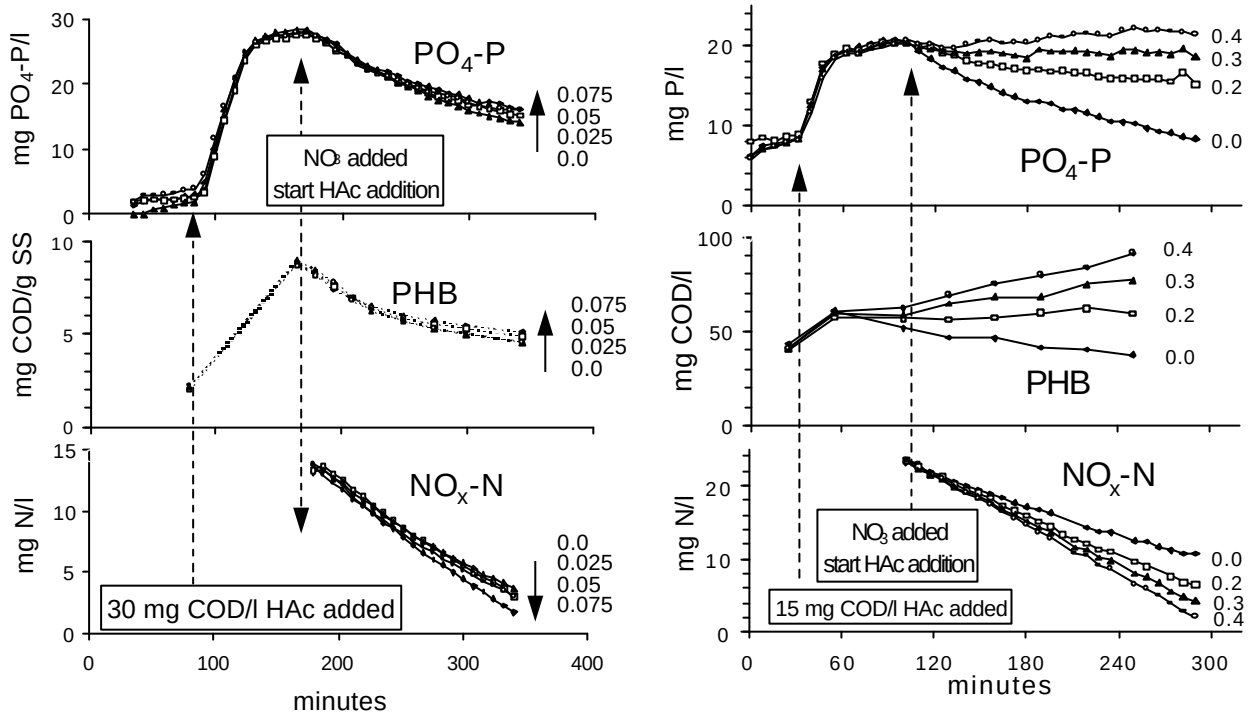

Figure 4.4-5. Exp. 3 (left) and Exp. 4 (right) examining various continuous acetate addition rates to anoxic activated sludge. The numbers to the right of the curves are acetate addition rates in mg COD$(l \cdot min)^{-1}$.

Table 4.4-2: Summary results of Exp. 4

	1	2	3	4	5	6
anoxic acetate [a)] addition rate	ΔPO_4-P [e)]	ΔHAc [b)]	ΔPHB [b)]	ΔHAc - ΔPHB [b)]	ΔNO_x-N [c)]	$\frac{(\Delta HAc - \Delta PHB)}{\Delta NO_x\text{-}N}$ [d)]
0	-10.4	0	-13.7	13.7	-10.5	1.3
0.2	-4.4	30	4.7	25.3	-13.3	1.9
0.3	-0.9	45	18.0	27.0	-15.0	1.8
0.4	1.6	60	27.0	33.0	-16.4	2.0

Table entries: a) mg COD/$(l \cdot min)^{-1}$; b) mg COD/l; c) mg N/l; d) mg COD/mg N; e) mg P/l

For the pilot plant, the low level addition rate of 0.025-0.075 mg COD $(l \cdot min)^{-1}$ for Exp. 3 translates to an inlet waste water COD content of approximately 10-20 mg COD/l. In this range the influence of the anoxic acetate addition was small but in the expected direction. Increasing acetate addition rate progressively decreased the phosphate uptake and PHB utilisation rates and increased the denitrification rate. The higher addition rates of 0.2-0.4 mg COD $(l \cdot min)^{-1}$ for Exp. 4 translate to pilot

plant inlet waste water COD contents of approximately 80-160 mg COD/l, and here the influence of the anoxic COD addition is more pronounced. The phosphate dynamics changed from a net uptake to a net release and the PHB dynamics changed from a net utilisation to a net storage with increasing acetate addition rate. Again of interest is that, for an addition rate of 0.2 mg COD $(l \cdot min)^{-1}$, a net P-uptake occurred with a net PHB storage.

Table 4.4-2 shows the amount of acetate added and the overall changes in nitrate, phosphate and PHB concentrations between 100 and 250 minutes during the anoxic period of Exp. 4. The 4th column represents the COD available for denitrification originating either from PHB or the continuously added acetate. The 6th column is the ratio of this COD to the nitrate consumed. The low values resulting here (the theoretical COD requirement for denitrification is 4-5 g COD/g N, Henze *et al*., 1997) indicate that other sources of COD, e.g. hydrolysis products, are also contributing significantly, and this extent is greater when acetate is not added. Other experiments (not shown) exhibited the same qualitative behaviour as the results shown here, with a marked change in P-uptake behaviour for anoxic acetate addition rates of 0.1 and greater. Rates of denitrification and the rates of change for PO_4-P and PHB differed among the experiments. This can be explained by differences in sludge activities, since the sludge was obtained from the pilot plant on different days. The acetate addition rate for which the transition from anoxic P-uptake to P-release occurred also differed among the experiments, lying in the range of 0.2 to 0.4 mg COD $(l \cdot min)^{-1}$.

According to the above results, the amount of phosphate taken up by denitrifying PAO in the anoxic zone is reduced by the introduction of an organic substrate readily taken up by the PAO. Hence, the addition of organic substrate to the anoxic zone appears to be detrimental to BPR. However, associated with the reduced P-uptake is a higher level of stored PHB and, based on anaerobic-aerobic experiments similar to Exp. 1 (not shown), the higher PHB level leads to a higher P-uptake rate in the subsequent aerobic zone of an anaerobic-anoxic-aerobic process. Exp. 5 was, therefore, performed in order to evaluate the effect of continuous HAc addition on P-removal in the combined anoxic-aerobic steps. The procedure was similar to Exp. 4 with the exception that HAc addition rates of 0, 0.05, 0.1 and 0.15 mg COD $(l \cdot min)^{-1}$ were employed and an aerobic period was included after the anoxic period. The continuous HAc addition was stopped at the start of the aerobic period.

Figure 4.4-6 a-c shows the results of Exp. 5. For the anoxic period, the behaviour is qualitatively the same as for Exp. 3 and Exp. 4. The aerobic P-uptake rate is increasing with an increasing level of PHB at the start of the aerobic period. This means that the reactor with the highest anoxic acetate addition rate exhibits the highest P-uptake rate in the subsequent aerobic period. Seen from the end of the combined anoxic-aerobic period, the four reactors are approximately equivalent in the amount of phosphate taken up and the PHB content of the sludge. This is illustrated by Figure 4.4-6 d), showing the phosphorus and nitrate removal for the reactors receiving continuous acetate addition, when taking the performance of the reactor without continuous acetate addition as a basis. While the P-removal is virtually the same for the reactors, a significant improvement in N-removal is achieved. However, the phosphate concentration at earlier points in time is higher with increasing HAc addition rate, meaning that the net P-removal with shorter aerobic periods is lower.

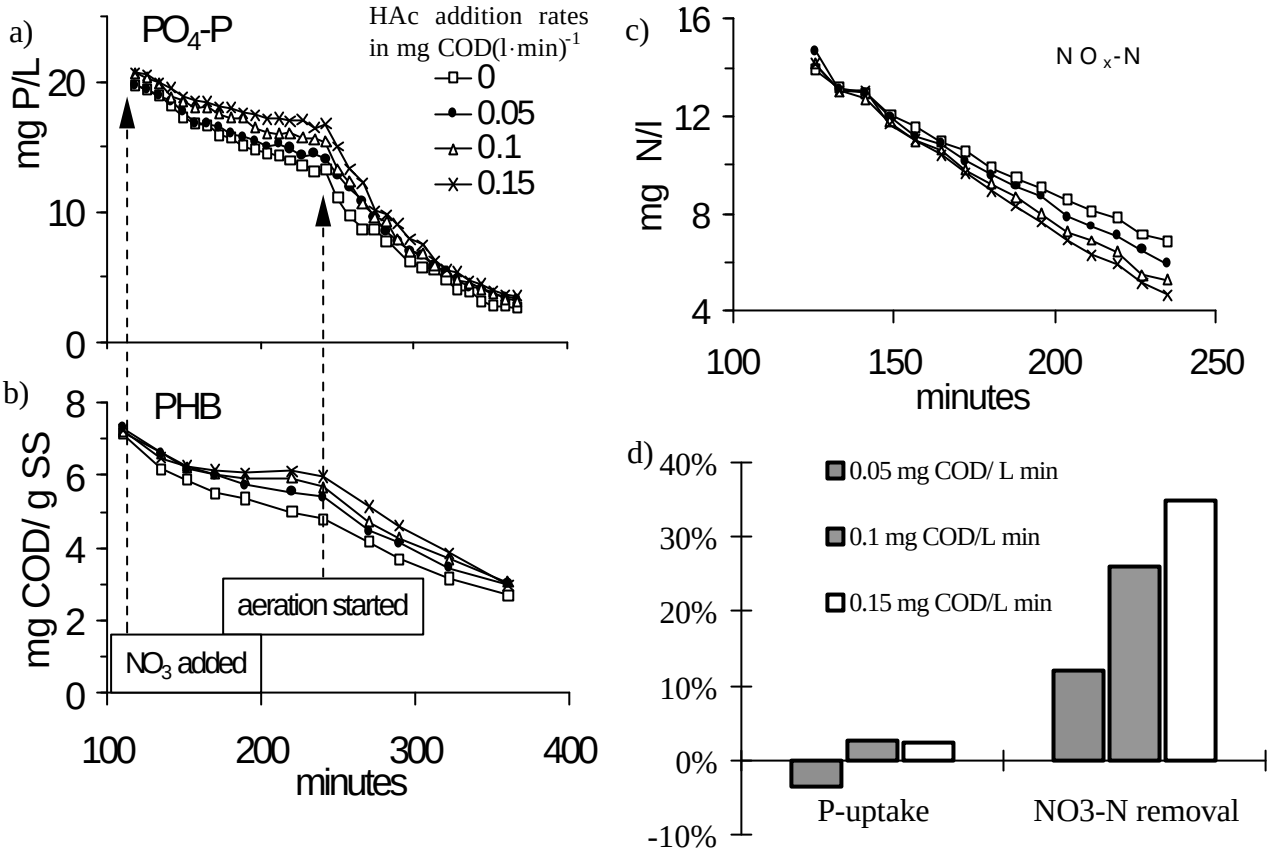

Figure 4.4-6. Exp.5 examining continuous HAc addition to sequential anoxic-aerobic P-uptake.
a) measured PO_4-P concentration in the liquid phase b) measured PHB concentration in the sludge
c) measured NO_X-N concentration in the liquid phase
d) P and N-removal of the reactors with continuous acetate addition in relation to the reactor without

4.4.1.4 Discussion

In this study sodium acetate was used as organic substrate since it is an easily degradable substrate typical of wastewater and formed in activated sludge processes by fermentation, and which readily promotes BPR activity. Consequently, the following discussion will refer to acetate as organic substrate. However, the points in the discussion are expected to apply to other BPR promoting substrates, e.g. other volatile fatty acids.

Exp. 1 supports previous findings discussed in the introduction that there exists denitrifying PAO and at least a fraction of the PAO present in the mixed culture studied here can take up phosphate using nitrate as electron acceptor and internally stored PHB as organic substrate. The rates of P-uptake, PHB utilisation and denitrification increase with increasing PHB level indicating that maintaining the PHB level high, e.g. by avoiding excessively long aerobic contact times or by supplemental organic substrate addition during weak waste water periods (Teichfischer, 1995; Temmink *et al.*, 1996), may lead to improved BPR performance.

A division of PAO in at least two groups, denitrifiers (DNPAO) and non-denitrifiers, has been hypothesised in the past (Kerrn-Jespersen and Henze, 1993; Comeau *et al.*, 1987; Vlekke *et al.*, 1988). This theory is supported by the phosphate pattern of Exp. 5 (Figure 4.4-6). The anoxic P-uptake due to DNPAO, using nitrate as an electron acceptor, slows down as their intracellular organic storage material becomes limiting. The non-denitrifying PAO still have their anaerobically produced intracellular organic supply intact and even increased throughout the anoxic period, and their activity leads to the increase in P-uptake upon start of aeration.

Similarly, the response to continuously added acetate under anoxic conditions seen in Exp. 2 through Exp. 5 can be explained on the basis of parallel or overlapping activities of denitrifying and non-denitrifying PAO. DNPAO are responsible for anoxic P-uptake and PHB utilisation whereas anoxic P-release and PHB accumulation occurs due to the non-denitrifying PAO. It is the relative speed of the processes which determine whether there is a net P-release or uptake and a net PHB utilisation or accumulation. This is dependent on the level of intracellularly stored PHB and, to a greater extent, on the rate of acetate availability during the anoxic phase. At higher acetate addition rates the anoxic P-release and PHB accumulation become dominant and mask the phosphorus uptake by DNPAO. Interesting is the observation that net P-uptake can occur with a net accumulation of PHB, and this can be explained by a difference between the P-uptake/PHB utilisation and P-release/PHB storage yield ratios. Another possible explanation is that DNPAO can use organic substrates directly for both growth and P-uptake using nitrate as electron acceptor, as indicated by Kuba *et al*. (1994).

Continuous acetate addition to the anoxic zone increases the denitrification rate and, correspondingly, the amount of nitrogen removed for a given anoxic hold-up time, and this was examined as a control handle to improve N removal in an alternating BIODENIPHO^{TM} process (Isaacs *et al.,* 1994). For BPR systems, an interesting question is how the organic substrate addition affects BPR performance. Regardless of whether the organic substrate is added as a supplemental carbon source or comes about due to organic conversions or carry over from the anaerobic zone, the following two situations can occur:

- At low addition rates a net anoxic P-uptake occurs but at a reduced rate compared to when no anoxic acetate is available. At the same time, less PHB is utilised and, therefore, since this PHB is available for subsequent utilisation in the aerobic zone, overall P-uptake may not be diminished by the acetate addition.
- At higher anoxic acetate addition rates a net P-release occurs during denitrification. As shown here in Exp. 5 and in previous work (Isaacs *et al*., 1993) this anoxic release is not necessarily detrimental to P-removal. The reason for this is that a PHB accumulation is associated with the P-release and, correspondingly, will result into a higher PHB level during the subsequent aerobic period. Exp. 1, Exp. 5 and other experiments which were performed similarly to Exp. 1 but with an aerobic period in place of the anoxic period (not shown) indicate that a higher PHB level indeed leads to a higher aerobic P-uptake rate.

In Exp. 5 all reactors with anoxic acetate addition exhibited a considerable increase in nitrate removal (up to 30 % for the highest HAc addition at the end of the denitrifying period) with no apparent negative effect on P-removal after a two hour aerobic period. However, for aerobic periods shorter than two hours the phosphate level in the reactors with anoxic acetate addition was higher than the reactor without anoxic acetate addition. This means that for an insufficiently long aerobic period (here, less than 2 hours) a lower net P-removal with anoxic acetate addition would have resulted. Apparently, P-removal performance depends on the interactions between the amount of phosphate released during denitrification, the aerobic P-uptake rate (which is apparently a function of internally stored PHB), and the aerobic residence time. Indeed, the more general question of how P-removal as a function of aerobic hold-up time is influenced by the amount of prior phosphate release and PHB storage (under both anaerobic and anoxic conditions) is currently not well understood but important to achieving optimal process design and control and, hence, is a subject for further investigation.

4.4.2 Additional Investigations

This section addresses some questions, arising from the evaluation of all the batch tests and not covered by the previous discussion.

Can the PHB storage due to anoxic acetate addition be attributed to the PAO?

This aspect is essential, when considering the anoxic acetate addition as a possible operational strategy for the stabilisation of biological nutrient removal processes. It is known that micro-organisms, other than PAO, can store PHB when an excessive amount of acetate is added in the anoxic or aerobic phase, i.e. when they experience a considerable surplus in COD availability (Dircks *et al.*, 1999). However, the amount of acetate added instantaneously in these investigations exceeds the amounts used in the present study by 2-3 orders of magnitudes (50-100 times higher). Furthermore the acetate addition in this study was performed continuously at low concentration levels, reducing further the probability for other organisms than PAO to store PHB.
It has been demonstrated in section 4.1 that the P-uptake rates are highly dependent on the PHB level. Hence, the observed increases in the P-uptake rates after stopping the acetate addition, are a clear indication, that the major part of the stored PHB can be attributed to the PAO.

Why are there cases of PHB storage without associated P-release?

In batch tests without acetate additions to the anoxic or aerobic phase, the observed phosphate and PHB pattern normally follow each other, i.e. PHB is accumulated during P-release and utilised during P-uptake. Several experiments in this study exhibit a deviation from this behaviour, when acetate is added in the anoxic phase. In experiment 6, depicted in Figure 4.4-7, this phenomenon can be seen, when comparing the concentration pattern of the reactors receiving 0.1 and 0.2 mg COD_{HAc} / L min, respectively. Both reactors exhibit about the same anoxic P-uptake, but differ significantly in their PHB concentration during the anoxic phase.

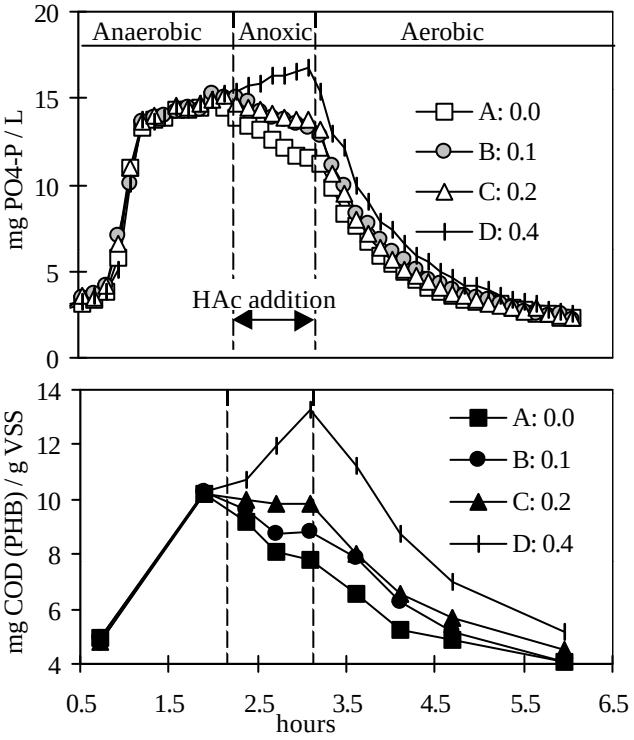

Figure 4.4-7 Exp. 6. Investigating various acetate addition rates to the anoxic phase, with subsequent aeration. Numbers to the right are addition rates in mg COD_{HAc} $(L_R \cdot min)^{-1}$.

As discussed in section 4.4.1.4 the measured concentration pattern can be explained on the basis of parallel or overlapping activities of denitrifying (DNPAO) and non-denitrifying (O2-PAO) phosphate accumulating organisms. According to the current understanding for anoxic conditions (s. chapter 2) the DNPAO have 4 possible sources for ATP supply (poly-P degradation, TCA cycle, oxidative phosphorylation and glycogen degradation) and two potential sources to provide the reducing equivalents,$NADH_2$, (TCA cycle, glycogen pool). Depending on the internal level of NADH, ATP and Acetyl-CoA, there might be no need for poly-P degradation to fulfil the energy requirements for acetate uptake and PHB storage. In these cases an overall P-uptake with concurrent PHB storage could occur in the reactor.

Under aerobic conditions all PAO possess the sources for ATP and NADH, mentioned above. Hence, when acetate is added during aeration, the probability of observing P-uptake along with PHB accumulation should increase. Experiment 7, displayed in Figure 4.4-8, was performed to address the difference responses when adding acetate continuously to anoxic and aerobic conditions respectively. Both reactors received 30 mg COD_{HAc}/L initially in the anaerobic phase. Acetate addition, 1mg COD_{HAc} $(L_R \cdot min)^{-1}$ for 30 minutes, was applied after being 45 min. into the aerobic and anoxic phase, respectively. The anoxic reactors shows immediate P-release, upon the start of acetate addition, whereas the aerobic reactors continues to exhibit P-uptake. For both reactors an accumulation of PHB is observed during this period, although to quite different degree. Competition for acetate between the different microbial groups is higher under aerobic conditions, essentially leading to less PHB stored by PAO.

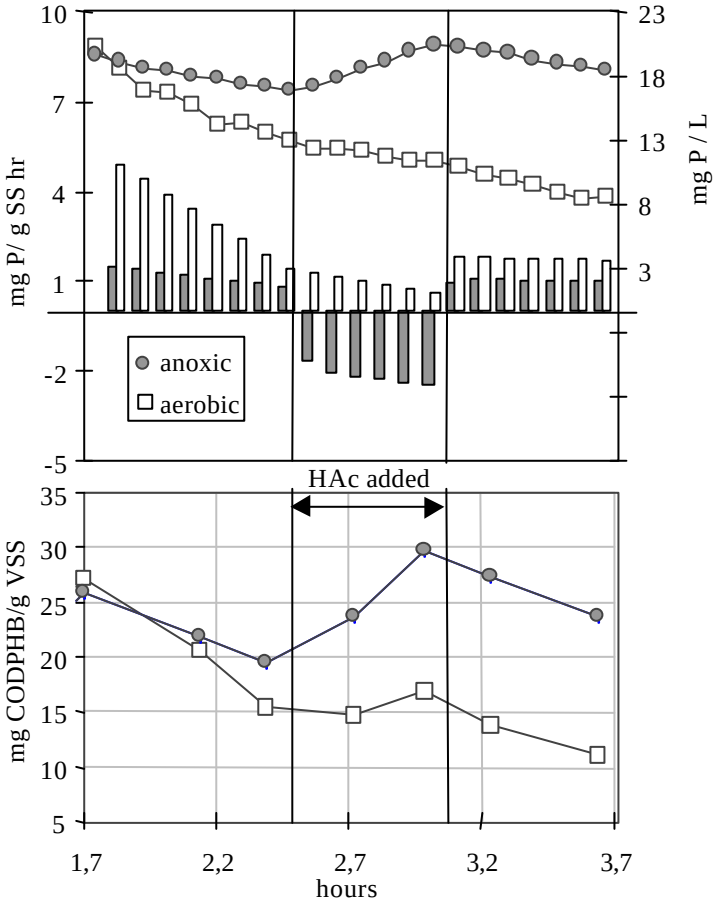

Figure 4.4-8. Exp.7. Comparing the response when adding 1mg COD_{HAc}/ L_R min to an anoxic and aerobic reactor.
a) P measurements and P-uptake(positive) / P-release(negative) rates.
b) PHB measurements for both reactors.

Once the acetate addition was stopped, both reactors showed ongoing P-uptake, at slightly increased and more stable rates. The P-release in the anoxic reactor is mainly due to the activity of the O2-PAO, acting as under anaerobic conditions. From the measurements in this experiment, it cannot be stated whether the DNPAO use the poly-P degradation as an energy source or not. Under aerobic conditions all PAO can make use of the respiratory chain, thus conditions can arise, as in this test, where P-release might not be required to satisfy the energy requirements.
These results underline the explanation for the possibility of P-uptake along with PHB accumulation, as a consequence of the underlying metabolic mechanism of the PAO.
In addition, it can be assumed that the temporary availability of acetate in the aerobic phase does not necessarily have a negative impact on BPR. But it should be stressed, that aerobic acetate addition cannot be regarded as an alternative to stabilise the BPR process, as long term application most definite would favour the growth of non-PAO in the system. Furthermore it offers no advantage concerning the improvement of denitrification.

General tendency of the phosphate pattern during continuous anoxic acetate addition.

The response of the sludge to anoxic acetate addition will depend on the rate of acetate addition and the length of the anoxic and aerobic phases. Apart from this, some characteristics of the sludge will also influence the response to a certain extent. Since the batch experiments were performed on different dates using activated sludge from a pilot scale plant fed with real wastewater these characteristics might have varied somewhat from experiment to experiment. Some of the relevant factors subject to variation include MLSS, the fractions of active denitrifying PAO, non-denitrifying PAO and denitrifiers without phosphate accumulating activity, the initial PHA content of the PAO, the initial organic substrate pool and the rate of hydrolysis of slowly biodegradable organic substrates. With the exception of MLSS/MLVSS and PHA none of these quantities were measured. In addition, as different anoxic and aerobic time intervals were applied in the various experiments, a direct comparison of the experiments is hardly possible. In order to gain at least some information about the tendency of the response of the sludge, the amount of phosphorus taken up (or released) within the first hour of anoxic conditions was calculated for each reactor. The results obtained were related to those of the control reactors (without anoxic acetate addition) of the corresponding experiments. For the purpose of comparing the different batch tests, these ratios were divided by the corresponding VSS value. Figure 4.4-9 shows the calculated ratios versus the acetate addition rate.
Despite the possible difference in the sludge characteristics, Figure 4.4-9 exhibits the general tendency of decreasing P-uptake with increasing rate of acetate addition. A switch from P-uptake to P-release occurs at a rate of around 0.3 mg COD_{HAc}/ L_R min added. This values does not necessarily guarantiee a complete phosphorus take up within one cycle(anaerobic/anoxic/aerobic), but might serve as an empirical value for avoiding anoxic P-release when adding acetate to the process.

During pilot plant operation a 'leakage' of up to 10 mg COD/L min of acetate from the anaerobic column to the anoxic reactor was observed for certain conditions. This corresponds to a values of around 0.05 mg COD_{HAc}/L_R min in Figure 4.4-9. According to the results obtained in this study, this "leakage' does not impose a negative impact on BPR – on the contrary, by improving denitrification and rising the PHB level, it seems to increase the stability of the overall nutrient removal process.

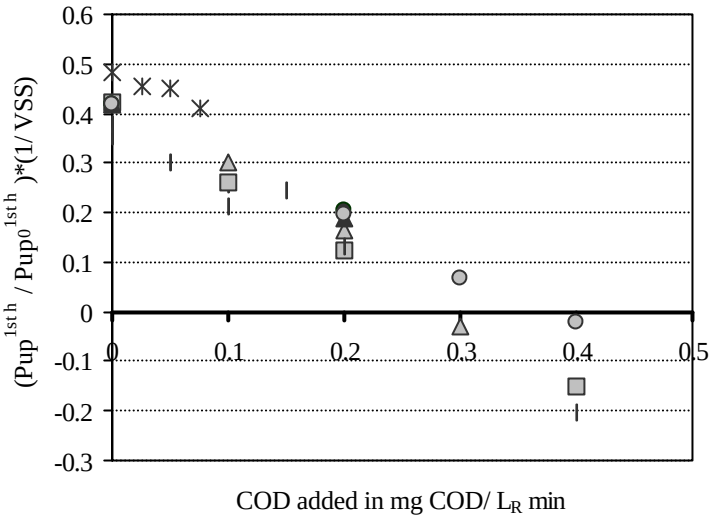

Figure 4.4-9. Ratio of the amount of P taken up/released in the reactors receiving HAc to the control reactor without HAc addition. The ratio is divided by the VSS for the purpose of comparing the different batch tests. The P-uptake/release was determined for the first hour of anoxic conditions.

4.4.3 Conclusion

Using acetate as model organic substrate, the effect of a continuous introduction of a BPR promoting organic substrate to the denitrifying reactor of a BPR process has been examined. Under anoxic conditions PHB is utilised and phosphate is taken up, which indicates that at least a fraction of the PAO can denitrify. The rates of anoxic P-uptake, PHB utilisation and denitrification increase with increasing initial level of intracellularly stored PHB. At low acetate addition rates a net anoxic P-uptake still occurs, but the P-uptake and PHB utilisation rates are reduced compared to when no anoxic acetate is available. At higher acetate addition rates a net P-release and a net storage of PHB occurs. For certain intermediate acetate addition rates there may be a net P-uptake while PHB accumulates. This seems to be due to the fact that under anoxic conditions the denitrifying fraction of PAO does not necessarily need the process of poly-P degradation as a source to fulfil their requirements for energy and reducing equivalents.

In all cases of anoxic acetate addition less PHB is utilised, thus leading to an increase in the P-uptake rates in the subsequent aerobic phase, due to the higher level of PHB available. Hence, overall P-uptake may not be diminished by the acetate addition.

The introduction of acetate to a denitrifying reactor in a BPR system increases the denitrification rate and, hence, is beneficial to N-removal. Furthermore it decreases considerably the risk of nitrate accumulation leading to a reduction in BPR performance due to nitrate introduction to the anaerobic zone. Whether or not the P-release which arises due to the introduction of acetate is detrimental to overall P-removal appears to be dependent on the interaction between the acetate addition rate, the aerobic P-uptake rate, which is a function of the PHB level, and the aerobic residence time.

The results obtained indicate that introduction of low levels (≈ 0.05 mg COD_{HAc}/ L_R min) of organic substrate to the anoxic zone, either due to organic conversions or carry over from the anaerobic zone, do not interfere with the BPR performance. Furthermore the use of external acetate addition to the anoxic phase can be proposed as a control handle in order to prevent nitrate accumulation and secondarily stabilise BPR.

4.4.4 References

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4.5 Conclusions on Cause and Effect Relationships

Each of the sections in chapter 4 dealt with a separate aspect concerning BPR. In order to provide a better overview, a summarising conclusion of the most important results is presented in this section.

All work conducted so far clearly shows that anoxic P-uptake occurred in a Biodenipho^{TM} pilot plant as well as in batch tests, which included including an anoxic period. PHA utilisation and phosphate uptake was observed under anoxic conditions, indicating that at least a fraction of the PAO can use nitrate as an electron acceptor for phosphate uptake.

The *significance of the PHA level* in the cells for anoxic and aerobic P-uptake as well as denitrification was demonstrated. Aerobic and anoxic P-uptake rates were shown to be highly dependent on the PHA level, and P-uptake rates under anoxic conditions were found to be 40 to 60 % of the aerobic ones. Batch tests revealed a saturation effect (max. P-uptake rate) with regard to PHA. Maximal aerobic P-uptake rates were in the order of 8 to 9 mg P / (g VSS h) whereas anoxic ones remained below 4 mg P / (g VSS h).

Denitrification rates also increased at higher internally PHA levels due to the increased activity of PAO under anoxic conditions. Contribution of PAO to overall denitrification was quite significant. During batch tests up to 50% of denitrification could be attributed to PAO.

However, it was observed that sudden increases in the COD load of the incoming waste water lead to temporary deterioration of BPR, despite an immediate increase of the measured PHA in the biomass. Evaluation of the PHA utilisation rate and the P-uptake rate indicate, that the yield of PHA to biomass might increase for the PAO upon sudden increase of the COD load, i.e. more carbon is directed to growth, resulting in less PHA available for P-uptake.

The *existence of two populations of PAO* (DNPAO and O2PAO) was strongly supported by the batch results obtained. The denitrifying part (DNPAO) exhibits the ability to use nitrate and oxygen as electron acceptors, whereas the second group (O2-PAO) use only oxygen.

A procedure based on the ratio of the initial anoxic and aerobic P-uptake rates exhibited the most reliable results in assessing the two fractions of PAO. Provided severe PHA limitation is reduced to a minimum, this method can be employed for detecting changes in the population distribution or anoxic BPR activity, that might take place due to changes in operational strategies. However the selection of an appropriate time interval for the estimation of P-uptake rates is a key factor that must be taken into account.

Investigating the *effect of nitrite on the PAO activity*, low nitrite concentration levels (≤ 4 mg N/L) have been shown not to interfere with BPR efficiency. The results obtained suggest that the denitrifying fraction of PAO is capable of the entire pathway of nitrate reduction to nitrogen gas.

At increasing nitrite concentrations severe interference with the PAO metabolism occured. Inhibition of the PAO metabolism was found to start at critical nitrite concentrations above 5 mg NO_2-N/L. The inhibition was not momentary, but lasted for at least several hours after nitrite exposure. Aerobic phosphate uptake was harmed severely as well at these NO_2-N levels and the P-uptake stopped completely after exposure to higher nitrite levels. In addition denitrification rates decreased, as at least the DNPAO stopped contributing to the overall denitrification.

In order to study the *impact on BPR of an easily biodegradable substrate present in the anoxic zone*, acetate was introduced to the denitrifying zone during batch tests. In all cases an increase in the

denitrification rate could be observed. At low acetate addition rates, reduced anoxic P-uptake and PHA utilisation rates are observed compared to conditions when no anoxic acetate is available. At higher acetate addition rates a net P-release and a net storage of PHA may occur. In all cases of anoxic acetate addition less PHA is utilised, thus leading to an increase in the P-uptake rates in the subsequent aerobic phase, due to the higher level of PHA available.

The experimental findings, summarised above, suggest certain consequences with respect to operation, control and modelling:

- ⇨ For plant operation it is advisable to keep the PHA pool at a continuously high level for maintaining BPR efficiency and improved denitrification. This suggests to employ appropriate aeration control to avoid unnecessary oxidation of the PHA pool. An increase of the PHA level can be achieved by adding VFA rich streams from pre-fermentation or hydrolysis units to the inlet of the anaerobic zone. Proper control of denitrification can reduce or avoid nitrate recirculation from the secondary clarifier to the anaerobic zone. This will ensure that in the anaerobic zone a maximal amount of VFA is utilised for BPR.
- ⇨ Sudden increases in the COD load of the incoming waste water should be avoided as they will cause temporary decrease of BPR performance. The use of preceding equalisation tanks can reduce the fluctuation of the incoming COD load and hence stabilise the BPR process. If external carbon sources are added to the system, the addition should be performed such that large steps upward in the COD load are avoided.
- ⇨ Under normal circumstances only little or no accumulation of nitrite is expected in alternating or re-circulating processes treating municipal waste water. However, nitrite accumulation may occur in certain cases, e.g. due to discharge of industrial wastes or exposure to high levels of ammonia. In theses cases, even for momentary exposure to elevated nitrite concentrations, activated sludge systems not acclimatised to nitrite will experience problems in BPR. Hence, discharge of sidestreams with high ammonia concentrations to the system call for a careful monitoring and eventually appropriate dilution.
- ⇨ Conditions might occur during which BPR promoting organic substrates can be present in the anoxic reactor. The results so far suggest that leakage of easily biodegradable COD from the anaerobic zone to the anoxic one is not necessarily interfering with satisfactory BPR. Furthermore they indicate also that external COD (VFA) addition to the anoxic zone can be used as a control handle to prevent nitrate accumulation in the system without negative impact on BPR.
- ⇨ Appropriate models for the BPR process should reflect the important results presented in this section, i.e. the PHA dependence of the P-uptake rates as well as the ability of PAO to use easily biodegradable COD during anoxic conditions.

The last two points, i.e. simultaneous presence of COD in the anoxic zone and its potential for control purposes as well as the required model modifications need further investigations. The following chapters will deal with intensified studies of these aspects at pilot plant scale and extend the investigations from batch tests to the performance of a continuous system (BioDeniPho®process).

5 EXTERNAL ADDITION OF ACETATE TO THE ANOXIC ZONE - PILOT PLANT BEHAVIOUR -

ABSTRACT

Investigation are presented, dealing with the response of a BioDeniPho® pilot plant to the continuous introduction of a BPR promoting organic substrate to the denitrifying zone.

The study addresses the effect of potential leakage of easy biodegradable COD from the anaerobic to the anoxic zone, as well as the use of a model based control routine for the external carbon source addition in order to control nitrate in the system. In addition to the control performance, focus is on the resulting phosphate dynamics and the limits induced by the goal of satisfactory phosphate removal.

The pilot plant experiments were performed over several cycles while monitoring the course of NO_X-N, NH_4-N, PO_4-P, PHB and PHV, COD and Acetate. The experimental period covered a time interval of approximately 2 months. The results are discussed in conjunction with the calculated P-uptake, PHB utilisation and denitrification rates.

No negative impact on BPR was noticed, at external acetate addition rates that were in the same order of magnitude as the detected flow (leakage) of COD from the anaerobic zone. The control routine applied proved to be suitable for nitrate control. A simple modification assured that phosphate accumulation in the plant due to the acetate addition was avoided, i.e. no increase in the phosphate concentration of the effluent. Anoxic activity of the PAO was maintained during the experimental period and checked by batch tests. Furthermore, the possibility of BPR stabilisation through external carbon source addition to the anoxic zone is discussed.

BPR deterioration was detected during some experiments and seemed to be due to sudden increases of the COD load in the inlet. In order to account for these scenarios too, control strategies could consist of a combination of the external carbon source addition with, for example, aeration time length control or equalisation of the inlet.

5.1 Introduction

The performances of processes for combined nitrogen and phosphorus removal from municipal wastewater in activated sludge processes are generally limited by the availability of carbon sources. A large part of the readily biodegradable COD (VFA) is taken up by the PAO in the anaerobic zone, while the slowly biodegradable part and the organics not used by PAO are needed/used for denitrification in the anoxic phases. Consequently, organic carbon sources and nitrate are simultaneously present in the anoxic reactor. The main part of these organics are not (or only to a low extent) (s. section 2) used by the PAO. However, situations are possible, in which BPR promoting organic substrates (mainly acetate and propionate) are also present in the anoxic reactor. Several circumstances might lead to such situations /conditions:

1. Flow of readily biodegradable C-sources from the anaerobic zone to the anoxic one, due to incomplete uptake of VFA as:
   a) the retention time in the anaerobic reactor is insufficient,
   b) the VFA uptake by PAO is limited (poly-P or glycogen limitation).
2. Ongoing conversion reactions in the anoxic zone (hydrolysis, fermentation), transforming slowly biodegradable C-sources into readily biodegradable and BPR promoting ones.
3. External addition of a C-source to control denitrification (Isaacs *et al.*, 1994a, Yuan *et al.*, 1996).

Scenario 3 can be used in processes for N-removal to compensate for periods of low COD/N ratios in the incoming wastewater as well as for periods of low temperature or unusually high nitrogen loads. The COD/N ratio, coming into the plant, needs to be sufficiently high in order to reduce the NO_X-N completely to nitrogen gas. Henze (1991) stated that theoretically 4.2 gCOD/g N is required for total nitrogen removal, including assimilation, when using glucose as a carbon source. In practice the COD/N ratio requirement is higher, with typical values lying in the range of 5-10 g COD/g N for combined nitrification denitrification plants (Henze, 1991). Apart from the COD/N ratio the denitrification rate is also influenced by the nature of the carbon source and the temperature of the mixed liquor. A comparison of the denitrification rates using different organic sources is presented by Henze *et al.*(1997), revealing a rate reduced by about 60%, when using raw waste water compared to acetate or methanol. Similarly, Tam *et al.* (1992) and Gerber *et al.* (1986) stated a declining rate, when investigating denitrification with acetate, methanol and glucose respectively. Hence it is well accepted that the highest rates are obtained with the most easily degradable forms of carbon sources.

Processes with combined biological P and N removal are expected to require an even higher COD to nutrient ratio for satisfactory nutrient removal, as carbon is needed for both processes, denitrification and dephosphatation. Furthermore they offer two potential locations for an external carbon source addition. With respect to the performance of the biological phosphorus removal the inlet of the anaerobic zone is often considered as the appropriate location. In this case most of the added COD will be used by the PAO in the system. Tests concerning the use of fatty acid from raw or primary sludge fermentation to promote BPR are reported in literature (Pitman *et al.*, 1992; Teichgräber *et al.*, 1995). Teichfischer (1995) and Krühne and Jørgensen, (1999) proposed an addition to the anaerobic zone to stabilise the BPR process, i.e. to overcome problems in phosphate removal due to influent dynamics. This approach, however, exhibits a time delay of the response for both P-removal

and denitrification, which is equal to the anaerobic residence time and might impose a problem for control tuning and hence closed loop performance. In addition, with respect to nitrogen removal, a contribution to improved nitrate removal by normal denitrifiers occurs only if nitrate is recycled to the anaerobic tank. This is due to the fact that the externally added substrate is only available in the anaerobic zone. As the PAO (phosphate accumulating organisms) take up the carbon anaerobically and store it internally, the amount of carbon available in the mixed liquor of the anoxic zone does not increase. Consequently also the contribution of normal denitrifyers to denitrification remains unchanged. Hence, most of the time, the improvement relies only on the action of the denitrifying PAO, which are not easily accessible. As a consequence this kind of control will increase the degree of complexity, as it has to be based on more complex models (e.g. model predictive control).
In the case of the anoxic addition, studied in this section, denitrifiers and PAO will compete for the carbon source. It is hard to predict which group of organisms will have an advantage in this competition. Due to the fact that the amount of normal denitrifiers exceeds by far the amount of PAO, it could be assumed that the denitrifiers have an advantage in the competition for acetate uptake. As the anoxic addition of acetate exhibits a direct response (almost no time delay) with respect to denitrification, it will be regarded as a primary support of the denitrification process,. In table 5.1-1 some aspects concerning the expected effect of the different location of the carbon additions are summarised.

Table 5.1-1. Expected effects of anaerobic COD addition versus anoxic COD addition.

Type of external C-addition	Anaerobic	Anoxic
Primary support of	*BPR*, increased C-availability in the anaerobic phase leads to an increase in PHA storage of PAO and thus to higher P-uptake rates.	*Denitrification by* a) normal denitrifiers, limitation by carbon availability is reduced. b) DNPAO, using nitrate as an ë-acceptor during acetate metabolism
Secondary support of	*Denitrification*, via a) Increased anoxic activity of PAO, due to an increased level of internally stored PHA. b) Normal denitrifiers, if nitrate is recycled to the anaerobic phase	*BPR*, via a) Increased PHA level in the plant. b) Reducing the amount of NOx-N recycled to the anaerobic zone.
Response time	Time delay, equal to the anaerobic residence time (assuming plug flow).	Denitrification : Direct response. BPR : Time delay = a) anoxic phase length. ≈ b) SRT in the plant.

In general, the interaction between nitrate, organic substrates and the denitrifying and phosphorus removing activity of the sludge are most likely to influence the process performance. Hence, gaining information about these interactions is a necessary basis for the development of process operation schemes and possible control strategies of the overall nutrient removal process.

In this study the focus is put on external addition of acetate to the anoxic zone in order to reproduce and investigate the three scenarios mentioned above. Results of batch experiments, performed to gain preliminary information about these scenarios were presented in section 4.4 (Meinhold *et al.*, 1998). It was concluded that low levels of acetate addition to the anoxic zone lead to a slight increase in the

denitrification rate without imposing a negative effect on BPR. At higher rates of acetate addition a decrease in the achieved net P-removal was observed. Whether or not the introduction of acetate is detrimental to overall P-removal appeared to be dependent on the interaction between the acetate addition rate, the aerobic P-uptake rate, which is a function of the PHA level, and the aerobic residence time. As the study was carried out in batch experiments, the conclusions drawn account only for a one cycle behaviour. But independent of the scenario (1-3) to be investigated, the response of the system over several cycles is of main interest. Hence, the experiments in this section were carried out in the pilot plant over several cycles in order to study a system operated similarly to real scale wastewater treatment plants.

The feasibility of the external carbon source addition as a control strategy for improved nitrogen removal has been presented by Isaacs et *al.* (1995). The current study adapts the presented strategy and examines its possible use for a combined N and P-removal process, putting the focus on the arising phosphorus dynamics, i.e. possible arising phosphate accumulation in the reaction tanks due to the external acetate addition.

Investigation of the experiments is further addressing the extent of secondary support for BPR as well as the extent of denitrification improvement. The former is expected to occur due to the efficient decrease of nitrate, thus reducing the amount of nitrate recycled to the anaerobic zone.

5.2 Material and Methods

5.2.1 Experimental Setup

The experiments were carried out using a BioDeniPho® pilot plant, operated with a four phase schedule as depicted in Figure 5.2-1. For general characteristic parameters of the pilot plant and its operation one is referred to Appendix 8.4. The pH was monitored in the anaerobic column as well as in one of the reaction tanks. It stayed rather constant for each location: anaerobic zone at 7.5 ± 0.1, anoxic phase : 7.2 ± 0.1, aeration phase: 7.7 ± 0.1. The temperature remained at 19 °C ±1 for all experiments. The concentrations of NH_4-N, NO_X-N and PO_4-P were monitored on-line, using a FIA system (Isaacs and Søeberg, 1998), with sampling ports as indicated in Figure 5.2-1. Further measurements for some experiments included:

a) Influent (incoming wastewater) : total and filtered COD, acetate.
b) End of anaerobic column and one reaction tank (T2): PHA, acetate and filtered COD at the beginning and end of each phase.
c) Return sludge: PHA, NO_X-N.

The external COD addition was performed with a calibrated peristaltic pump, fed to the outlet of the anaerobic column, thus directing the flow of the acetate addition always to the corresponding anoxic reactor, according to the phase schedule. The amount of COD added was checked by weighing the bottles, containing the COD stock solution, at appropriate time intervals. Scenarios with high nitrate concentration in the plant were induced by imposing a step load of ammonia either in front or after the anaerobic column. Ammonia was added as NH_4Cl solution. The stock solutions for the additions (NH_4-N and COD) were concentrated, such that the influence on the overall flow through the plant was negligible (increase of less than 2%).

The overall length of the aeration period in each tank was set to a constant duration of 30 minutes to ensure that the dissolved oxygen (DO) was depleted before switching to the anoxic phase. Furthermore, the fixed aeration period enables an easier comparison between the different experiments.

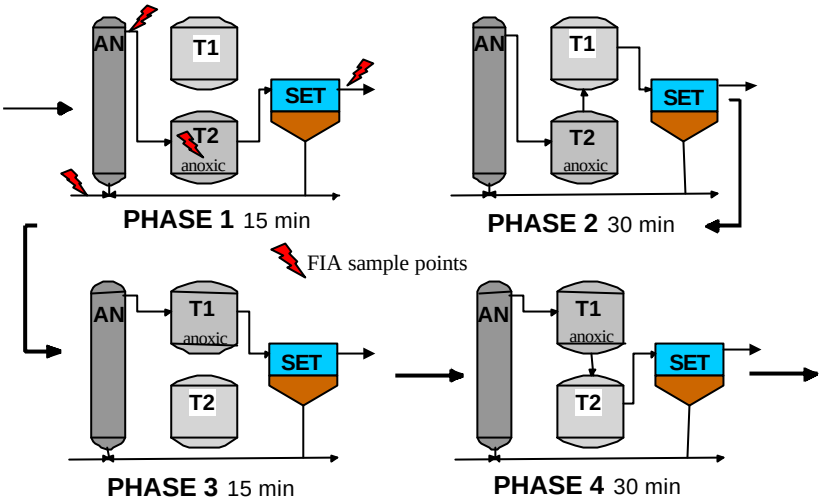


Figure 5.2-1. Phase schedule of the pilot plant, including FIA sample points ( ).

Analytical methods

Ammonia nitrogen (NH_4-N), nitrate plus nitrite (NO_X-N) and ortho-phosphate (PO4-P) were analysed with FIA (Pedersen *et al.*, 1990). PHB and PHV were measured as described in Smolders *et al.*, (1994) with minor modifications. MLSS, MLVSS and COD were determined according to APHA Standard Methods (1985) and acetate by gas chromatography.

Experimental investigations

The experiments are divided in two sections according to the type of acetate addition performed. The part with *constant addition rates* was performed primarily in order to compare the response of the BioDeniPho process with the results obtained from batch experiments (section 4.4, Meinhold *et al.*, 1998). In the second section the external acetate addition to the anoxic zone was implemented in a simple control routine (strategy) to *control denitrification* using variable addition rates. The limiting frame of this strategy, imposed by the aim of complete P-removal, is illustrated and discussed.

Batch tests/assays were performed at the begining and the end of each experimental period, applying a procedure according to section 4.2 to obtain a measure for the activity of the denitrifying fraction of PAO (DNPAO). This is expected to give at least an indication of the effect of anoxic acetate addition on the anoxic performance of the PAO on a medium term basis (approximately 4 weeks).

5.2.2 Control Strategy and Algorithm applied

The denitrification process is in most cases limited by the availability of carbon in a readily metabolisable form. Isaacs and Henze (1995) demonstrated that the denitrification rate can be immediately increased by adding either acetate or hydrolysed sludge as a carbon source to the denitrifying zone. For optimal control of nitrate removal, the external carbon addition rate has to be adjusted carefully according to the needs for complete denitrification. Insufficient dosing will result in high nitrate concentrations in the effluent and in the return stream. Exceeding the carbon requirements for denitrification will increase the costs dramatically due to a higher carbon usage, a higher sludge production and an increased oxygen demand. Existing strategies are aiming at the control of the C/N ratio to the anoxic zone. Possibilities for control of post denitrification by appling external carbon dosing are presented by Londong 1992 and Hoen et *al*. 1996. For pre-denitrification (Yuan et *al*., 1996) or alternating systems (BioDenitro™, Isaacs *et al*., 1995) similar strategies are proposed, using the nitrate concentration in the anoxic zone itself as a control variable.

For the experiments concerning the control of denitrification presented in this work, the control strategy proposed by Isaacs *et al*.(1995) is adopted. In the following structure and derivations of the basic equations are illustrated. For reasons of simplicity the NO_X-N will be represented by N.
The structure of the control algorithm is presented schematically in Figure 5.2-2. The strategy uses a model based methodology, employing two simple types of models. The *prediction model* is based on mass balances for nitrate. It determines the existing (background) denitrification rate and the desired rate for a given set point of the nitrate concentration to be reached at the end of the denitrification period. The *relational model* relates the denitrification rate to the rate of carbon addition. As this relationship will change gradually in time, a recursive adaptation of the parameters is applied. The different lines in Figure 5.2-2, refer to the occurrences during different periods :

- thin lines : actions at the start of the tank's denitrification period.
- thick lines : actions at the end of the denitrification phase,
- dotted line : illustrates the general possibility to include the determination of the background denitrification rate in the adaptation routine.

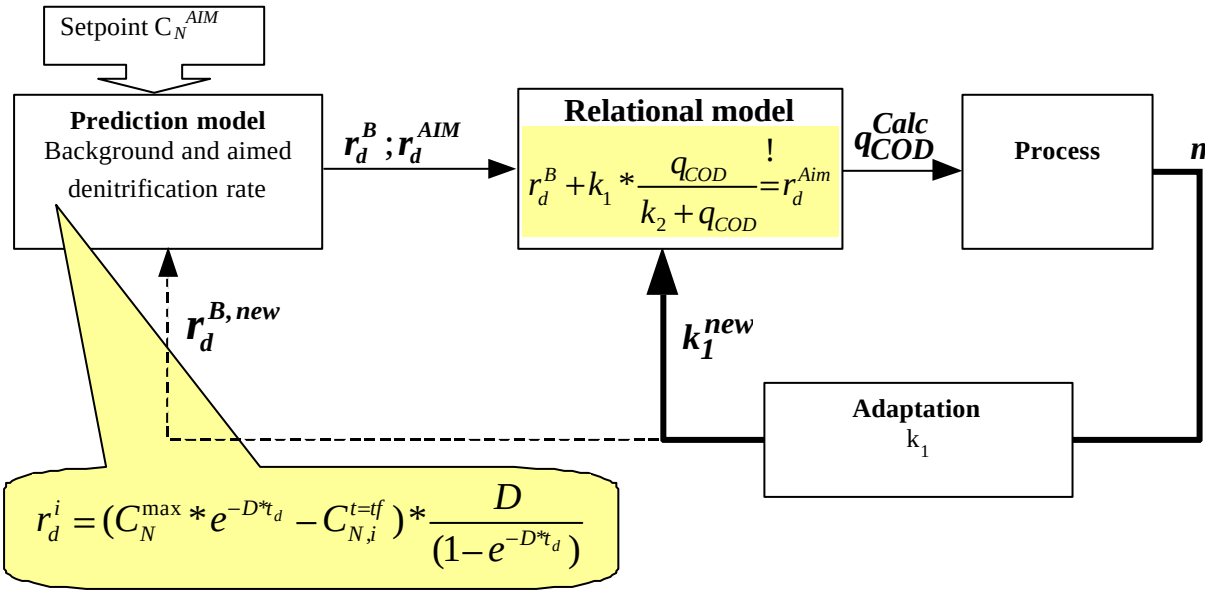


Figure 5.2-2. Schematic diagram of the control strategy for the external addition of carbon

Equation (eq 5.1) represents the general balance around one reaction tank for NO_X-N, provided no nitrate is coming from the anaerobic zone.

$$\frac{dC_N}{dt} = -D * C_N - r_d \qquad \text{(eq 5.1).}$$

Solution : $$C_N^{t=t_f} = C_N^{\max} * e^{-D*t_d} - \frac{r_d}{D} * (1 - e^{-D*t_d}) \qquad \text{(eq 5.2).}$$

Its solution is represented by equation (eq 5.2) using the following notation

t_f = end of the time allocated to denitrification

t_d = time available for denitrification

C^{max}_N = maximum concentration of NO_X-N at the start of the denitrification period.

By rearranging the above equation and setting the time allocated for denitrification to the length of a half cycle (t_f = t_d = 45min) the *background denitrification rate* can be determined (eq 5.3). The measurements are taken from the last cycle of the plant operation without COD addition.

$$r_d^B = (C_N^{\max} * e^{-D*t_d} - C_N^{t=tf}) * \frac{D}{(1 - e^{-D*t_d})} \qquad \text{(eq 5.3)}$$

The *desired denitrification rate* is calculated by inserting the setpoint for the NO_X-N concentration to be reached in the reaction tank at the end of the anoxic phase (replacing $C_N^{t=tf}$ with C_N^{AIM}):

$$r_d^{Aim} = (C_N^{\max} * e^{-D*t_d} - C_N^{Aim}) * \frac{D}{(1 - e^{-D*t_d})} \qquad \text{(eq 5.4).}$$

The decision whether carbon should be added is made by a simple comparison of the two determined rates. In case of $r_d^{Aim} > r_d^B$ the addition is started; when the inequality is not fulfilled the control signal q_{COD} remains at its minimum value of 0.

The empirical relationship between the denitrification rate as a function of the acetate addition rate is described by Isaacs *et al.* (1995), as follows:

$$r_d^{Calc} = r_d^B + r_d^{COD} = r_d^B + k_1 * \frac{q_{COD}}{k_2 + q_{COD}} \qquad \text{(eq 5.5)}$$

The equation for the carbon addition rate is thus given by equation (eq 5.6).

$$q_{COD}^{Calc} = k_2 * \left[\frac{k_1}{r_d^{Aim} - r_d^B} \right]^{-1} \qquad \text{(eq 5.6)}$$

Since changes in temperature, waste water composition and sludge activity will cause a change in the influence of q_{COD} on r_d with time, a frequent updating of the parameters is needed. For simplicity reason and because k_2 is difficult to estimate accurately, only k_1 is submitted to adaptation. This can be done by applying a recursive least square algorithm as described in Isaacs *et al.*, (1995). The basic approach, applied in this work, is to calculate the observed denitrification rate according to (eq 5.7.

$$r_d^{Obs} = (C_N^{\max} * e^{-D*t_d} - C_N^{\min}) * \frac{D}{(1 - e^{-D*t_d})} \qquad \text{(eq 5.7).}$$

This will be used in re-estimating the value for k_1 according to:

$$\hat{k}_1 = \frac{q_{COD}}{k_2} * (r_d^{Obs} - r_d^B) \qquad \text{(eq 5.8)}$$

As this procedure relies on only 2 measurements points, the risk of inducing 'wrong' updates, due to measurement inaccuracy should be taken into account. Hence, in all the experiments performed, the updated value (k^t) for k_1 was obtained by the conservative approach of choosing the average between the old (k^{t-1}) and the re-estimated value.

$$k_1^t = \frac{k_1^{t-1} + \hat{k}_1}{2} \qquad \text{(eq 5.9)}$$

The recursive update is only invoked if external carbon has been added, as otherwise no information concerning k_1 is available. In the latter case a re-evaluation of the background denitrification rate, r_d, is performed.

The starting value for k_1 was obtained by using the measurements of appropriate sets of batch experiments from section 4.4 in combination with equation (eq 5.8). The background denitrification rate, r_d^B, was calculated from the control reactor without anoxic COD addition, while r_d^{Obs} corresponds to the observed rate in the reactors receiving acetate during the anoxic phase. An average value of k_1 = 0.165 mg N/(L min) was determined and applied as a starting value to each experiment.

The value for k_2 was set to 0.95 mg COD/ (L_R min) and held constant as suggested by Isaacs *et al.* (1995).

5.3 Results

5.3.1 Constant Addition of Acetate and the Effect on BPR.

Several experiments were performed applying a constant acetate addition rate, covering the interval from 0.05 to 0.6 mg COD_{HAC}/L_R min. This corresponds to a COD concentration of about 13 to 160 mg COD_{HAC}/L in the stream flowing from the anaerobic zone to the anoxic one. The lower limit of this interval is approximately equivalent to the maximum concentration of acetate 'leaking' from the anaerobic column. During the plant operation before and in between the experiments, a leakage of 1-10 mg COD_{HAC} /L from the anaerobic to the anoxic zone was noticed. This matches an external addition rate to the anoxic zone of about 0.0004 to 0.04 mg COD_{HAC}/L_R min. The results of the experiments carried out with an addition rate below 0.1 mg COD_{HAC}/L_R min (not shown) were in line with results of the corresponding batch tests, presented in section 4.4. A slightly improved denitrification efficiency was observed (up to 10%), along with only slightly higher P- dynamics in the tanks. No negative effect of the external COD addition on BPR could be seen, i.e. no rise of the PO_4-P-concentration in the effluent occurred due to the extra carbon added. Hence it can be concluded that no direct negative impact occurs due to leakage of limited amount of VFA into the anoxic zones, within the time interval investigated (24-36 hours).

In the following the results of 3 representative experiments with constant external addition rates will be shown, each one characterised by its specific conditions. Their results will be used to illustrate the different aspects of the response to the external acetate addition, as well as the important factors influencing the plant performance. The experiments **Exp.A** and **Exp.B** differ in the level and dynamics of the COD in the inlet and hence also in the amount of P released in the anaerobic column and in the level of PHA throughout the plant. The applied acetate addition rate to the anoxic zone was the same for both experiments (0.14 mg COD_{HAC}/L_R min). The results of experiment (**Exp.C**) will be used to demonstrate the effect of a rather high acetate dosing (0.6 mg COD_{HAC}/L_R min).

For experiment **Exp.A** the concentration pattern for PO_4-P, NH_4-N and NO_X-N at the end of the anaerobic zone and in the effluent, are shown in Figure 5.3-1. The depicted time interval covers also the period before and after the COD addition. As nitrate concentration in the anaerobic zone is virtually 0, it is not depicted in the corresponding figure. The same accounts for NH_4-N in the effluent. The grey shaded area illustrates the period of COD addition to the anoxic phases.

Although no COD and PHA measurements are available for this experiment, the phosphate concentration in the anaerobic zone offers a certain insight into the conditions of the plant. For more than the first half of the experiment the P-release (difference of P in the inlet and at the end of the anaerobic zone) is unusually high (≈ 32 mg P/L), surpassing the one observed during 'normal' plant operation (15-20 mg P/L) with up to 50%. This situation can be attributed to high COD content, especially BPR promoting organics, in the influent and low nitrate concentration in the recycle stream. Consequently, also relatively high PHA content can be assumed in the plant. In addition, no major increase of the COD content in the inlet during the experiment can be assumed. This is illustrated by the rather constant or only declining phosphate concentration at the end of the anaerobic zone and an almost constant influent concentration of phosphate (not shown). Ammonia was added just after the anaerobic zone over the whole length of the experiment, causing an accumulation of nitrate in the tank and in the effluent prior to and also after the period of the COD

addition. The decrease of nitrate in the effluent can be attributed to the effect of the external acetate addition, exhibiting a time delay of around 4 hours.

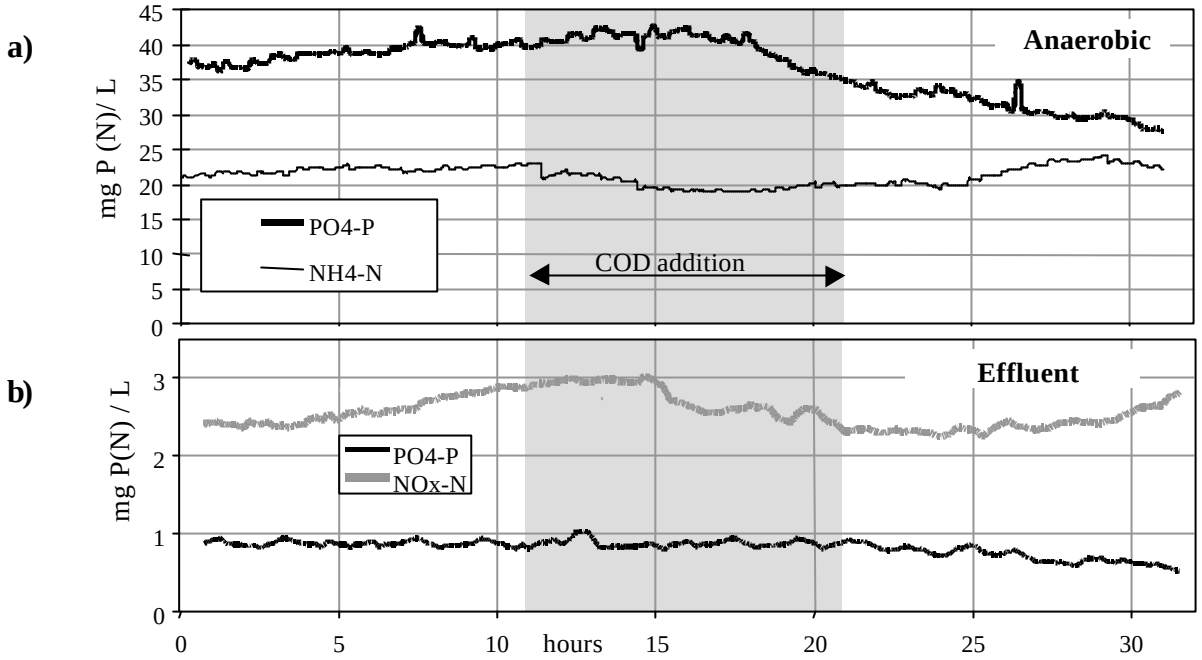


Figure 5.3-1.**Exp.A**: const. addition of 0.14 mg COD_{HAC}/L_R min to the anoxic zone.
a) PO_4-P and NH_4-N in the anaerobic column b) PO_4-P and NO_X-N in the effluent

Looking at the phosphate pattern of the effluent, no increase of the phosphate concentration can be detected after the start of the anoxic acetate addition. The decrease in phosphate towards the end of the experiment (after 22h) is most likely due to the declining phosphate concentration in the anaerobic column, which is probably caused by a decrease of the inlet COD (not measured), resulting in a decreased amount of phosphate sent to the anoxic/aerobic tanks.

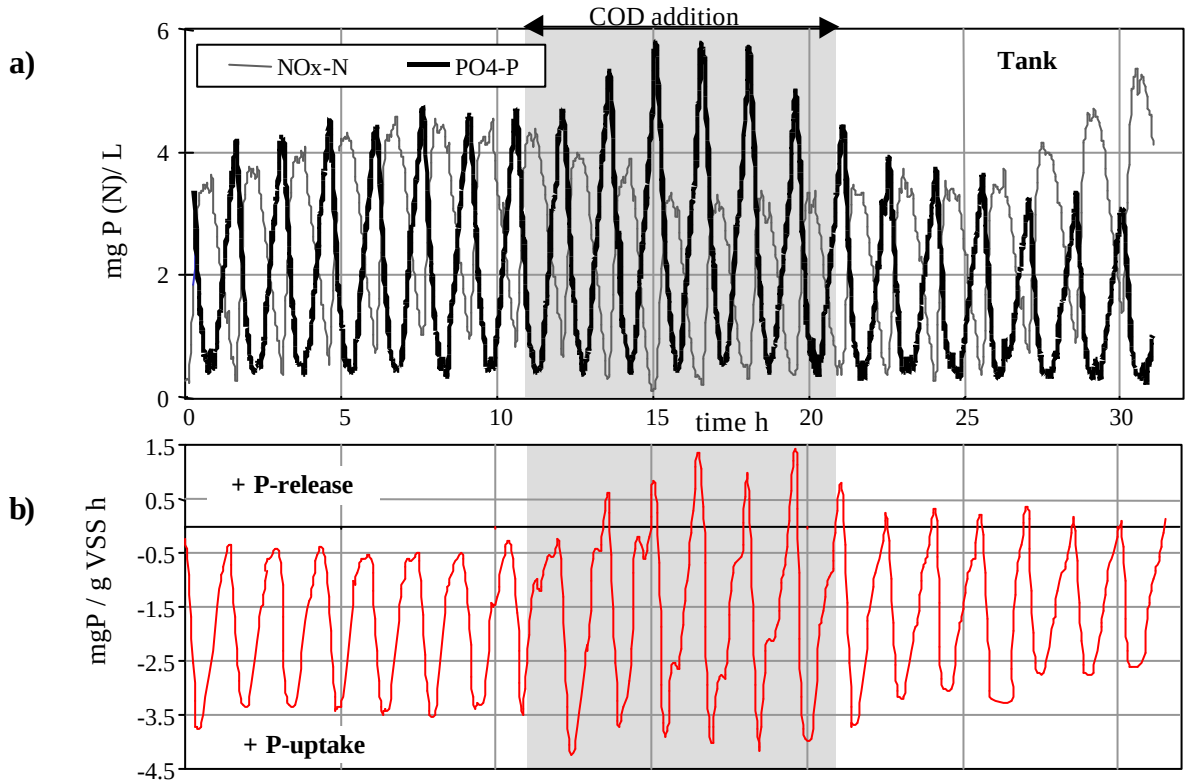


Figure 5.3-2. **Exp.A**: const. addition of 0.14 mg COD_{HAC}/L_R min to the anoxic zone (grey shaded).
SS=3.8g/L VSS 2.9g/L a) PO_4-P and NO_X-N in tank2 b) P-uptake/release rates in tank2

High levels of phosphate and PHA in the anaerobic column are conditions, that lead to distinct and relatively high P-dynamics in the reaction tanks, as depicted in Figure 5.3-2a (P-pattern prior to acetate addition). Upon the start of the COD addition no differences in the phosphate pattern of the tank can be observed during the first cycle, but the nitrate content starts to decrease due to a higher denitrification. During the subsequent cycles the phosphate dynamics in the tank increase, i.e. higher phosphate concentrations are reached at the end of each anoxic phase, achieving still approximately the same final concentration at the end of each cycle. These higher levels of phosphate, reached at the end of each anoxic cycle, are mostly due to the disappearance of nitrate, thus establishing anaerobic conditions. Anaerobic phosphate release is higher than under anoxic conditions, often illustrated by a bending point in the concentration curve. This is supported by the variation of the P-uptake/release rates, shown in Figure 5.3-2b, which were calculated based on mass balances around the reaction tank. The occurrence and increase of P-release can be observed with the beginning of the second cycle of the acetate addition period (P-rate >0). The P-release due to anaerobic conditions in the tanks might be detrimental to the achievable P-elimination, as it increases the amount of P to be removed in the tanks within one cycle. In this experiment, however the P-removal efficiency was not severely affected, probably due to a sufficient aeration period duration and high P-uptake rates, caused by high PHA levels.

The observed increase of the aerobic P-uptake rates (Figure 5.3-2b) upon the start of external acetate addition is relatively low. Only approximately 0.5 mg P/g VSS h higher uptake rates are noticed. A possible explanation could be related to the already high PHA content in the sludge. As shown in section 4.1 and Petersen *et al.* (1998) the increase of the P-uptake rates becomes smaller at a certain PHA level (saturation effect), i.e. further increase of the PHA level induces only a marginal increase in the P-uptake rate.

The second experiment, **Exp. B**, is characterised by a strong variation (increase) of the COD load, being the major difference compared to Exp. A. Experiment B, was started at 'normal' COD loading of the plant. But a quite drastic increase in the COD of the incoming wastewater occurred during the experimental period, reaching similar conditions towards the end as in Exp. A. Figure 5.3-3 shows the corresponding concentrations of PHA, phosphate in the anaerobic column and total COD as well as filtered COD in the influent.

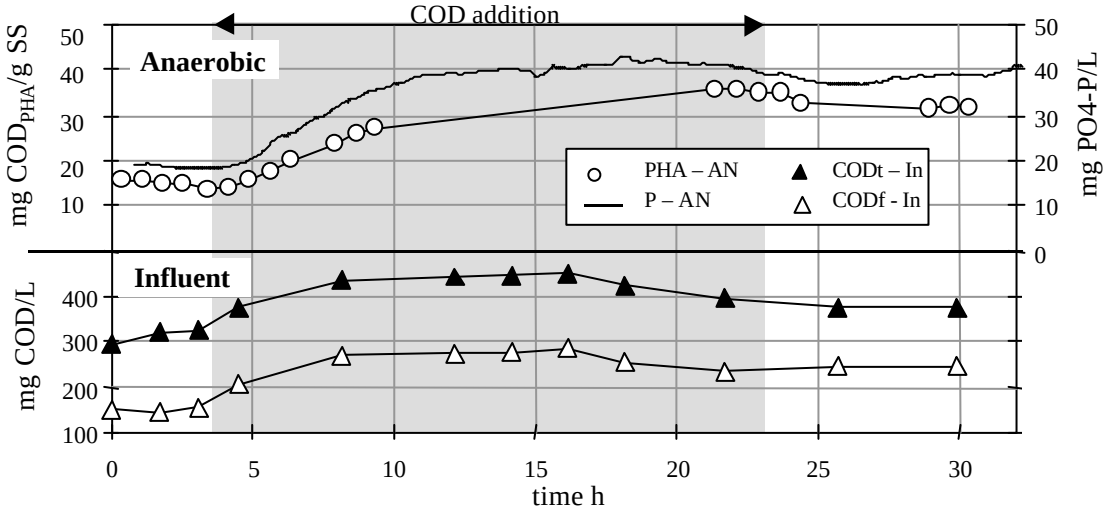


Figure 5.3-3. **Exp.B**: constant addition of 0.14 mg COD_{HAC}/L_R min to the anoxic zone (grey shaded). CODt and CODf in the influent; PO_4-P, PHA, in the anaerobic zone SS = 3.57 g/L, VSS = 2.75 g/L

Considering the hydraulic retention time of the anaerobic zone of 66 min, the phosphate and PHA patterns exhibit a similar behaviour as the COD in the inlet. The rise in the COD load represents a rather 'sudden' and steep increase. The phosphate concentration at the end of the anaerobic zone doubles within only five hours. Observations of the pilot plant process during periods without external carbon source addition (not shown), have revealed that such an increase leads to temporary deterioration of the BPR. A similar response was observed during Exp. B as represented by Figure 5.3-4, showing the nutrient concentrations in the reaction tank and in the effluent.

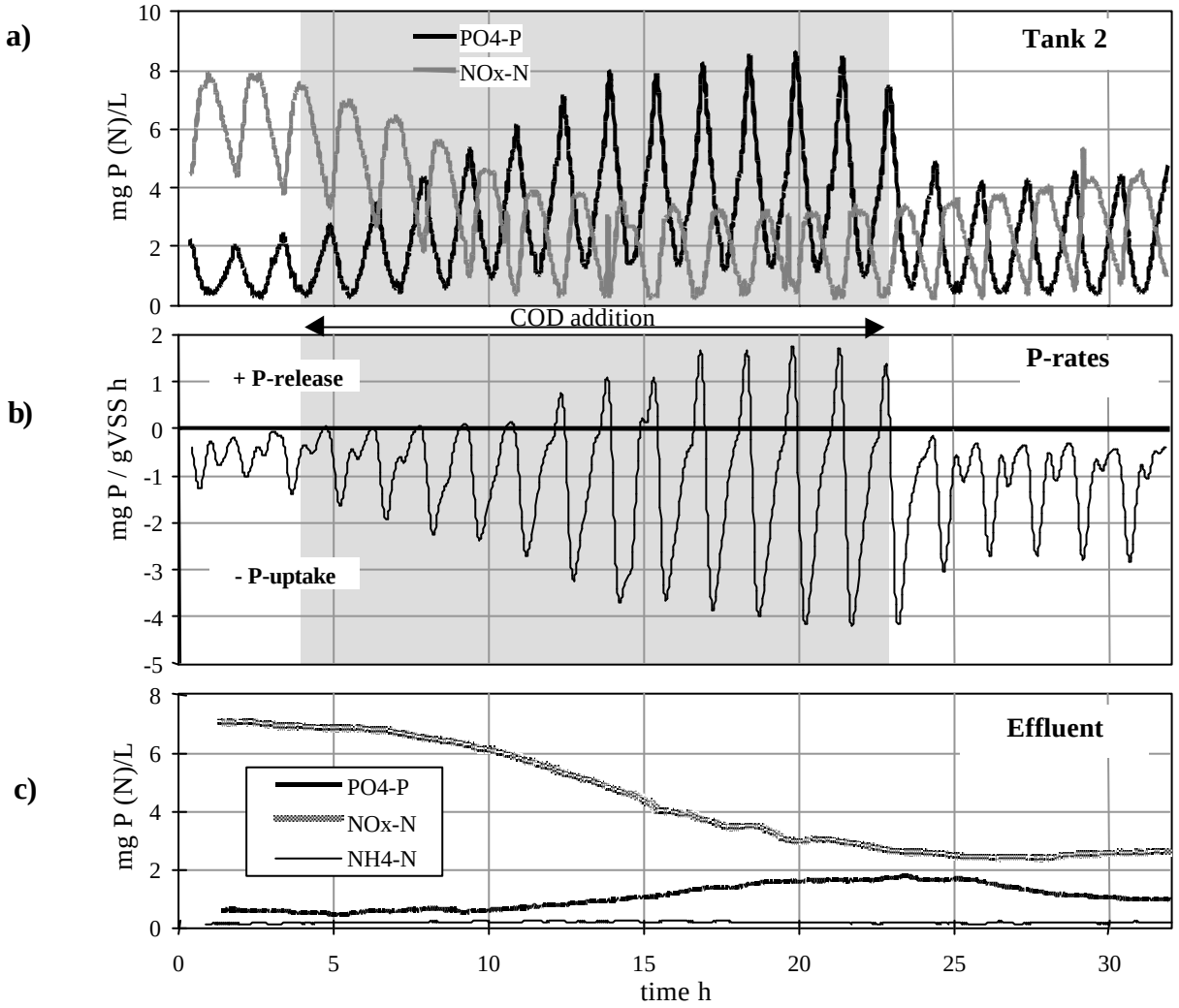


Figure 5.3-4. **Exp.B**: constant addition of 0.14 mg COD_{HAC}/L_R min to the anoxic zone.

SS = 3.57 g/L, VSS = 2.75 g/L

a) PO_4-P and NO_X-N in tank2 b) P-release/uptake rates c) PO_4-P, NH_4-N and NO_X-N in the effluent

With regard to denitrification, an immediate impact on the nitrate concentration is noticed once the external acetate addition is started. Towards the end of the addition period the effluent concentration of NO_X-N has decreased to approximately 40% of its starting value.

In contrast to experiment Exp. A., an increase in the phosphate concentration in the tank as well as in the effluent occurred. Several effects are possible, which contribute to this rise in the general level of phosphate. First, the drastic increase in the COD content of the inlet is known to promote such a deterioration. Second, although not observed in Exp. A, the external acetate addition to the anoxic phase could have contributed to this behaviour. This would be most likely due to the fact that, again, once nitrate disappeared, anaerobic conditions arose in the tanks during the 'anoxic' phase. This causes the P-release, induced by the acetate addition, to increase. Consequently, as the addition has

been performed over a longer period than in Exp. A, the anaerobic P-release in the tanks could result in a more expressed BPR deterioration. In addition, as being at elevated phosphate concentration, it is also possible that the aeration period was insufficient for complete P-uptake (s. discussion in section 1.1). The extent to which the acetate addition contributes to the rise in the general level of phosphate cannot be quantified precisely, but the results indicate that this type of addition should be avoided, once anaerobic conditions are established in the tanks, i.e. once the nitrate concentration reaches 0 mg/L.

The anaerobic P-release in the tank starts with the 4th cycle of the addition period: This becomes apparent, when looking at the calculated phosphate rates in one of the tanks (Figure 5.3-4b). Furthermore, during this experiment, a substantial difference in the P-uptake rates can be observed. Starting from around 1 mg P/ gVSS h, maximum rates of around 4.5 mg P/ gVSS h are reached towards the end of the addition period, being similar to the values in Exp.A. The gradual increase of the P-uptake rates from cycle to cycle is caused by the steady increase of the PHA level in the tank, as depicted in Figure 5.3-5a. This increase can be caused by both, the general increase in COD in the inlet and by the external acetate addition. The contribution of each factor is hard to estimate. However, the sudden drop in the PHA level after the stop of the acetate addition indicates, that its contribution was notable. Similar to phosphate, the establishment of an anaerobic period in the reaction tanks during the addition period has resulted in a significant increase of the PHA accumulation. This is illustrated by Figure 5.3-5b, showing the calculated accumulation and/or utilisation of PHA for each phase of the operational schedule.

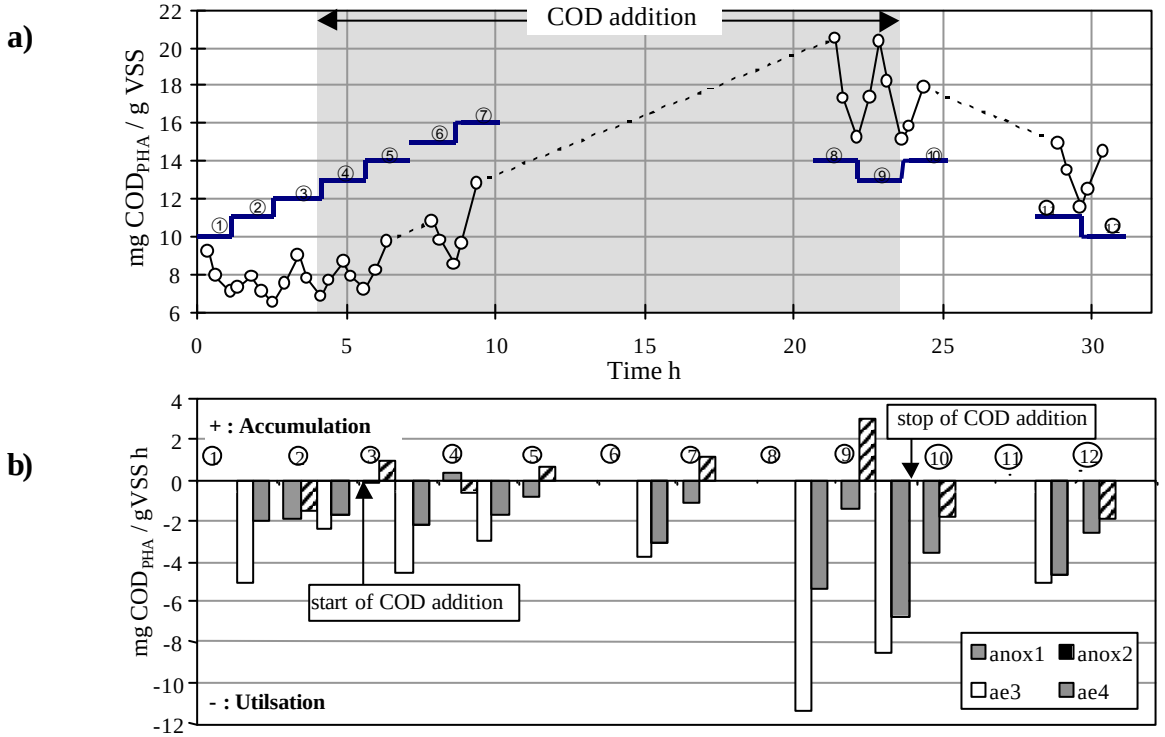


Figure 5.3-5. **Exp.B**: constant addition of 0.14 mg COD_{HAC}/(L_R min) to the anoxic zone.

a) PHA in tank2 b) PHA utilisation/accumulation rates for the 4 phases based on mass balances around the tank Numbers refer to the different cycles (each starting with the first anoxic phase); SS = 3.57 g/L, VSS = 2.75 g/L

As some PHA measurements are lacking, the cycles are numbered in both figures for easier referencing (each cycle is considered to start with the first anoxic phase). During the cycles no.1 through no.6, no anaerobic conditions arose, thus the anoxic PHA accumulation was marginal, if it

occurred at all. Cycle no.9 is the only cycle with anaerobic conditions, where all PHA measurements are available. Here the PHA accumulation in the second phase of the anoxic period (4 phase schedule) was about three times as high as in the cycles without anaerobic conditions.
Once the external COD addition was stopped, only PHA utilisation along with P-uptake occurred during the anoxic phase (Figure 5.3-4b). Furthermore, it should be pointed out that the PHA utilisation rates increased with increasing level of PHA, similar to the observations for the batch experiments in section 4.4.
Both experiments (Exp. A and Exp. B) together illustrate that, in addition to the external acetate addition, the variation of the COD in the inlet does have a major impact on the dynamics in the reaction tanks and consequently on the effluent concentration. It seems that a sudden rise in the inlet COD contributes to a high extent to BPR deterioration, whereas the external acetate addition, can be performed avoiding negative impacts on BPR.

Exp.3 was performed to investigate a relative high rate of external acetate addition (0.6 mg COD_{HAC}/L_R) over a short time period. As the COD concentration in the influent stayed rather constant (Figure 5.3-6), its influence on the system behaviour is only minor. Hence, the observations made can be regarded as characteristic for the system response to high addition rate levels.

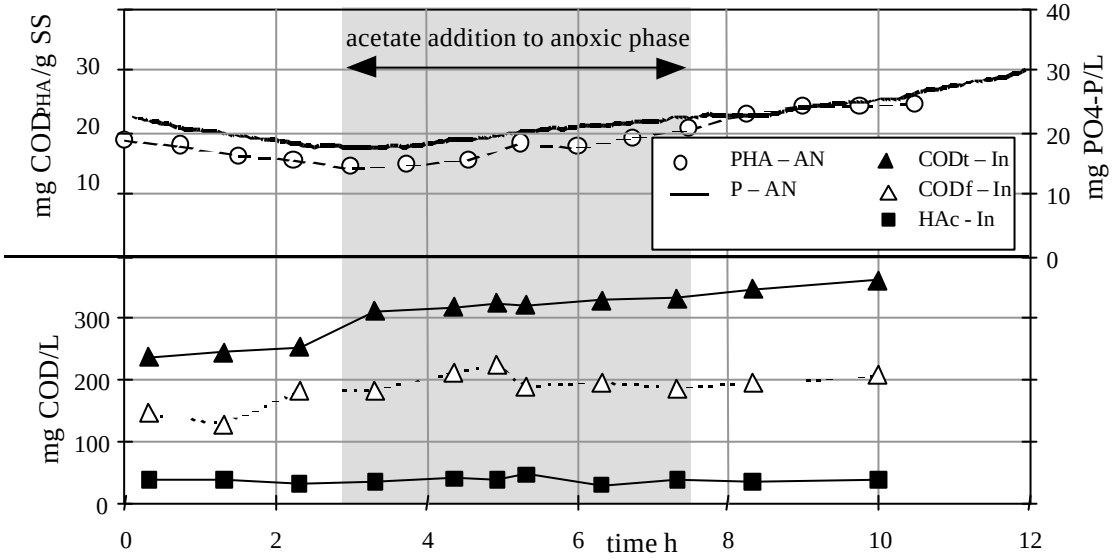


Figure 5.3-6. **Exp.C**: Constant addition of 0.6 mg $COD_{HAC}/(L_R$ min) to the anoxic zone.
CODt, CODf and HAc in the **influent**; PO_4-P, PHA, in the **anaerobic** zone SS = 3.37 g/L, VSS = 2.71 g/L

The period of external acetate addition covered three cycles and the results obtained for phosphate, nitrate and PHA are presented in Figure 5.3-7a-c. As expected, the nitrate concentration starts to decrease immediately, when the acetate addition is invoked. Already within the third cycle of the addition period, nitrate has disappeared, resulting in anaerobic conditions in the tank. The bending point in the phosphate pattern, as well as in the corresponding rate curve, clearly marks this situation. The consequences are more expressed at this level of addition rate and a remarkable increase in the phosphate and PHA concentration in the tank can be noticed. Additionally, the phosphate concentration in the tank illustrates that, at this level of acetate addition rate, phosphate is accumulated in the system, along with an increase of the PHA level, even in the cycles without anaerobic conditions. This is further underlined by the rates for phosphate, shown in Figure 5.3-7b, revealing a significant release of phosphate under anoxic conditions, i.e. with nitrate still present.
Due to the rising PHA level the (aerobic) P-uptake rates increase as long as acetate is added. It is also

noteworthy, that the P-uptake rates are not only higher during the period of C-addition, but also during the two cycles following immediately after the addition was stopped. This is caused by the PHA level, which, for a certain time period, remains above the one measured at the start of the experiment.

In Figure 5.3-7b the average denitrification rate for each half cycle is also depicted. While acetate was added, the denitrification rate increased by approximately 80%. It dropped back to its starting value within 2-3 cycles after the stop of the external C-addition.

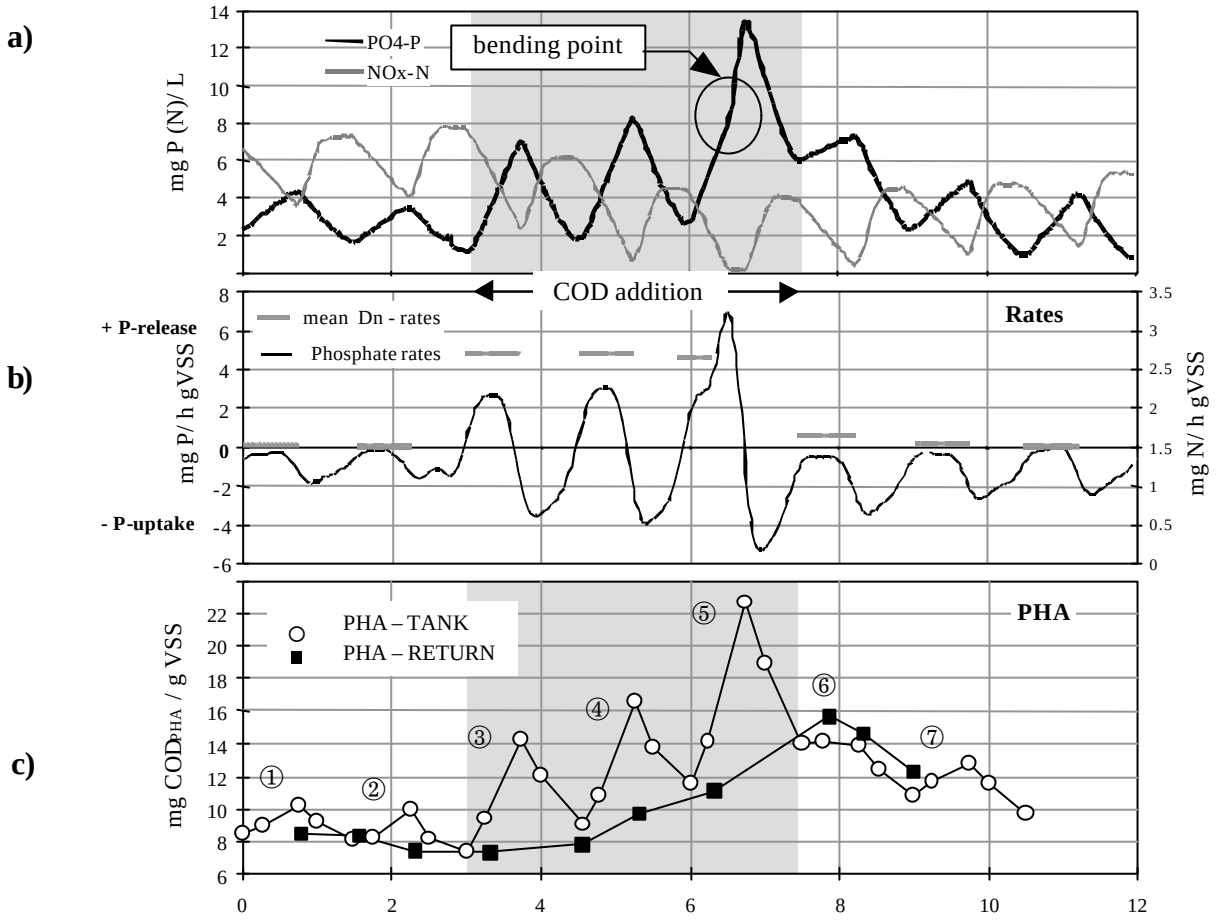


Figure 5.3-7. **Exp.C**: Tank 2, addition of 0.6 mg COD_{HAC}/(L_R min) to the anoxic zone (grey shaded).
SS = 3.37 g/L, VSS = 2.71 g/L

a) P and NO_X-N in tank2 b) P-hosphate rates and average denitrifcation rate c) PHA in tank2 and in the returnstream.
The numbers refer to the different cycles during Exp. C

The PHA content in the return sludge, Figure 5.3-7c, exhibits a remarkable increase, doubling its values within 3 cycles. This increase could be explained by two phenomena. First, the external acetate addition leads to a general increase in PHA, as more PHA is accumulated per cycle than used. The second phenomenon is based on the decrease of the nitrate concentration in the settler. Nitrate in the settler normally induces denitrification by DNPAO, using their internally stored PHA. If the nitrate concentration is now significantly reduced in the reaction tanks, less PHA will be utilised in the settler and will therefore be available for P-uptake, when recycled via the anaerobic column to the reaction tanks.

The nitrate concentration in the return sludge is depicted together with the other nutrient concentrations in the effluent in Figure 5.3-8. The nitrate concentration in the return sludge drops astonishingly fast. In general denitrification in the settler can occur due to the activity of the denitrifying fraction of PAO (DNPAO) using internally stored PHA and also due to normal

denitrifiers if a carbon source is available (organic substrate present in the liquid phase or produced by endogenous processes). Measurements verified that the COD sent to the settler remained at a minimum, hence the denitrification in the settler must be attributed mainly to the DNPAO. A possible explanation for the increased denitrification in the settler could be that denitrification in the settler was severely limited by the low level of PHA in the DNPAO before the start of the acetate addition. As the level of PHA increases during the experiment it causes also an acceleration of the denitrification during the settling process. In addition less nitrate is sent to the settler, resulting overall in a fast decrease of the NO_X-N concentration in the return sludge.

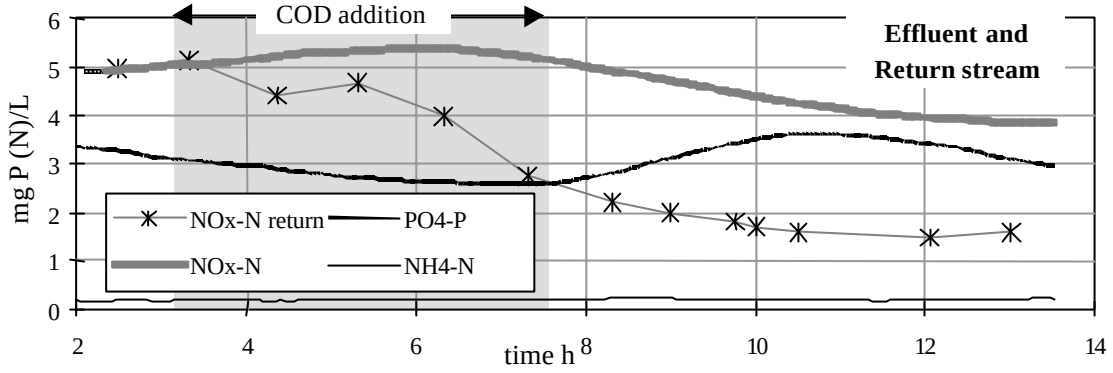


Figure 5.3-8. **Exp.C**: Outlet; addition of 0.6 mg COD_{HAC}/(L_R min) to the anoxic zone (grey shaded) PO_4-P, NH_4-N , NO_X-N in the effluent and NO_X-N in the return stream.

The actual decrease of nitrate in the effluent is not as high as in the Exp.B for example, because here the addition was only performed for 3 cycles (certain buffer effect of the settler). A longer addition period would probably have lowered the nitrate content in the effluent significantly, but focus during these experiments was mainly put on the phosphate dynamics. The phosphate concentration in the effluent is the result of the described P accumulation in the reaction tanks. The outlet concentration, being elevated for more than 5 hours, underlines the BPR deterioration in this experiment.

PHA accumulation and utilisation rates were calculated based on mass balances around the tank for the whole experimental period (Figure 5.3-9). As the sampling was performed at the start of each phase, these rates represent the average over each phase. They illustrate and support the findings mentioned above. The accumulation in the anoxic phases during the acetate addition goes along with the observed P-release and the increase in the aerobic utilisation rates corresponds to the higher aerobic activity of the PAO.

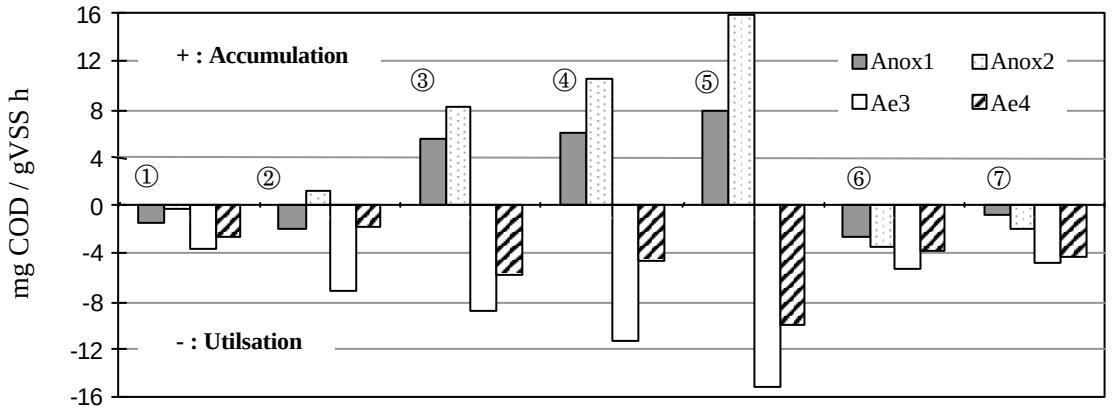


Figure 5.3-9. **Exp.C**: PHA utilisation/accumulation rates in tank2.

Rates for the 4 phases based on mass balance around the tank; SS = 3.37 g/L, VSS = 2.71 g/L
The numbers refer to the different cycles during Exp. C

It is noteworthy that during the second anoxic phase of the second cycle PHA is accumulated though no P is being released. This corresponds to similar situations observed during the batch experiments in section 4.4.2. It seems that under certain conditions, no or less poly-P degradation is needed to fulfil the energy requirement for acetate uptake and PHA storage.

Exp.C revealed clearly that there is a critical level of the external acetate addition rate, at which BPR will deteriorate and result in increased P concentration in the effluent. The observed increase in PHA and P-uptake rates were not able to compensate the increased amount of P being released during the anoxic phases. As a consequence, if the external addition of acetate to the anoxic zone is to be used as a control strategy, a routine for avoiding phosphate accumulation has to be implemented.

It has to be kept in mind that for all three experiments presented here the time period of aeration allocated for P-uptake was kept constant. In general it is possible that the aeration period was insufficient to prevent phosphate accumulation. A variable, adjusted aeration period could reduce the effect of BPR deterioration, but not prevent it totally (s. discussion in section 5.4).

5.3.2 Control of Denitrification and its Effect (limitting frame) on BPR;

Several experiments have been performed applying the control algorithm described in section 1.1.1 to scenarios of high ammonia loading. By adding a NH_4CL solution to the inlet of the anaerobic column, the ammonia load was kept at 180 to 200% of its original starting value for a time period of several hours. Imposing such a step-increase of ammonia leads to a corresponding increase in the NO_X-N concentration in the reaction tanks, upon which the controller reacts. Focus was put on the arising phosphate dynamics due to the external acetate addition and the possible capability to maintain sufficient phosphate removal capacity. Hence, the experiments presented here differ in the settings of the controller to avoid phosphate accumulation in the effluent. Simple rules or hard constraints were implemented to test the potential of these simple modifications.

In **Exp. D**., shown in Figure 5.3-10 and 5.3-11, a default value of 0.5 mg NO_X-N /L was applied for the aimed nitrate concentration within the controller routine (concentration in the tanks at the end of the anoxic phase). This corresponds to almost complete nitrate removal within the time allocated for denitrification: In addition it should also prevent the occurrence of anaerobic conditions if the actual denitrification rate should exceed the calculated one. As the preceding section has shown that phosphate is likely to be accumulated at acetate addition rates above 0.5 mg COD_{HAC} /(L_R min), a limiting frame (hard constraint) was put on the addition rate routine, using 0.5 mg COD_{HAC}/(L_R min) as the maximum allowable addition rate.

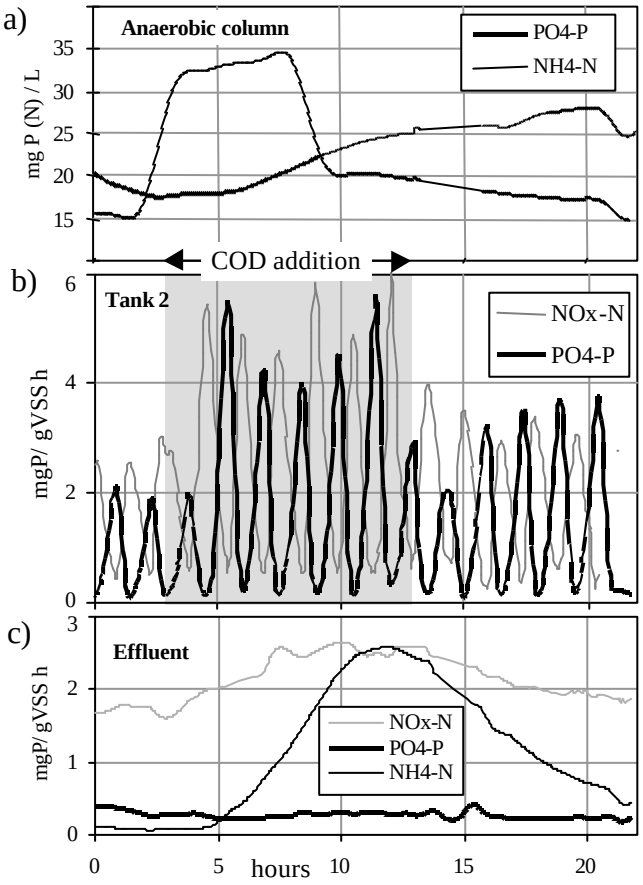


Figure 5.3-10 **Exp D**. Controlling denitrification at rising ammonia loading.

a) NH4-N and PO4-P at the end of the anaerobic column b) Response in tank 2, Nox-N and PO4-P

c) Effluent concentration of NH4-N, PO4-Pand NOx-N gray shaded area : period of COD addition

Controller: C_N^{AIM}= 0.5 mg N/L, $q_{COD,\,max}$ = 0.5 mg COD/(L_R min); k_2 = 0.95 mg COD/ (L_R min)

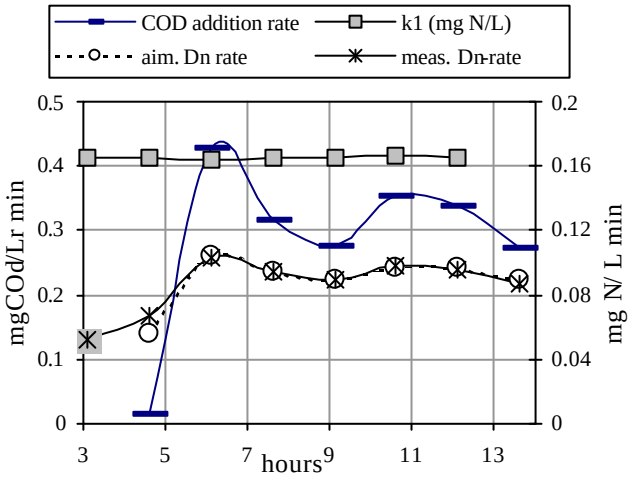


Figure 5.3-11. **Exp D**.

Right axis : calculated Cod add:ition rate

Left axis: evolution of k1 and denitrification rates.

✳: denitrification rate without COD addition

In Figure 5.3-10a the concentration of ammonia and phosphate at the end of the anaerobic column are shown. The time period of elevated ammonia load can clearly be seen. Phosphate rises slowly over the course of the experiment, suggesting that no deterioration of the process is to be expected due to a sudden increase in the COD load of the inlet. Consequently the response observed in the reaction tanks (Figure 5.3-10b) can be mainly attributed to the action of the controller. Once high nitrate concentrations are detected in the tank, significant acetate addition rates are applied, causing the phosphate dynamics to be more expressed: The magnitude of the phosphate peaks is 2 to 2.5 times higher than at the beginning of the experiments. Still virtually all phosphate is removed within one cycle, keeping the effluent concentration below 0.5 mg P/L during the whole experimental period (Figure 5.3-10c).

According to the phase schedule (Figure 5.2-1) the mixed liquor is always sent to the settler during the peak concentrations of nitrate. As these increase, due to more ammonia being converted to nitrate, a slight increase of nitrate in the effluent is observed during the high loaded period (Figure 5.3-10c). However, the increased denitrification, due to the controlled addition of acetate, prevents a further accumulation of nitrate in the system. The NO_X-N concentration reached at the end of phase 2 of each cycle lies always within the range of 0.4 to 0.6 mg N/L, which corresponds well to the chosen setpoint of the aimed concentration of 0.5 mg N/L. Consequently the increase in the effluent and hence also the amount of nitrate recycled to the anaerobic column are kept at a minimum.

The rise of the ammonia concentration in the outlet (Figure 5.3-10c) illustrates that nitrification was not complete, because of an insufficient aeration time, which was fixed at 30 minutes.

The control moves are depicted in Figure 5.3-11. Despite having to deal with nitrate concentration twice as high as before the increase of the ammonia load, the acetate addition rates stay always below the maximal allowed rate of 0.5 mg $COD_{HAC}/(L_R$ min). They remain within the range, where no accumulation of phosphate is expected to occur, based on the results from section 5.3.1. The depicted denitrification rates represent the average over the whole anoxic period of each cycle. The fact that the aimed rate and the actual observed one only show insignificant differences underlines the accuracy of the control routine, at least for the time period tested. The grey shaded cross in Figure 5.3-11 represents the background denitrification rate, determined for the last cycle before the external COD addition started. An increase in the denitrification rate of up to 80% can be noticed during the time of controled COD addition, illustrating the significant improvement achievable in NO_X-N removal.

Furthermore, also the evolution of the k1 is depicted in Figure 5.3-11 (values related to the right hand axis). The parameter was re-estimated after each cycle with COD addition according to the procedure described in section 1.1.1, but no major changes occurred in its values.

Exp. E was performed for two reasons. The first objective was to record the response of the system to a high ammonia load without controller. The second one aimed at creating a scenario, in which the controller is forced to realise acetate addition rates in the range where P accumulation was observed in section 5.3.1. Both objectives were realised by applying a sequence of high ammonia loads. During the first one no control was utilised, resulting in an elevated nitrate concentration. As the controller was started just before the second period of high ammonia load, it was confronted with a critical situation of already high nitrate concentration in the system plus an additional increase in the ammonia load.

The control algorithm was slightly changed for this experiment. Instead of the fixed allowable maximum acetate addition rate a routine was implemented, adjusting the addition rate in case of phosphate accumulation being observed in the reaction tank. The principle of this simple routine is depicted in Figure 5.3-12. No upper limit for the addition rate is implemented at the start of the controller. Therefore the value, calculated by the relational model, is directly applied for the starting cycle (cycle n-1). Before performing the addition for the following cycle (cycle n), the measurements of phosphate in the tank are used to determine if accumulation of phosphate in the tank has occurred during the last cycle. In case of no accumulation, the calculated acetate addition rate, $q_{COD,n}^{Calc}$, is applied to the process. If accumulation is detected, the employed addition rate will be adjusted to 70% of the actual calculated one for this cycle. This induces a change (rise) in the value for the aimed nitrate concentration from its default value of 0.5 mg N/L. Hence, this simple routine represents a trade off between nitrate and phosphate removal, putting higher weight on the performance of the later one.

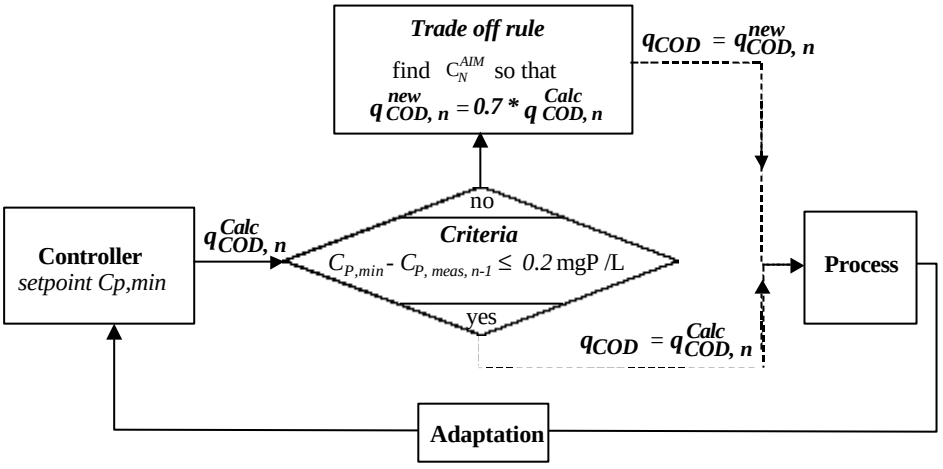


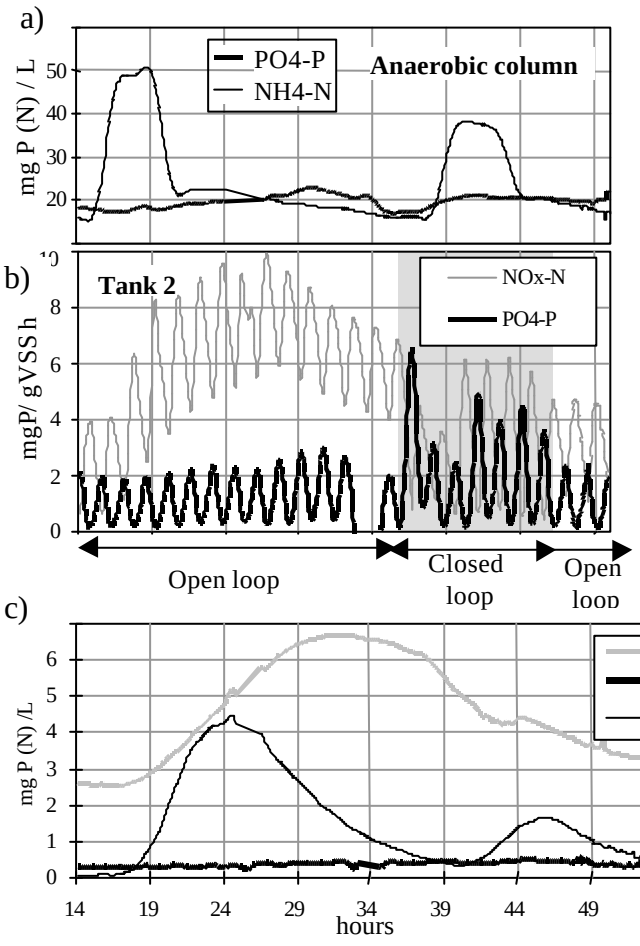
Figure 5.3-12. Trade off rule applied to avoid P accumulation due to high COD addition rates.

The concentration patterns of ammonia and phosphate in the anaerobic column are depicted in Figure 5.3-13a. Similar as in the previous experiment, it can be assumed that only the controller action affects the system's output, as the concentration of phosphate in the anaerobic column remained almost constant (no major changes in the influent COD). Upon the first rise in the ammonia load, nitrate in tank 2 rises for approximately 10 hours (Figure 5.3-13b). Only after the addition of ammonia stopped and after complete nitrification in the tanks is reached (data not shown) the nitrate concentration starts to decline slowly. But elevated nitrate concentrations are noticed for a period of 20 hours. Phosphate removal remains during this period at a satisfactory level, although a slight increase in the baseline in the reaction tank (line going through the point of minimum P concentration) can be noticed.

After 35.5 hours into the experiment, the controller was started. Maximum nitrate concentrations were still around 7 mg N/L in the tanks. As the default value for the aimed nitrate concentration was set to 0.5 mg N/L, a rather high COD addition rate (0.9 mg $COD_{HAC}/(L_R$ min) was applied by the controller. This caused the nitrate concentration to decrease to 0.8 mg N/L towards the end of the first half cycle. As expected, the magnitude of the phosphate dynamics increased immediately and an accumulation of phosphate was detected at the end of this cycle. Consequently, the 'trade off rule' was applied for the following cycle and resulted in the re-establishing of complete P-removal at the expense of a slight increase in the final nitrate concentration (approx. 1.2 mg N/L). The 'trade off

routine' was applied another time, when the second rise in the ammonia load showed its effect on the nitrate concentration in the tank (Cycle #4-5 of the controlled period). Similar satisfactory results were achieved as during the first time. As the experiment was stopped after the 7th cycle of external acetate addition, the phosphate dynamics dropped back to their original levels and the denitrification rate declined significantly.

The pattern of the nutrient concentrations in the effluent, shown in Figure 5.3-13c, reflect the behaviour observed in the reaction tank. An accumulation of ammonia is observed twice, as nitrification remained incomplete in the reaction tanks during the periods of elevated ammonia load. As both periods exhibit approximately the same nitrification rate (not shown) and thus the same nitrate 'production rate', the two scenarios are directly comparable. Nitrate exhibits a significant increase during the uncontrolled period, whereas the effect of the second high ammonia load is reduced to a minimum by the control action. Furthermore, also the high nitrate content due to the first scenario is reduced remarkably fast to a minimum level. Phosphate remains throughout the whole experiment below 0.5 mg P/L. Probably due to the buffer capacity of the settler, imposing an equalising effect, the accumulation of phosphate observed twice in the reaction tank, is not noticeable in the effluent.



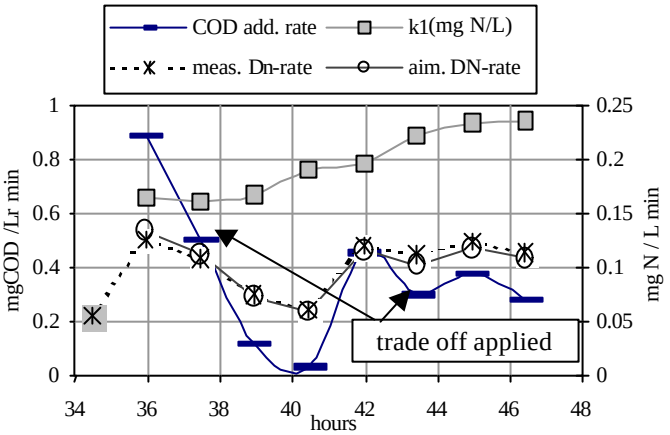


Figure 5.3-14. **Exp E.**
Right axis : calculated Cod addition rate
Left axis: evolution of k1 and denitrification rates
✳ : denitrification rate without COD addition

Figure 5.3-13. **Exp E.** Response to elevated ammonia loading, without and with controller acting
a) NH4-N and PO4-P at the exit of the anaerobic column b) Response in tank 2, Nox-N and PO4-P
c) Effluent concentration of NH4-N, PO4-Pand Nox-N
Controller: default C_N^{AIM} = 0.5 mg N/L ➪ Phosphate trade off routine.

Figure 5.3-14 illustrates the control moves during the experiment (Exp.E). The different nitrate concentration levels the controller had to deal with are reflected by the changes in the addition rate

applied. As the controller was started at already high nitrate concentration in the system, also high COD addition rates were needed to achieve (almost) complete denitrification. With decreasing nitrate level in the tanks, also the COD addition rate is decreasing. Upon the second rise in the nitrate load the addition rate increases again, resulting in denitrification rates more than twice as high as the ones before COD addition. Apart from the nitrate level, also the criterion for avoiding P-accumulation influences the value for the acetate addition rate, leading to a reduction of the actual rate applied. The two periods, for which the 'trade off' rule (applied addition rate = 70% of the calculated one) was employed are marked by the arrows in the figure.

Concerning the denitrification rate, no significant difference is noticed between the aimed denitrification rate and the actual measured one, similar to in Exp.D. The evolution of the parameter k_1, though, is quite different during this experiment. A gradual increase from its starting value of 0.165 mg/L to around 0.24 towards the end of the experiment is noticed. Despite this quite drastic change over a relatively short time period, no negative effect on the performance of the controller could be detected. The same observations were also made during other experiments (not shown). These results indicate that the conservative approach in re-estimating the new k1 value exhibits sufficient accuracy in tracking the drift of the parameter without imposing a problem on the performance of the control routine.

5.4 Discussion

General aspects

Since acetate is an easily degradable substrate typical for wastewater and which readily promotes BPR, it was used in these investigations as organic substrate for the external COD addition to the anoxic zone. Consequently, the following discussion will refer to acetate as organic substrate. However, the points in the discussion are expected to apply to other BPR promoting substrates, e.g. other volatile fatty acids.

Qualitatively, the response to the external acetate addition to the anoxic zone over several cycles in the pilot plant is similar compared to the batch experiments (section 4.4). The denitrification rate increased in all cases of introduction of acetate to the denitrifying zone. Anoxic P-uptake and PHA utilisation rates are reduced at low acetate addition rates compared to when no acetate is available during anoxic phases. At higher acetate addition rates a net P-release and a net storage of PHA may occur. In all cases of anoxic acetate addition, overall less PHA is utilised, thus leading to an increase in the P-uptake rates in the subsequent aerobic phase, due to the higher level of PHA available.

Since the improvement of denitrification due to external addition of an organic source is a rather well investigated subject, the focus in the following discussion will be put on the system's response with respect to overall phosphate removal.

Deterioration of BPR

Any circumstances leading to a rise in the phosphate concentration of the effluent are considered as a deterioration of the BPR performance. These situations are characterised by more phosphate being accumulated in the tanks during the anoxic phases (phase 1 and 2 of a cycle) than taken up during the subsequent aerobic phase (phase 3 and 4). The external addition of a BPR promoting substrate to the anoxic zone involves the potential to increase the possibility of BPR deterioration. Therefore it needs

particular attention. However, several conditions (e.g. aeration time, COD variation of the inlet) might also contribute to such incidents. Therefore, only experiments, in which the effect of the external COD addition on BPR can be isolated, are taken into consideration at this stage. The influence of a varying aeration time was avoided by keeping the length of the aeration period constant for the whole experimental duration. Hence, experiments that exhibited no major changes in the concentrations of the incoming waste water (e.g. Exp. A, C and E) were evaluated.

A critical acetate addition rate, ranging between 0.35 to 0.4 mg COD / (L_R min), was determined for those experiments, in which no anaerobic conditions occurred during the period allocated for denitrification,

Application of *Acetate addition rates below* these values, exhibited no negative effect on the effluent phosphate concentration. Carry over of BPR promoting organic substrates from the anaerobic zone to the anoxic phase were detected equivalent to addition rates of up to 0.04 mg COD / (L_R min). Consequently, they do not represent a potential risk for BPR deterioration.

Higher Acetate addition rates than the critical one lead to an accumulation of phosphate in the system along with a rise of the average PHA level. Despite the increasing PHA level, phosphate removal was incomplete. Two circumstances could be responsible for this behaviour:

1) *Insufficient aeration time.*
   It seems reasonable that, due to the increased amount of phosphate to be removed, the time assigned for P-uptake and nitrification could have been insufficient for complete P-removal in some cases.
2) *Temporary imbalance between phosphate release and uptake.*
   Even at sufficient aeration time, a temporary imbalance between P- release and P-uptake could provoke such a response. This has been observed upon rather sudden variations (increase) of the available amount of COD coming to the plant (Isaacs *et al*., 1994b, Carucci *et al*., 1999, Filipe *et al*., 2001 and own observations). An increase in the available amount of acetate causes an immediate increase in the amount of phosphate released and in the amount of PHA stored. At constant pH (Smolders *et al*., 1994 ; Liu *et al.*, 1996b), both reactions are linearly dependent on the uptake of VFA (Wentzel *et al*., 1989a). An imbalance occurs because the P-uptake rate does not increase by the same magnitude as the preceding P-release. High acetate addition rates, inducing anoxic P-release in the tanks, may well generate such a situation. Two explanations are possible for this phenomenon, but from the available measurements it cannot be stated which is more likely to occur or whether both are appropriate:
   a) *Change in the intracellular flow of carbon*. Upon the suddenly increased amount of PHA stored, the intracellular flow of carbon in the PAO organisms could change, directing more carbon towards the growth process. Hence, less PHA would remain for P-uptake (see also section 4.1.3).
   b) *Difference in the kinetics for P-release and P-uptake as described by Filipe et al., (2001).* In contrast to the P-release rate, being linearly dependent on the acetate uptake rate, the dependency of the P- uptake rate on the PHA content follows saturation kinetics (s. section 4.1). The increase in the uptake rate with rising PHA content is lower compared to the increase of the P-release rate. Furthermore, the amount of PAO in the system, which strongly influences the uptake rate, does not exhibit a direct response to short term variation of the VFA loading. Hence, less phosphate is taken up than previously released.

As the critical addition rate will most likely be dependent on the sludge conditions and operational parameters, it is expected to change in time and evidently from system to system. However, the results obtained during this study illustrate that the range of allowable addition rates represents a feasible operating window for the control of denitrification without causing BPR to deteriorate.

Furthermore, the occurrence of anaerobic conditions in the reaction tanks during the period allocated for denitrification and acetate addition should be avoided. Experiments A, B and C reveal, that the amount of phosphate released will increase drastically during anaerobic conditions, inducing the problem of insufficient P-uptake at applied acetate addition rates even below the critical one. As a consequence a controller for COD addition to the anoxic zone must incorporate a routine which avoids anaerobic conditions or/and stops acetate addition once these conditions arise.

Overall, the external acetate addition seems to be a minor factor regarding the cause of BPR deterioration, if controlled carefully. Indeed, as discussed above, elevated phosphate concentration in the effluent only due to the acetate addition can even be entirely avoided by appropriate means. Evaluating all pilot plant experiments it becomes evident, that the COD content in the inlet and its variation has a far greater impact on the development of phosphate and PHA levels in the plant. Experiments, that exhibited a rather drastic increase in the COD (VFA) concentration of the influent (similar to Exp.B), resulted in BPR deterioration, also in cases without the addition of external organics to the anoxic zone. This phenomenon is attributed to effects such as the imbalance of P-release in the anaerobic zone and P-uptake in the subsequent zones, as discussed above. If, during such a scenario, external acetate addition is applied, it will involve the risk of increasing the deterioration level. This is illustrated by experiments A and B: In both cases the same addition rates were applied. The sludge conditions can be expected to be very similar as the experiments were performed within a short period of time (both experiments were performed within 3 days). The experiment (B), during which a drastic increase in the COD load is experienced, exhibits an elevated phosphate concentration in the outlet for a certain period of time. On the other side, experiment A, not influenced by a sudden COD increase in the inlet, shows satisfactory phosphate removal, i.e. no rise in the effluent concentration. The exact impact of the external acetate addition can not be determined for these experiments, as the time period of the applied addition varied. But it can still be concluded that sudden increases of the COD in the inlet will lead inevitably to a lower value for the critical acetate addition rate. Whether reducing the applied addition rate during such conditions will completely avoid BPR deterioration remains questionable, but it should be kept at a minimum.

Effect of PHA level on the P-uptake rates

The dependency of the phosphate uptake rates on the PHA level is one of the important factors for (long term) satisfactory BPR performance in activated sludge systems (section 4.1, Brdjanovic *et al.*,1998 and Petersen *et al.*, 1998). In Figure 5.4-1 the initial aerobic P-uptake rates are shown as a function of the initial PHB level measured in one of the tanks of the pilot plant. For comparison, results obtained from batch experiments (section 4.1) are also depicted. A clear deviation between the two systems can be noticed, exhibiting lower uptake rates at the same level of PHB for the pilot plant system. Differences in the concentration of PAO in the sludge are unlikely to be the reason for this large deviation. Estimation of the amount of PAO in the sludge during side experiments (batch tests) revealed differences of only 5 to 10 % between the two systems. The estimation was performed using the observed P-release rate during anaerobic conditions, the VSS concentration, the observed

ratio of P/HAc and the rate constant for PHA storage according to the ASM2 default value (Henze *et al.*, 1995).

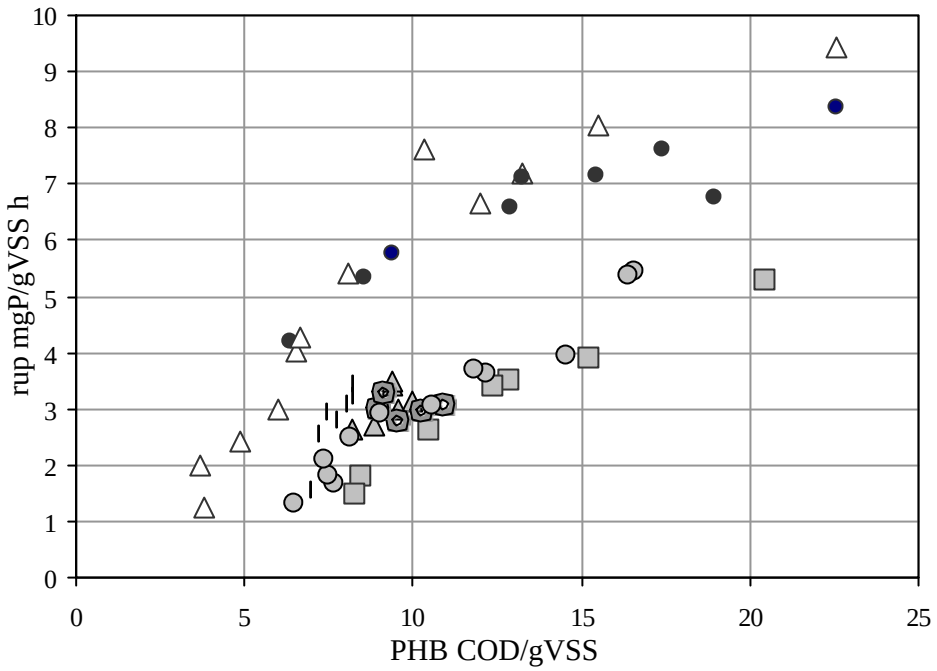


Figure 5.4-1. Initial aerobic P-uptake rates as a function of the initial PHB content.
Comparing batch test and pilot plant results.
black : batch tests (from section 5.1); white symbols: from Petersen *et al*., 1998 grey symbols: pilot plant.

A possible explanation for this behaviour could be the difference in the processes themselves (batch vs. BioDeniPho™). The different methods of operation lead to a different distribution of the internal storage products (PHA, poly-P and glycogen) in the PAO. This distribution is not accessible as the measured values (e.g. PHA concentration) represent only the average in the sludge. Assuming, for example, approximately the same amount of PAO in both systems, the P-uptake rate is mainly influenced by the internal storage pools of the bacteria. In the pilot plant process the reactor receives during the phases 1 and 2 for 45 minutes the mixed liquor from the anaerobic column. For a flow rate of 3 L/min this is equivalent to 135 L of mixed liquor per cycle, which corresponds to around 18 % of the total reactor volume. Hence, during one cycle also only 18 % of the PAO present in the tank have a high PHA and a low poly-P content. This part of the PAO will exhibit the highest P-uptake rates. However, they may not be able to compensate the low uptake rates of the fraction of PAO, whose uptake rates are more limited by low PHA and high poly-P content. Furthermore, the PAO fraction with high PHA content may well be already within the region of the saturation effect observed in the P-uptake kinetics, i.e. the P-uptake rates are independent of the PHA content.

In contrast to that, the PHA level is more evenly distributed in the batch tests. The whole sludge is submitted always to the same conditions at the same time, i.e. all the biomass experiences first the anaerobic and then the P-uptake phase. Consequently all PAO present in the reactor have taken up approximately the same amount of acetate, resulting in (more) evenly distributed levels of PHA, poly-P and glycogen. This seems to lead to higher P-uptake rates observed in the reactor, compared to the ones in the pilot plant at the same level of measured PHB content.

The interference of other bacteria capable of storing PHA without phosphate dynamics, e.g. GAO (Cech and Hartmann, 1993, Liu *et al*., 1996a and Satoh *et al*., 1994), causing a decrease in the P-uptake rates at fixed levels of PHA, is unlikely. Batch tests carried out in between the pilot plant experiments revealed ratios (P-release to acetate taken up, PHB stored to acetate taken up) corresponding to the existing understanding of the PAO metabolism. Consequently the PAO can be regarded as the predominant bacterial group in the system, capable of anaerobic PHA storage.
In general these findings illustrate the risk involved, if results obtained from batch experiments will be directly transferred to pilot plant or full scale processes. Furthermore they show that the distribution of the measured components such as PHA, plays an important role on the performance of the process. This distribution is unfortunately not accessible. Hence, if batch tests are specifically carried out to obtain more information about a system, which differs significantly in the operation from the batch procedure, the sludge should be submitted only to the phase of interest. If, for example, the aerobic phase is of interest, the sludge should be taken out of the plant just before the aeration phase and transported to the batch reactors, applying there the aeration phase. This would assure the same distribution of internally stored products in the batch test as in the plant, leading to representative results for the process design studied.

Effect of COD addition on denitrification.

The level of denitrification rate depends on several factors such as, the amount and the composition of the carbon sources coming to the plant, the sludge distribution (amount of denitrifiers, DNPAO and O2PAO), the growth of the denitrifying micro-organisms during the experimental phase and the activity of the DNPAO. In Figure 5.4-2a the average denitrification rates as a function of the external COD addition rate are depicted over the course of several pilot plant experiments.

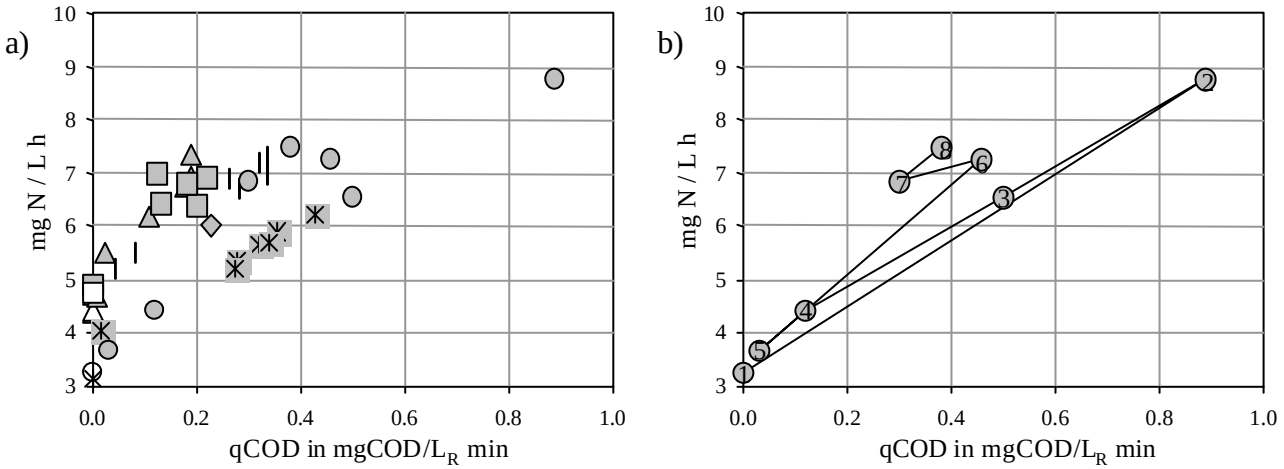


Figure 5.4-2. Denitrification rates during pilot plant tests – averaged over the anoxic time period

a) Average denitrification rates as a function of the external COD addition rate
white symbols : without acetate addition grey symbols: rates during external acetate addition
Different symbols refer to the different pilot plant experiments

b) Denitrification rates of Exp. E, numbered according to point of time (cycle #) in the experiment

The influencing factors will differ from experiment to experiment, and maybe even during one experiment. Therefore, when comparing different experiments, it is unlikely that a certain increase in the denitrification rate can be related to a specific value of the rate of acetate addition. Nevertheless, it can be stated that increases of 50 to 80 % in the denitrification rate were achieved through COD addition, without causing phosphate to accumulate.

In Figure 5.4-2b the denitrification rates of experiment E are numbered according to point of time (cycle #) in the experiment. The pattern suggests that the denitrification rate for a fixed addition rate can increase with time. It cannot be stated from the available measurements whether this effect is due to growth of normal denitrifiers, due an increasing activity of DNPAO or simply because more organic substrate is transferred from the anaerobic to the anoxic zone. But it illustrates that a simple control routine, as described in section 6.2.2, must contain a frequent, recursive updating of the corresponding parameters in order to deal with the changing denitrification activity of the sludge.

Besides resulting in lower nitrate effluent concentration, the external COD addition may induce further advantages for the overall process performance:

a) Improved settling of the sludge. Denitrification in the settler results in rising N2-gas bubbles, which may interfere with the settling sludge. Strong denitrification may even cause floating sludge in the settler. Reducing the amount of nitrate sent to the settler, reduces the denitrification along with N_2 production in the settler to a minimum. Hence, the sedimentation of the sludge should be improved or at least stabilised.
b) Reduced substrate competition in the anaerobic column. If nitrate is present in the anaerobic column, due to re-circulation from the settler, the 'normal' denitrifiers will use part of the incoming COD for denitrification, reducing the amount of COD available for BPR (Gerber *et al.*, 1987). Hence, the control of nitrate, resulting in a minimum nitrate concentration in the return-sludge, will stabilise biological phosphorus removal.
c) Possible inhibition of the fermentation of complex soluble COD to SCFAs (short chain fatty acids) due to the presence of nitrate in the anaerobic zone is avoided or at least reduced to a minimum.

Assuming a constant background denitrification rate over the period of the experiment, the amount of nitrate removed due to the external substrate addition can be estimated. Based on this assumption the ratio of COD added to NO_X-N removed can be calculated according to equation 6.4.1.

$$C/N = \frac{q_{COD}}{(r_d - r_{d)}^B} \qquad \text{(eq 5.10)}$$

The Yield (C/N ratio) determined this way varies between 4-6.5 g COD_{HAc}/g NO_X-N. Theoretically 1.26 mol HAc/ mol NO_3-N is required for total nitrogen removal by 'normal heterotrophic denitrifiers (assuming $C_5H_7NO_2$), including assimilation, when acetic acid is used as a carbon source (Henze *et al.*,1997). This corresponds to 5.4 gCOD/g N, which is about the average of the range determined above. A comparison of these values will give only a slight indication for which process the carbon is mainly used, as too many uncertainties exist. For example, comparing the activity of one group of biomass (normal denitrifier) with the activity of 3 groups (denitrifier, DNPAO and O2-PAO) induces already a certain inaccuracy. A clear distinction for which process the carbon is used, is not possible as the denitrification and phosphate removing processes are coupled due to the action of the DNPAO. Furthermore the assumption of a constant background denitrification rate will not hold for the majority of the experiments, as indicated by the discussion of Figure 5.4-2b.

Aspects of stabilisation of BPR performance

The external addition of an organic substrate to the anoxic zone is primarily regarded as a support of NO_X-N removal. However, two potential aspect were expected to induce a stabilisation effect on the

BPR performance. Both of them are based on keeping the PHA content of the PAO at a sufficient or even gradually increasing level:

a) *Direct increase* of the PHA level in the tanks from cycle to cycle.
   A slow but steady increase of PHA, caused by the external addition, would avoid possible limitation of the P-uptake rate. During the experiments, characterised by satisfactory phosphate removal, no such effect was noticed, i.e. all PHA accumulated during the anoxic phases of a cycle, was used in the subsequent aerobic P-uptake phase. An increase of the PHA level on a cycle to cycle basis was only observed in experiments, exhibiting an accumulation of phosphate over several cycles (BPR deterioration).
   It should be noted though, that applying low acetate addition rates to the anoxic zone during low loading conditions might be in favour of BPR. It represents a means to keep PHA at a certain level, when the COD in the inlet becomes severely limited. This should reduce the effect of BPR deterioration upon the re-establishment of normal conditions (fast rise in the inlet COD). As this specific scenario was not investigated, no definitive conclusions can be drawn.
b) *Long term* increase of the PHA level by reducing the amount of nitrate recycled.
   By minimising the amount of nitrate recycled with the return sludge, substrate competition in the anaerobic zone is reduced and the amount of organic substrate available for BPR is increased.
   This aspect displays its effect on a long term basis (days), by stabilising the PHA pool and possibly leading to increased growth of the PAO (further enrichment). The extent of stabilisation will probably be very much dependent on the conditions during which the addition is applied

Based on the current investigation the support for BPR is expected to occur only on a long term base. Experiments with the controller acting over several days to weeks will be necessary to completely verify this.

Though not leading consistently to a support of BPR, the following aspects should be noted:

- The occurrence of PHA accumulation without associated P-release, as observed in batch experiments (Meinhold *et al.*, 1998), was also detected occasionally during the pilot plant experiments. It is possible that conditions arose, which reduced the need of the PAO to supply energy via the poly-P degradation for the acetate uptake. But it might also be the net result of the overlay of the processes of P-release/PHA storage and P-uptake/PHA consumption of the two groups, DNPAO and O2-PAO. Also a combination of both explanation is possible. Based on the available measurements no definite conclusions can be made.
- The calculation of the PHA utilisation or accumulation rates in the different phases revealed for the majority of experiments, that an overall net storage of PHA during anoxic conditions only occurred together with a net P-release, predominantly leading to increasing phosphate concentration in the effluent. Hence, during controlled acetate addition (no BPR deterioration), the net PHA utilisation (as an superposition of PHA degradation and PHA storage processes) in the anoxic zone is reduced, but net PHA accumulation is not expected to occur.
- Denitrification in the settler can be mainly attributed to the DNPAO, using their PHA pool as a carbon source to reduce nitrate to nitrogen gas. This is underlined by the observation of a rapid decline of nitrate in the return sludge, once the PHA level rises in this zone (experiment C).

Effect of acetate addition on the fraction of denitrifying PAO

DNPAO and O2PAO exhibit different metabolism under anoxic conditions (section 2.1.3.2). The O2-PAO are assumed to act under anoxic condition the same way as under anaerobic conditions. The metabolic activity of DNPAO in the simultaneous presence of nitrate and acetate, however, is not known exactly. Uptake of acetate and storage as PHA, making full use of the TCA cycle, is one possibility (Filipe *et al.*, 1997). But there exists no fundamental reason against a direct usage/growth on acetate, omitting the storage of PHA. Also a combination is possible. As a consequence it cannot be predicted whether the external addition of acetate induces a potential advantage or disadvantage (wash-out) for the DNPAO. Hence, it is of interest to follow their activity during the experimental phases. Information about the denitrifying fraction of PAO was obtained, by comparing the initial P-uptake rates, exhibited in aerobic and anoxic batch reactors. For the exact description of the procedure one is referred to section 4.2. The batch tests were carried out prior to and after each of the experimental pilot plant periods involving external acetate addition to the anoxic zone. The periods differ in their duration (period I - 22 days and period II - 16 days). Both periods are characterised by the circumstances, that during more than 60% of the time, external acetate addition at varying rates, was applied to the system. The results of the assay for both periods are listed in Table 5.4-1.

Table 5.4-1 Results of bioassay for the denitrifying fraction of PAO

PAO fraction	Experimental period I		Experimental period II	
	before	after	before	after
DNPAO	59 %	73 %	57 %	53 %
O2-PAO	41 %	27 %	43 %	47 %

Whereas almost no changes occurred after the experimental period II, a considerable increase of DNPAO activity was determined after period I. It should be stressed however, that the results represent only rough indications. As the inlet to the pilot plant was not controlled (only constant flow-rate), conditions occurred that also might have an impact on the distribution of the PAO fractions (dilution due to rain events, weekend effects, high loaded situations etc.). Hence it cannot be concluded that the external COD addition favours the development of DNPAO. But since both periods differ from 'normal' operation in the external acetate addition, it can be stated that overall no decrease in DNPAO was induced by the introduction of acetate to the anoxic zone.

Control of denitrification and its limiting frame for BPR

With regard to *improved nitrogen removal*, the results obtained underline the feasibility of the external carbon source addition as a control strategy, as presented by Isaacs *et al*., (1994a, 1995). As illustrated by the results of experiment D (Figure 5.3-11) and experiment E (Figure 5.3-14), the aimed denitrification rates, calculated by the controller and the measured rates exhibit no critical deviation from each other. This is supported by Figure 5.4-3, showing the given set point as well as the actually achieved nitrate concentration at the end of each cycle for both experiments.

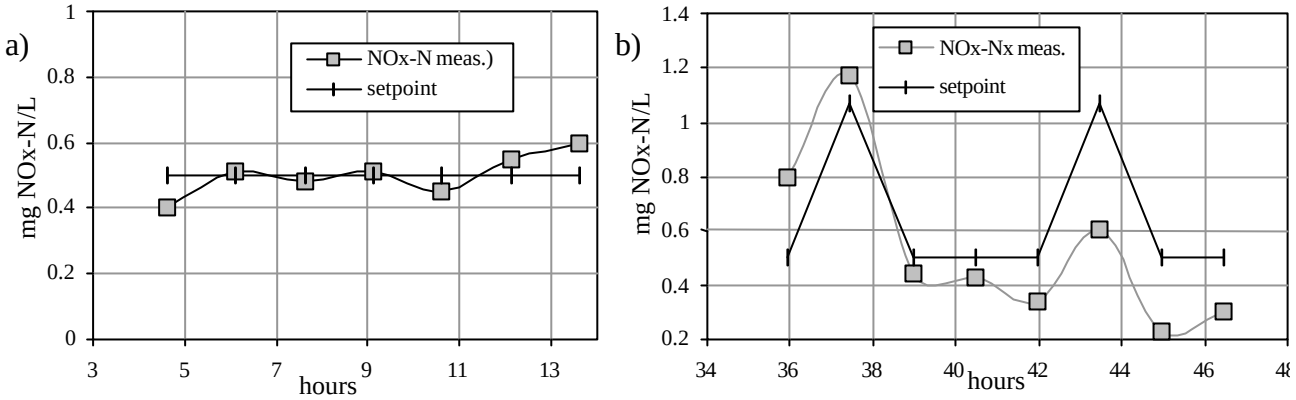


Figure 5.4-3. Set-point tracking : NO_X-N conc. at the end of a cycle : a) Exp. D and b) Exp. E

Taking into account the measurement inaccuracy of approximately ± 0.1 mg N/L, the deviations, during the time period studied, remain within reasonable bounds.

In fact, the quality of the set point tracking is mainly dependent on the quality of the recursive estimation method applied for the parameter k_1, as all drifts or inaccuracy of the whole control unit are lumped into the re-estimation procedure. The main conditions, causing a drift in the system are :

- Changes in the background denitrification rate, induced by shifts of the sludge conditions, its composition or the activity of the DNPAO.
- Changes in the pump accuracy.

The simple and conservative approach of the parameter estimation, presented in section 5.22, proved to produce reasonable results within the time period studied. It exhibited sufficient accuracy in tracking the drift of the parameter k_1 without imposing a problem on the performance of the control routine. By keeping the initial value for k_1 fixed for all experiments, the ability of the procedure to deal with an inaccurate starting value could be tested. The results underlined, that the procedure guarantees satisfactory results also in cases of inaccurate initial values for k_1. Whether a critical drift occurs, when applied on a long term base, remains to be investigated. If this occurs, a more sophisticated estimation procedure will have to be utilised (Isaacs *et al.*, 1995)

Concerning the *denitrification rate,* increases of up to 80% were noted. But more important during all experiments with controlled addition complete denitrification to the set-point was achieved in the tanks. This ensured that an accumulation of nitrate was prevented and its concentration in the return sludge reduced to a minimum. Measurements (not shown) verified, that no excessive acetate was added, i.e. no acetate was carried over to the aerobic phase.

During all experiments acetate was used as a *supplementary carbon source*. Due to the cost of acetate this is economically not feasible. In practice acetate might be substituted with similar carbon sources. Kristensen and Jørgensen (1990), for example, found that hydrolysate obtained from biologically hydrolysed sludge induces similar denitrification rates as obtained with acetate. Furthermore they state that hydrolysate from thermally or chemically hydrolysed sludge gave rates which were approximately half the ones obtained with acetate. Thornberg *et al.*, (1995) have demonstrated the feasibility of the addition of hydrolysed secondary sludge to the anaerobic zone for BPR improvement/promotion.

With regard to the *accumulation of phosphate,* the introduction of acetate to the phases allocated for denitrification involves in general two critical scenarios. The first one is the occurrence of anaerobic conditions in the reaction tanks (illustrated by the experiments A-C). The second one is the application of high external acetate addition rates due to high initial nitrate concentrations. Both

might induce more phosphate being released in the phase assigned for denitrification than being taken up in the subsequent aerobic phase. The controller predicts the denitrification as a function of the added acetate sufficiently well (Exp D and E) to prevent anaerobic conditions. Hence, only a routine is needed that avoids too high addition rates. In order to achieve this, phosphate measurements are obligatory, indicating whether phosphate accumulates or not. As the experimental set-up in this study included a fixed duration for the aeration period, a routine was chosen that adjusts the maximal allowable addition rate. This approach includes a *trade-off* between satisfactory BPR and complete denitrification in critical situations. If phosphate accumulation is detected, the applied addition rate will be reduced to 70% of the theoretically calculated one for the corresponding cycle. This leads to a lower denitrification rates but ensures satisfactory P-removal performance. The value of 70% is based on experimental observations and not optimised, but exhibited satisfactory results during all experiments. During most of the experiments this trade-off rule had to be applied for maximal two subsequent cycles. Hence, this routine is only needed during critical situations, which do not occur too often. The predominant time interval of pilot plant testing was characterised by 'normal conditions', i.e. the acetate addition rates stayed within the range where no P-accumulation in the plant is to be expected. As a consequence no constraint for COD addition rate needs to be imposed in these situations.

The trade-off routine, described above, represents a simple rule-based approach. A further extension could be the addition of the control/adjustment of the aeration period. Whether this could lead to similar results was not tested, but it has to be kept in mind that conditions can occur in which even a prolonged aeration period does not lead to complete P-uptake.

In situations where BPR deteriorates due to a sudden increase of COD concentration in the inlet, the external addition of acetate to the anoxic zone might contribute to an even further increase of the phosphate accumulation. Though the controller was not designed to counteract these conditions, it should certainly be able to prevent an additional increase of phosphate accumulation due to the introduction of acetate, i.e. reduce the addition to a minimum or even stop it entirely. If this can be achieved, the proposed control system seems suitable as a sole control routine to prevent nitrate accumulation with a potential for long term BPR stabilisation. Further investigations will have to show whether the controller is able to deal with highly dynamic COD load in the inlet, by using this or a similar simple rule based approach. Research addressing this subject as well as the long term effect on the biomass composition and the possible BPR stabilisation will have to be carried out.

More promising though for the general goal of controlling nitrogen and phosphorus removal will probably be a more complex strategy, including the presented approach as one part. For example, a combination of the external carbon source addition with aeration time length control and /or equalisation of the inlet load (Filipe *et al.*, 2001) will be more suited also to counteract BPR deterioration due to the sudden increase of the COD in the inlet.

5.5 Summary and Conclusion

The investigations performed in this study addressed the effect of a continuous introduction of a BPR promoting organic substrate to the denitrifying zone of a BPR process. Acetate has been applied as model organic substrate to the anoxic phases of a pilot plant, operated according to the

BioDeniPho™ concept. Furthermore the suitability of the external acetate addition as a control handle to avoid nitrate accumulation and its impact on phosphate removal were studied.

No major differences were detected in the qualitative response of the pilot plant over several cycles and the observations made in batch experiments. The introduction of acetate to the denitrifying zone induces in all cases an increase in the denitrification rate. At low acetate addition rates, reduced anoxic P-uptake and PHA utilisation rates are observed compared to conditions when no anoxic acetate is available. At higher acetate addition rates a net P-release and a net storage of PHA may occur. In all cases of anoxic acetate addition less PHA is utilised, thus leading to an increase in the P-uptake rates in the subsequent aerobic phase, due to the higher level of PHA available.

Quantitative comparisons of the aerobic P-uptake rates in batch and pilot plant tests revealed lower aerobic P-uptake rates for the pilot plant process at the same level of PHA measured in the sludge. This observation could be explained based on the difference in the operation mode of a batch with a sequence of anaerobic/anoxic/aerobic phases and the alternating operation schedule of a BioDeniPho plant. These results underline the potential risk involved when comparing data from different process configurations.

Occasional leakage of readily biodegradable COD from the anaerobic zone to the anoxic one was found not to be detrimental to the nutrient removal performance. A set of experiments were carried out to represent this scenario, employing constant low addition rates (below 0.1 mg COD_{HAC} / L_R min), resulting in slight improvements of the denitrification and no detectable negative effects on the BPR performance.

The implementation of a simple model based control strategy for adjusting the acetate addition rate to the need for denitrification proved to be feasible to prevent nitrate accumulation in the system. This approach, however, may lead to addition rates, at which more phosphate is released during the anoxic phase than taken up in the subsequent aerobic phase. Critical addition rates were determined at around 0.5 COD_{HAC} / (L_R min) for the experimental set-up used. Hence, there is an obvious need to control the maximal allowable addition rate in order to prevent BPR to deteriorate. A simple trade off routine between phosphate removal and denitrification proved to be very effective. In case phosphate accumulation was observed in one cycle, only 70 % of the calculated addition rate was applied for the following cycle. Complete P-removal was re-establish at once at the expense of a slight increase of the nitrate concentration.

Application of this control routine avoided accumulation of nitrate in the plant and lead to a considerable reduction of the amount of nitrate recycled with the return sludge. Consequently a minimisation of the substrate competition in the anaerobic zone between denitrifiers and PAO is ensured. This is of importance, for example, for low loading conditions. During these conditions ammonium is normally fully oxidised and,without control, nitrate would accumulate in the system. Controlling denitrification avoids this accumulation and consequently adds to the stability of the nutrient removal performance.

Problems in the BPR performance occurred if the amount of phosphate in the anaerobic column rose rather quickly due to a sudden increase of COD in the influent. In order to account for this or similar scenarios, in addition to the nitrate control, a combination of control routines will be necessary. A control strategy combining the proposed control method with aeration time length control and/or equalisation of the influent load should represent a promising approach.

Within the time period tested no negative effect were determined concerning the PAO activity, i.e. aerobic and anoxic P-uptake rates. Further investigations will have to be carried out regarding the long term effect of the external carbons source addition on BPR and on the microbial composition of the sludge as well as addressing the question whether a stabilisation effect can be achieved on a long term basis.

5.6 References

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6 MODELLING BIOLOGICAL PHOSPHORUS REMOVAL

ABSTRACT

The study addresses the investigation of an existing model for biological nutrient removal from municipal waste water. Using a priori knowledge and experimental results areas of model deficiency are indicated with respect to BPR and a revised/extended model is proposed.

The investigation focuses on the ability of the model to predict the phosphorus uptake as a function of the initial PHA level. Revised rate expressions are implemented for poly-phosphate storage and PHA utilisation of the phosphate accumulating organisms (PAO). Furthermore the process of anoxic acetate uptake and storage as PHA (approach from Filipe *et al.*, 1997) is added to the model. Both aspects are essential, as they have been observed to occur in praxis. Simulations are evaluated with data from an alternating type pilot plant, covering a time period of several cycles. The revised model exhibits an improved prediction quality with regard to the nutrient and internal PHA concentration and is able to capture PHA limited P-uptake as well as the effect of acetate flow into the anoxic phase on BPR dynamics. For the investigations only a few parameter had to be adjusted and the proposed extensions lead overall to only five additional parameters compared to the original model.

In a second step, the revised model is extended to two groups of PAO, according to the electron acceptor used. The simulation study assesses the ability of DNPAO, capable of using both nitrate and oxygen, to compete successfully in BPR systems to purely aerobic PAO (O2PAO). It is proposed that the proliferation of DNPAO is relying to a certain extent on external impacts, such as the influent composition (presence of DNPAO). However, growth being depending only on internal cell storage materials (PHA) represents a severe restriction in the model.

Introduction

Application and utilisation of mathematical models in wastewater treatment have increased considerably and nowadays models represent a major tool within all aspects of engineering. Models are used with a large variety of purposes, such as:

- *training* of operators and engineers,
- *understanding* of the underlying mechanisms,
- *static design* of systems : rules for full scale design (ATV, 1991; Wentzel *et al*., 1990),
- *dynamic design* : development, analysis, implementation of control and operating strategies.
- *optimal experimental design*: techniques for rational design of experiments aiming at maximising the reliability of model selection, parameter estimation and model validation (Vanrolleghem and Van Daele, 1994; Vanrolleghem and Dochain, 1998).
- *communication :* using the model as a common ground, conceptualising knowledge.

Depending on the intended use of the model, the model exhibits a large variety in complexity and in the degree of physical interpretability of the individual model components.

In this work the focus is on the improved understanding of the process itself, leading to the investigation of a model with increased complexity but keeping specific components physically interpretable, i.e. following the approach of 1st-engineering principle (use of continuity, mass balances etc). The first section presents a short introduction to the methodology of model investigation. The subsequent sections deal the refinement and modification of an existing model to be performed for an improved description of the biological nutrient removal process. In a first step the relationship between phosphate uptake rates and initial PHA level is implemented in the model. In a second step this modified model is extended to account for the process of anoxic acetate uptake by PAO. The last section presents a simulation study performed for two groups of PAO (DNPAO and O2PAO), in which conditions are investigated that might have a potential influence on DNPAO proliferation in the system.

6.1 Introduction to Model Investigation

6.1.1 Model Structure Characterisation within the Frame of System Identification

This section is intended to give a short introduction to relevant aspects of system identification; for detailed information one is referred to literature (e.g. Ljung, 1987, 1994; Van Impe *et al.,* 1998, Jørgensen, 1994).

Models can be categorised in many different ways. Differentiating the model types according to which degree they reflect the understanding of the basic mechanism of the system is a very useful classification (Caswell, 1976). Blackbox models (and neural networks) aim at producing observable outputs from the inputs without implementing further knowledge of physical or internal relationships between the system inputs and outputs. They are often successfully used in control applications.

Whitebox models offer the possibility for physical interpretation of the mathematical equation and their parameters and for including new gained knowledge of the system mechanism.

Greybox models, being an intermediate between white and blackbox models, exhibit to a varying degree the attributes of both types mentioned above. The use of hybrid models, i.e. a combination of

first principle modelling and, for examples, neural networks, has grown with the increase in computing capacity and measurement availability over the last years .

Modelling can be seen as an iterative cycle in which experiments play an important role. They are used to indicate aspects of model deficiency, leading to the implementation of new knowledge into the model, which again is tested against experimental data. Furthermore the data is of course also used in parameter estimation and model validation. Making optimal use of existing information in order to identify the most adequate model is the task of *system identification* (*SI*), consisting of the iterative steps shown in Figure 6.1-1 (e.g. Carstensen *et al*., 1998].

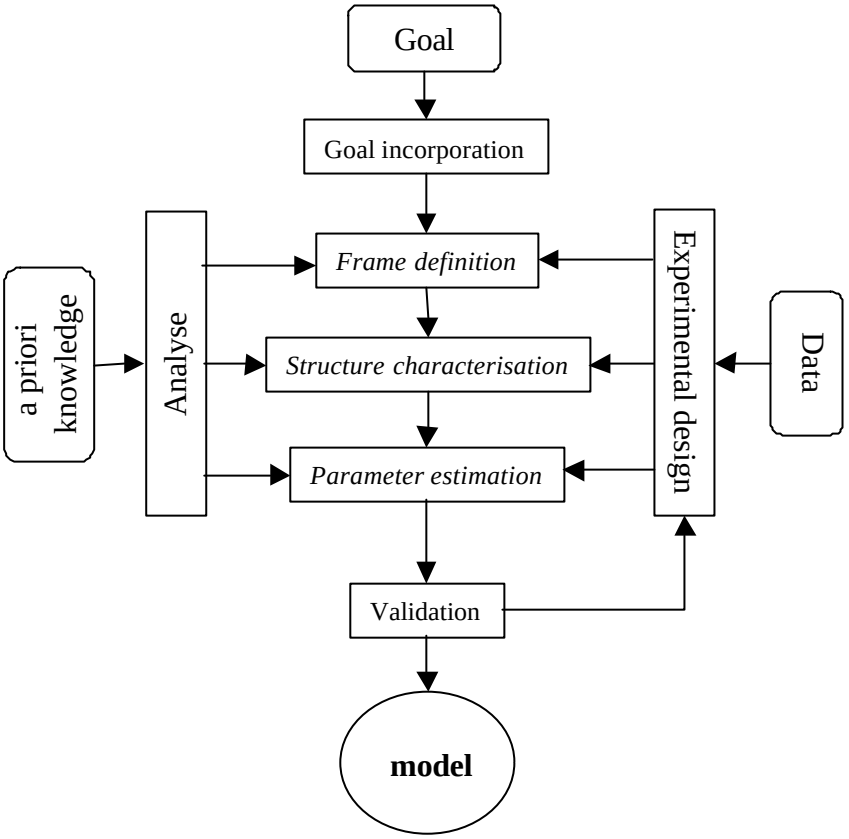


Figure 6.1-1 System Identification cycle from Vansteenkiste and Spriet (1982)

Frame definition : choose the system boundaries, input/output variables, type of models.
Structure characterisation : choose candidate model(s), level of model complexity, and determine the functional relationships between variables.
Parameter estimation : find numerical values for the constants in the functional relationships.
Validation : confront the model with new data, reflecting the purpose it was built for.

Whereas the developed methodology for *SI* is applicable to (and sometimes automated for) most physical and chemical systems, biological models in general exhibit particular characteristics, causing some problematic aspects (Vansteenkiste and Spriet, 1982). Often the *a priori*-knowledge is insufficient, leading to an increase in the recursive work between model structure and experimental data. For identification and application (parameter estimation / simulation) complex numerical algorithms have to be applied, being time and computing intensive, because of the non-linear behaviour of the models with regard to state variables as well as to the parameters. Furthermore the lack of some methods for the non-linear cases, e.g. the theoretical identifiability, poses more problems. In order to overcome these problems, including the insufficient measurement capability

and the fact that some parameters are highly correlated with each other, research activity in this field has increased significantly over the last years (e.g. Petersen *et al.*, 2001; Kops *et al*., 1999; Julien *et al*., 1998).
The following section can not be regarded as an application of the existing mathematical methodology of *SI* to modelling biological wastewater treatment. The main part of the work deals with the first steps of structural characterisation, using a priori knowledge and experimental results to indicate areas of model deficiency and to propose revised/improved model structures.

6.1.2 Model Variety and Choice of the Model

As biological phosphorus removal (BPR) has been a subject of research for more than the last ten years, almost inevitably a variety of models have been developed and proposed. (e.g. Henze *et al.*, 1995; Henze *et al.*, 1998; Smolders *et al.*, 1994; Smolders *et al.*,1995); Kuba *et al.*, 1996; Murnleitner *et al.*, 1997; Brdjanovic (1998); Barker and Dold (1997 a, 1997b); Pramanik *et al.*1999; Maurer and Boller, 1998). Most models rely on first engineering principle and differ in the assumed mechanism of the process or in their aim, e.g. including the mechanism of chemical precipitation (e.g. Maurer and Boller, 1998).
The quality of model prediction is often dependent on the chosen prediction horizon, i.e. the ability of the model to represent the large interval of time constants of the processes involved. The prediction of soluble components concentrations might be sufficient on a short term basis but fail on a long term basis as the slow dynamics of the process, e.g. the state of the biomass, are not captured correctly. In addition, some behaviour under critical conditions, such as COD 'shock-loads' in the inlet or extreme starving conditions due to dilution effects, might neither be captured by the model.
Biological nutrient removal processes of municipal waste water treatment are almost permanently running under C-limitation, i.e. starvation condition/stress for the micro-organisms. In addition, the content of these organic sources in the inlet/feed varies strongly, daily and seasonally. As BPR is highly dependent on the dynamics of the internal storage components of the PAO, reliable prediction of phosphate removal depends on the correct modelling of these state variables. The BPR-model developed at the TU Delft (e.g. Murnleitner *et al.*, 1997), further referred to as TUD-model, has been developed based on a metabolic structure of the currently assumed mechanism. Therefore it is expected to describe the PHA and glycogen dynamics more accurately, offering the possibility to capture limitations due to PHB and glycogen. In the ASM2 versions all internally stored carbon is lumped into a PHA pool, thus considering only 2 major internal storage pools (poly-P and PHB) (Henze *et al.*, 1995; 1998), which induces problems when comparing experimental data of PHB with model prediction.
As basis for the work described here the combination of the TUD-model with the ASM2 (ASM2/TUD) as presented by Brdjanovic (1998) is chosen, taking also the processes of N-removal, hydrolysis and fermentation etc. into account.

6.1.3 Aim of Investigaton

Experimental investigations (secton 4.1, Petersen *et al.*, 1998, Meinhold *et al.* 1998, Temmink *et al.*, 1996) show that the interaction between PHA (internal C-storage) dynamics and phosphorus dynamics is one key issue with regard to phosphorus removal from municipal wastewater. The process runs most of the time C-limited, i.e. also PHA will be limiting P-removal as illustrated in

section 4.1. The present investigation focuses on the ability of the model to predict the phosphorus uptake as a function of the PHB level. This is of interest as fluctuations of the PHA content occur due to varying inlet conditions as well as due to the use of external acetate addition as a possible actuator in control strategies (section 5).

The correct estimation of PHA and its interaction with phosphate is relevant for almost the whole range of time constants (minutes to weeks) exhibited by BPR (see section 2.2.2). It is important to predict the phosphate release/uptake as a function of the PHA level on a cycle to cycle time scale in order to predict the short term P-removal capacity. Furthermore the PHA content needs to be predicted also well over a longer period of some weeks, to capture the development (growth/decay) of the PAO correctly and consequently to predict long term P-removal.

A second step addresses the need to account for the process of anoxic acetate uptake by PAO when modelling BPR. This process is of relevance and does appear quite often, as shown and discussed in section 5. An approach for anoxic acetate uptake, presented in literature (Filipe and Daigger, 1997), is discussed and implemented into the refined model and tested with appropriate pilot plant data.

As the focus during this investigation is put on the qualitative ability of the model to represent the specific interactions between phosphorus uptake and internally stored PHA, time periods of one to several cycles of the pilot plant are taken into account In addition potential 'weak spots' and important aspects as prerequisites for the task of full dynamic calibration and long term investigation will be pointed out (s. section 6.2.3).

Unless indicated differently, simulations and experimental data refer to PHB, being the major component of PHA. However, ‘PHA’ is kept as an index in the model notation and when discussing general dependencies and influences.

6.2 Qualitative Investigation – Proposed Extension to the ASM2/TUD model

6.2.1 P-uptake as a Function of PHA

In literature the combination of the ASM2 with the TUD-model has been applied to full scale plants, reaching a satisfactory prediction capability for the dissolved components phosphate, nitrate and ammonia (Veldhuizen *et al.*, 1999) even without any calibration (Brdjanovic,1998). Brdjanovic pointed out that the prediction quality of the polymers (PHA and glycogen) was less than for the soluble components, strongly suggesting to include the internal storage products (glycogen, poly-P and PHA) in any calibration procedure. In order to make use of the theoretical potential of the model to predict the process behaviour also during limitation of the internal storage compounds, their dynamic behaviour and interaction with the soluble components have to be taken into account. In the following investigation the focus is put on PHB and phosphate interaction.

Figure 6.2-1 illustrates the ability of the ASM2/TUD model (Brdanovjc, 1998) to predict the dynamics of the nutrients in the liquid phase (NH_4-N, PO_4-P,NO_X-N) in the pilot plant, only adjusting the initial conditions and the key parameters parameters k_{PP}, k_{GLY}, η_{NO3}, q_{fe} (Table 6.2-1).

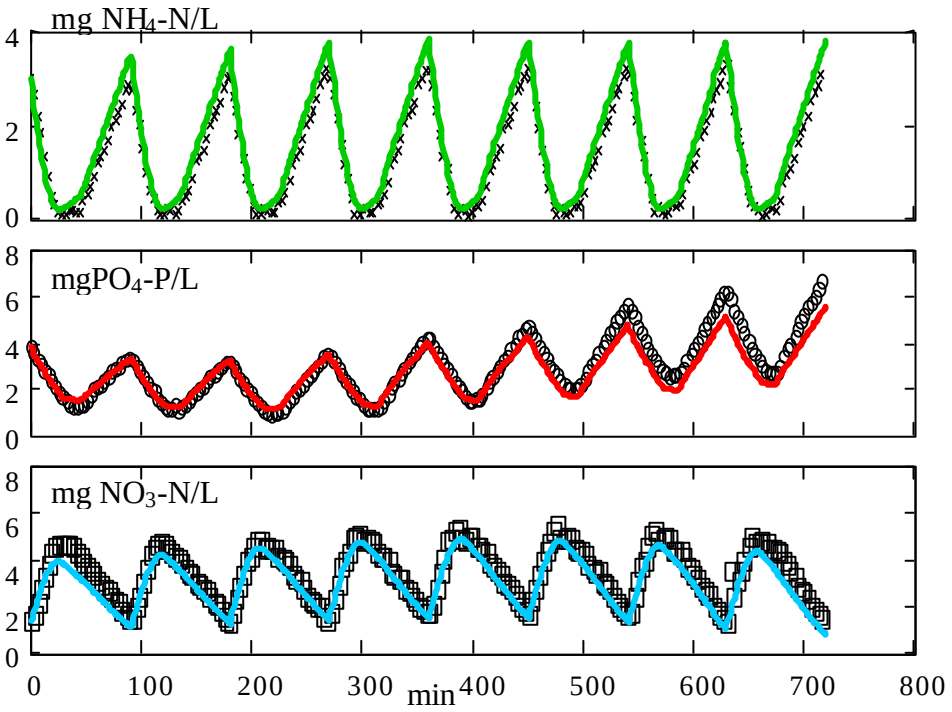


Figure 6.2-1 Nutrient pattern in one reaction tank of the pilot plant.
Solid lines: simulation with ASM2-TUD; symbols: measured concentrations

Table 6.2-1 Kinetic parameter adjusted for simulation with ASM2/TUD.

Parameter	Values			Unit	Description
	applied	Brdanovjc,1998	Murnleitner *et al.*, 1997		
k_{PP},	0.11	0.11	0.45	gP/gCOD d	poly-P formation rate
k_{GLY}	0.45	0.15	1.09	gCOD/gCOD d	Glycogen formation rate
η_{NO3}	0.4			-	anoxic reduction factor
q_{fe}	1.2	1	3 (default ASM2)	gCOD/gCOD d	max. fermentation rate

The corresponding PHB pattern is shown in Figure 6.2-2. The qualitative behaviour over the time period of 800 minutes is followed quite well, but the amplitude within the cycle-dynamics is predicted too large, i.e. the amount of PHB coming from the anaerobic column (storage of PHB) and also the PHB degradation in the tank is overestimated. The simulated PHB pattern exhibits a deviation of up to 250% and more for the prediction of PHB decrease (phase 1&2) and increase (phase3 &4, s. appendix 8.2.1 for phase schedule)

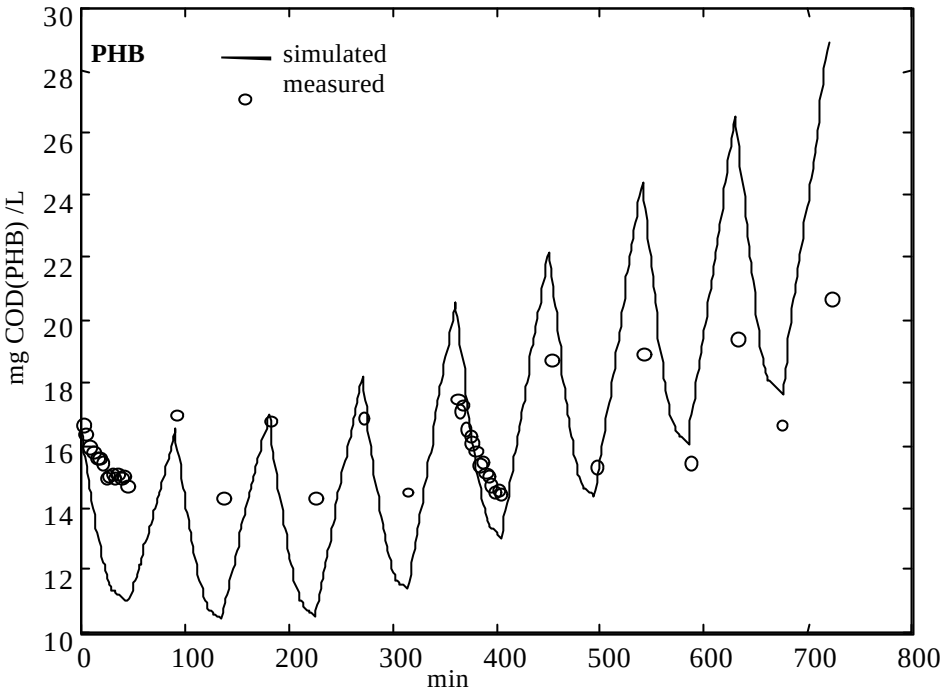


Figure 6.2-2 PHB pattern in one reaction tank of the pilot plant; simulation with ASM2-TUD.

The wrong prediction of the PHB dynamics does not effect the phosphate patterns, as the ASM2/TUD model does not incorporate any dependency of the poly-P storage on the PHB level In equation 6.1 and 6.2 the rate expressions for PHA lysis and poly-P storage of the ASM2/TUD model are depicted (the terms M_j^i are refering to the corresponding switching functions for saturation effects, see appendix 8.5.4).

$$r_{PP} = k_{PP} * \frac{1}{f_{PP}} * \frac{S_{O2}}{K_{O2}^{P} * g_{PP} + S_{O2}} * \frac{S_{PO4}}{K_{PPO4}^{P} + S_{PO4}} * X_{PAO} \qquad \text{(eq 6.1)}$$

$$r_{pha} = k_{PHA} * (f_{PHA})^{2/3} * \frac{S_{O2}}{K_{O2}^{P} + S_{O2}} * M_{NH4}^{P} * M_{ALK}^{P} * M_{PO4}^{P} * X_{PAO} \qquad \text{(eq 6.2)}$$

The biological phosphate removal part of this model has been validated by Murnleitner *et al.,* (1997), resulting in fairly good prediction of the measured data. But the data for validation was taken from a lab-scale reactor fed with synthetic wastewater, using a high acetate load (400 mgCOD, exact composition see Smolders, 1995; Smolder *et al.*, 1995), which is about 10-20 times higher than the amount of acetate observed during pilot plant operation with municipal wastewater in the present investigation. Under such highly loaded operating condition no PHA limitation on P-uptake is to be expected. Furthermore the PHA utilisation at this acetate level might show a different behaviour than at the level normally observed in the pilot plant or in full scale plants.

It is evident that, when incorporating a PHA dependency in the poly-P uptake process, also the dynamic behaviour of PHA has to be checked and possibly revised for improved prediction.
Concerning the poly-P storage two possible extensions were taken into consideration, whereas for the PHA degradation three different approaches were tested:

Table 6.2-2. Terms varied in the rate expressions for poly-P storage and PHB degradation.

Poly-phosphate storage	$r_{PP} = ... * \left(f_{PHA}\right)^{\frac{2}{3}}$	eq 6.3.
	$r_{PP} = ... * \frac{f_{PHA}}{f_{PHA} + p}$	eq 6.4.
PHA degradation	$r_{PHA,deg} = ... * \left(f_{PHA}\right)^{\frac{2}{3}}$	eq 6.5.
	$r_{PHA,deg} = ... * \frac{f_{PHA}}{f_{PHA} + q}$	eq 6.6.
	$r_{PHA,deg} = ... * \left(f_{PHA} - f_{PHA,min}\right)^{\frac{2}{3}}$	eq 6.7.

For poly-P storage eq 6.3 was chosen, as it represents the same dependency on PHB as the rate expression for the glycogen formation rate in the TUD model and avoids the introduction of an additional parameter. For comparison, extension eq 6.4, representing the 'classical Monod term approach,' was also investigated.
For the PHA (PHB) degradation/lysis three expressions were taken into consideration, eq 6.5 representing the original one from the TUD model, eq 6.6 using a Monod term and eq 6.7 incorporating a certain minimal PHB fraction, not available for phosphorus uptake (e.g. Temmink *et al.*, 1996; Petersen *et. al.*, 1998).
Simulations were run for batch tests with an aerobic P-uptake phase as well as a test with anoxic uptake phase. Prior to the batch test simulations, estimates of unknown states (glycogen) and unknown initial conditions (e.g. initial biomass composition) were obtained from pilot plant simulation using the ASM2/TUD model and parameters presented in table 6.2.1. No mathematical criteria were used for choosing the most appropriate approach, as this decision could be made just by evaluating their qualitative behaviour. The models were eavaluated, focussing on the initial part of the phosphate curves in the batch experiments and the corresponding PHB patterns. The chosen model was checked against pilot plant data later on.
Caution has to be taken when using these two different sources of experimental data, as they represent different time frames concerning the period of actual phosphate uptake as well as the level of initial PHB content. The pilot plant was operated with a 30 min aeration time, whereas in the batch experiments uptake periods of up to 4 hours were applied. The 'optimal' model should be able to represent both cases, but if this is not achievable, the goal must be reformulated, i.e. redefining the frame definition. In this investigation prediction of experimental batch data was tested, but the main interest and goal was the ability to predict phosphorus removal for the conditions observed in the pilot plant. These conditions are close to the ones in full scale plants, i.e. low initial PHB content in the aerobic/anoxic tanks and aerobic P-uptake periods with a time interval of less than 45 min (initial uptake rates).
In the following the obtained results and the conclusion drawn will be illustrated using a aerobic batch experiment, as the investigation of the anoxic behaviour supported the same conclusions.

For the whole time period (uptake periods of up to 4h) of the batch tests, all combinations of the rate expression from Table 6.2-2 result in rather poor quality for the phosphate and PHB pattern (not shown), simulating all 4 reactors with the same set of parameters. Simulations with different amount of PAOs and different initial content of internal storage compounds (poly-P, Glyc) to account for possible glycogen and/or poly-P limitation did not reveal any improvements in the qualitative prediction. This indicates that effects such as prolonged uptake periods are not captured correctly by the model. Further evaluation of the rate expressions deals with the prediction quality for the linear part of the phosphorus uptake curves (initial rates), which corresponds to up to 80 min of aerobic uptake period for the experiment discussed here. For the discussion of the experimental patterns the reader is referred to section 4.1.

When restricting the interval of interest to the linear part of the phosphate uptake curves, the prediction quality becomes acceptable. The different rate structures exhibit a different degree of spread out (Figure 6.2-3) of the 4 P-curves, which was taken as one criterion for the decision of the most appropriate one. Fitting for example one P-uptake curve, by adjusting the 'half-saturation coefficient' p and/or kpp, depending on which combination of rate expressions was used, leads to a certain deviation for the other three curves. Figure 6.2-3 shows the phosphate concentration at different initial PHB levels (4 reactors in parallel) for two different rate expression for poly-P storage.

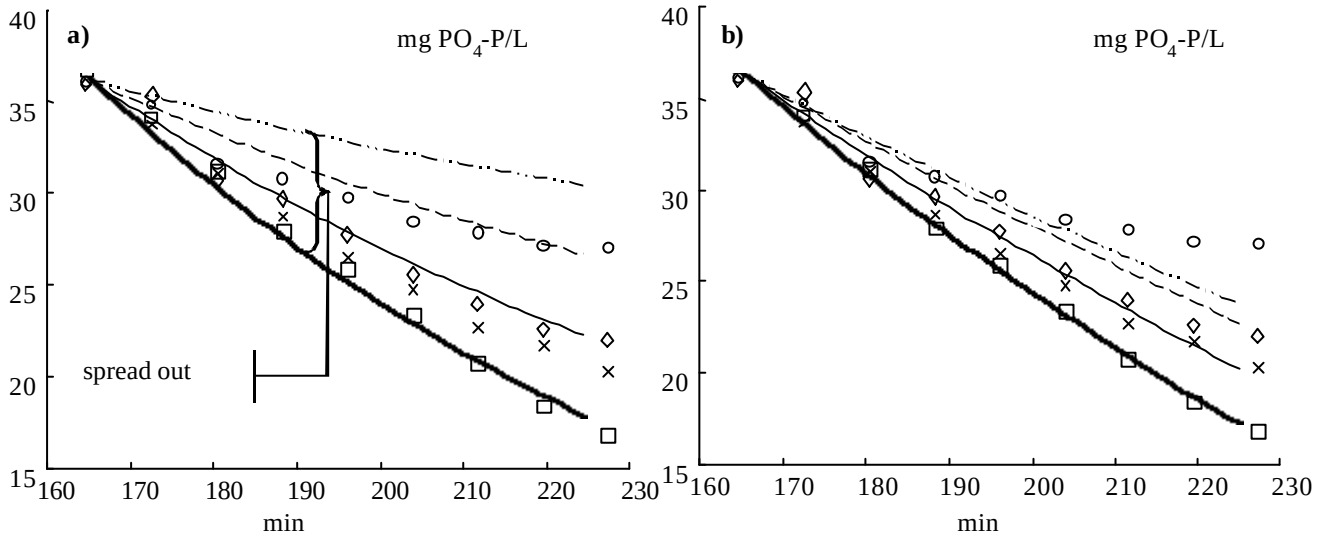


Figure 6.2-3 Phosphate pattern, aerobic uptake. a): $r_{PP} = ... * (f_{PHA})^{\frac{2}{3}}$ b): $r_{PP} = ... * \frac{f_{PHA}}{f_{PHA} + p}$

Anaerobic –aerobic batch test with four reactors in parallel. Each reactor received a different initial amount of acetate at the start of the anaerobic phase. Before the start of aeration the orthophosphate concentrations in the reactors were brought to the same level by external addition of KH_2PO_4

In all three PHA degradation expressions the rate is only dependent on the PHA concentration, as one of the three internal storage compounds. In the original expression (eq 6.22), a term for ammonia limitation was included (M^P_{NH4}). This is due to the way the model was developed; PHA is first transformed into biomass (PHA storage) and from there 'distributed' to the other processes (PHA lysis) (Murnleitner *et al.*, 1997). The use of this limiting term, however, leads to a slowing down and almost stop of the predicted PHB degradation when ammonia concentration is close to zero, which is not in line with the experimental observation, of both, batch and pilot plant data. Here no such

influence of ammonia on PHA utilisation and P-uptake was observed (section 4 and 5.2). As a consequence the limiting term for ammonia (M^P_{NH4}) was eliminated from the rate expressions for PHA degradation.

The expression (eq 6.7), incorporating the idea of a minimal PHB concentration not available for the PHB storage/degradation dynamics, revealed the best qualitative behaviour over the whole range of initial PHB levels for the first 90 min uptake period (Figure 6.2-4). The parameter $f_{PHA,\ min}$ was set to 0.01 mg COD (PHB)/mg COD (PAO), representing the same order of magnitude as suggested by Petersen *et al.,* (1998).

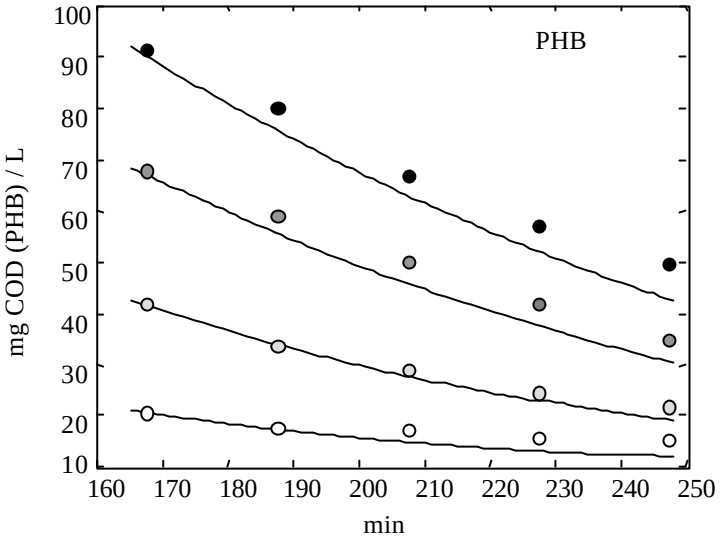


Figure 6.2-4: PHB pattern in the aerobic period of a batch test with $r_{PHA,\ deg} = ... * \left(f_{PHA} - f_{PHA,min}\right)^{\frac{2}{3}}$.

Anaerobic –aerobic batch test with four reactors in parallel, receiving a different initial amount of acetate at the start of the anaerobic phase. Depicted are the first 90 minutes of the aerobic phase.

Based on the investigation of several batch tests, the rate expressions, presented below (including the parameters modified) were chosen as the most appropriate ones.

$$r_{pha,deg.} = k_{PHA} * \frac{S_{O2}}{K^P_{O2} + S_{O2}} * M^P_{ALK} * M^P_{PO4} * \left(f_{PHA} - f_{PHA,min}\right)^{\frac{2}{3}} * X_{PAO} \qquad \text{(eq 6.8)}$$

$$r_{PP,stor.} = k_{PP} * \frac{1}{f_{PP}} * \frac{S_{O2}}{K^P_{O2} * g_{PP} + S_{O2}} * \frac{S_{PO4}}{K^P_{PPO4} + S_{PO4}} * \frac{f_{PHA}}{f_{PHA} + p} * X_{PAO} \qquad \text{(eq 6.9)}$$

k_{pp},	0.1	Poly-P formation rate	gP/gCOD d
k_{GLY}	0.4	Glycogen formation rate	gCOD/gCOD d
η_{NO3}	0.4	Anoxic reduction factor	-
$f_{PHA,\ min}$	0.01	Minimum PHB content in PAO	gCOD/gCOD

Table 6.2-3 : Revised rate expression for PHB degradation and poly-P storage and parameters

Simulating the same pilot plant scenario as in Figure 6.2-1 with the revised model structure results in a similar quality for the soluble components of interests (Figure 6.2-5), but shows a significant improvement in predicting the PHB dynamics. Figure 6.2-6 shows the PHB dynamics of the revised model in comparison to the ASM2/TUD, illustrating the achievable improvements. Apart from slight changes in the parameter k_{pp} and k_{GLY}, the same parameter values were used for both simulations. Despite the discrepancy observed for the simulation of the batch experiments the revised model

exhibits a significant increase in the quality of the prediction of the pilot plant behaviour. This underlines the importance of the implementation of the functional relationship between phosphorus uptake and the level of PHA concentration and of refining the rate expression for PHA degradation.
It should be noted that a full calibration will most likely result in a further improvement of the prediction quality, enabling to extend the prediction horizon.
The steps taken for the 'manual calibration' prior to the pilot plant simulation are summarised together with some additional information about calibration aspects in section 6.2.3.

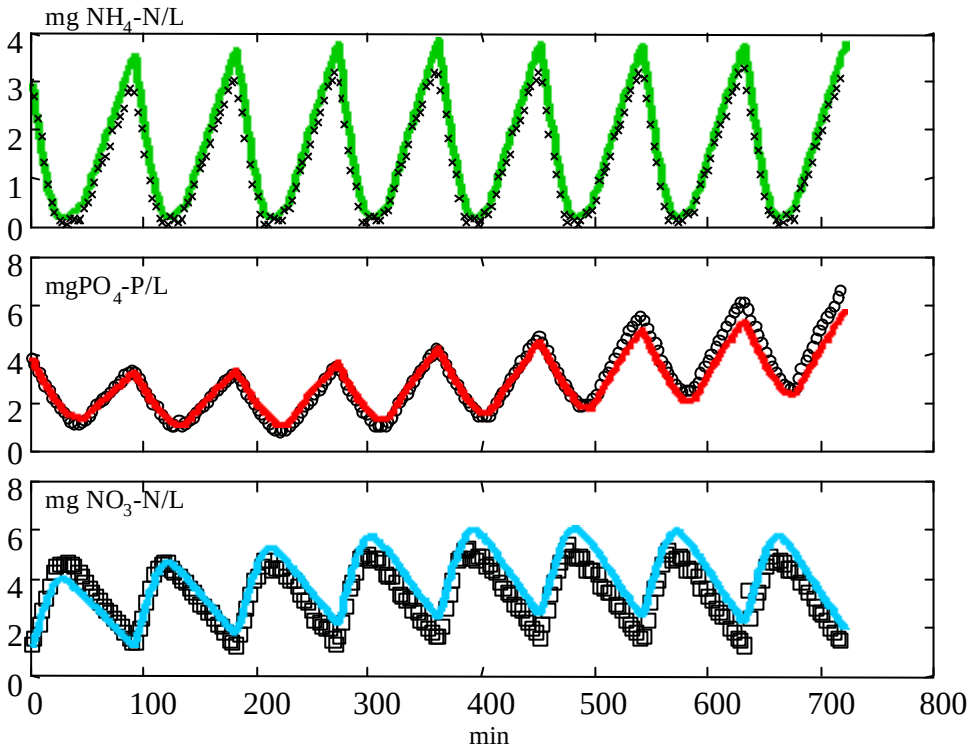


Figure 6.2-5 : pilot plant prediction of the revised model.

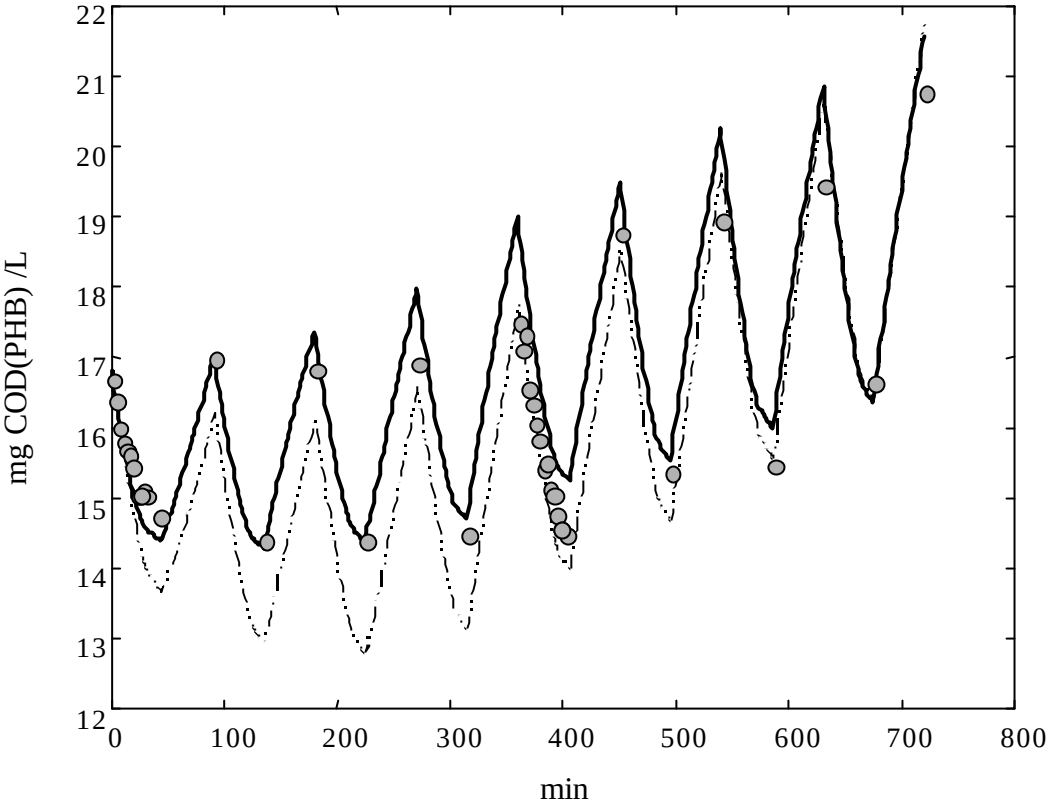


Figure 6.2-6 : PHB pattern , comparing ASM2-TUD and revised model

6.2.2 Acetate Addition to the Anoxic Phase

Introduction of acetate to the anoxic zone might be due to ongoing conversion reactions (hydrolysis and fermentation) within the anoxic phase or due to 'overflow' from the anaerobic zone. The latter case can be caused by insufficient anaerobic retention time, poly-P or glycogen limitation in the anaerobic compartment. The importance of the process of anoxic acetate uptake by PAO has already been illustrated in section 4.4 and 5.

The ASM2/TUD model does not account for a possible anoxic acetate uptake by PAO, as the rate expression for acetate uptake/PHB storage is limited to anaerobic conditions (eq 6.10):

$$r_{PHA,stor.} = q_S^{max} * \frac{S_A}{K_{PA} + S_A} * I^P_{O2} * I^P_{NO3} * M^P_{GLY} . * M^P_{PP} * X_{PAO} \qquad \text{eq 6.10}$$

The rate expression could be 'activated' for anoxic or aerobic conditions simply by removing the inhibition terms I^P_{NO3} and I^P_{O2}, but it is unlikely that this would represent the underlying mechanism. If an electron acceptor is present, the micro-organisms will be able to make use of the TCA cycle for fulfilling the requirements for energy and reducing equivalents. Thus they do not need the ED-pathway, which is supplying the reducing equivalents under anaerobic conditions (section 2.1.3). Evidently, the change in the pathways used, should be represented by the rate expressions and stoichiometry of the model. For this work the approach presented by Filipe and Daigger (1997) is implemented in the revised model from Chapter 6.2.1 and tested with an appropriate pilot plant scenario.

In Figure 6.2-7 this approach is illustrated. Acetate is transported over the cell membrane and activated to acetyl-CoA, which is further converted to PHB. With poly-P degradation, the TCA cycle and the oxidative phosphorylation, three sources of ATP are considered. The reducing equivalents,$NADH_2$, are provided by the TCA cycle. It is assumed that the $FADH_2$ produced is equivalent to $NADH_2$ (Smolders *et al.*, 1994b). In this model the internal glycogen is not involved in the mechanisms of anoxic acetate uptake.

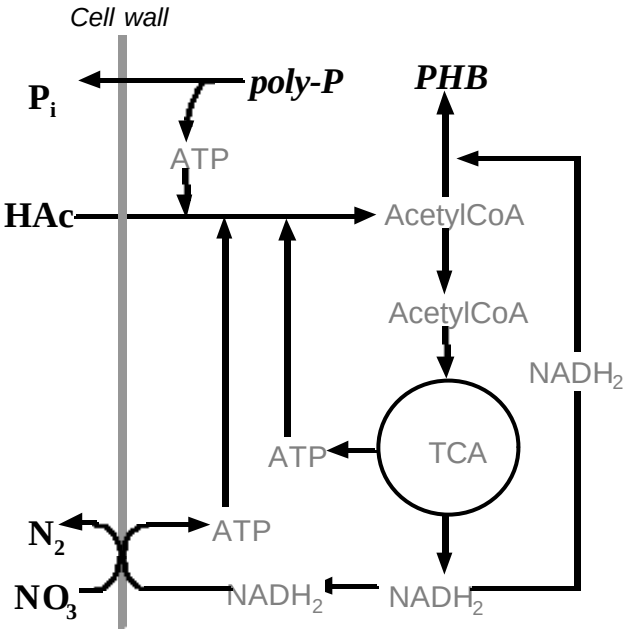


Figure 6.2-7 : anoxic HAc uptake (Filipe and Daigger, 1997).

The implementation of this approach leads to the following changes in the rate expressions for the storage of PHB (eq 6.11 and eq 6.12). The values for the stoichiometry parameters used in the simulation are the ones suggested by Filipe and Daigger (1997) and listed in Table 6.2-4.

$$r_{PHA,AN} = q_S^{max} * \frac{S_A}{K_{PA} + S_A} * I_{O2}^P * I_{NO3}^P * M_{GLY}^P * M_{PP}^P * X_{PAO} \qquad \text{eq 6.11}$$

$$r_{PHA,ANOX} = q_S^{max} * \eta_{NO3}^P * \frac{S_A}{K_A^P + S_A} * I_{O2}^P * M_{NO3}^P * M_{PP}^P * X_{PAO} \qquad \text{eq 6.12}$$

The scenario shown in Figure 6.2-8 represents a situation, in which the presence of acetate in the anoxic zone leads to phosphorus release. Based on mass balances it can be shown that during the first three cycles, the increase in the measured phosphorus concentration exceeds the possible increase just due to dilution. Hence acetate must have been present, resulting in PHB storage and phosphorus release in the reaction tank during the anoxic phases. Simulation, using the model without the process of anoxic acetate uptake (results not shown), were not able to predict the concentration pattern of the first cycles correctly. As a consequence the prediction quality over the whole time period was influenced negatively.

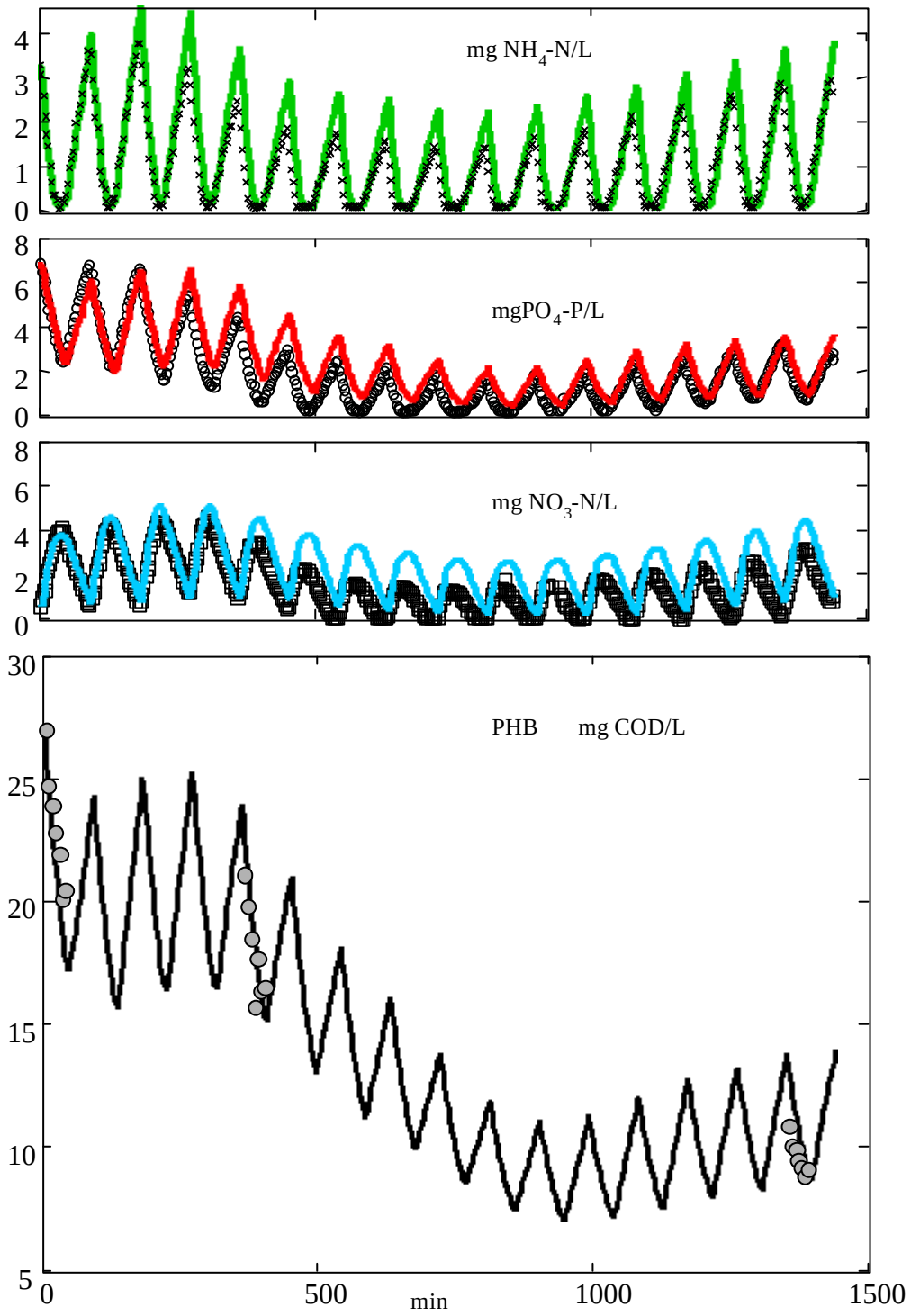


Figure 6.2-8 : Reaction tank of the pilot plant, simulation with the revised model (PHA, poly-P modification and anoxic acetate uptake according to Filipe and Daigger (1997).

Results obtained, using the revised model with anoxic acetate uptake included, are shown in Figure 6.2-8. The stoichiometric parameters used for the anoxic acetate uptake are listed in Table 6.2-4, all other parameters remained unchanged (s. appendix 8.4). As during all pilot plant simulation, ammonia was overestimated, due to a too high predicted ammonia 'production' in the anaerobic phase (s. chapter 6.2.3). Hence also nitrate shows a deviation. This problem should be easily overcome when performing a full calibration. Phosphate and PHB patterns are predicted quite well, clearly demonstrating the need to incorporate anoxic acetate uptake in the model structure, in

addition to the functional relationship between P-uptake and PHB level. Overall the results demonstrate the feasibility of the approach used.

Process	S_A	S_{NO3}	S_{PO4}	X_{PP}	X_{PHA}
anox. PHA storage	-1	$-Y^{DN}_{NO3}$	Y^{DN}_{PO4}	$-Y^{DN}_{PO4}$	Y^{DN}_{PHA}
		-0.04	0.31	-0.31	0.9
	gCOD	gN/gCOD	gP/gCOD	gP/gCOD	gCOD/gCOD

Table 6.2-4. Stoichiometric parameters used for anoxic acetate uptake

Simulation results for a constant external acetate addition to the anoxic zone are shown in Figure 6.2-9. A similar scenario to the one describe in section 5.3 (Exp.C, figure 5.3-7) was created. The pilot plant was simulated with a constant influent composition, reaching satisfactory nutrient removal, upon which a NH_4-N shock load was applied (by doubling the NH4-N inlet concentration) with the intention to cause a nitrate accumulation. After reaching a nitrate concentration of the same order as in the experiment, the constant addition of acetate (0.6 mg COD/L_R min) was initiated. Figure 6.2-9 exhibits the same qualitative behaviour, as observed during the experiments: upon the start of the external acetate addition the denitrification rate increases significantly due to a higher activity of denitrifiers and PAO. At the same time the slope of the increase in phosphate concentration during anoxic conditions increases, as anoxic acetate uptake induces a certain phosphorus release. The P-release increases considerably as soon as all nitrate is consumed (bending point) and anaerobic conditions are established. The dynamics of PHB are also shown and found to follow the phosphate dynamics as long as acetate is added. Due to the increase in the PHB level (5-10%/cycle), the phosphorus uptake rates are also increasing. Once the acetate addition is turned off, the phosphate concentration level drops rapidly to its former level, whereas the decline in PHB concentration is slower and its concentration remains for the 4 subsequent cycles at a higher level than before the acetate addition.

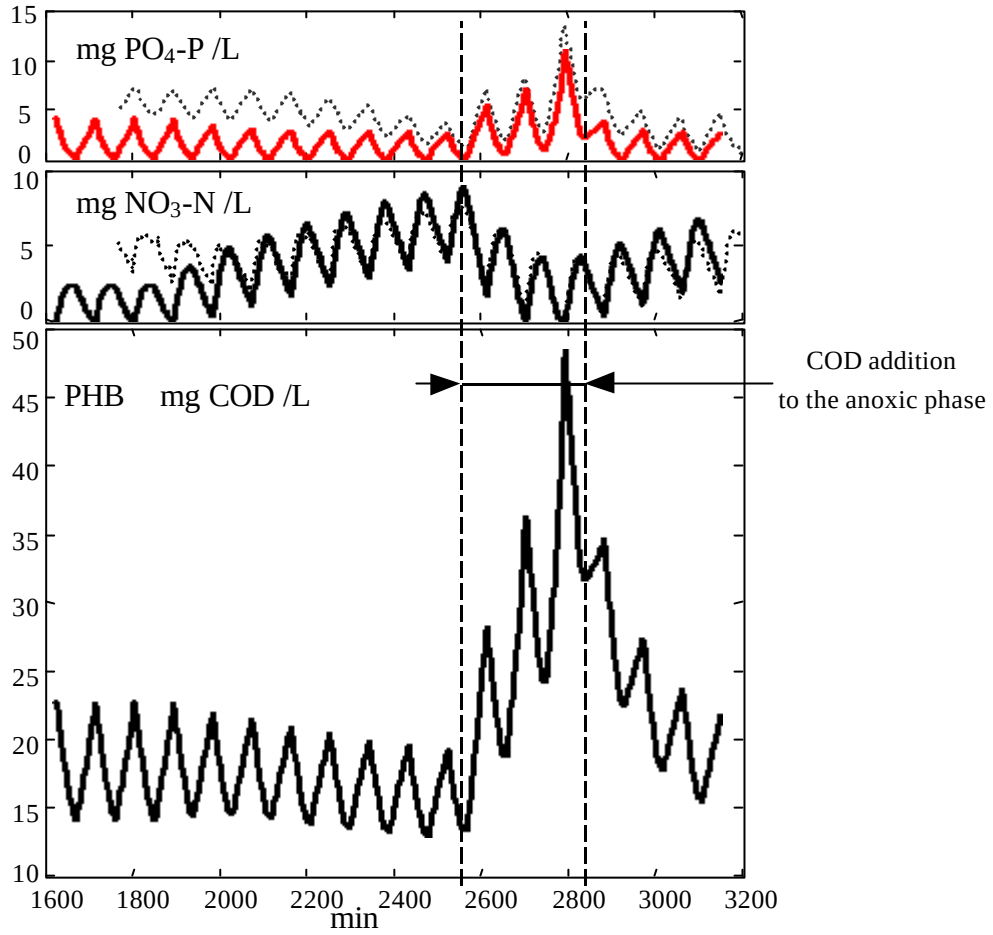


Figure 6.2-9 Simulation study : external acetate addition to the anoxic phase of the reaction tanks. Dotted line : measurements from a similar experiment.

The intention was to illustrate the capability of the modified model to capture qualitatively the effect of continuous acetate addition. Although no attempt has been made to fit the simulation to the experimental data from section 5.3, the revised model exhibits concentration patterns very close to the ones experimentally observed (see dotted line in Figure 6.2-9). Simulations with a constant addition below 0.1 mg COD(HAc)/ L_R min (results not shown) resulted in negligible increase of the phosphorus concentration in the effluent within the firsts cycles (0.3 mg P/L), and subsequently exhibited an improved and stable P-removal. Decrease in the removal efficiency occurred only when nitrate was used up before the end of the anoxic period, so that anaerobic conditions arose, or if addition rates above 0.4 mgCOD/L_R min were applied. All simulation results, obtained so far, reflect to a high extent the conclusions drawn in the experimental part (s. chapter 5), i.e. the possibility to increase the denitrification capacity without detrimental effect on the BPR process by anoxic acetate addition. This aspect underlines once more the feasibility of the approach applied here. Once calibrated properly, the model should not only be more accurate, but also feasible for implementation in control strategies involving external addition of acetate to the process.

6.2.2.1 Discussion of the approach for anoxic acetate uptake

Filipe and Daigger (1997) developed the stoichiometric model, using a metabolic approach, including observations and results reported in literature (Chuang *et al.*, (1996), Kuba *et al.*, (1994), Wentzel *et al.*, (1989). The model is based on the current understanding of the process, in which the

TCA cycle is expected to be fully operative under anoxic conditions and is regarded as the source for the reducing equivalents. The phosphorus release, induced by acetate uptake, is associated with the need for ATP, necessary to activate acetate to acetyl-CoA and the need for energy to transport acetate through the cell membrane (Smolders *et al.*, 1994a). Furthermore the electron transport chain is fully operative during anoxic growth, and excess reducing equivalents, generated in the TCA cycle, can be used to produce ATP. The stoichiometry proposed by Filipe and Daigger (1997) for the anoxic acetate uptake, shown in eq 6.13, is dependent on three parameters (X, δ, α_1). By setting the P/O ratio (δ) according to observations from Kuba *et al*., (1996c) to 1, and by calculating the amount of ATP necessary to transport 1 mmol-C of acetate (represented by α_1 = 0.11), using results of Smolders *et al*. (1995) and Kuba *et al*. (1996), the authors reduced the unknown parameters to a single one : X, representing the amount of PHB that is accumulated per acetate taken up by the cell.

$$\begin{aligned} &-CH_2O-\left[\left(\alpha_1+0.5X\right)-\delta\left(2-2.25X\right)\right]HPO_3-\left(0.8-0.9X\right)HNO_3 \\ &+\left[\left(1.4-\alpha_1-1.7X\right)+\delta\left(2-2.25X\right)\right]H_2O+XCH_{1.5}O_{0.5}+\left(1-X\right)CO_2 \\ &+\left[\left(\alpha_1+0.5X\right)-\delta\left(2-2.25X\right)\right]H_3PO_4+\left(0.4-0.45X\right)N_2=0 \end{aligned} \qquad \text{eq 6.13}$$

(Filipe and Daigger 1997)

Considering two extreme cases for the source of ATP (no or all ATP is produced in oxidative phosphorylation), the authors calculated the interval for possible values of X to [0.69;0.89] (C-mol PHB / C-mol HAc). Kuba *et al.* (1994) estimated this parameter to be equal to 0.8 mmol-C PHB/mmol-C acetate), being in the predicted range.

Accepting the values for δ and α_1, the stoichiometric parameters of the model used in the simulation can be calculated on COD basis as follows :

α_1=0.11	$Y_{PO4}^{DN}=\left[\left(\alpha_1+0.5X\right)-\delta\left(2-2.25X\right)\right]*\frac{31}{32}$		g P/g COD
$\delta=1$	$Y_{NO3}^{DN}=\left[-\left(0.8-0.9X\right)\right]*\frac{14}{32}$		gN/gCOD
$X\in[0.78;1]$	$Y_{PHA}^{DN}=X*\frac{36}{32}$	with $X\in[0.78;1]$	GCOD/gCOD

The advantage of this approach is that the process of anoxic acetate uptake can be implemented in the model resulting in only one additional parameter. Its values are bounded by the mentioned interval, giving hard constraints for this parameter in automated parameter estimation procedures.

One question that might arise is whether glycogen is involved in this process or not. Not considering glycogen might be a special case of reality, as the contribution of glycogen to energy and $NADH_2$ requirements could be possible. The mechanism (pathways) used, probably depends more or less on the level of ATP, Acetyl-CoA and reducing equivalents Depending on their level (and the requirement for them) the mechanisms, i.e. the sources, might vary (glycogen participation, P-release, oxidative phosphorylation). If the level of Acetyl-CoA is sufficiently high, glycogen might be involved, if not, i.e. at low acetate concentration, contribution of the glycogen pool is possible.

The advantage of accepting no glycogen involvement is the simplification of the distribution of carbon (acetate) in the cell and as a result a process description dependent on only one unknown parameteris obtained. The simulations so far have shown, that using this approach, is adequate to predict experimental data obtained from pilot plant operation.

6.2.3 Aspects of Pilot Plant Simulation and Calibration

Pilot plant simulations were initiated using the ASM2/TUD model and parameters from Brdjanovic (1998) performing steady state simulations in order to obtain an estimate of the unknown states (glycogen) and unknown initial conditions (initial biomass composition). Subsequently, these were used for the simulation of pilot plant behaviour, fitting key parameters and initial conditions to a set of measurements, by performing a rough parameter tuning using a priori knowledge (section 6.2.1). This included rough calibration for VSS and sludge retention time, followed by a stepwise checking for nitrification, denitrification similar as suggested by Henze *et al.*(1998). Aiming at a qualitative investigation, no attempt was made to achieve a full dynamic calibration. Consequently as few parameter as possible were changed from their ASM2 default values. The parameters for phosphorus removal (Y_{PO4}, Y_{PHA}, k_{pp}, k_{GLY}, *p*) were adjusted according to experimental observation (batch tests and pilot plant data). The initial poly-P content was calculated as shown in eq 6.14 :

$$X_{PP,initial} = P_{total} - VSS*0.02 \qquad \text{eq 6.14}$$

The total phosphorus (P_{total}) was measured at the end of the aerobic period, when the phosphorus concentration in the liquid phase was virtually down to 0, thus representing the amount of total phosphorus in the biomass Furthermore it was assumed that 2% of the biomass (as VSS) is phosphorus not bounded as poly-P. Consequently the difference between these two terms can be taken as the poly-P concentration (in mg/L) of the PAO. The value obtained (88 mg P/L) is well in the reasonable range for the system considered. For a sludge sample from an enriched PAO culture 95% of the ash of can be regarded as poly-P (Van Loosdrecht, 1998). This is not applicable to the current system, as the sludge consists of a variety of micro-organisms and inert materials, which would lead to a significant over-estimation of the poly-P content in the system.

Although focus was on the uptake period during this investigation, key parameters for the anaerobic phase (Y_{PO4}, Y_{PHA} and q_{fe}) had to be adjusted according the experimental observation in order to obtain adequate predictions. For the yields of phosphate released to acetate (Y_{PO4}) and for PHA storage to acetate (Y_{PHA}) the average values from the experimental part (s. section 4) were taken. The chosen Y_{PO4} value (0.5 mg P/mg COD) lies within the range of the default value when adjusted to the corresponding pH (Smolders *et al.*, 1994a). Depending whether only PHB or PHA, as a sum of PHB and PHV, was considered in the investigation, the values for Y_{PHA} were found to be 1.0 mg COD (PHB)/mgCOD for PHB and 1.3 mg COD(PHA)/mg COD for PHA. The obtained value for the PHA yield coefficient is lower than the default value (1.5 mg COD(PHA)/mg COD). A possible explanation could be that there is not a strict separation between the possible biochemical models (section 2.1.3). This is also illustrated by the investigation of Pereira *et al.*, (1996), using nuclear magnetic resonance (in vivo 13^C-NMR and 31^P-NMR experiments) to study the pathways behind biological phosphorus removal. They suggest that in addition to the ED pathway (Embden-Doudoroff) also the TCA cycle is still active under anaerobic conditions, leading to lower yield values than obtained theoretically for the ED-pathway alone.

The maximum fermentation rate (q_{fe}) was subsequently adjusted , using acetate, phosphorus and PHB data from the anaerobic phase. A list of the parameter values used is presented in appendix 8.3.

Care should be taken with regard to simulating the dissolved oxygen concentration (DO). Simulation that were performed accidentally with a wrong DO set-point (not shown) predicted the depletion of DO, after switching off the aeration, about 5 min prior to the experimentally observed pattern.

During this time period the simulated ammonia concentration started to rise again, resulting in false ammonia and consequently also false nitrate prediction. This deviation added up to increasingly worse prediction with increasing simulation time. Similar observations were made for phosphorus. In addition, when combining experimental data for ammonia, nitrate and phosphate with the DO-signal, one has to account for the different time constants of the measurements.

Long term simulation have to rely on a proper (full) calibration of the model and on the use of an improved settler model including reaction in the settler and a better description of the settling process. As described in literature (Henze *et al.,* 1998; Brdjanovic, 1998) a detailed wastewater characterisation is a definite prerequisite for a proper calibration. Furthermore an adequate sampling frequency of the inlet wastewater is essential, as relatively fast changes in the $COD_{filtered}$ and HAc content, exhibitting an immediate impact on the BPR dynamics, were observed during this investigation. A sampling frequency of at least every 15 min. for COD and VFA is suggested, unless it can be assured that no sudden changes occur in the system studied.

The present investigation as well as the work from Brdjanovic (1998) revealed that model parameters being able to describe a plant behaviour failed to do so for batch tests. Brdjanovic,(1998) reported inconsistencies with regard to the nitrification process which were similar to the ones observed here. In the present work the specific growth rate of nitrifiers had be increased from 1 (default) to 1.3 1/d for plant prediction. These values exhibited a too high nitrification rate in the batch tests, which could not be compensated by adjusting the initial amount of nitrifiers within a reasonable frame. Similar problems were encountered with regard to phosphorus removal (s. section 6.2.1), where only the initial rates of the batch tests were reflected sufficiently well by the chosen parameters. If results from batch tests are used one should make sure that the conditions applied to the batch experiments are similar to the ones in the plant (PHB level and distribution, s. also section 5.4, length of uptake phase and possible limitation by internal storage pools). In addition, (future) full calibration procedure should make use of experimental glycogen data, which were not available for this investigation. This would also assure correct prediction of this third internal storage pool.

In general, the data scarcity induces an important problem for the parameter estimation step. Identifiability of model parameters, i.e. the possibility to give a unique value to each parameter in a mathematical model, is a problem in each bioprocess modelling effort (e.g. Holmberg, 1982; Jeppsson and Olsson, 1993; Nihtila and Virkkunen 1977). The parameters presented here represent a good initial 'guess' for automated estimation procedures, reducing the time needed to find the set of parameters, which is fulfilling the chosen criteria.

6.3 Assuming the Existence of 2 PAO groups – Simulation study

Experimental results from literature (e.g. Kerrn-Jespersen and Henze, 1993; Bortone *et al.*, 1996; Meinhold *et al.*, 1999 (section 4.2)) suggest the possibility of two different groups of PAO, which differ in their ability to use either only oxygen (O2-PAO) or oxygen and nitrate (DNPAO) as electron acceptor. Simulation studies reported in literature (e.g. Filipe and Daigger , 1997) did not succeed in maintaining both groups in the system, as, depending on the sludge retention time, one group was washed out.

In this section of the study an extended model is used to investigate circumstances that might have a potential influence on DNPAO proliferation in the system and to address the difficulties encountered when extending the model to include two groups of PAO.

The model structure and stoichiometry, obtained when extending the revised model from chapter 6.2 to two groups, is presented in appendix 8.5. The approach is based on the current understanding of the mechanism of one group (Murnleitner *et al.*, 1997) and subsequently extended to two groups, using the same kinetic expressions as developed in section 6.2 (incl. the anoxic acetate uptake).

The items addressed in this study are (i) the impact of having a certain amount of DNPAO in the influent and (ii) the effect of the continuous presence of acetate in the anoxic phase. It is accepted that a certain amount of 'normal' denitrifiers are found in most inlet waters (e.g. Henze *et al.*, 1995). As it seems likely that a fraction of these denitrifiers is capable of inducing BPR activity, the presence of DNPAO in the inlet water represents a reasonable assumption. Concerning the second aspect, the operation of the BioDenipho pilot plant has illustrated that acetate or at least BPR inducing substrate might well be present in the anoxic stage (e.g. Figure 6.2-8). Hence, both circumstances can occur on a regular basis in real scale and might impose a significant impact on the development of the PAO distribution in the system.

Simulations

Two simulation sets were performed, differing mainly in the applied value for the yield coefficient Y_{PHA} (PHA storage to acetate utilised) for anaerobic conditions. In set 1 the theoretical value of 1.5 gCOD/gCOD (Brdjanovic,1998) was applied, whereas in set 2 Y_{PHA} was lowered to 1.3, representing the average of the experimental values determined (section 4.1). Table 6.3-1 list the exact scenarios applied in each simulation set.

Table 6.3-1. Simulation sets and applied scenarios

Set		Scenarios		Parameter
1	a)	No DNPAO in the inlet;	no external acetate addition.	default, Y_{PHA} = 1.5
	b)	1mg DNPAO2 in the inlet; [2)]	no external acetate addition.	
	c)	No DNPAO in the inlet;	anoxic acetate addition. [1)]	
2	a)	No DNPAO in the inlet;	no external acetate addition	default, Y_{PHA} = 1.3
	b)	2mg DNPAO2 in the inlet; [2)]	no external acetate addition.	
	c)	No DNPAO in the inlet;	anoxic acetate addition. [1)]	
	d)	2mg DNPAO2 in the inlet; [2)]	anoxic acetate addition. [1)]	

[1)] as 0.1mg COD_{SA}/L_R min to the anoxic phase [2)] as mg COD_{DNPAO}/L

Simulations were performed for the BioDeniPho plant (s. appendix 8.4) with a steady influent in order to better identify the impacts of the scenarios investigated. Plant parameters applied were the

ones listed in appendix 8.4. Focus in this study is put on the distribution of O2PAO and DNPAO, including their internal storage pools, after having simulated a time period corresponding to at least three to six sludge ages (21 d).

As the experimental data reflect only the sum of PAO and their PHA content, it cannot be used directly to determine the initial conditions concerning the BPR related variables of the 2 group model. Hence, the initial conditions were assumed equal for both groups of PAO, to avoid favouring one group by the choice of the initial conditions. Thereby it was verified, that the sum of the corresponding components were in the range of the ones observed either during simulation with one group of PAO (section 6.2) or/and during the experimental phases (section 5 and 6).

In Table 6.3-2 the applied inlet- and initial conditions are presented. The kinetic parameters employed are listed in appendix 8.5 and correspond, with the exception of Y_{PHA}, to the ones determined in section 6.2.

Table 6.3-2. Inlet conditions and important initial conditions for simulation set 1 and set 2

Plant							
Return flow rate	1.5	L/min	Sludge age	21 d			
Inlet flow rate	1.5	L/min	Temperature	20°C			
					Initial		
Influent			*Influent*		*Anaerobic*	*Tank 1*	
So	0	mg/L	Xh	10	1000	1000	mg COD /L
Sf	190	mg COD /L	XO2PAO	0	350	350	mg COD /L
Sa	50	mg COD /L	XO2pp	0	100	70	mg P /L
Snh4	30	mg N/L	XO2pha	0	60	25	mg COD /L
Sno3	0	mg N/L	XO2gly	0	100	70	mg COD /L
Spo4	4	mg P/L	XDNPAO	0[1]	350	350	mg COD /L
Si	0.4*Sa	mg COD /L	XDNpp	0	100	70	mg P /L
Salk	0.4*Sa	mg COD /L	XDNpha	0	60	25	mg COD /L
Xi	0.35*Sa	mg COD /L	XDNgly	0	100	70	mg COD /L
Xs	2*Sa	mg COD /L	Xaut	0	75	75	mg COD /L
			Xtss	10	2400-2900	2400	mg COD /L

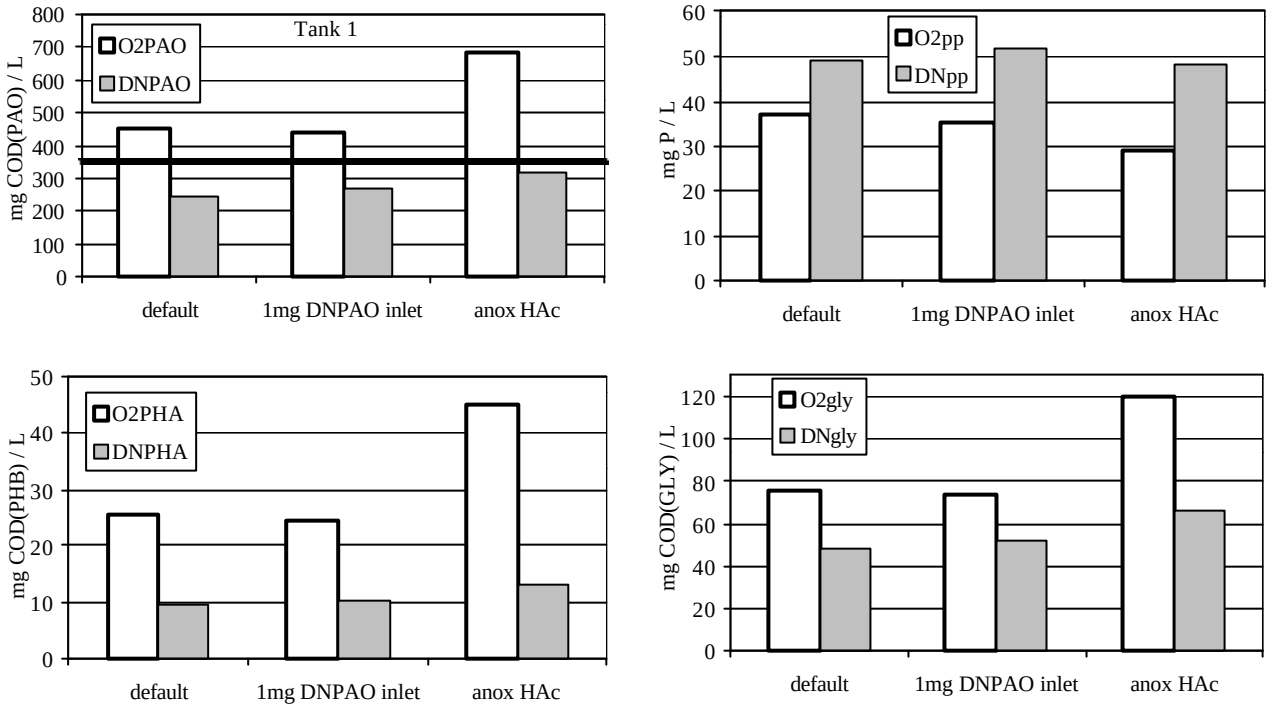
[1] subject to change, depending on the scenario simulated

Results

The results of simulation set 1 are depicted in Figure 6.3-1, showing the final concentrations of the PAO groups and their storage compounds in one reaction tank (end of the anoxic phase) after simulating for 65 days. The simulations were stopped at this time, as the results underline the tendency for a decrease in the amount of DNPAO for the three scenarios, although steady state is not yet reached at this point. The difference between the two groups becomes greater with ongoing simulation time, finally leading to a wash-out of the DNPAO (simulation not shown).

According to the model, the evolution of the two groups, i.e. their growth, relies only on the rate of PHA lysis, being dependent on the PHA level itself. Hence, two factors play an important role in the competition of the two groups: 1) the amount of PHA being built up and 2) the effectiveness in using the PHA for growth, i.e. the yield coefficient of PHA to biomass. The O2-PAO are assumed to store PHA under anaerobic and anoxic conditions with the same rate. The DNPAO store PHA under anoxic conditions at a reduced rate, thus building up a smaller PHA pool than the O2-PAO within the same time period. Furthermore, the yield coefficient (PHA/biomass) is reduced under anoxic

conditions compared to aerobic ones (s. also Copp and Dold, 1998). As a consequence, the ability of the DNPAO to use nitrate as an electron acceptor represents rather a disadvantage within the model. This is reflected by the results shown in Figure 6.3-1, exhibiting a significant smaller amount of PHA stored by the DNPAO and hence also a smaller amount of DNPAO in the system. This accounts for all three scenarios presented. Although it seems that, adding DNPAO to the inlet (1mg COD_{DNPAO}/L) or adding acetate to the anoxic zone, has a slight positive impact of the evolution of DNPAO in the system, both scenarios also resulted in a wash-out of DNPAO after longer simulation time.



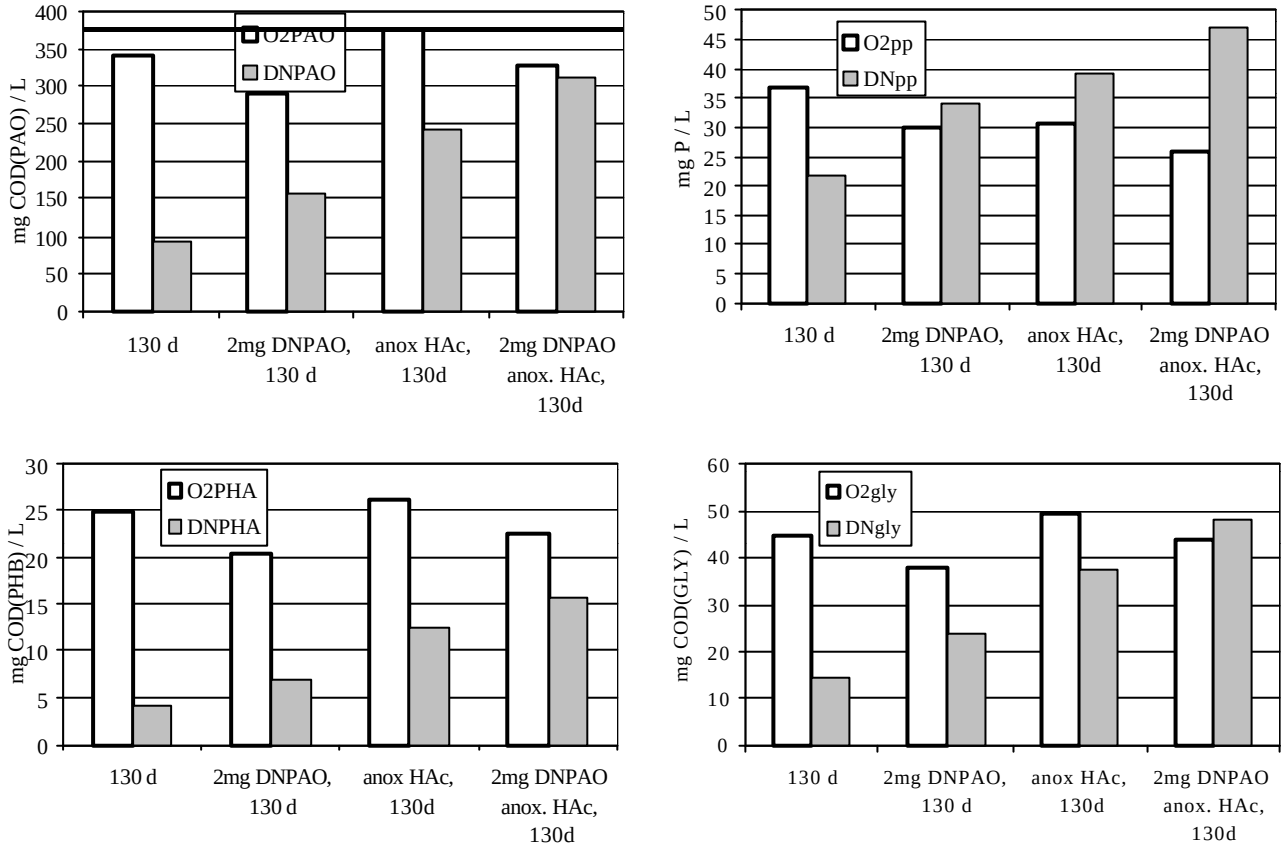
default	No DNPAO in the inlet, no external acetate addition to the anoxic phase
1mgDNPAO :	1mg COD_{DNPAO}/L in the influent, no external acetate addition
anox. HAc:	No DNPAO in the inlet; 0.1mg COD_{SA}/L_R min to the anoxic phase.

Figure 6.3-1. **2 group - Simulation set 1**: PAO, PHA, poly-P and glycogen distribution in one reaction tank, after simulating for 65 days, using default parameters and Y_{PHA}=1.5. Black line = initial value of DNPAO & O2PAO.

For the second set of simulations, the yield coefficient of anaerobic PHA storage from acetate (and the anoxic one for O2PAO) was set to Y_{PHA}=1.3 g COD_{PHA}/g COD_{HAc}, as determined during the experimental work. This will reduce the disadvantage of the DNPAO (Y_{PHA}^{DN}=0.9 g COD/g COD) during anoxic PHA storage to a certain extent. However, this alone is not expected to prevent the wash-out of DNPAO. The simulations included as scenarios a) certain amount of DNPAO in the inlet, b) presence of acetate in the anoxic zone and c) a combination of scenario a) and b). The amount of DNPAO in the inlet (2 mg/L) was doubled compared to simulation set 1, but still remained within a realistic range. In Figure 6.3-2 the results of the simulations, concerning the distribution of PAO and their internal storage pools, are depicted again for one reaction tank (end of anoxic phase). The simulation time was prolonged (130 days) compared to simulation set 1, in order to better identify the evolution of the variables of concern.

Reducing the value for Y_{PHA} prolongs the time period, that DNPAO are able to stay in the system, but, as expected, final wash out is not prevented. This tendency can be clearly seen for the '*default*' scenario. It is not so evident for the scenarios '*2mgDNPAO* 'and '*anox. HAc*', but prolonged simulations (not shown) exhibited an ongoing decrease in the DNPAO fraction. Hence, the assumption of a complete wash-out when reaching steady state seems very reasonable. However, comparing the results of these two scenarios with the '*default*' one, illustrates that the DNPAO benefit to a certain extent from these imposed conditions.

The O2PAO generally manage to stay in the system, but exhibit a slight decrease from their initial value, except for the scenario with anoxic acetate addition. This behaviour can also mainly be attributed to the lower Y_{PHA} value applied during simulation.



default	No DNPAO in the inlet, no external acetate addition to the anoxic phase
2mgDNPAO :	2mg COD_{DNPAO}/L in the influent, no external acetate addition
anox. HAc:	No DNPAO in the inlet; 0.1mg COD_{SA}/L_R min to the anoxic phase.
2mg DNPAO, anox. HAc	2 mg COD_{DNPAO}/L in the influent; 0.1mg COD_{SA}/L_R min to the anoxic phase

Figure 6.3-2. **2 group - Simulation set 2**: PAO, PHA, poly-P and glycogen distribution in one reaction tank, after simulating for 130 days, Y_{PHA}=1.3. Black line = initial value of DNPAO & O2PAO.

The most interesting results are obtained for the combination of anoxic acetate addition with DNPAO entering the system with the influent. Only a marginal difference between the amount of O2PAO and DNPAO are predicted. Whereas the level of PHA stored by the DNPAO is still smaller than the one of the O2PAO, the difference between these two levels is considerably reduced compared to the other scenarios. Furthermore it is interesting to note that the DNPAO exhibit for the first time during all simulation trials a larger amount of glycogen stored compared to the O2PAO.

The results of this last scenario indicate strongly that DNPAO entrainement with the influent combined with the presence of acetate in the anoxic zone might play an important role in enhancing the amount of DNPAO in the system. This is supported by the fact that the concentrations of all components, predicted during the simulation, exhibit reasonable values. This, of course, is necessary if conclusions are to be drawn from these results. Figure 6.3-3 shows the model components in reaction tank 1 over the last cycles of the simulation set 2 (of day 130). The influent composition chosen correspond to conditions that should induce satisfactory nutrient removal in the pilot plant. The concentration pattern of ammonia, nitrate plus nitrite and phosphate, depicted in Figure 6.3-3 illustrate that this 'criterion' is fulfilled. Furthermore, the sum of the corresponding components for the two groups of PAO (PHA, poly-P, glycogen and amount of PAO) coincides also well with the levels, observed during pilot plant operation. Moreover, with regard to the estimation procedure for the DNPAO and O2PAO (section 4.2) also the approximate 50% of DNPAO predicted by the simulation are in the appropriate range. Hence, it seems justified to deduct certain tendencies from these simulation results.

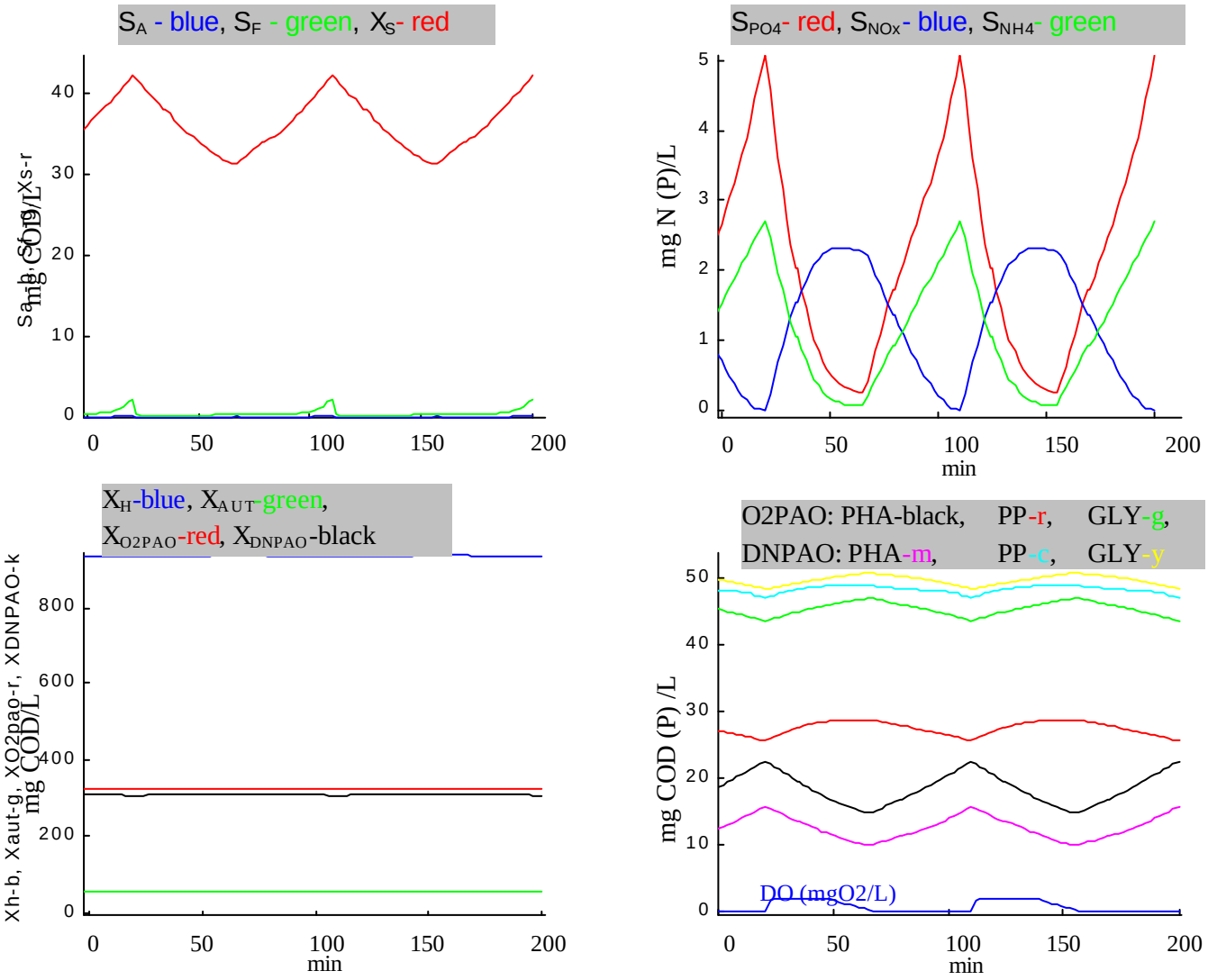


Figure 6.3-3 Concentration patterns in tank 1 one over the last 200 minutes of day 130 of the simulation. Scenario applied: 2mg COD_{DNPAO}/L in the inlet; 0.1mg COD_{SA}/L_R min to the anoxic phase

Although the results of these simulations should be taken as indications and not as definite conclusions, the results obtained strongly point out important aspects, influencing the ability of the

DNPAO to stay in the system. The presence of micro-organisms capable of anoxic P-uptake in the influent seems to be reasonable and exhibits a direct effect on the amount of DNPAO in the system. Moreover, accepting the predicted distribution of the internal storage products, conditions under dynamic operation might arise that favour DNPAO even further. Due to lower amount of poly-P and glycogen stored, the anaerobic PHA uptake of O2PAO might become limited by poly-P or glycogen shortage. The DNPAO, on the other side, are still able to store PHA due to their larger poly-P and glycogen pools and hence gain a temporary advantage over the O2PAO.

Overall, it seems that the presence and the proliferation of DNPAO is not only relying on the appropriate model structure, but is also upon on a series of external impacts, such as the influent composition and its variation in time.

However, with regard to the model structure, the fact that the growth depends only on cell internal storage materials (PHA) represents a severe restriction in the model. There exist no fundamental reason against a direct growth on organic substrate in the presence of an electron acceptor and future research may lead to further extensions. This is particularly important for anoxic conditions, since during these phases it is well possible that organics (VFA) are present, hence inducing temporary advantages for DNPAO.

The extension to two groups of PAO, allows the model to assess and predict situations, in which certain processes connected to BPR, might be limited for only one group but not for the other. In such conditions the macroscopic observed (= measured) response of BPR cannot be explained by a one-group model, whereas the model with DNPAO and O2PAO might be helpful for understanding the underlying cause.

However, currently there are no analytical methods to determine the distribution of internal storage compounds between the two groups. Hence, establishing initial conditions (and subsequent calibration), based on analytical measurements is not possible. In addition, up to date, there exist no ‘100%’ proof of the existence of DNPAO and O2PAO, although several investigations point strongly in this direction (s. section 4.2).

As a consequence, modelling of the two groups remains a theoretical study for now, but clearly illustrating the need for further research.

6.4 Conclusion

An existing model for BPR (combined ASM2/TUD) has been revised regarding its potential to account for the process of anoxic acetate uptake by PAO and for the relationship between phosphate uptake rates and initial PHA level. The rate expression for phosphorus uptake and PHA storage have been modified accordingly. The obtained, revised model exhibits a significant improvement of the prediction capability concerning the dynamics of the dissolved components, NH_4-N, NO_X-N, PO_4-P, as well as the dynamics of the internal storage compound PHA for operation with real municipal wastewater. Essential aspects such as the effect of acetate presence during the anoxic phase on BPR are captured by this model. It has been illustrated that these new proposed extensions are necessary, if the prediction of the nutrient removal processes shall cover as many scenarios as possible, occurring during waste water treatment. The refined model resulted in only 5 additional parameters to be estimated. Their values, used in this study, represent good initial values for a future, possibly automated parameter estimation procedure. Future work should involve testing the model against experimental glycogen data, which were not available for this investigation.

In a second step the revised model has been extended to two groups of PAO, differing in their ability to use either only oxygen (O2-PAO) or oxygen and nitrate (DNPAO) as electron acceptor. Focus during pure simulation studies, was put on external disturbances, that might have a potential impact on the proliferation of the DNPAO. In most cases a wash-out of the DNPAO was predicted by the model. However, the simulations illustrated that the entrainment of DNPAO into the system via the influent combined with the presence of acetate in the anoxic zone, impose an important influence on the ability of the DNPAO to compete successfully with O2PAO.

A situation could be captured in which for example the P-uptake of one group is limited due to different distribution of internal storage products. However, the model applicability is severely restricted due to a lack of measurements for differentiating the distribution of the internal storage pools between the two groups of PAO. This lead to a lack of possibilities for determination of initial conditions and hence also for calibration and validation.

While applying a ‘one-group’ model might be sufficient for most practical applications, the extension to two groups offers a research-tool for improved understanding of the underlying mechanisms. Future research should be performed within this area and should also include the possibility of direct growth on external substrate by PAO, in particular during anoxic conditions, which represents currently a severe restriction in the existing models.

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7 SUMMARY AND CONCLUSION

Biological phosphorus removal (BPR) was investigated, putting the focus on the factors influencing the behaviour of the phosphate accumulating organisms (PAO) under denitrifying conditions. In particular the interactions in the anoxic zone of a combined nitrogen and phosphorus removal activated sludge process were addressed and their consequences on operation and performance evaluated.

The research of this thesis was subdivided into different steps, involving experimental phases as well as model evaluations. Process behaviour and performance were monitored in batch and pilot plant (alternating BioDeniPho type) experiments at different imposed conditions, using liquid phase and internal storage compounds (PHA) measurements. Model evaluation addressed the refinement and modification to be performed for an improved description of the biological nutrient removal process.

- **Experimental work with focus on anoxic conditions and its governing phenomena.**

(1) Dependency of anoxic and aerobic P-uptake on the PHA content.

a) Under anoxic conditions PHB is utilised and phosphate is taken up, which indicates that at least a fraction of the PAO can use nitrate as an electron acceptor for phosphate uptake. Uptake rates under anoxic conditions were found to be 50 to 60 % of the aerobic ones.

b) Aerobic as well as the anoxic P-uptake rates have been shown to be highly dependent on the PHA level in the cells. In this study a saturation effect (max. P-uptake rate) with regard to PHA started to occur at a level of around 0.15 mg COD_{PHB}/mg COD_{PAO}. Maximal aerobic P-uptake rates at these PHA levels during batch tests were in the order of 8 to 9 mg P / (g VSS h) whereas anoxic ones remained below 4 mg P / (g VSS h).

c) Quantitative comparisons of the aerobic P-uptake rates in batch and pilot plant tests revealed lower aerobic P-uptake rates for the pilot plant process at same level of PHA measured in the sludge. This observation presumably relies on the different PHA-distribution in the sludge of the two systems, being based on the difference in the operation mode between a batch reactor (anaerobic/anoxic/aerobic sequence) and the alternating scheme of a BioDeniPho plant.

d) Overall denitrification also improves at higher internally stored PHA level, due to the increased activity of PAO also under anoxic conditions. Contribution of PAO to overall denitrification was quite significant. During batch tests up to 50% of denitrification could be attributed to PAO. In full scale or pilot plant operation the relative contribution of the PAO to denitrification will be less, as denitrification by 'normal' denitrifiers (non PAO) will be higher due to an increased availability of extracellular COD sources during anoxic conditions.

e) The PHA content not or less available for biodegradation was estimated in the order of 0.01 g COD_{PHA}/g COD_{PAO}, being in the same order of magnitude as values reported in literature.

f) A sudden increase in the anaerobic COD load leads to a temporary decrease in the phosphate removal capacity, despite an immediate increase of the measured PHA in the biomass. Evaluation of the PHA utilisation rate and the P-uptake rate indicate, that the yield of PHA to biomass might increase for the PAO upon sudden increase of the COD load, i.e. more carbon is directed to growth, resulting in less PHA available for P-uptake.

(2) Two groups of PAO (DNPAO and O2PAO) and possible ways to assess their activity.

a) Batch results obtained, supported by simulations, strongly substantiate the theory of two groups of PAO. The changes of phosphate and PHA pattern in a sequence of anaerobic-anoxic-aerobic phases could only be explained by the existence of two groups of PAO: the denitrifying part (DNPAO) able to use nitrate and oxygen as electron acceptors, and the second group (O2-PAO) only capable of oxygen utilisation.

b) Several procedures for assessing the two fractions were tested. The method based on the ratio of the initial anoxic and aerobic P-uptake rates exhibited the most reliable results. Provided severe PHA limitation is reduced to a minimum, the method proposed will find best use in detecting changes in the population distribution or anoxic BPR activity, that might take place due to changes in operational strategies. In the period studied the fraction of DNPAO estimated ranged between 40 to 60 %, with an exceptional high of 70 %.

c) The selection of an appropriate time interval for the estimation of P-uptake rates is a key factor that must be taken into account. A fixed procedure should be used to avoid introducing unnecessary variability in the estimation of PAO fractions. For comparison purposes the batch tests should always be performed under identical conditions.

(3) The effect of nitrite, as an intermediate in nitrification and denitrification, on the PAO activity.

a) Only little or no accumulation of nitrite is expected in alternating or re-circulating processes under normal circumstances due to the higher rate of nitrite reduction compared to nitrate reduction.

b) At low concentration levels ($\leq$ 4 mg N/L) nitrite has been shown to be suitable as electron acceptor in a similar manner as nitrate with respect to P-uptake and PHA utilisation. This suggests that the denitrifying fraction of PAO is capable of the entire pathway of nitrate reduction to nitrogen gas. Employing nitrite, nitrate and mixtures of both (with NO_2-N $\leq$ 4 mg N/L) resulted in the same performance with regard to anoxic phosphate uptake rates.

c) At increasing nitrite concentrations severe interference with the PAO metabolism occurs, causing PHA utilisation and anoxic phosphate uptake to cease. A critical nitrite concentration was found to be in the range of 5 to 8 mg NO_2-N/L, being apparently dependent on the sludge conditions. The inhibition is not momentary, but lasts for at least several hours after the nitrite exposure. Aerobic phosphate uptake is damaged severely as well at these NO_2-N levels and the P-uptake stops completely after exposure to slightly higher levels of nitrite. Denitrification rates decreased, as at least the DNPAO stopped contributing to the overall denitrification.

d) BPR at higher nitrite concentrations has been reported in literature. Hence, adaptation of the sludge to nitrite exposure might occur, increasing the acceptable, critical nitrite concentration. But activated sludge systems not acclimatised to nitrite will experience problems in BPR,

when nitrite might accumulate, even momentarily, for example due to discharge of industrial wastes or exposure to high levels of ammonia, favouring nitrite formation.

(4) The impact of an easily degradable substrate present in the anoxic zone on BPR.

a) The introduction of acetate to the denitrifying zone induces in all cases an increase in the denitrification rate. At low acetate addition rates, reduced anoxic P-uptake and PHA utilisation rates are observed compared to conditions when no anoxic acetate is available. At higher acetate addition rates a net P-release and a net storage of PHA may occur. In all cases of anoxic acetate addition less PHA is utilised, thus leading to an increase in the P-uptake rates in the subsequent aerobic phase, due to the higher level of PHA available.

b) Introduction of low levels of organic substrate to the anoxic zone, either due to organic conversions or carry over from the anaerobic zone, do not interfere with the BPR performance. Carry over of BPR promoting organic substrates were detected equivalent to addition rates of up to 0.04 mg COD / (L_R min). A set of experiments were carried out, employing constant low addition rates (≤ 0.1 mg COD_{HAC}/L_R min), resulting in slight improvements of the denitrification and no negative effects on the BPR performance.

c) A critical acetate addition rate, ranging between 0.35 to 0.4 mg COD / (L_R min), was determined for those experiments, in which no anaerobic conditions occurred during the period allocated for denitrification. Higher addition rates lead to an accumulation of phosphate in the system along with a rise of the average level of PHA. Despite the increasing level of PHA, phosphate removal was incomplete.

d) For certain intermediate acetate addition rates, occasionally a net P-uptake was detected along with PHA accumulation. This seems to be due to the fact that under anoxic conditions the denitrifying fraction of PAO do not necessarily need the process of poly-P degradation as a source to fulfil their requirements for energy and reducing equivalents.

♦ **Suitable Operational and control strategies**

(1) Control of denitrification by external COD addition to the anoxic phase.

a) Nitrate accumulation in the system is known to be detrimental to BPR. The implementation of a simple model based control strategy, adjusting the acetate addition rate to the need for denitrification, proved to be feasible to prevent nitrate accumulation in the system. It must be ensured, however, that high addition rates are avoided, at which more phosphate is released during the anoxic phase than taken up in the subsequent aerobic one. A simple trade off routine between phosphate removal and denitrification proved to be very effective to prevent BPR deterioration due to the external acetate addition: in case phosphate accumulation was observed in one cycle, only 70 % of the calculated COD addition rate was applied for the following cycle. Complete P-removal was re-established at once at the expense of a slight increase in the nitrate concentration.

b) Application of the modified control routine prevented accumulation of nitrate in the plant and considerably reduced the amount of NO_X-N recycled with the return sludge. Consequently a minimisation of the substrate competition in the anaerobic zone between denitrifiers and PAO

is ensured, adding to the stability of the nutrient removal performance. Control of the external addition was accurate enough, to avoid excessive acetate addition, i.e. no acetate was carried over to the aerobic phase.

c) During all experiments with controlled addition, complete denitrification down to the set-point was achieved in the reaction tanks. An increase of the denitrification rates of 50 to 80 % was noted, without causing phosphate to accumulate.

d) No negative effects were determined concerning the PAO activity, i.e. aerobic and anoxic P-uptake rates, within the time periods tested (2 to 3 weeks). Furthermore no decrease in DNPAO, determined by the ratio of anoxic/ aerobic P-uptake rates, was induced by the introduction of acetate to the anoxic zone.

(2) Further aspects to include in control/operational strategies for BPR systems.

a) Increased stabilisation of the process can be reached by assuring a high PHA content in the cells, inducing high P-uptake rates. For plant operation these observations / results advise :
   - to avoid unnecessary oxidation of the PHA pool, due to excessive aeration. Control of the dissolved oxygen concentration and of the (adjustable) aeration time supports the prevention of partial depletion of the internal PHA stores.
   - to increase the stability of the process, due to maintaining the internal PHA content at a higher level, by the use of pre-fermenters or hydrolysate to add external BPR promoting substrate to the inlet of the process (anaerobic or anoxic zones).

b) Sudden increases in the inlet COD load lead to temporary deterioration of BPR performance:
   - The use of preceding equalisation tanks can reduce the fluctuation of the COD load and thus counteract the BPR deterioration due to sudden COD increase in the influent (e.g. or dilution (rain events) or after low loading during weekends).
   - When adding external BPR promoting substrate to stabilise the process, e.g. use of pre-fermenters or hydrolysate, a sudden increase in the COD load should be avoided, i.e. the addition should be performed continuously with a slowly rising rate, instead of allowing a step upward in the COD load.

♦ Model evaluation and modification

Model refinement and modification have been performed based the combination of ASM2 (Activated Sludge Model No 2) and the TU Delft model.

(1) Modelling one group of PAO – essential aspects for improved prediction capability.

a) Model refinement addressed its potential to account for the process of anoxic acetate uptake by PAO and for the relationship between phosphate uptake rates and internal PHA level. The rate expressions for phosphorus uptake and PHA storage have been modified in order to reflect the two aspects mentioned above. Significant improvement of the prediction capability was obtained, being able to capture situations like the limitation of P-removal due to low PHA content and the impact on BPR by the carry-over of BPR promoting organic substrates from the anaerobic zone. Both scenarios are equally important for correct model predictions, as they are encountered on a regular basis during the operation with real municipal wastewater.

b) The refining of the model resulted in 5 additional parameters to be estimated: 2 kinetic and three stoichiometric ones. As the focus was put on the qualitative ability of the model to represent the specific interactions, a full calibration was not performed. However, only few parameters had to be adjusted to obtain good agreement with the observed pilot plant behaviour. The values presented in this study represent good initial values for a future, possibly automated, parameter estimation procedure.

c) With respect to operation and control including the external carbon source addition to the anoxic zone, the presented model represents a valuable base for implementation in advanced model predictive control strategies.

(2) Simulating two groups of PAO – influences on the proliferation of DNPAO in the system.

a) The refined model has been extended to two groups of PAO. Applying constant influent conditions, realistic results were achieved concerning soluble and particulate components compared to measurements from the pilot plant. Pure simulations illustrate, that the entrainment of DNPAO into the system via the influent combined with the presence of acetate in the anoxic zone, impose an important influence on the ability of the DNPAO to compete successfully with O2PAO.
Furthermore the results indicate that variation in the influent might induce a temporary advantage for the DNPAO: due to the higher amount of glycogen and poly-P stored by DNPAO, there is less risk for a limitation of the acetate uptake process compared to O2PAO.

b) The 2-group model offers new explanations for certain critical situations, due to different distribution of internal storage products. However, the applicability is severely restricted due to lack of measurements to differentiate the distribution of the internal storage pools between the two groupsw of PAO. This leads to a lack of possibilities for determination of initial conditions and hence also for calibration and validation. Hence, the application of the model with 2 groups remains restricted to theoretical investigations, still offering a research-tool for improved understanding of the underlying cause and effect relationships.

♦ Suggestions for Future Research

(1) Further research is needed concerning the behaviour of PAO upon sudden increases of the COD load in the inlet. Experimental investigation should be directed to the subject of possible unbalanced growth of PAO, i.e. a change in the metabolic carbon flow upon changes in substrate availability.

(2) Investigation concerning the biochemical mechanism of PAO for the usage of nitrite as well as for the inhibition cases is desirable, requiring defined experimental conditions such as known substrate and biomass composition and measurement of the corresponding variables.

(3) Control and operational strategies:

a) The long term effect of the controlled external carbon source addition to the anoxic zone on BPR and on the microbial composition of the sludge will have to be further evaluated. This includes addressing the question if a stabilisation effect can be achieved on a long term basis.

b) Integration of the proposed control method of external C-addition in a more global control strategy for BPR should be evaluated, i.e. combination with aeration control and/or equalisation.

c) The return sludge rate might also offer the possibility to counteract a certain COD load increase, as the same load is distributed to more sludge within the same time interval. This strategy is of course limited by constraints from plant operation (sludge blanket etc.) and its impact on the other compartments of the plant have to be evaluated carefully.

(4) Mathematical modelling of BPR in activated sludge,

a) Future work should involve full calibration of the proposed, refined model, including testing against experimental glycogen data, which were not available for this investigation.

b) The model structure should be re-evaluated with respect to the possibility of direct growth on external substrate by PAO, in particular during anoxic conditions, which represents currently a severe restriction in the existing models. These investigations will require adequate experimental research.

(5) 2 groups of PAO

a) No definite techniques are yet available to access the microbial groups responsible for BPR, but new microbial techniques, such as in-situ analysis via gene probes etc., might be useful in order to specify exactly the microbial distribution and to clarify the hypothesis of 2 major groups of PAO.

b) In case the existence of DNPAO and O2PAO can be proven and analytical methods to determine their quantity and their internal storage pools are established, the efforts to model BPR with 2 groups of PAO should be intensified.

8 APPENDIX

8.1 List of Abbreviations

ADP	adenosine diphosphate
ASM1, ASM2, ASM3	Activated sludge models *No. 1* (= Henze et al., 1987), *No. 2* (= Henze et al., 1995 a), *No. 3* (= Gujer et al., 1998)
ATP	adenosine triphosphate
BPR	Biological Phosphorus Removal
C_i	concentration in the liquid phase of dissolved component i
COD	chemical oxygen demand
DNPAO	denitrifying phosphate accumulating organisms
EMP-pathway	Embden-Meyerhof-Parnas pathway (glycosis)
ETC	(respiratory) electron transport chain
FIA	flow injection analysis
GLY	glycogen
NADH	nicotinamide adenine dinucleotide
O2PAO	fraction of PAO only able to use oxygen as an electron-acceptor
PAO	Phosphate Accumulating Organism
PHA	polyhydroxyalkanoates, organic storage product in PAO
PHB	poly-β-hydroxybutyrate, organic storage product in PAO
PHV	poly-β-hydroxyvalerate, organic storage product in PAO
P_I or PO_4-P	inorganic phosphate, orthophosphate (H_nPO4_{n-3})
P_t	total phosphate (organic and inorganic compounds)
SBR	sequencing batch reactor
SCFA	short chain fatty acid
SS	Suspended solids
TCA-cycle	tri carboxylic acid cycle
VFA	Volatile fatty acids
VSS	Volatile suspended solids

8.2 Experimental Facilities

8.2.1 Pilot Plant

The pilot plant utilised in this study consists of a pilot scale BIODENIPHO plant. This is a biological C-N-P removal activated sludge process, which is based on an alternating operation principle and is a registered trademark of Krüger A/S, Denmark,. A scheme of the plant is shown in Figure 8.2-1. AN is a 200 l vertical cylindrical-tube anaerobic reactor and SE is a 1000 l final settler. T1 and T2 are two 800 l aerobic/anoxic reactors equipped with mechanical agitators and air diffusers. On/off control is employed to maintain dissolved oxygen during an aerated period around a setpoint of 2 mg O_2/l. Real waste water obtained from the Lundtofte treatment plant serves as feed to the plant. Incoming wastewater and return-sludge are mixed in 1:1 volumetric ratio before entering the anaerobic column. The two sequential steps in N removal, nitrification (aerated) and denitrification (no aeration), are performed alternating in the two reactors T1 and T2 by periodically adjusting the flow path and aeration pattern according to a cyclic operating schedule. The four phase schedule employed in the pilot plant is also shown in Figure 8.2-1. During the work performed in this study the total period of aeration within one cycle was set to 30 minutes (instead of 45 min.), thereby avoiding the risk of dissolved oxygen being carried over into the phase allocated for denitrification.

In addition to dissolved oxygen, pH (end of anaerobic column) and temperature, nutrient concentrations are automatically measured in the pilot plant at four different locations in the process (inlet, exit of anaerobic zone, in one tank, effluent), using a FIA measurement system described below.

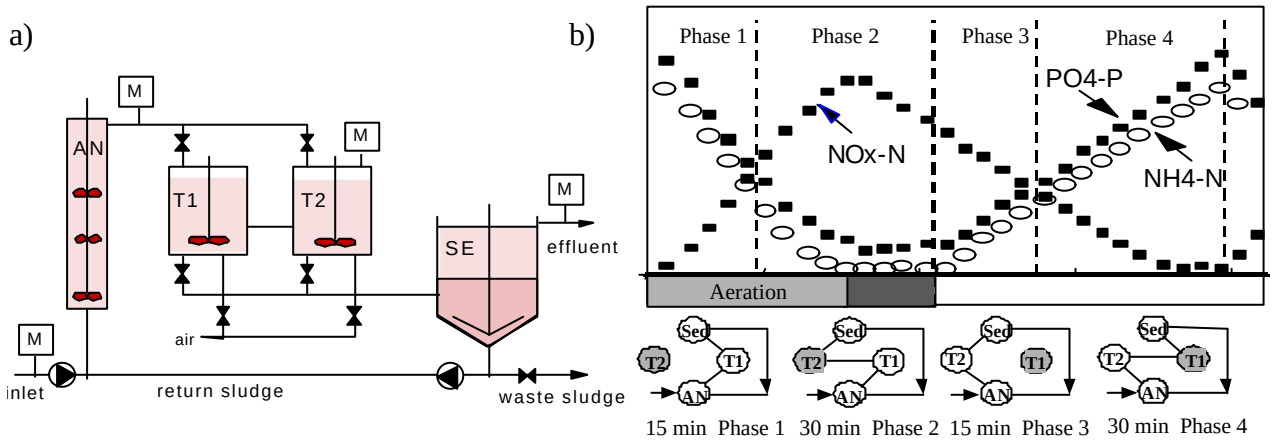

Figure 8.2-1.: a) Pilot scale Biodenipho plant. **M** denotes FIA measurement points.
b) Nutrient concentrations in **tank 2**; 4 phase cyclic operating schedule. A shaded reactor is nitrifying (aerated) and an unshaded tank is denitrifying (anoxic).

The excess sludge withdrawal was automated, performed discontinuously during 24 h according to the set point (amount of sludge to be withdrawn per day) set in the supervising control system.
Further operating parameters are given in Tab. 8-1 below.

Tab. 8-1 Operating parameters of the pilot plant (average values)

Sludge age	20 – 22 d	Average temperature	18 - 20 °C
Feed and return flow	1.5 L/min	Ratio SS_{tank} / $SS_{settler}$	2

8.2.2 Experimental Batch Set-up

In addition to the pilot plant, a laboratory scale setup consisting of batch reactors was utilised for studies requiring more defined conditions. The experimental set-up is illustrated in Figure 8.2-2 and consisted of four 5 litre Plexiglas cylindrical batch reactors. Each reactor was equipped with a motor driven stirrer. The reactors were covered with a Plexiglas lid but were not airtight. During the course of an experiment nitrogen gas was sparged to just above the liquid surface to exclude atmospheric oxygen and maintain anaerobic/ anoxic conditions. Aerobic periods were initiated by sparging compressed air through a diffuser at the bottom of the reactors. Chemical addition was performed by pipette or, in case of continuous addition, with a calibrated peristaltic pump. The pH was manually controlled to 7.0±0.1 through additions of 1.0 M HCl or 0.5 M NaOH throughout the course of the experiments. A pH of 7 was chosen to minimise chemical precipitation of phosphate based on an experimental study indicating that phosphate disappearance due to precipitation in this experimental set-up is minor if pH does not rise much above 7. The temperature of the bulk liquid during the course of the experiments remained constant and varied for all experiments between 18 an 20°C.

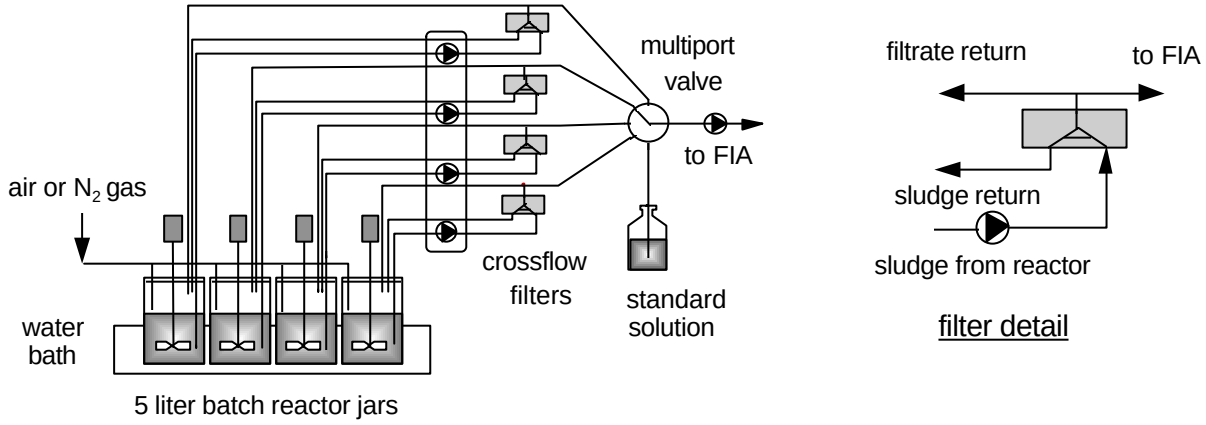

Figure 8.2-2. Schematic diagram of the experimental batch set-up

Automatic measurement of nitrate plus nitrite (NO_x-N), phosphate (PO_4-P) and ammonia (NH_4-N) or nitrite (NO_2-N), was performed using a modified version of flow injection analysis (FIA). Mixed liquor from each reactor was continuously pumped through a crossflow filter unit (pump: 4 channel Watson Marlow 505S; peristaltic tubing: 6.4 × 1.6 Maprene; transport tubing: 5 × 1.5 PVC; filter membrane: DOW Denmark ETNA20A; filter area: 36 cm^2; all tubing sizes are bore diameter × wall thickness in mm.) and back to the reactor. The filtrate from each filter unit was selected for analysis in turn by means of a multiposition valve. When not selected for injection, the filtrate was returned to the reactor from where it originated by means of 1.6 × 0.8 PFTE tubes. These tubes also served as sample storage buffers since the filtrate flowrates normally were slightly less than the pumping rate to the FIA system. The FIA system measured all three species in a given sample in parallel every 1.5 minutes. The four reactors were measured periodically in turn along with a standard solution (adapted to the range applied in the experiments), giving a measurement frequency for each reactor of 7.5 minutes (6 minutes when only 3 reactors were employed).

Unless indicated differently, the protocol common to each batch experiment was as follows. Activated sludge was obtained from a Biodenipho^{TM} pilot plant treating municipal wastewater. The sludge was obtained on the day each experiment was performed, therefore sludge characteristics

varied somewhat from experiment to experiment. Before taking the sludge, the pilot plant reactor was first isolated without aeration until nitrate was totally consumed. Four liters of sludge were then transferred to each of the four batch reactors, which immediately thereafter were stirred and placed under nitrogen gas. For some experiments the aqueous phosphate concentration level was raised by adding potassium phosphate. Unless indicated differently, most of the experiments were initiated with an anaerobic PHA-uptake/ phosphate-release step by adding sodium acetate (HAc) and maintaining the reactors anaerobic until the phosphate release associated with acetate uptake was complete in all reactors. Subsequently either anoxic or aerobic conditions were initiated.

8.2.3 Automatic Process Monitoring

A monitoring system, based on flow injection analysis (FIA) combined with cross flow filtration for sample preparation has been applied for the simultaneous measurement of ammonia (NH_4-N), oxidized nitrogen (NO_x-N) and phosphate(PO_4-P). Cross-flow filtration ensured that the samples are free of particles (ultrafiltration membrane with a molecular weight cut at 2 x 105 daltons). The significant characteristics of this FIA system include robustness, a low reagent consumption rate, a wide linearity range without a sample dilution step, and a fast measurement cycle, where a measurement of all three analytes is made every 1.5 minutes.

Automatic process monitoring of the pilot scale BIODENIPHO process was performed at four process locations (INlet, at the exit of the ANaerobic zone, in the aeration tank T2, OUTlet,(s. Figure 8.2-1). To provide continually process information at a sufficiently high frequency and with minimal time delay, the following sampling cycle was applied: STD, T2, IN, T2, AN, T2, OUT, T2. As each measurement required only 1.5 minutes, one cycle took approximately 14 minutes, starting with a calibration each time (standard solution). As a consequence, the nutrient concentrations in the reaction tank, T2, are measured every 3 minutes and for the other locations every 14 minutes. Batch experiments were monitored by connecting the batch set-up to the monitoring system, allowing to run 4 reactors in parallel with a measurement point every 7.5 minutes. The quality of the automated 1 point calibration was verified on a regular basis, by performing an external calibration via a standard row measurement. For a detailed description of the set-up of the measurement system and its characteristics the reader is referred to Isaacs and Søeberg (1998).

Measurement principles:

PO_4-P: Phosphate forms a complex with molybdate(VI) ions, which are reduced to the blue molybdate(V) ions in a second step. The intensity of the colour is photometrically measured at 660 nm.

NH_4-N: The analysis relies on ammonia's property as a base. The pH of the sample volume is changed to about 13 by injection into the basic reagent. Due to its pKa value (9.25) all of the dissolved ammonium ions in the sample are converted to ammonia gas ($NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$). As the sample volume passes through the gas diffusion module, a portion of the ammonia gas diffuses through a gas permeable membrane into a buffer/indicator solution (reagent with weak pH buffer, adjusted to pH 6.8, containing a pH sensitive indicator). Ammonia, causes a change in the pH and in the indicator colour, being monitored at 590nm.

NO_X-N: This analyser measures both nitrate and nitrite. In a first step, nitrate is reduced to nitrite by the surface catalytic activity of cadmium-copper-amalgam. Subsequently, nitrite and sulfanilamide from an added reagent react to form a diazonium salt. This salt together with N-(1-naphtyl)-ethylendiamin, is converted to a diazo dye, to be detected by a spectrophotometer at 540 nm.

For the measurements of NO2-N only, the same set up as for nitrate was used, omitting the cadmium column.

8.2.4 Off-line Analysis

PHB and PHV were measured from samples collected manually. The procedure for sample collection consisted of withdrawing 30 ml of mixed liquid followed by immediate centrifugation (3 min. at 3500 rpm) and immediate freezing of the sludge pellet. The pellets were then freeze dried before further analysis. The procedure of the analysis was performed with slight modifications according to Foglia and Henze (1995), being based on Smolders *et al.*, (1994):

> Under mildly acidic conditions, PHB is depolymerised into 3-hydroxybutyrate (3_HB), which is further convertyed into 3-hydroxy propyl ester and extracted into CH_2Cl_2 to be analysed by Gas-liquid chromatography. The lysis of the cells, the depolymerisation of PHB and the esterification of 3-HB occur during one single reaction. The volatile propyl ester extracted in $CH_2Cl_2$is isolated and quantified by GC analysis.

MLSS and MLVSS were determined according to APHA Standard Methods (1985).

Acetate was determined via gas chromatography.

APHA (1985). Standard Methods for Examination of Water and Wastewater. 16th edition, American Public Health Association, Washington D.C.

Foglia A. and Henze M. (1995) Analysis of PHB in Activated Sludge by Gas Chromatography Method - Use of the GC Vega 600. Distributed from the Department of Environmental Engineering at the Technical University of Denmark.

Isaacs S. and Søeberg H. (1998). Flow Injection Analysis for On-line Monitoring of a Wastewater Treatment Plant. In: *Advanced Instrumentation, Data Interpretation and Control of Biotechnological Processes.* Eds. Van Impe J., Vanrolleghem P. and Iserentant D., Kluwer Academic Publishers, Dordrecht, Netherlands, pp.1-39.

Smolders G.J.F., van der Meij J., van Loosdrecht M. C. M. and Heijnen J. J. (1994). Model of the anaerobic metabolism of the biological phosphorus removal process: stoichiometry and pH influence. *Biotechnol. Bioeng.* **42**, 461-470.

8.3 Stoichiometry and Kinetics for Modelling

8.3.1 Stoichiometric Matrix, Coefficients and Parameters

Definition of Stoichiometric Coefficients in the Model Matrix

- Stoechiometric coefficients for S_{NH4}

$c_{123n} = i^N_{XS} - i^N_{SI} * fsi - (1-fsi) * i^N_{SF}$
$c_{46n} = i^N_{Sf} / Y_H - i^N_{BM}$
$c_{57n} = - i^N_{BM}$
$c_{8n} = i^N_{SF}$
$c_{9n} = i^N_{BM} - i^N_{XI} * f_{XIH} - (1-f_{XIH}) * i^N_{XS}$
$c_{12n} = (-i^N_{BM} / Y_{PHA}{}^O)$
$c_{13n} = (i^N_{BM} / Y_{PP}{}^O)$
$c_{14n} = (i^N_{BM} / Y_{GLY}{}^O)$
$c_{15n} = (i^N_{BM} * m^O_{PAO} / m_{O2})$
$c_{16n} = (-i^N_{BM} / Y_{PHA}{}^{NO})$
$c_{17n} = (i^N_{BM} / Y_{PP}{}^{NO})$
$c_{18n} = (i^N_{BM} / Y_{GLY}{}^{NO})$
$c_{19n} = (i^N_{BM} * m^{NO3}_{PAO} / m_{NO3})$
$c_{20n} = - i^N_{BM} - 1/Y_{AUT}$
$c_{21n} = i^N_{BM} - i^N_{XI} * f_{XIA} - i^N_{XS} * (1-f_{XIA})$
$c_{22n} = 0$

- Stoechiometric coefficients for S_{PO4}

$c_{123p} = i^P_{XS} - i^P_{SI} * f_{SI} - (1-f_{SI}) * i^P_{SF}$
$c_{46p} = i^P_{Sf} / Y_H - i^P_{BM}$
$c_{57p} = - i^P_{BM}$
$c_{8p} = i^P_{SF}$
$c_{9p} = i^P_{BM} - i^P_{XI} * f_{XIH} - (1-f_{XIH}) * i^P_{XS}$
$c_{12p} = - i^P_{BM} / Y_{PHA}{}^O$
$c_{13p} = i^P_{BM} / Y_{PP}{}^O - 1$
$c_{14p} = i^P_{BM} / Y_{GLY}{}^O$
$c_{15p} = i^P_{BM} * m^O_{PAO} / m_{O2}$
$c_{16p} = - i^P_{BM} / Y_{PHA}{}^{NO}$
$c_{17p} = i^P_{BM} / Y_{PP}{}^{NO} - 1$
$c_{18p} = i^P_{BM} / Y_{GLY}{}^{NO}$
$c_{19p} = i^P_{BM} * m^{NO3}_{PAO} / m_{NO3}$
$c_{20p} = - i^P_{BM}$
$c_{21p} = i^P_{BM} - i^P_{XI} * f_{XIA} - i^P_{XS} * (1-f_{XIA})$
$c_{22p} = Y^{DN}_{PO4}$

- Stoechiometric coefficients for T_{SS}.

$c_{123t} = -i^{TSS}_{XS};$
$c_{4t} = i^{TSS}_{BM}$
$c_{5t} = i^{TSS}_{BM}$
$c_{6t} = i^{TSS}_{BM}$
$c_{7t} = i^{TSS}_{BM}$
$c_{9t} = i^{TSS}_{XI} * f_{XIH} + i^{TSS}_{XS} * (1-f_{XIH}) - i^{TSS}_{BM}$
$c_{10t} = -3.23 * Y_{PO4} + 0.6 * Y_{PHA} - 0.84 * Y_{GLY}$
$c_{11t} = -3.23$
$c_{12t} = i^{TSS}_{BM} / Y_{PHA}{}^O - 0.6$
$c_{13t} = - i^{TSS}_{BM} / Y_{PP}{}^O + 3.23$
$c_{14t} = - i^{TSS}_{BM} / Y_{GLY}{}^O + 0.84$
$c_{15t} = - i^{TSS}_{BM} * m^O_{PAO} / m_{O2}$
$c_{16t} = i^{TSS}_{BM} / Y_{PHA}{}^{NO} - 0.6$
$c_{17t} = - i^{TSS}_{BM} / Y_{PP}{}^{NO} + 3.23$
$c_{18t} = - i^{TSS}_{BM} / Y_{GLY}{}^{NO} + 0.84$
$c_{19t} = - i^{TSS}_{BM} * m^{NO3}_{PAO} / m_{NO3}$
$c_{20t} = i^{TSS}_{BM}$
$c_{21t} = i^{TSS}_{XI} * fxia + i^{TSS}_{XS} * (1-fxia) - i^{TSS}_{BM}$
$c_{22t} = -3.23 * Y^{DN}_{PO4} + 0.6 * Y^{DN}_{PHA}$

- Stoechiometric coefficients for Salk alkalinity

$c_{123a} = c_{123n}/14 - c_{123p}*(1.5/31)$
$c_{4a} = c_{46n}/14 - c_{46p}*(1.5/31)$
$c_{5a} = c_{57n}/14 - c_{57p}*(1.5/31) + 1/(64*Y_H)$
$c_{6a} = c_{46n}/14 - c_{46p}*(1.5/31) + (1- Y_H)/(14*2.86* Y_H)$
$c_{7a} = c_{57n}/14 - c_{57p}*(1.5/31) + (1- Y_H)/(14*2.86* Y_H) + 1/(64*Y_H)$
$c_{8a} = c_{8n}/14 - c_{8p}*(1.5/31) - 1/64$
$c_{9a} = c_{9n}/14 - c_{9p}*(1.5/31)$
$c_{10a} = -Y_{PO4}*(1.5/31) + 1/64$
$c_{11a} = (-1.5/31)$
$c_{12a} = c_{12n}/14 - c_{12p}*(1.5/31)$
$c_{13a} = c_{13n}/14 - c_{13p}*(1.5/31)$
$c_{14a} = c_{14n}/14 - c_{14p}*(1.5/31)$
$c_{15a} = c_{15n}/14 - c_{15p}*(1.5/31)$
$c_{16a} = c_{16n}/14 - c_{16p}*(1.5/31) - (1- Y_{PHA}^{NO})/(14*2.86* Y_{PHA}^{NO})$
$c_{17a} = c_{17n}/14 - c_{17p}*(1.5/31) + 1/(14*2.86* Y_{PP}^{NO})$
$c_{18a} = c_{18n}/14 - c_{18p}*(1.5/31) + (1- Y_{GLY}^{NO})/(14*2.86* Y_{GLY}^{NO})$
$c_{19a} = c_{19n}/14 - c_{19p}*(1.5/31) + (1/2.86)/14$
$c_{20a} = c_{20n}/14 - c_{20p}*(1.5/31) - (1/Yaut)/14$
$c_{21a} = c_{21n}/14 - c_{21p}*(1.5/31)$
$c_{22a} = -Y_{PO4}^{DN}*(1.5/31) + Y^{DN}_{NO3}/14+1/64$

Stoichiometric Parameters of the Activated Sludge Model valid for 20 C

Default values from literature

	Value	Unit	Definition	Reference
i^{TSS}_{XS}	0.75	gTSS/gCOD	Ratio of TSS to Xs	Henze *et al*., 1995
i^{TSS}_{BM}	0.90	gTSS/gCOD	Ratio of TSS to biomass (X_H, X_{PAO}, X_{AUT})	Henze *et al*., 1995
i^{TSS}_{XI}	0.75	gTSS/gCOD	Ratio of TSS to Xi	Henze *et al*., 1995
i^{N}_{SI}	0.01	gN/gCOD	N content of inert soluble COD (Si)	Henze *et al*., 1995
i^{N}_{SF}	0.03	gN/gCOD	N content of soluble COD (Sf)	Henze *et al*., 1995
i^{N}_{XI}	0.03	gN/gCOD	N content of inert particulate COD (Xi)	Henze *et al*., 1995
i^{N}_{XS}	0.04	gN/gCOD	N content of particulate COD (Xs)	Henze *et al*., 1995
i^{N}_{BM}	0.07	gN/gCOD	N content of biomass (X_H, X_{PAO} and X_{AUT})	Henze *et al*., 1995
i^{P}_{SI}	0.00	gP/gCOD	P content of inert soluble COD (Si)	Henze *et al*., 1995
i^{P}_{Sf}	0.01	gP/gCOD	P content of soluble COD (Sf)	Henze *et al*., 1995
i^{P}_{XI}	0.01	gP/gCOD	P content of inert particulate COD (Xi)	Henze *et al*., 1995
i^{P}_{XS}	0.01	gP/gCOD	P content of particulate COD (Xs)	Henze *et al*., 1995
i^{P}_{BM}	0.02	gP/gCOD	P content of biomass	Henze *et al*., 1995
f_{SI}	0.0	gCOD/gCOD	Fraction of Si from hydrolysis	Henze *et al*., 1995
f_{XIA}	0.1	gCOD/gCOD	Fraction of inert COD from lysis (fxih=fxia)	Henze *et al*., 1995
f_{XIH}	0.1	gCOD/gCOD		Henze *et al*., 1995
Y_H	0.63	gCOD/gCOD	Yield of heterotrophic biomass	Henze *et al*., 1995
Y_{AUT}	0.24	gCOD/gCOD	Yield of autotrophic biomass (Xh)	Henze *et al*., 1995
Y_{PO4}	0.36	gP/gCOD	Yield coeff. (PO4/HAc)	Smolders
Y_{PHA}	1.50	gCOD/gCOD	Yield coeff. (PHA/HAc)	Smolders
Y_{GLY}	0.50	gCOD/gCOD	Yield coeff. (glycogen/HAc)	Smolders
m_{ATP}	0.456	mol_{ATP}/ (mol_{PAO} d)$^{-1}$	ATP consumption for maintenance	Murnleitner *et al*., 1997
δ	1.80	mol/mol	Amount of ATP produced per NADH	Murnleitner *et al*., 1997
Y_{PHA}^{O}	$\frac{1.04*(\eta+1)}{\eta+0.23}$	gCOD/gCOD	Yield coeff. (PHA/biomass; Aerobic)	Murnleitner *et al*., 1997
Y_{PP}^{O}	$\frac{0.9*(\eta+1)}{0.064*\eta+0.446}$	gP/gCOD	Yield coeff. (PP/biomass; Aerobic)	Murnleitner *et al*., 1997
Y_{GLY}^{O}	$\frac{0.93*(\eta+1)}{0.446*(2*\eta+1)}$	gCOD/gCOD	Yield coeff. (GLY/biomass; Aerobic)	Murnleitner *et al*., 1997
Y_{PHA}^{NO}	$\frac{1.04*(0.5*\eta+1)}{0.5*\eta+0.23}$	gCOD/gCOD	Yield coeff. (PHA/biomass; Anoxic)	Murnleitner *et al*., 1997
Y_{PP}^{NO}	$\frac{0.9*(0.5*\eta+1)}{0.06*\eta+0.446}$	gP/gCOD	Yield coeff. (PP/biomass; Anoxic)	Murnleitner *et al*., 1997
Y_{GLY}^{NO}	$\frac{0.93*(0.5*\eta+1)}{0.446*(\eta+1)}$	gCOD/gCOD	Yield coeff. (GLY/biomass; Anoxic)	Murnleitner *et al*., 1997
Y_{NO3}^{DN}	0.4	gN/gCOD	Yield coeff. (NO3/Acetate; Anoxic)	Filipe and Daigger (1997)
Y_{PO4}^{DN}	0.31	gP/gCOD	Yield coeff. (PO4/Acetate; Anoxic)	Filipe and Daigger (1997)
Y_{PHA}^{DN}	0.9	gCOD/gCOD	Yield coeff. (PHA/Acetate; Anoxic)	Filipe and Daigger (1997)

	Process	S_{O2}	S_F	S_A	S_{NH4}	S_{NO3}	S_{PO4}	S_I	S_{ALK}	X_I	X_S	X_H
	1. Aerobic Hydrolysis		$1-f_{SI}$		$c_{1,N}$		$c_{1,P}$	f_{SI}	$c_{1,a}$		-1	
	2. Anoxic Hydrolysis		$1-f_{SI}$		$c_{2,N}$		$c_{2,P}$	f_{SI}	$c_{2,a}$		-1	
	3. Anaerobic Hydrolysis		$1-f_{SI}$		$c_{3,N}$		$c_{3,P}$	f_{SI}	$c_{3,a}$		-1	
	HETEROTROPHIC ORGANISMS : X_H											
	4. Growth on S_F	$1-\frac{1}{Y_H}$	$-\frac{1}{Y_H}$		$c_{4,N}$		$c_{4,P}$		$c_{4,a}$			1
	5. Growth on S_A	$1-\frac{1}{Y_H}$		$-\frac{1}{Y_H}$	$c_{5,N}$		$c_{5,P}$		$c_{5,a}$			1
	6. Denitrification with S_F		$-\frac{1}{Y_H}$		$c_{6,N}$	$\frac{-(1-Y_H)}{2.86 \cdot Y_H}$	$c_{6,P}$		$c_{6,a}$			1
	7. Denitrification with S_A			$-\frac{1}{Y_H}$	$c_{7,N}$	$\frac{-(1-Y_H)}{2.86 \cdot Y_H}$	$c_{7,P}$		$c_{7,a}$			1
	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_{XI}	$1-f_{XI}$	-1
	PHOSPHATE ACCUMULATING ORGANISMS : X_{O2PAO} & X_{DNPAO}											
Anaerobic	**10. Storage X^{O2}_{PHA}**			-1			Y_{PO4}		$c_{10,a}$			
Anaerobic	**10a Storage X^{DN}_{PHA}**			-1			Y_{PO4}		$c_{10,a}$			
Anaerobic	**11. Maintenance X_{O2PAO}**						1		$c_{11,a}$			
Anaerobic	**11a Maintenance X_{DNPAO}**						1		$c_{11a,a}$			
Aerobic	**12. Lysis PHA^{O2PAO}**	$\frac{1}{Y^O_{PHA}}-1$			$c_{12,N}$		$C_{12,P}$		$c_{12,a}$			
Aerobic	**12a Lysis PHA^{DNPAO}**	$\frac{1}{Y^O_{PHA}}-1$			$c_{12a,N}$		$c_{12a,P}$		$c_{12a,a}$			
Aerobic	**13. Storage X^{O2}_{PP}**	$-\frac{1}{Y^O_{PP}}$			$c_{13,N}$		$C_{13,P}$		$c_{13,a}$			
Aerobic	**13a Storage X^{DN}_{PP}**	$-\frac{1}{Y^O_{PP}}$			$c_{13a,N}$		$c_{13a,P}$		$c_{13a,a}$			
Aerobic	**14. X^{O2}_{GLY} formation**	$1-\frac{1}{Y^O_{GLY}}$			$c_{14,N}$		$C_{14,P}$		$c_{14,a}$			
Aerobic	**14a X^{DN}_{GLY} formation**	$1-\frac{1}{Y^O_{GLY}}$			$c_{14a,N}$		$c_{14a,P}$		$c_{14a,a}$			
Aerobic	**15. Maintenance X_{O2PAO}**	-1			$c_{15,N}$		$C_{15,P}$		$c_{15,a}$			
Aerobic	**15a Maintenance X_{DNPAO}**	-1			$c_{15a,N}$		$c_{15a,P}$		$c_{15a,a}$			
	DNPAO											
Anoxic	**16. Lysis PHA^{DNPAO}**				$c_{16,N}$	$\frac{1-Y^{NO}_{PHA}}{2.86\,Y^{NO}_{PHA}}$	$c_{16,P}$		$c_{16,a}$			
Anoxic	**17. Storage X^{DN}_{PP}**				$c_{17,N}$	$-\frac{1}{2.86\,Y^{NO}_{PP}}$	$c_{17,P}$		$c_{17,a}$			
Anoxic	**18. X^{DN}_{GLY} formation**				$c_{18,N}$	$-\frac{1-Y^{NO}_{GLY}}{2.86\,Y^{NO}_{GLY}}$	$c_{18,P}$		$c_{18,a}$			
Anoxic	**19. Maintenance**				$c_{19,N}$	$-\frac{1}{2.86}$	$c_{19,P}$		$c_{19,a}$			
	22 Storage PHA^{DNPAO}			**-1**	$c_{22,N}$	**$-Y^{DN}_{NO3}$**	**Y^{DN}_{PO4}**		**$c_{22,a}$**			
	NITRIFYING ORGANISMS : X_{AUT}											
	20. Growth	$-\frac{4.57-Y_{AUT}}{Y_{AUT}}$			$c_{20,N}$	$\frac{1}{Y_{AUT}}$	$c_{20,P}$		$c_{20,a}$			
	21. Lysis				$c_{21,N}$		$c_{21,P}$		$c_{21,a}$	f_{XI}	$1-f_{XI}$	

	Process	X_{O2PAO}	X^{O2}_{PP}	X^{O2}_{PHA}	X^{O2}_{GLY}	X_{DNPAO}	X^{DN}_{PP}	X^{DN}_{PHA}	X^{DN}_{GLY}	X_{AUT}	X_{TSS}
	1. Aerobic Hydrolysis										$c_{1,t}$
	2. Anoxic Hydrolysis										$c_{1,t}$
	3. Anaerobic Hydrolysis										$c_{1,t}$
	HETEROTROPHIC ORGANISMS : X_H										
	4. Growth on S_F										$c_{4,t}$
	5. Growth on S_A										$c_{5,t}$
	6. Denitrification with S_F										$c_{6,t}$
	7. Denitrification with S_A										$c_{7,t}$
	8. Fermentation										
	9. Lysis										$c_{9,t}$
	PHOSPHATE ACCUMULATING ORGANISMS : X_{O2PAO} & X_{DNPAO}										
Anaerobic	**10. Storage X^{O2}_{PHA}**		$-Y_{PO4}$	Y_{PHA}	$-Y_{GLY}$						$c_{10,t}$
Anaerobic	**10a Storage X^{DN}_{PHA}**						$-Y_{PO4}$	Y_{PHA}	$-Y_{GLY}$		$c_{10a,t}$
Anaerobic	**11. Maintenance X_{O2PAO}**		-1								$c_{11,t}$
Anaerobic	**11a Maintenance X_{DNPAO}**						-1				$c_{11a,t}$
Aerobic	**12. Lysis PHA^{O2PAO}**	$\frac{1}{Y^{O}_{PHA}}$		-1							$c_{12,t}$
Aerobic	**12a Lysis PHA^{DNPAO}**					$\frac{1}{Y^{O}_{PHA}}$		-1			$c_{12a,t}$
Aerobic	**13. Storage X^{O2}_{PP}**	$-\frac{1}{Y^{O}_{PP}}$	1								$c_{13,t}$
Aerobic	**13a Storage X^{DN}_{PP}**					$-\frac{1}{Y^{O}_{PP}}$	1				$c_{13a,t}$
Aerobic	**14. X^{O2}_{GLY} formation**	$-\frac{1}{Y^{O}_{GLY}}$			1						$c_{14,t}$
Aerobic	**14a X^{DN}_{GLY} formation**					$-\frac{1}{Y^{O}_{GLY}}$			1		$c_{14a,t}$
Aerobic	**15. Maintenance X_{O2PAO}**	$-\frac{m^{PAO}_{O2}}{m_{O2}}$									$c_{15,t}$
Aerobic	**15a Maintenance X_{DNPAO}**					$-\frac{m^{PAO}_{O2}}{m_{O2}}$					$c_{15a,t}$
Anoxic	**DNPAO**										
Anoxic	**16. Lysis PHA^{DNPAO}**					$\frac{1}{Y^{NO}_{PHA}}$		-1			$c_{16,t}$
Anoxic	**17. Storage X^{DN}_{PP}**					$-\frac{1}{Y^{NO}_{PP}}$	1				$c_{17,t}$
Anoxic	**18. X^{DN}_{GLY} formation**					$-\frac{1}{Y^{NO}_{GLY}}$			1		$c_{18,t}$
Anoxic	**19. Maintenance**					$-\frac{m^{DNPAO}_{NO3}}{m_{NO3}}$					$c_{19,t}$
Anoxic	**22 Storage PHA^{DNPAO}**						$-Y^{DN}_{PO4}$	Y^{DN}_{PHA}			$c_{22,t}$
	NITRIFYING ORGANISMS : X_{AUT}										
	20. Growth									1	$c_{20,t}$
	21. Lysis									-1	$c_{21,t}$

8.3.2 Rate Equations

Process	Rate equation
	HYDROLYSIS
1. Aerobic hydrolysis	$K_h * M^L_{O2} * \frac{f_S}{K^L_X + f_S} * X_H$
2. Anoxic hydrolysis	$K_h * \eta^L_{NO3} * I^L_{O2} * M^L_{NO3} * \frac{f_S}{K^L_X + f_S} * X_H$
3. Anaerobic hydrolysis	$K_h * \eta_{fe} * I^L_{O2} * I^L_{NO3} * \frac{f_S}{K^L_X + f_S} * X_H$
	HETEROTROPHIC ORGANISMS : X_H
4. Growth on Sf	$\mu_H * \frac{S_{O2}}{K^H_{O2} + S_{O2}} * \frac{S_F}{K_F + S_F} * \frac{S_F}{S_A + S_F} * M^H_{NH4} * M^H_{PO4} * M^H_{ALK} * X_H$
5. Growth on Sa	$\mu_H * \frac{S_{O2}}{K^H_{O2} + S_{O2}} * \frac{S_A}{K^H_A + S_A} * \frac{S_A}{S_A + S_F} * M^H_{NH4} * M^H_{PO4} * M^H_{ALK} * X_H$
6. Denitrification with Sf	$\mu_H * \eta^H_{NO3} * I^H_{O2} * \frac{S_F}{K_F + S_F} * \frac{S_F}{S_A + S_F} * \frac{S_{NO3}}{K^H_{NO3} + S_{NO3}} * M^H_{NH4} * M^H_{PO4} * X_H$
7. Denitrification with Sa	$\mu_H * \eta^H_{NO3} * I^H_{O2} * \frac{S_A}{K^H_A + S_A} * \frac{S_A}{S_A + S_F} * \frac{S_{NO3}}{K^H_{NO3} + S_{NO3}} * M^H_{NH4} * M^H_{PO4} * X_H$
8. Fermentation	$q_{fe} * I^H_{O2} * I^H_{NO3} * \frac{S_F}{K_{fe} + S_F} * M^H_{ALK} * X_H$
9. Lysis	$b_H * X_H$

	AUTOTROPHIC ORGANISMS
20. Growth	$\mu_{AUT} * \frac{S_{O2}}{K^N_{O2} + S_{O2}} * \frac{S_{NH4}}{K^N_{NH4} + S_{NH4}} * M^N_{PO4} * M^N_{ALK} * X_{Aut}$
21. Lysis	$b_{AUT} * X_{AUT}$

NOTE: The rate equation for PAO, below, are given for both groups (O2PAO and DNPAO). The actions to be taken, if one or two groups are to be simulated are the following:

- ❑ 1 group of PAO
- O2PAO are set to zero in the system, i.e. equation 10 to 15 are taken out of the system.
- The reduction factor under anoxic conditions, η^P_{NO3}, has to be adjusted to the system (0.4 in this study).

- ❑ 2 groups of PAO
- Equations for O2PAO (10 to 15) are activated.
- The reduction factor under anoxic conditions, η^P_{NO3}, has to be set to 1.

	PHOSPHATE ACCUMULATING ORGANISMS:
O2-PAO	
10. An PHA storage	$q_S^{max} * \frac{S_A}{K_A^P + S_A} * I_{O2}^P * M_{O,GLY}^P * M_{O,PP}^P * X_{O2PAO}$
11. An maintenance	$m_{AN} * I_{O2}^P * M_{O,PP}^P * X_{O2PAO}$
12. Aerobic lysis of PHA	$k_{PHA} * \frac{S_{O2}}{K_{O2}^P + S_{O2}} * M_{ALK}^P * M_{PO4}^P * \left(f_{PHA}^{O2} - f_{PHA,min}\right)^{2/3} * X_{O2PAO}$
13. Aerobic storage PP	$k_{PP} * \frac{1}{f_{PP}^{O2}} * \frac{S_{O2}}{K_{O2}^P * g_{PP} + S_{O2}} * \frac{S_{PO4}}{K_{PO4}^P + S_{PO4}} * \frac{f_{PHA}^{O2}}{f_{PHA}^{O2} + p} * X_{O2PAO}$
14. Aerobic glyc. formation	$k_{GLY} * \frac{1}{f_{GLY}^{O2}} * (f_{PHA}^{O2})^{2/3} * \frac{S_{O2}}{K_{O2}^P + S_{O2}} * X_{O2PAO}$
15. Aerobic maintenance	$m_{O2} * \frac{S_{O2}}{K_{O2}^P + S_{O2}} * M_{O,PHA}^P * X_{O2PAO}$
DNPAO	
10a. Anaerobic PHA storage	$q_S^{max} * \frac{S_A}{K_A^P + S_A} * I_{O2}^P * \mathbf{I_{NO3}^P} * M_{Dn,GLY}^P * M_{Dn,PP}^P * X_{DNPAO}$
11a. Anaerobic. maintenance	$m_{AN} * I_{O2}^P * I_{NO3}^P * M_{DN,PP}^P * X_{DNPAO}$
12a. Aerobic PHA lysis	$k_{PHA} * \frac{S_{O2}}{K_{O2}^P + S_{O2}} * M_{ALK}^P * M_{PO4}^P * \left(f_{PHA}^{DN} - f_{PHA,min}\right)^{2/3} * X_{DNPAO}$
13a. Aerobic storage PP	$k_{PP} * \frac{1}{f_{PP}^{DN}} * \frac{S_{O2}}{K_{O2}^P * g_{PP} + S_{O2}} * \frac{S_{PO4}}{K_{PO4}^P + S_{PO4}} * \frac{f_{PHA}^{DN}}{f_{PHA}^{DN} + p} * X_{DNPAO}$
14a. Aerobic glyc. formation	$k_{GLY} * \frac{1}{f_{GLY}^{DN}} * (f_{PHA}^{DN})^{2/3} * \frac{S_{O2}}{K_{O2}^P + S_{O2}} * X_{DNPAO}$
15a. Aerobic maintenance	$m_{O2} * \frac{S_{O2}}{K_{O2}^P + S_{O2}} * M_{DN,PHA}^P * X_{DNPAO}$
16. Anoxic PHA lysis	$k_{PHA} * \boldsymbol{h}_{NO3}^P * \left(f_{PHA}^{DN} - f_{PHA,min}\right)^{2/3} * \frac{S_{NO3}}{K_{NO3}^P + S_{NO3}} * I_{O2}^P * M_{ALK}^P * M_{PO4}^P * X_{DNPAO}$
17. Anoxic storage of PP	$k_{PP} * \boldsymbol{h}_{NO3}^P * \frac{1}{f_{PP}^{DN}} * \frac{f_{PHA}^{DN}}{f_{PHA}^{DN} + p} * \frac{S_{NO3}}{K_{NO3}^P * g_{PP} + S_{NO3}} * \frac{S_{PO4}}{K_{PO4}^P + S_{PO4}} * I_{O2}^P * X_{DNPAO}$
18. Anoxic glyc. formation	$k_{GLY} * \boldsymbol{h}_{NO3}^P * \frac{1}{f_{GLY}^{DN}} * (f_{PHA}^{DN})^{2/3} * \frac{S_{NO3}}{K_{NO3}^P + S_{NO3}} * I_{O2}^P * X_{DNPAO}$
19. Anoxic maintenance	$m_{NO3} * \frac{S_{NO3}}{K_{NO3}^P + S_{NO3}} * M_{Dn,PHA}^P * I_{PO2} * X_{DNPAO}$
22. Anoxic HAc uptake	$q_S^{max} * \boldsymbol{h}_{NO3}^P * \frac{S_A}{K_A^P + S_A} * I_{O2}^P * M_{NO3}^P * M_{Dn,PP}^P * X_{DNPAO}$

8.3.3 Switching Functions (Saturation and Inhibition)

$$M^{L}_{O2} = \frac{S_{O2}}{K_{LO2} + S_{O2}} \qquad M^{P}_{O,GLY} = \frac{X^{O2}_{GLY}}{K^{P}_{GLY} + X^{O2}_{GLY}} \qquad M^{P}_{DN,GLY} = \frac{X^{DN}_{GLY}}{K^{P}_{GLY} + X^{DN}_{GLY}}$$

$$M^{L}_{NO3} = \frac{S_{NO3}}{K_{LNO3} + S_{NO3}} \qquad M^{P}_{O,PP} = \frac{X^{O2}_{PP}}{K^{P}_{PP} + X^{O2}_{PP}} \qquad M^{P}_{DN,PP} = \frac{X^{DN}_{PP}}{K^{P}_{PP} + X^{DN}_{PP}}$$

$$M^{N}_{ALK} = \frac{S_{ALK}}{K^{N}_{ALK} + S_{ALK}} \qquad M^{P}_{O,PHA} = \frac{X^{O2}_{PHA}}{K^{P}_{PHA} + X^{O2}_{PHA}} \qquad M^{P}_{DN,PHA} = \frac{X^{DN}_{PHA}}{K^{P}_{PHA} + X^{DN}_{PHA}}$$

$$M^{N}_{PO4} = \frac{S_{PO4}}{K^{N}_{PO4} + S_{PO4}}$$

$$M^{H}_{ALK} = \frac{S_{ALK}}{K^{H}_{ALK} + S_{ALK}} \qquad M^{P}_{ALK} = \frac{S_{ALK}}{K^{P}_{ALK} + S_{ALK}} \qquad M^{P}_{PO4} = \frac{S_{PO4}}{K^{P}_{PO4} + S_{PO4}}$$

$$M^{H}_{NH4} = \frac{S_{NH4}}{K^{H}_{NH4} + S_{NH4}} \qquad M^{P}_{NH4} = \frac{S_{NH4}}{K^{P}_{NH4} + S_{NH4}} \qquad M^{P}_{NO3} = \frac{S_{NO3}}{K^{P}_{NO3} + S_{NO3}}$$

$$M^{H}_{PO4} = \frac{S_{PO4}}{K^{H}_{PO4} + S_{PO4}}$$

$$I^{L}_{O2} = \frac{K^{L}_{O2}}{K^{L}_{O2} + S_{O2}} \qquad I^{H}_{O2} = \frac{K^{H}_{O2}}{K^{H}_{O2} + S_{O2}} \qquad I^{P}_{O2} = \frac{K^{P}_{O2}}{K^{P}_{O2} + S_{O2}}$$

$$I^{L}_{NO3} = \frac{K^{L}_{NO3}}{K^{L}_{NO3} + S_{NO3}} \qquad I^{H}_{NO3} = \frac{K^{H}_{NO3}}{K^{H}_{NO3} + S_{NO3}} \qquad I^{P}_{NO3} = \frac{K^{P}_{NO3}}{K^{P}_{NO3} + S_{NO3}}$$

8.3.3.1 Specific ratios

$$f_S = \frac{X_S}{X_H}$$

$$f^{O2}_{PHA} = \frac{X^{O2}_{PHA}}{X_{O2PAO}} \qquad f^{DN}_{PHA} = \frac{X^{DN}_{PHA}}{X_{DNPAO}}$$

$$f^{O2}_{PP} = \frac{X^{O2}_{PP}}{X_{O2PAO}} \qquad f^{DN}_{PP} = \frac{X^{DN}_{PP}}{X_{DNPAO}}$$

$$f^{O2}_{GLY} = \frac{X^{O2}_{GLY}}{X_{O2PAO}} \qquad f^{DN}_{GLY} = \frac{X^{DN}_{GLY}}{X_{DNPAO}}$$

8.3.4 Kinetic Parameters

Default values from literature

	Value	Unit	Definition	Reference
HYDROLYSIS				
K_h	3	1/d	Hydrolysis rate constant	Henze et al., 1995
η^L_{NO3}	0.6		Anoxic hydrolysis reduction factor	Henze et al., 1995
n_{fe}	0.1		Anaerobic hydrolysis reduction factor	Henze et al., 1995
K^L_{O2}	0.2;	$gO2/m^3$	Saturation/Inhibition coeff. for oxygen	Henze et al., 1995
K^L_{NO3}	0.5	gN/m^3	Saturation/Inhibition coeff. for NOx-N	Henze et al., 1995
K^L_X	0.1	gCOD/gCOD	Saturation coeff. for particulate COD	Henze et al., 1995
HETEROTROPHIC ORGANISMS				
μ_H	6	1/d	Maximal growth rate on substrat	Henze et al., 1995
q_{fe}	3	gCOD/gCOD.d	Maximun fermentation rate	Henze et al., 1995
η^H_{NO3}	0.8		Reduction factor for denitrification	Henze et al., 1995
b_H	0.4	1/d	Lysis rate constant	Henze et al., 1995
K^H_{O2}	0.2	$gO2/m^3$	Saturation/Inhibition coeff. for oxygen	Henze et al., 1995
K_F	4	$gCOD/m^3$	Saturation coeff. for growth on Sf	Henze et al., 1995
K_{fe}	20	$gCOD/m^3$	Saturation coeff. for fermentation of S	Henze et al., 1995
K^H_A	4	$gCOD/m^3$	Saturation coeff. for acetate	Henze et al., 1995
K^H_{NO3}	0.5	gN/m^3	Saturation/Inhibition coeff. for nitrate	Henze et al., 1995
K^H_{NH4}	0.05	gN/m^3	Saturation coeff. for Snh4 as nutrient	Henze et al., 1995
K^H_{PO4}	0.01	gP/m^3	Saturation coeff. for Spo4 as nutrient	Henze et al., 1995
K^H_{ALK}	0.1	$molHCO_3/m^3$	Saturation coeff. for alkalinity	Henze et al., 1995
PHOSPHORUS ACCUMULATING ORGANISMS				
q_S^{max}	9.67	gCOD/gCOD.d	Acetate consumption rate	Smolders, 1995
m_{AN}	0.05	gP/gCOD.d	Anaerobic maintenance coefficient	Smolders, 1995
m_{NO3}	0.02	gN/gCOD.d	Anoxic maintenance coefficient	Murnleitner *et al.*, 1997
m_{O2}	0.06	gCOD/gCOD.d	Aerobic maintenance coefficient	Murnleitner *et al.*, 1997
m^{NO3}_{PAO}	0.06	gCOD/gCOD.d	Biomass consumption for anoxic maintenance	Murnleitner *et al.*, 1997
m^O_{PAO}	0.07	gCOD/gCOD.d	Biomass consumption for aerobic maintenance	Murnleitner *et al.*, 1997
K^P_A	4.0	$gCOD/m^3$	Saturation coefficient for acetate	Henze et al., 1995
K^P_{NO3}	1.4	gN/m^3	Saturation coefficient for nitrate	Murnleitner *et al.*, 1997
K^P_{O2}	0.2	$gCOD/m^3$	Saturation coefficient for oxygen	Henze et al., 1995
K^P_{ALK}	0.1	$molHCO_3/m^3$	Saturation coefficient for alkalinity	Henze et al., 1995
K^P_{NH4}	0.05	gN/m^3	Saturation coefficient for ammonium	Henze et al., 1995
K^P_{PO4}	0.01	gP/m^3	Saturation coefficient for phosphate for growth	Murnleitner *et al.*, 1997
K^P_{PHA}	0.01	$gCOD/m^3$	Saturation coefficient for PHA	Henze et al., 1995
K^P_{GLY}	0.01	$gCOD/m^3$	Saturation coefficient for glycogen	Henze et al., 1995
K^P_{PP}	0.01	gP/m^3	Saturation coefficient for polyphosphate	Henze et al., 1995
g_{PP}	0.1		Nitrate sensitivity factor for poly-P formation reduction factor for affinity constant	Murnleitner *et al.*, 1997
k_{PHA}	7.55	gCOD/gCOD.d	PHA decay rate	Murnleitner *et al.*, 1997

k_{PP}	0.45	gP/gCOD.d	Poly-P formation rate	Murnleitner *et al*., 1997
k_{GLY}	1.09	gCOD/gCOD.d	Glycogen formation rate	Murnleitner *et al*., 1997
η^{P}_{NO3}	0.4		Reduction factor under anoxic conditions, to be set to 1 if 2 groups of PAO are modeled	
NITRIFIERS				
μ_{AUT}	1	1/d	Maximal growth rate of X_{AUT}	Henze et al., 1995
b_{AUT}	0.15	1/d	Decay rate	Henze et al., 1995
K^{N}_{O2}	0.5	$gO2/m^3$	Saturation/Inhibition coefficient for oxygen	Henze et al., 1995
K^{N}_{NH4}	1	gN/m^3	Saturation coefficient for Snh4	Henze et al., 1995
K^{N}_{ALK}	0.50	$molHCO_3/m^3$	Saturation coefficient for alkalinity	Henze et al., 1995
K^{N}_{PO4}	0.01	gP/m^3	Saturation coefficient for Spo4	Henze et al., 1995

8.3.5 Parameters Values adjusted for the Simulation in this Study

Parameter	Values			Unit	Description
	applied	Other authors			
q_{fe}	1.2			gCOD/gCOD.d	max. fermentation rate
		1	Brdanovjc,1998		
		3	Henze et al., 1995		
k_{PP},	0.11			gP/gCOD d	poly-P formation rate
		0.11	Brdanovjc,1998		
		0.45	Murnleitner *et al.*, 1997		
k_{GLY}	0.45			gCOD/gCOD d	Glycogen formation rate
		0.15	Brdanovjc,1998		
		1.09	Murnleitner *et al.*, 1997		
η_{NO3}	0.4		For 1group of PAO		anoxic reduction factor
	1		For 2 group of PAO		
Y_{PO4}	0.4			gP/gCOD	Yield coeff. (PO4/HAc)
		0.36			
Y_{PHA}	1.0		For 1 group simulation	gCOD/gCOD	Yield coeff. (**PHB**/HAc)
	1.3				Yield coeff. (**PHA**/HAc)
	1.3 or 1.5		For 2 group simulation		with PHA=PHB+PHV
		1.50	Murnleitner *et al.*, 1997		
q_S^{max}	6.67			gCOD/gCOD.d	Acetate consumption rate
		9.67	Smolders, 1995		
K_F	2			$gCOD/m^3$	Sat. coeff. for growth on Sf
	4		For 2 group simulation		
		4	Henze et al., 1995		
K^H_A	2			$gCOD/m^3$	Saturation coeff. for acetate
		4	Henze et al., 1995		
$f_{PHA,\,min}$	0.01	-	-	gCOD/gCOD	minimum PHA content in PAO
p	0.005	-			In: $r_{PP} = ... * \frac{f_{PHA}}{f_{PHA} + p}$
μ_{AUT}	1.3			1/d	Maximal growth rate of X_{AUT}
		1	Henze et al., 1995		

Process	S_A	S_{NO3}	S_{PO4}	X_{PP}	X_{PHA}
anox. PHA storage	-1	$-Y^{DN}_{NO3}$	Y^{DN}_{PO4}	$-Y^{DN}_{PO4}$	Y^{DN}_{PHA}
		-0.04	0.31	-0.31	0.9
	gCOD	gN/gCOD	gP/gCOD	gP/gCOD	gCOD/gCOD

Table 8.3-1. Stoichiometric parameters used for anoxic acetate uptake