Stellingen

behorende bij het proefschrift "A metabolic model of the biological phosphorus removal; *stoichiometry, kinetics and dynamic behaviour*" door Gertjan Smolders.

1. Milieu-biotechnologisch onderzoek dient zich niet zozeer te richten op het werken met een rein-cultuur maar op het werken met een rein-metabolisme.
   Dit proefschrift

2. Het herhaaldelijk citeren van een aanname maakt deze vanzelf een vaststaand feit.
   Hooymans, C.M. Diffusion coupled with bioconversion in immobilized systems, use of an oxygen microsensor.
   Noorman, H. Methodology on monitoring and modelling of microbial metabolism.

3. De meeste eigenschappen van *Acinetobacter* 210A komen niet overeen met de verschijnselen waargenomen in de biologische fosfaatverwijdering.

4. Aangezien de opname van fosfaat energie kost, vermindert de zuurstofopnamesnelheid in afwezigheid van fosfaat en wordt deze ten onrechte toegeschreven aan een verminderde groeisnelheid.
   Wentzel, M.C. et al., 1989. Enhanced polyphosphate organism cultures in activated sludge systems. Part II & III, Water SA, 15, 2 71-102.

5. De experimenteel waargenomen variatie in fosfaat/acetaat ratio's bewijst dat alle voorgestelde modellen met vaste fosfaat/acetaat ratio's onjuist zijn.
   Wentzel, M.C., et al. 1991. Evaluation of biochemical models for the biological excess phosphorus removal, Wat. Sci. Tech., 23, 567-765: *Comparing the theoretical values* [...] *with those in the literature, there is support for all the proposed models.*

6. Het samenvoegen van PHB en glycogeen tot één niet meetbare modelcomponent zoals in het actiefslib model No.2, leidt tot een model dat niet experimenteel valideerbaar is.

7. Het samenvoegen van PHB en glycogeen tot één modelcomponent zoals in het actiefslib model No.2, leidt tot een model dat de remming van de acetaatopname ten gevolge van glycogeenuitputting niet zal kunnen beschrijven.

8. Wachtgeld is het geld waar AIO's 4 jaar op hebben moeten wachten.

9. De huidige wasmiddeltechnologie is er vooral op gericht elkaar om zeep te helpen.

10. De essentie en het voordeel van tijd is dat niet alles tegelijk gebeurt.

11. Iets is alleen schitterend als het in het licht gehouden wordt.

12. Het overmatig in goede banen leiden van de natuur leidt onvermijdelijk tot rampen.

13. Iets nieuws kun je niet bedenken.

14. Het aanhouden van een toetsverdeling voor computers die oorspronkelijk ontwikkeld werd om te voorkomen dat de hamertjes van de typemachine in elkaar zouden lopen, is op zijn minst achterhaald.

A metabolic model of the biological phosphorus removal

stoichiometry, kinetics and dynamic behaviour

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Proefschrift

ter verkrijging
van de graad van doctor
aan de Technische Universiteit Delft, op
gezag van de Rector Magnificus Prof. ir. K.F. Wakker,
in het openbaar te verdedigen ten overstaan van een commissie,
door het College van Dekanen aangewezen,
op maandag 10 april 1995
te 16.00 uur
door

Gerardus Johannes Franciscus SMOLDERS

landbouwkundig ingenieur

geboren te Arnhem

Dit proefschrift is goedgekeurd door de promotor:
Prof. dr. ir. J.J. Heijnen

Dr. ir. M.C.M van Loosdrecht
heeft als begeleider in belangrijke mate aan het totstand komen van het proefschrift bijgedragen.

Samenstelling promotiecommissie:

Prof. dr. ir. W. Verstraete (Universiteit van Gent)
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Prof. dr. ir. J. Tramper (Landbouw Universiteit Wageningen)
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Prof. dr. W. Gujer (EAWAG Dubendorf, Zwitserland)

This study was carried out at the Department of Biochemical Engineering, Delft, University of Technology, The Netherlands.

De sporen! zei Poeh. *Er is een derde dier bij de andere twee gekomen*!
Poeh! riep Piglet, Denk je dat het nog een Woezel is?
Nee, zei Poeh, want het maakt andere sporen. Het zijn òf Twee Woezels en één, mogelijkerwijze, Wizzel, òf Twee, mogelijkerwijze, Wizzels en één, als het er een is, Woezel.

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Introduction

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Introduction

General

Phosphorus removal from waste water is generally seen as a control strategy to prevent eutrophication of surface waters. In the past, phosphorus was a limiting nutrient with regard to algal growth in fresh- and coastal waters. Due to eutrophication, which is the enrichment of ecosystems with nutrients as a result of human activities, the limitation of phosphate was removed causing a severe disturbance of ecological processes. The coastal waters of the Netherlands are highly eutrophic; about 50 % of the nutrients in the coastal zone is of human origin.[16] The occurrence of very low oxygen tensions in large areas (± 10.000 km^2) in the German Bight [26] was subscribed to the increased primary production caused by eutrophication. These facts have contributed to the resolution of the North Sea Countries to decrease the discharge of N and P in 1995 with 50 %.

The main contributors to the phosphorus load on fresh surface water are cross-border rivers, domestic and industrial waste water and phosphate run-off from cultivated land. The majority of phosphorus originates from phosphate fertilizers and human and animal foodstuffs. The accumulated concentration of phosphorus in the Dutch environment in 1990 is thought to be the lowest at any time in the previous twenty years. Imports by the cross-border rivers have more than halved as compared with 1980. In 1990, the amount of phosphorus in household detergents was fairly negligible. Sales of artificial fertilizers have fallen and the production of manure is also tending to decline, for a part due to a reduction in the use of phosphate additives in mixed animal feed.

The phosphorus accumulation in fresh water (approx. 18 mln kg P per year) was considerably less in 1990 than in the preceding years. Despite the sharp decrease in the amount of phosphate in household detergents, domestic sewage is still a major source of phosphate pollution of fresh surface water. Soil run-off and leaching have increased and now equal the discharge of domestic sewage. The load of phosphates on the surface water via the effluent from sewage plants decreased by approximately 45 % between 1987 and 1990, see figure 1.

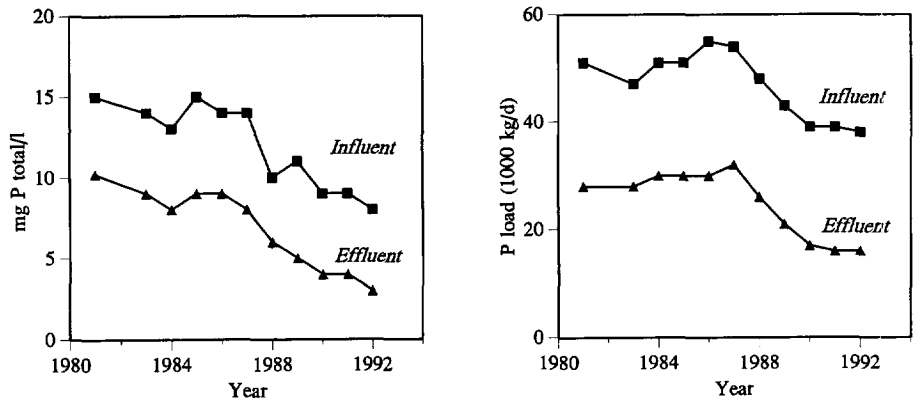

Figure 1. *Effect of the introduction of phosphorus-free detergents on the P-discharge of waste water treatment plants. Influent and effluent phosphate concentrations (left), and total phosphorus load of waste water treatment plants.*

This is a result of the increased use of detergents either low in phosphates or phosphate free. The number of sewage treatment plants with facilities for phosphate removal rose from 21 in 1985 to 34 in 1990.[33]

Although the yearly accumulated amount of phosphate is decreasing and the phosphate removal facilities in waste water treatment systems are increasing, there is still a net accumulation of 18 mln kgP per year. To decrease the phosphate concentration in the surface waters, besides decreasing soil run-off in agriculture, also phosphorus removal from waste water will have to increase. The effluent requirements concerning phosphorus will become more stringent in 1995. Depending on the capacity of the waste water treatment plant, the effluent requirement will be 1 mg P/l for plants with a treatment capacity of over 100.000 p.e. and 2 mg P/l for plants with a lower capacity.

Phosphorus removal from waste water

For the removal of phosphate from waste water, several methods can be applied of which chemical precipitation and biological phosphorus removal are the most commonly used. Precipitation of phosphate with chemicals like alum, ferric chloride and lime is a conventional method. The application of precipitation with chemicals is a reliable and fairly simple technique. The method has significant disadvantages of which the most important one is the high cost of chemicals when low phosphate concentrations are required.

Significantly more chemical sludge is produced, that may overload existing sludge handling equipment, the produced sludge does not dewater as well as conventional sludge, and higher sludge treatment and disposal costs can be expected. By the addition of chemicals to the waste water an undesired increase of the salt content of the effluent is obtained. The chemicals requirement is dependent on the total phosphorus concentration of the waste water and the desired effluent quality. The chemical dosage increases as shown in figure 2, when the desired phosphate effluent concentration is decreased. When the phosphate effluent demands increase, so will the required amounts of chemicals and the costs of the overall precipitation method will therefore increase. Precipitation of phosphorus is a method with a number of difficult to estimate variable costs, like chemicals and sludge treatment, but only low investment cost.

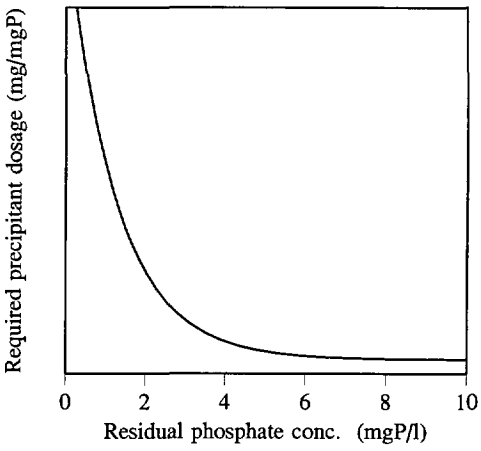

Figure 2. *Chemicals requirement as a function of the desired P-effluent concentration.*

Biologically, phosphorus can be removed in the activated sludge process in two ways. A significant amount of the influent phosphate is removed through the stoichiometric coupling to microbial growth in a system, because all organisms require phosphate for the production of new biomass. In excess of the phosphate that is strictly needed for growth, biological P-removal takes place by microbial uptake of phosphate and storage as polyphosphate. Levin

and Saphiro,[19] introduced the term "luxury uptake" to describe the ability of activated sludge to remove more phosphate than that required for growth.

The advantage of the biological phosphorus removal is that the P-removing organisms are able to take up phosphorus to very low effluent concentrations, without increasing costs. The disadvantages of the biological P-removal are however the complexity of the process which is still not completely understood. This hinders the design and operation of P-removing activated sludge systems. The operation of a P-removing activated sludge system is critical because the amount of stored phosphate present in the system represents a large amount compared to the influent load. When for instance the whole system would become anaerobic due to an aeration failure, this accumulated phosphorus could be released from the system in the environment. Another disadvantage of the biological P-removal process is that the storage of phosphate by P-removing organisms (polyP organisms) remains reversible. After the phosphate uptake and storage as polyphosphate, the organisms are still able to degrade the polyphosphate and release the phosphate again. Sludge handling after the P-removal process becomes therefore very important. Phosphate release by the polyP organisms should be prevented during the sludge handling, because it would lead to new environmental problems afterwards. Combinations between biological and chemical P-removal are also possible and closer to optimal in this respect. The P-removing organisms are used to take up the phosphate in the process to get a low effluent phosphorus value, while the phosphate is released from the biomass in a separate stripper tank, leading to a liquid stream with a high phosphate concentration which is then removed by chemical precipitation.

Microbiology of the biological phosphorus removal

General

Biological phosphorus removal from waste water is based on the enrichment of activated sludge with phosphate accumulating organisms. These organisms are able to store phosphate inside the cell as polyphosphate during aerobic conditions. Polyphosphate serves as an energy source enabling the organism to store substrate during anaerobic conditions. When enough P-removing organisms are accumulated in the sludge of an activated sludge system, all phosphate is taken up during the aerobic phase and a phosphate free effluent is obtained. The stored phosphate leaves the process inside the biomass in the waste sludge flow or is stripped from the biomass and can be precipitated with chemicals or regained in crystallised form. The

problem of obtaining a well performing phosphate removing process can be simply formulated as: how to make the conditions in the system such that especially growth of P-organisms is favoured ?

The first reports on treatment plants with more phosphate removal than expected date from 1959.[29] From studies on full scale installations it appeared that biological phosphorus removal occurred in plug flow type installations where the return sludge was added at the start of the reactor.[21, 32, 39] These installations were high loaded, resulting in low oxygen concentrations at the inlet region. Barnard[3] discovered that activated sludge plants designed for nitrogen removal, with nitrification in the aerobic phase and denitrification in the anaerobic phase were also capable to remove high concentrations of phosphate. Since that time, a considerable body of literature concerning the prerequisites and the mechanism of biological phosphate removal in the activated sludge process has been published. Literature reviews on biological phosphate removal have been presented by Barnard,[5] Toerien,[30] Wentzel[37] and Jenkins.[15]

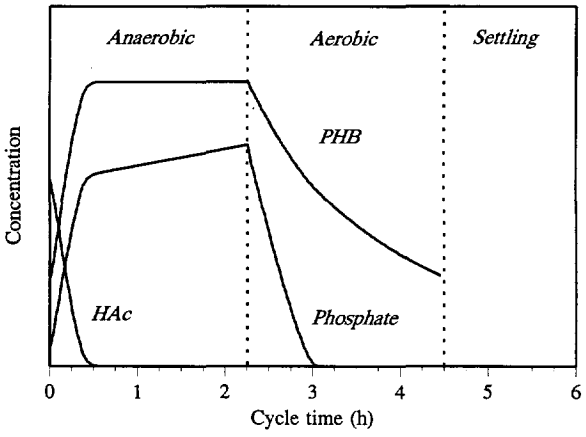

Figure 3. *Change in concentration during the biological phosphorus removal in a sequenced batch process after addition of acetate.*

Key aspects

The key aspects observed in waste water treatment systems with biological phosphorus removal are the anaerobic consumption of lower fatty acids (mainly acetate) and subsequent conversion to poly-ß-hydroxy alkanoates (PHA, like PHB: poly-ß-hydroxy butyrate or PHV: -valerate) by the sludge while phosphate is released to the fluid. When oxygen is provided

during the aerobic phase, the released phosphate is very quickly taken up by the sludge and PHB is consumed, see figure 3. During the aerobic phase, the organisms are capable of accumulating more phosphate than released during the anaerobic phase, resulting in the net uptake of phosphate.

Biochemical mechanism

Fuhs and Chen[11] provided the earliest microbial and biochemical concepts of enhanced P removal. After studies into the phenomena of P-uptake and release, they concluded that a single microorganism, or closely related species, were responsible for the phenomenon. These bacteria accumulated both PHB and polyP, the former serving as a source of energy for polyP accumulation. In studying the nutritional requirements of the polyP accumulating bacteria, they found that volatile fatty acids, in particular acetic acid, were the preferred substrate.

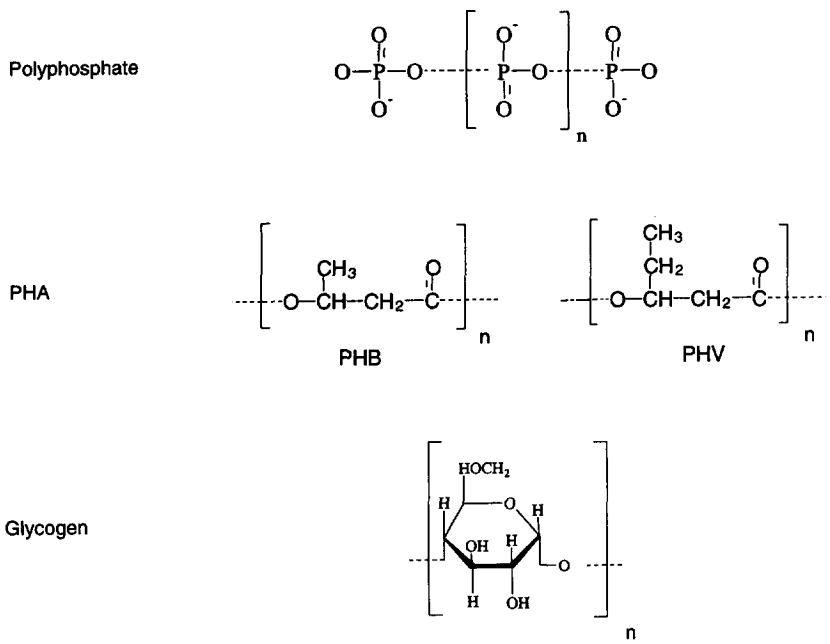

Figure 4. *Storage products of the biological P-removal metabolism.*

Based on observations in activated sludge systems showing biological P-removal, hypothetical models were proposed by Nicholls,[24] Rensink,[27] and Marais.[21] These models did not fully explain the biochemical mechanism. They agreed upon the anaerobic/aerobic sequence as

favourable for the growth of the organisms that accumulated polyphosphate during the aerobic phase. In the anaerobic phase, glucose,[24] volatile fatty acids produced during this phase,[27] or substrate from the sewage,[21] were supposed to be stored in the bacteria as carbon reserves.

More detailed biochemical models describing the mechanism of the biological phosphorus removal have been developed afterwards. Basically these models can be reduced to two models which are different in the source of reduction equivalent needed for the synthesis of PHB during anaerobic conditions. One of the models is the Comeau/Wentzel model[7, 35] the other one is the Mino model.[1, 23] According to these models the biological P-removal is based on several internally stored compounds: polyphosphate, PHA and glycogen (Mino). Figure 4 shows the composition of the different polymers involved in the polyP metabolism.

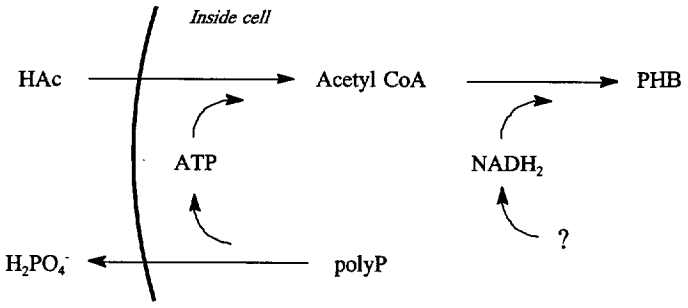

Figure 5. *Mechanism of the anaerobic acetate consumption.*

Anaerobic metabolism

During the anaerobic phase, the polyP organisms take up lower fatty acids (mainly acetate) and store these as poly-hydroxy-alkanoates (PHA, mainly PHB). For the anaerobic uptake and conversion of acetate to PHB, in the first step to acetylCoA, ATP is required while for the conversion of acetylCoA to PHB $NADH_2$ is required, see figure 5.

ATP generation

The anaerobic metabolism of the biological phosphorus removal is based on the ability of the P-removing organisms to use polyphosphate as an energy source. This enables the organism

to generate the energy required for the conversion of acetate to acetyl CoA during anaerobic conditions:

$$polyP_n + ADP \longleftrightarrow polyP_{n-1} + ATP$$

The storage of phosphate in intracellular volutin granules as polyphosphate is quite common and occurs in both prokaryotes as eukaryotes. According to Kulaev,[18] polyP might play an important role in several cellular functions. PolyP can serve as a storage of phosphate, magnesium or potassium as well as a storage of energy. Polyphosphate is a linear molecule and has the structure as depicted in figure 4. The negative charge is compensated by a metal ion (Mg^{2+} or K^+). The pathway for the biosynthesis of polyP in the biological P-removal is still not elucidated.

$NADH_2$ generation

The source of $NADH_2$ required for the conversion of acetylCoA to PHB during anaerobic conditions has been the main point of controversy in the past. According to the Wentzel model, $NADH_2$ is produced during anaerobic conditions by the conversion of part of the acetate in the tricarboxylic acid cycle (TCA), see figure 6.

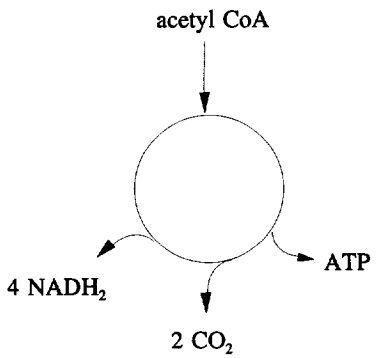

Figure 6. *Generation of reduction equivalents during anaerobic conditions according to the Comeau/Wentzel model.*

Mino[23] and Arun[1] did not agree with this Comeau/Wentzel model. They stated that the TCA cycle can not be operative during anaerobic conditions, because one of the problems in the anaerobic operation of the TCA cycle is the formation of $FADH_2$. This compound can not be used in the PHB synthesis and can only be regenerated with an external electron acceptor. Mino and Arun hypothesized that reduction equivalents are produced from the conversion of internally stored glycogen through the Emden-Meyerhoff-Parnas pathway to PHB.
In the degradation of glycogen $NADH_2$ and ATP are produced as shown in figure 7. Until now, the mechanism of the anaerobic production of reduction equivalents for conversion of acetate to PHB in the biological phosphorus removal is still under discussion. The required amount of energy for the conversion of acetate to acetylCoA is fixed. Therefore, it is expected that the ratio between phosphate release and acetate uptake during anaerobic conditions is constant, but different for the hypothetic models of Mino and Wentzel. For the Wentzel model a phosphate/acetate ratio of 0.5 P-mol/C-mol acetate is expected whereas for the Mino model a ratio of 0.25 P-mol/C-mol is expected. From experimental observations no such constant ratio seems to exist. A wide range of values for the phosphate/acetate ratio have been reported (see chapter 2). However, no explanation was found in literature for the variation in the experimentally observed phosphate/acetate ratio, and due to this variation, no discrimination could be made between the two proposed models for $NADH_2$ production.

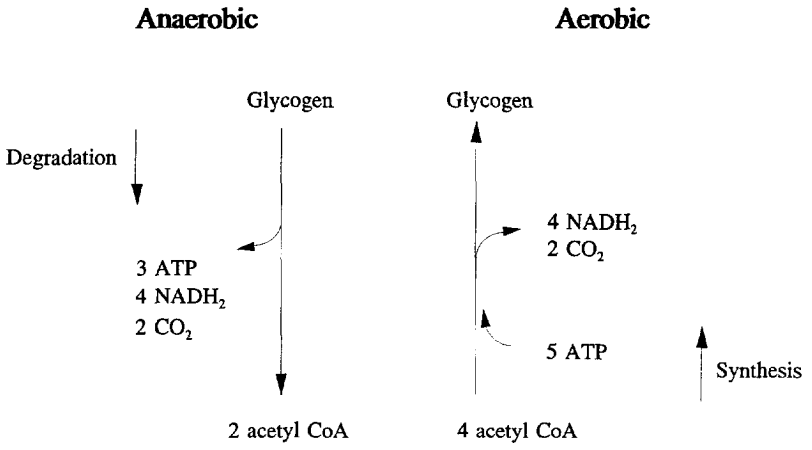

Figure 7. Generation of reduction equivalents during anaerobic conditions from glycogen degradation according to the Mino model, and synthesis of glycogen during aerobic conditions.

Aerobic metabolism

Although the actual phosphate uptake takes place during this phase, the aerobic metabolism of the biological phosphorus removal process was never given much attention. During this phase growth of the P-removing organisms takes place on internally stored PHA, which is not a common feature performed by bacteria. Subsequently, phosphate is taken up and stored as polyphosphate. Wentzel[36] has performed experiments during the aerobic phase with enriched activated sludge. The phosphate uptake and oxygen consumption during the aerobic phase were measured, while nitrification took also place during these experiments. Wentzel observed a decrease in oxygen consumption after phosphate was taken up during the aerobic phase, and concluded that when phosphate was completely consumed the P-removing organisms became phosphate limited and therefore their growth rate decreased. Wentzel did not measure whether the PHB conversion and biomass production decreased.

Pure culture

Until now no pure culture has been isolated that can be held responsible for the biological P-removal process. In fact, biological P-removal is still a poorly understood biological removal process. In almost all other processes, the organisms responsible for the phenomena observed in activated sludge have been isolated, identified and shown to perform their functions both in pure culture and in activated sludge, and to be present in activated sludge in sufficient numbers to carry out these functions. The same cannot be said for the biological P-removal.[15] Pure culture experiments on *Acinetobacter* to determine the kinetics and the metabolism with respect to biological phosphorus removal have differed in several important aspects from the circumstances in activated sludge. The isolation of the organisms has been performed on agar discs under constant aerobic, anaerobic or anoxic conditions. The conditions observed in biological P-removal in activated sludge (anaerobic acetate uptake and aerobic growth) have rarely been duplicated during the isolation. Experiments have been performed at much higher growth rates than even the highest operation rate of biological P-removing plants; and in none of the pure culture experiments the isolated organism has been grown in anaerobic/aerobic cycles with acetate consumption during the anaerobic phase as experienced in the biological P-removal. Because of these differences the key aspects concerning biological phosphorus removal have not been duplicated yet with a pure culture.

For a long time, *Acinetobacter* spp were seen as the microorganisms responsible for the biological P-removal process. However, a number of differences exist between the conversions shown with pure *Acinetobacter* cultures and the waste water experiences. Fuhs and Chen[11]

showed that *Acinetobacter* aerobically stored PHB and polyP. However, in phosphate uptake and release experiments, acetate uptake occurred aerobically and was accompanied by a phosphate decrease associated with aerobic growth. Deinema[8] showed that *Acinetobacter* 210A, isolated from activated sludge, could aerobically take up both phosphate and COD, and internally form inorganic polyP. Anaerobic conditions were not evaluated however. Later, Deinema[9] showed that aerobically batch cultured *Acinetobacter* spp containing 7-8% P released only 10-20% of the accumulated polyP when incubated anaerobically for 24 h with acetate, lactate and in the absence of carbon substrate. The uptake of the added substrates was not measured in these experiments. Continuously cultured, aerobically grown *Acinetobacter* 210A released phosphate in the presence of acetate, lactate, ethanol, and CO_2 but no PHB accumulation was shown. PHB accumulation of *Acinetobacter* 210A was shown by Bonting[6] during aerobic conditions under phosphate limitation. This is however completely different from the situation in the biological P-removal where PHB is produced with the uptake of acetate and release of phosphate during anaerobic conditions.
Clearly, the studies on the P-metabolism in *Acinetobacter* spp seem to be of limited relevance for the biological phosphorus removal process.

Biological phosphorus removal process configurations

Systems for enhanced biological P-removal

The prerequisites for biological phosphorus removal are the recirculation of sludge through an anaerobic and aerobic phase in the system, and the introduction of influent in the anaerobic reactor which has to be a plug flow type of reactor.[21,32,39] Based on simple observations of the process a lot of different process types were developed in the past. These process alternatives can be divided into two main groups: mainstream and side-stream processes. Mainstream processes are characterized by the fact that the anaerobic phase is in the waterline of the process and the phosphate is removed inside the P-removing organisms. In the side-stream process, the anaerobic phase is in the sludge line of the process and the P-removing organisms are used to concentrate the phosphate which is finally removed through precipitation. An anaerobic tank is placed in the sludge line to select for P-removing organisms. In the anaerobic tank in the side-stream process, phosphate is released through addition of influent or acetate, and precipitated chemically after separation.

Mainstream processes

Many process types were developed based on the mainstream process. If the process is combined with nitrogen removal, one or more anoxic zones are required. Anoxic means that not oxygen but nitrate is present. The combination of nitrogen removal with the phosphorus removal process has led to a number of variations on the mainstream process, foremost different in the incorporation of the anoxic zone in the process.

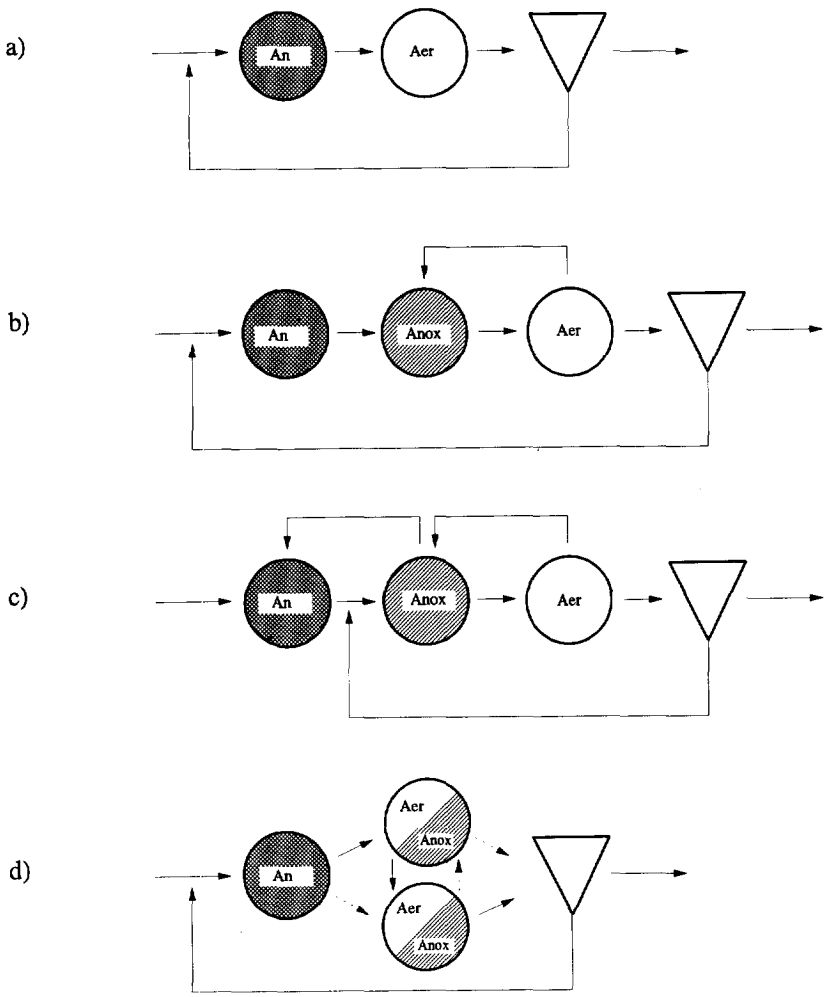

Figure 6. Mainstream processes. a) A/O process; b) A/O process with N-removal; c) UCT process; d) Biodenipho process; An: anaerobic, Anox: anoxic, Aer: aerobic.

The process configuration of a mainstream process in which only phosphate and no nitrogen is removed, is shown in figure 6a. An anaerobic reactor is placed in front of an aerobic reactor and the return sludge is mixed with the influent in the anaerobic phase. This process is called the A/O process which stands for anaerobic/oxic, and was developed in the mid seventies in the United States.[14]

If nitrogen removal has to be incorporated into the process the simplest process configuration is obtained when an anoxic reactor is placed between the anaerobic and aerobic reactor, see figure 6b. Nitrification takes place in the aerobic phase, and the nitrate produced during this phase, is returned to the anoxic reactor where denitrification takes place.

One of the major disadvantages of this process configuration is the high recycle ratio between the aerobic and anoxic reactor which is required to keep the nitrate content of the effluent low. The nitrate still present in the return sludge does disturb the anaerobic phase, because the COD consumption during the denitrification interferes with the COD consumption for the biological P-removal. The configuration found in figure 6b is called an A/O process with N-removal or a modified phoredox process and was developed by Barnard.[3]

To eliminate the effect of nitrate in the anaerobic phase, the return sludge is added to the anoxic reactor and an extra recycle is introduced between the anoxic and anaerobic reactor. This concept was first developed by Ekama et.al.[10] and was called the University of Cape Town (UCT) process (figure 6c).

The problem of the high recycle ratio required for the denitrification was avoided in the biodenipho process (Arvin[2]), see figure 6d. This process is based on the same concept as depicted in figure 6b, except that the anoxic/aerobic reactor is alternating. After nitrification in the aerobic phase, the aeration is stopped and the whole reactor becomes anoxic for denitrification. The recycle of large water volumes through the process is avoided in this way.

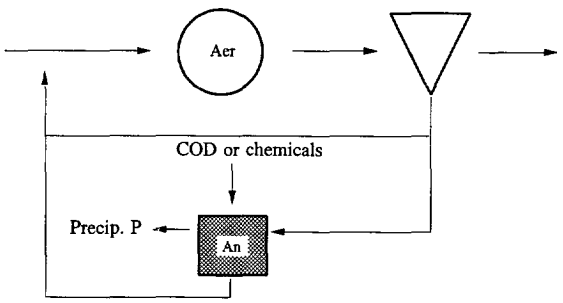

Figure 7. *Side-stream process: Phostrip process.*

Side-stream

Side-stream processes are characterized by the fact that the anaerobic phase is present in the sludge line and not in the water line. In the anaerobic tank phosphate is released from the P-removing organisms, sometimes by addition of acetate, and precipitated chemically after seperation. Another difference with the mainstream process is that the phosphate leaves the system precipitated or crystallized. The phostrip process was the first side-stream process developed by Levin[20] in the sixties (figure 7).

Performance

Although knowledge of the biological P-removal process was still limited only, as shown above, a variety of systems for the biological P-removal was developed, which performed all quite reasonable. The problem concerned with the variety of process configurations is that good concepts for the design and comparison of the performance of these process types are not available. Most design rules are obtained from practical experience. With the increased demands on the phosphate effluent concentration, the evaluation of these process types becomes more important in combination with a need for a better understanding of the process. This understanding can be found in a model of the biological phosphorus removal process.

Modelling of the P-removal

Previous models

As long as the mechanism underlying the biological P-removal remained unclear only kinetic models for the biological P-removal were developed, predicting the rates of P-uptake and acetate uptake without a proper validation of the biochemical reactions underneath.[31, 34] The first biochemistry based mathematical model of the biological P-removal was developed by Wentzel.[35, 36] This model described the mechanism of acetate uptake and conversion to PHB with the production of reduction equivalents through the TCA cycle. The model predicted a constant ratio for the anaerobic phosphate/acetate ratio and did not explain the wide range of ratios found experimentally. One of the major assumptions of the model was that the phosphate uptake during the aerobic phase was stoichiometrically coupled to growth. For this reason two growth regimes were considered: a high growth rate when phosphate was present extracellularly and a limited growth rate when phosphate was absent in the liquid.
The kinetic relations of the model were based on the VSS in the system while the VSS represents the sum of biomass, PHB (and glycogen). The content of the storage compounds

during the anaerobic/aerobic phase are highly dynamic and the growth kinetics should therefore not be based on the total biomass.

During aerobic conditions it was assumed in the model that PHB was completely consumed and used for growth. Although this is an useful simplifying assumption from a modelling point of view, the actual situation is different.

In the model of Wentzel, the biomass decay mechanism was used to predict the death rate of P-removing biomass. The use of decay in case of the P-removal results in a subsequent release of storage products to the fluid when the biomass dies and falls apart. External concentrations of a number of storage compounds will therefore appear. These concentrations have to be incorporated into the model and additional degradation or conversion reactions are required to avoid accumulation of these compounds. The model therefore contains a rather large number of parameters.

The model was experimentally validated in an acetate fed, labscale system at three different sludge ages. The acetate and phosphate conversions were measured as well as the oxygen consumption. PHB was not measured. In the experiments, the P-removing organisms were always supplied with a surplus of phosphate. Phosphate was therefore always present in the effluent, and thus the maximal P-uptake capacity of the organisms was determined. Operation of a P-removing system at the maximal P-uptake capacity is not recommended however, because spare P-uptake capacity of the organisms is required to be able to maintain full P-removal when a peak load of phosphate enters the system.

Application of models

A model is a simplification of reality that can be used to understand or predict some aspects that match reality. Reality itself is far to complex to be understood in all detail and simplifications will always be necessary; the kind and number will depend on the application of the model. Different models will be required for different applications: to get a better understanding of a process, in the design, optimization or in the actual control of the process. An example of this problem is found in the modelling of waste water treatment processes. Biological waste water treatment processes are based on the activity of microorganisms, and consequently modelling of these processes starts with the description of these microbial activities. The biochemical and microbial knowledge of microorganisms in general, and the organisms present in the specific system, is very extensive and can not be incorporated completely into a model because this would require too many parameters. Over-simplification of the system by regarding all organisms as capable to perform all conversions, described with one biomass and the same yield on different substrates, will result in a model that does not

accurately describe the process and does not supply enough understanding of the process. The need exists for a modelling approach that uses the fundamental biochemical and microbiological knowledge maximally, with a minimal number of parameters.

Such an intermediate form of a model for the design and optimization of a process is found in the macroscopic system description. The macroscopic description of systems, as well as the model structure of reaction engineering is based on the possibility of treating systems in terms of macroscopic variables, e.g. concentrations of chemical compounds, amount of energy, temperature and pressure. Two types of macroscopic system descriptions can be distinguished: the black-box model and the grey-box model. In the black-box model, the process is considered as a black-box exchanging only compounds like substrate and products with the environment. A black-box shows how a system behaves under certain circumstances. What exactly is happening under these circumstances remains completely unknown. In a grey-box, insight exists in the behaviour of the interior of the system. If one starts investigating a system, the system as a whole is considered a black-box, but after some experimentation some mechanisms or sub-systems can be elucidated. This process can continue, if one considers one of the subsystems as a new system. Important is, however, that the final subsystems are always black-boxes. An example of this is the microbial cell. First the whole cell had to be considered as a black-box. Subsequently, organelles were discovered which were later in turn divided in subsystems describing in more detail the reactions of the metabolism.

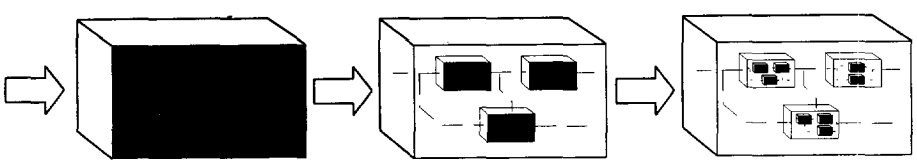

Figure 10. *Black boxes.*

It may be wondered how a grey-box can be predictive at all, if it is only a collection of black-boxes. The main reason for this predictive character is that although the surroundings of the cell can change considerably, the surroundings of the organelles remain fairly constant.[28] Further the subsystems are simpler than the system itself. The behaviour of the subsystem can

be described by a model that may have been tested extensively. When a grey-box model of a microbial metabolism is developed it is called a metabolic model.

Metabolic model

A metabolic model is based on the principle that the metabolism of organisms is composed of a limited number of universal metabolic pathways. It is therefore easier to model these metabolic pathways then each separate microorganism. From a thermodynamic point of view, these pathways will require a certain minimal amount of energy, while through selection, microorganisms try to increase their efficiency and approach this minimum. The amount of energy required in these pathways could therefore be considered as more or less constant, which ultimately leads to a constant stoichiometry.

In a metabolic model, the conversions of components observed at the outside of the organism are reduced to a number of internal characteristic reactions of the metabolism. Examples of these internal reactions are in case of the biological phosphate removal, the storage of PHB, the polyphosphate synthesis or the production of ATP in the oxidative phosphorylation. In general these internal reactions are studied extensively and have, for the most, a fixed stoichiometry.

In addition to the exchangeable compounds defined in a black-box model, non-exchangeable compounds which are only active inside the cell (metabolic intermediates, reduction equivalents and ATP) are also considered in a metabolic model. A set of linear relations follows from metabolic constraints. With the metabolic approach maximal use is made both of the biochemical knowledge of a system, and the conservation principles of compounds and elements through which a minimal number of stoichiometric parameters is needed to describe the system. Also a minimal number of reaction rates is obtained leading to a minimal number of kinetic expressions. Therefore it appears to be highly attractive to follow the strategy of metabolic modelling for the biological phosphorus removal process.

Scope of the thesis

From the preceding sections, it appeared that the biological phosphorus removal is a complex process, for the main part based on internal storage compounds. The metabolism of the phosphorus removal is only partly understood, while a wide scala of system types are available. To improve the design and optimization of the biological phosphorus removal process, the understanding of the process will have to be improved.

The aim of this thesis is to achieve a better comprehension of the biological phosphorus removal process through the development of a mathematical model based on the metabolism. First, a biochemical concept of the metabolism will be developed to establish all relevant reactions and their stoichiometry. This will be the basis of the metabolic model in which the kinetics will be incorporated. The biological phosphorus removal is an outstanding process for the development of a metabolic model because almost all relevant conversions take place internally and are not dependent on external concentrations. Therefore, it is expected that when the metabolism of the P-removal is properly understood the model can be applied to a wide variety of different system configurations.

One of the reasons why the metabolism of the biological phosphorus removal is still poorly understood, is that most research is performed with waste water as influent. The multitude and variability of substrates present in waste water combined with the complexity of the biological P-removal, complicates the elucidation of the metabolism. To explain a metabolism, the ideal situation is found when research can be performed with synthetic substrate and a pure culture. No pure culture of the P-removal process is available, and therefore an enrichment culture is used for the research. This is achieved by choosing the process conditions for the phosphorus removal such, that only P-organisms will grow. Only the main substrate (acetate) is therefore supplied with the influent and interaction with denitrification during anaerobic conditions is omitted by avoiding nitrification in the system.

A large set of measurements has to be performed in each phase of the process to be able to set up the redox- and carbon balances. This can elucidate wether all relevant components and reactions are incorporated in the model or that some component is missing. Storage compounds like PHB and glycogen have to be measured extensively for this reason. From the measured results the stoichiometric parameters and kinetic expressions will be derived. Fixed stoichiometric parameters for biomass, polyP, PHB and glycogen formation are obtained in this way.

Outline of the thesis

This thesis describes the development and validation of a mathematical process model based on the metabolism of the biological phosphorus removal process from waste water. In the development of the model, the relevant components and stoichiometry of the reactions during the anaerobic and aerobic conditions have to be established first.
Chapter 2 focuses on the energetics and stoichiometry of the anaerobic metabolism. The main question during this phase is the source of reduction equivalents that enables the polyP organisms to take up substrate in the absence of an external electron acceptor. Also an explanation has to be found for the wide range of phosphate release/acetate uptake ratios observed experimentally. From measurements a clear answer was obtained regarding this topic.
In chapter 3, the energetics and stoichiometry of the aerobic phase are determined. Internally stored PHB is used for growth, storage of phosphate and glycogen formation. Relevant metabolic reactions for this phase are defined and the stoichiometry for the sub-reactions is established and quantified using the coupling of all these conversions to oxygen consumption. The uptake of phosphate and storage as polyphosphate is shown to have a direct effect on the oxygen consumption in the aerobic phase.
In Chapter 4 the kinetic expressions for the reactions of the anaerobic and aerobic phase are derived. It is shown that the PHB content of the cells determines primarily the growth rate during the aerobic phase. With the kinetic expressions, the dynamic behaviour of all components including the storage products are described and experimentally verified over one anaerobic/aerobic cycle at a single growth rate. It is also shown that the rates should be defined relative to the active biomass and not to the total biomass including the storage products.

An unusual aspect of the internal storage of substrate in the biological phosphorus removal, is how the organism controls the growth rate. If the PHB content is the main factor that controls growth rate, the kinetic parameters will be the same during the anaerobic/aerobic cycle as well as over a wide range of growth rates. To validate the metabolic model in steady state over a wide range of PHB contents, experiments are performed at different growth rates (chapter 5). Variation of the growth rate has a large effect on the biomass concentration in the reactor. If the volumetric substrate conversion remains constant, the specific rates and storage content will increase when the biomass concentration is decreased. Variation of the

biomass concentration by variation of the sludge age of the system will therefore have large effects on the content of storage products. It was shown that one set of kinetic parameters is capable to describe the measured conversions of all components observed in the reactor as a function of the growth rate.

In chapter 6, the metabolic model is applied to a number of non-steady state situations during the start up of the process. The competition with other heterotrophic organisms is studied during the start-up phase as well as the effect of different loading regimes on the growth. The uptake of substrates during anaerobic conditions, while other aerobic organisms are inactive, results in an advantage of the polyP organisms during aerobic conditions. PolyP organisms can use their internal substrate while external substrate may be absent. Other aerobic organisms are not capable to grow during the aerobic phase and are washed out from the system. When a system is started with a small inoculum of polyP organisms this effect can be studied. Because the content of the storage products during a start up is very dynamic, it is a challenging situation to test a model.

Finally in chapter 7, a steady state analysis is developed using the stoichiometric parameters of the dynamic model. This analysis can be used for the evaluation of different process configurations and the necessity of a stripper tank.

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Anaerobic stoichiometry

2

Stoichiometry and pH influence of the anaerobic metabolism

G.J.F. Smolders, J. van der Meij, M.C.M. van Loosdrecht, J.J. Heijnen

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

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Introduction

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

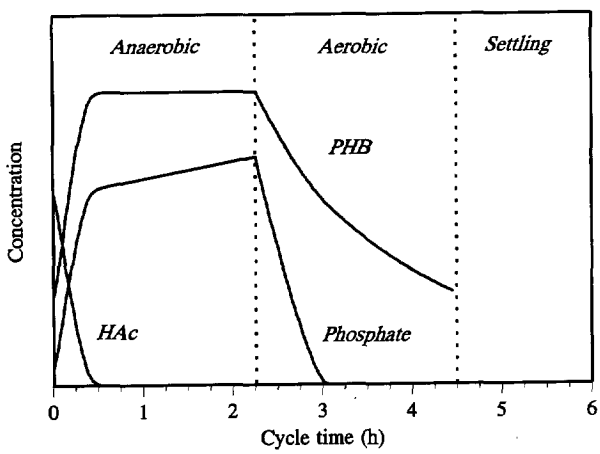

Figure 1. *Change in concentrations during the biological phosphorus removal in a sequenced batch process after addition of the influent.*

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

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

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

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

Table I. Phosphate/acetate ratios found in literature.

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

Thermodynamic consideration of the acetate transport

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

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

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

Where:

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

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

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

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

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

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

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

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

Metabolic model of the anaerobic metabolism

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

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

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

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

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

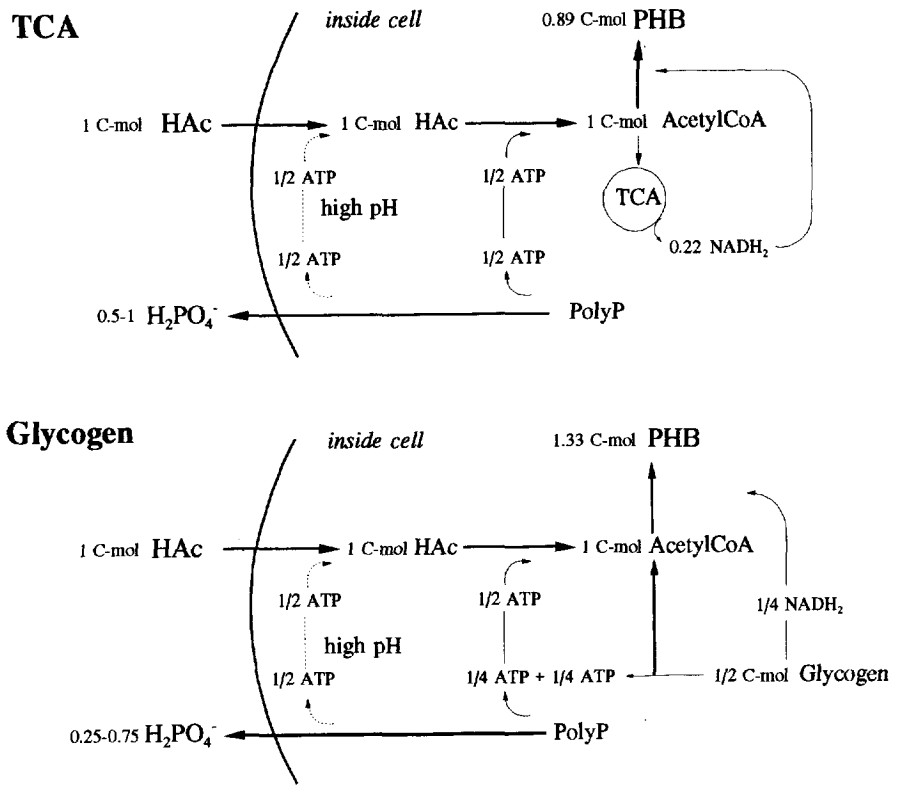

Figure 2. *Schematic representation of the metabolic models: the TCA cycle and glycogen as source of reduction equivalents and the energy requirements of the pH dependent acetate transport.*

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

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

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

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

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

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

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

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

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

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

TCA cycle as source of $NADH_2$

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

Glycogen as source of $NADH_2$

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

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

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

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

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

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

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

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

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

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

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

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

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

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

Media

Sterilized synthetic medium was used containing per litre: 0.85 g $NaAc \cdot 3H_2O$ (400 mgCOD/l) as carbon source, 107 mg NH_4Cl (28 mgN/l), 75.5 mg $NaH_2PO_4 \cdot 2H_2O$ (15 mgP/l), 90 mg $MgSO_4 \cdot 7H_2O$, 36 mg KCl, 14 mg $CaCl_2 \cdot 2H_2O$, 1 mg yeast extract, 0.3 ml nutrient solution. The nutrient solution contained per litre: 1.5 g $FeCl_3 \cdot 6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4 \cdot 5H_2O$, 0.18 g KI, 0.12 g $MnCl_2 \cdot 4H_2O$, 0.06 g $Na_2MoO_4 \cdot 2H_2O$, 0.12 g $ZnSO_4 \cdot 7H_2O$, 0.15 g $CoCl_2 \cdot 6H_2O$, 10 g EDTA.

Analyses

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

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

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

Experimental setup

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

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

Results

Steady state operation

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

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

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

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

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

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

CO_2/HAc ratio

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

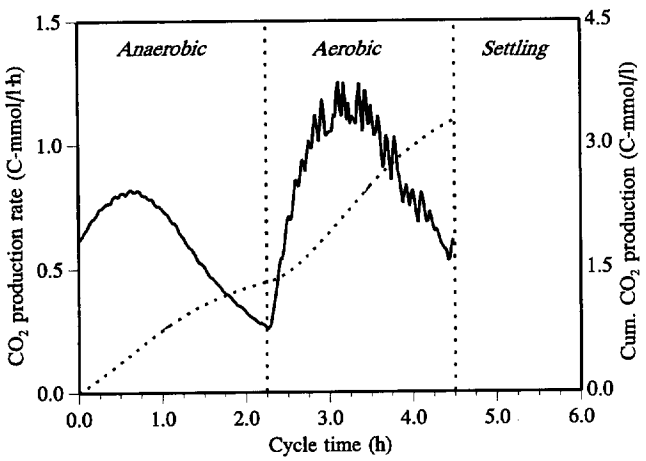

Figure 3. CO_2 production during a cycle in the SBR, —— CO_2 production, ····· cumulative CO_2 production. 6 C-mmol/l acetate was added.

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

PHB/HAc ratio

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

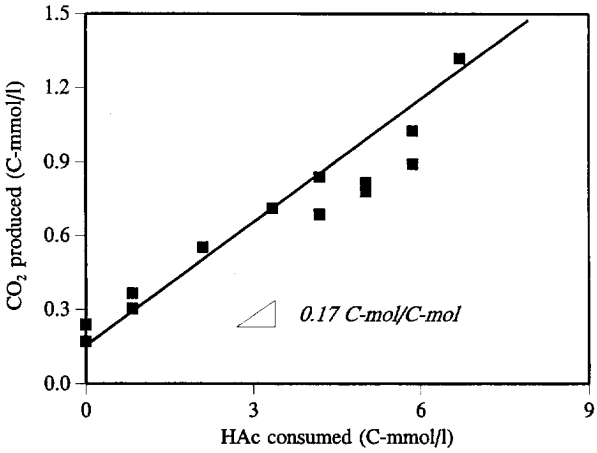

Figure 4. *CO_2 production per C-mol acetate consumped as measured in batch experiments with increasing acetate concentrations.*

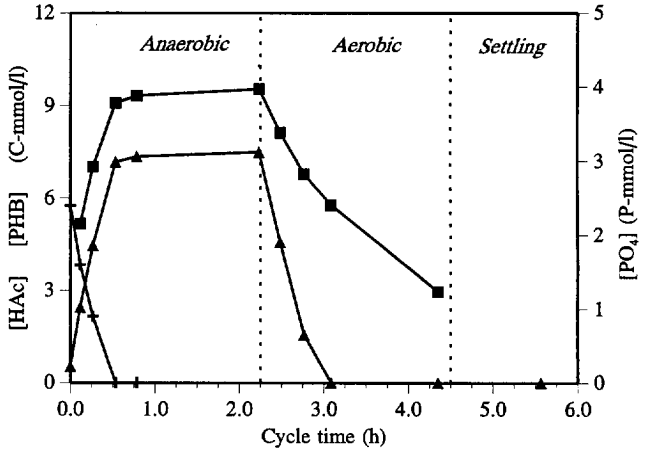

Figure 5. *PO_4 (▲), HAc (+) and PHB (■) profiles during a cycle. Initial acetate concentration 5.75 C-mmol/l, phosphate 0.24 P-mmol/l.*

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

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

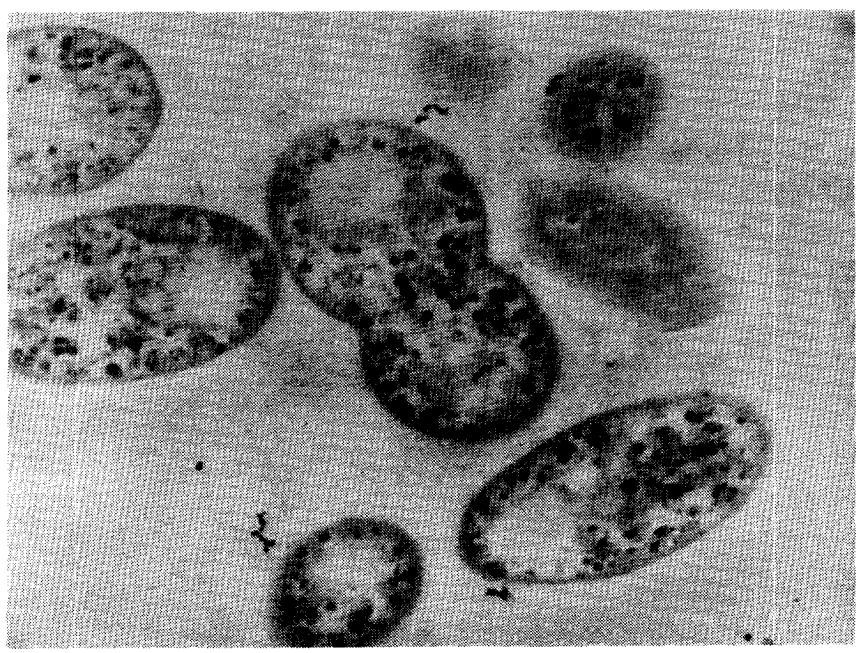

Figure 6. *Electron microscopic detection of glycogen in the sludge at the end of the aerobic phase. The dark spots are stained glycogen granules. Polyphosphate is not present anymore since it disappeared from the cells during the fixation and staining procedure. Magnification 48.000x.*

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

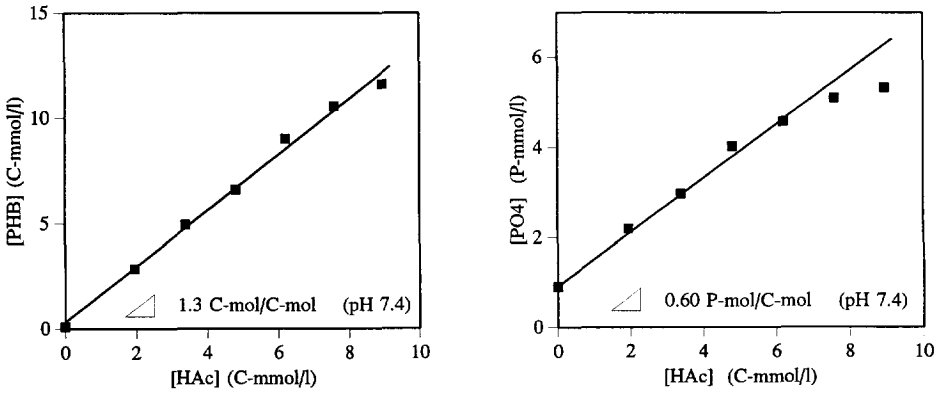

Figure 7. PHB production (left) and phosphate release (right) per C-mol acetate consumed as measured in batch experiments with increasing acetate concentrations; pH 7.4

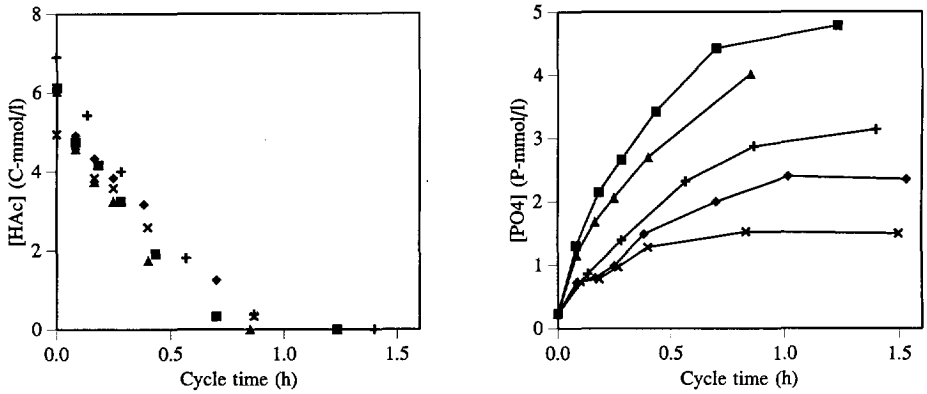

Figure 8. Acetate uptake (left) and P-release (right) at different pH-values. Average initial acetate concentration 6 C-mmol/l, MLSS 3.2 g/l, pH 5.8 (×), pH 6.4 (♦), pH 7.0 (+), pH 7.8 (▲), pH 8.2 (■).

Phosphate/HAc Ratio and the pH effect

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

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

Discussion

Carbon balance

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

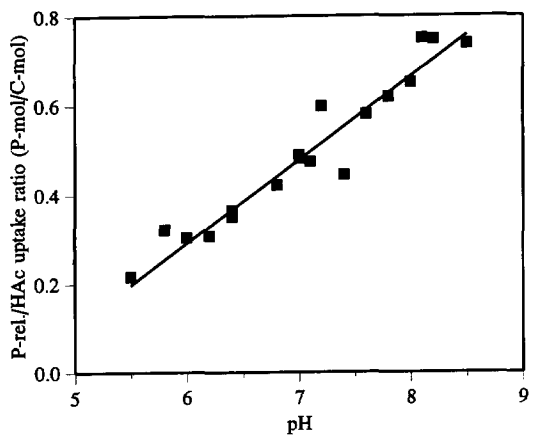

Figure 9. *P-release as a function of the pH. The plotted line was the calculated line based on the increase in $\Delta\psi$ across the cell membrane with increasing pH, according to equation (8).*

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

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

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

ATP balance

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

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

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

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

Thermodynamic considerations

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

$$Y_{P/HAc} = (1/4 + \alpha_1) / \alpha_2 = 1/4 + \alpha_1 \quad \text{(molATP/C-mol)} \tag{5}$$

Combination with equation 4 gives:

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

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

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

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

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

Consequences

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

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

Conclusions

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

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

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

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

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Nomenclature

α_1	ATP required for uptake of acetate	(molATP/C-mol)
α_2	ATP generated in the degradation of polyphosphate	(molATP/P-mol)
$\Delta G°''$	transport energy	(kJ/mol)
n	charge of the transported acetate per C-mol	(-)
η	efficiency	(-)
Δp	proton motive force (pmf)	(kJ/mol)
$\Delta \psi$	electric potential difference over the cellmembrane	(kJ/mol)
r	reaction rate	(mol/m^3· h)
v	internal reaction rate	(mol/m^3· h)
R	gasconstant	(kJ/K· mol)
T	temperature	(K)

subscripts

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

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Aerobic stoichiometry

3

Stoichiometry of the aerobic metabolism

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

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Introduction

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

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

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

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

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

Metabolic model

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

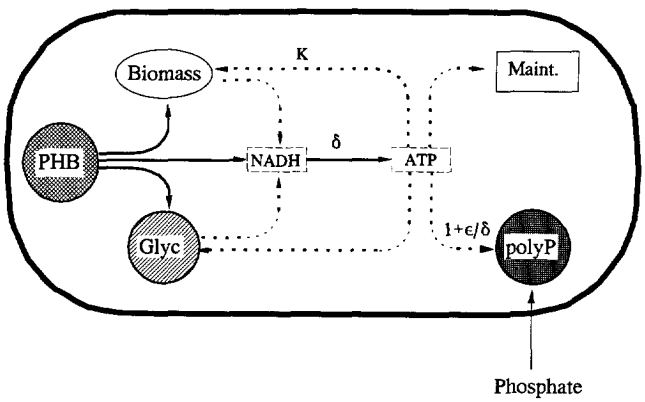


Figure 1. *Aerobic metabolism.*

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

***Reaction 1:* PHB catabolism**

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

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

***Reaction 2:* Oxidative phosphorylation**

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

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

***Reaction 3:* Biomass synthesis from PHB**

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

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

***Reaction 4a:* Phosphate transport**

The transport of phosphate across the cell membrane is a process which requires energy. Phosphate is a negatively charged ion and has to be taken up against the electric potential difference over the cell membrane. Positive ions required for the polyP synthesis (Mg^{2+} and K^+) are taken up without energy costs.[6, 11] The energy used for the transport of phosphate is generated by the import of protons which are subsequently exported over the cell membrane in the oxidation of $NADH_2$.[6, 11] Therefore a certain amount of phosphate, ϵ, can be transported

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

$$- H_3PO_{4\ out} - 1/\epsilon\ NADH_2 - 1/2\epsilon\ O_2 + H_3PO_{4\ in} + 1/\epsilon\ H_2O = 0 \qquad [4a]$$

Reaction 4b: **Polyphosphate synthesis**

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

$$H_3PO_{4\ in} - \alpha_3\ ATP + \underset{polyP}{HPO_3} + H_2O = 0 \qquad [4b]$$

Reaction 5: **Glycogen production**

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

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

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

PHB conversion rate

The conversion rates can be expressed as a function of the internal reaction rates by a set of linear equations with parameters composed of the stoichiometric coefficients of the internal

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

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

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

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

and α the metabolic reaction matrix:

$$\alpha = \begin{bmatrix} -1 & 0 & -1.27 & 0 & 0 & -4/3 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & -0.20 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & -0.015 & 1 & -1 & 0 \\ 0 & -1/2 & 0 & -\frac{1}{2\epsilon} & 0 & 0 \\ 1 & 0 & 0.27 & 0 & 0 & 1/3 \\ -1.5 & 1 & -0.385 & \frac{1}{\epsilon} & 1 & -5/6 \\ 1/2 & \delta & -(K+\frac{m_{sp}}{\mu}) & 0 & -\alpha_3 & -5/6 \\ 2.25 & -1 & 0.615 & -\frac{1}{\epsilon} & 0 & 1 \end{bmatrix}$$

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

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

If the well known[12] assumption is made that no net accumulation of $NADH_2$ and ATP takes place, $r_{ATP} = 0$ and $r_{NADH2} = 0$. Further, the assumption is made that the internal ortho-phosphate concentration is kept constant and therefore $r_{p\,in} = 0$. Now we have a system with 15 unknown rates ($v_1..v_5$ and $r_{phb}..r_w$) and 12 linear equations. With the measurement of 15-12 = 3 of the unknown rates, all remaining conversion rates in the system can be described. The

following unknown rates were chosen: r_x, r_{pp} and r_{gl}. The overall equation for the conversion of PHB in the aerobic phase then becomes:

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

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

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

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

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

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

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

Oxygen conversion rate

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

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

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

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

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

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

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

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

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

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

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

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

Determination of the stoichiometric coefficients of the metabolic model

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

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

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

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

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

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

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

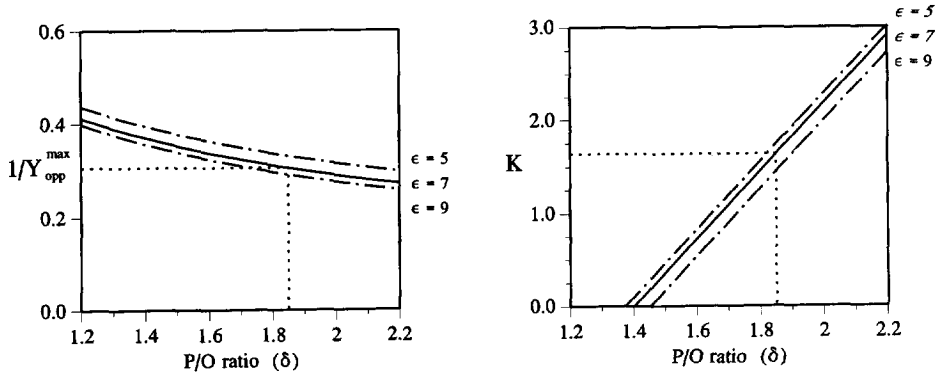


Figure 2. Dependency of Y_{opp}^{max} (left) and the polymerisation coefficient, K, (right) on the P/O ratio, δ, and the phosphate transport coefficient, ϵ.

Determination of the maintenance coefficient, m_{ATP}

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

Determination of the polymerisation coefficient, K

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

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

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

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

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

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

Media

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

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

Analyses

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

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

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

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

Measurement of the respiration rate in the aerobic phase

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

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

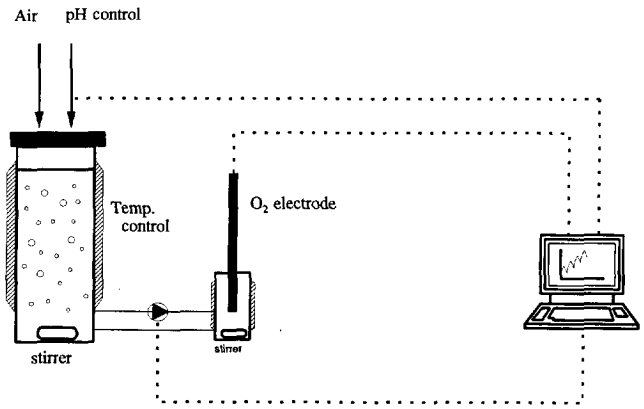


Figure 3. Schematic representation of the experimental setup for the continuous measurement of the oxygen consumption rate.

Results

SBR measurements

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

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

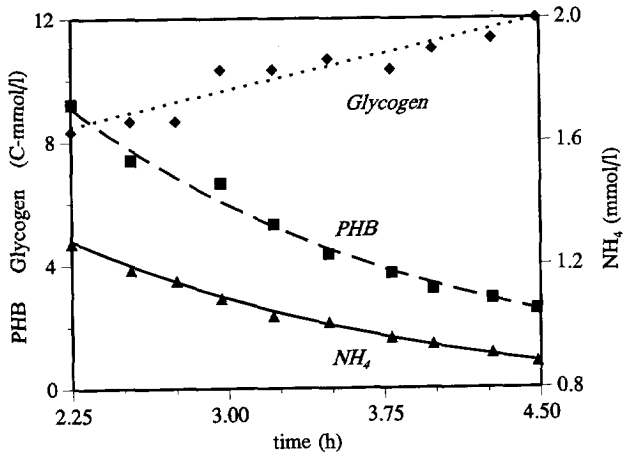


Figure 4a. Conversion of PHB (■), NH4 (▲) and glycogen (♦) during the aerobic phase in the SBR.

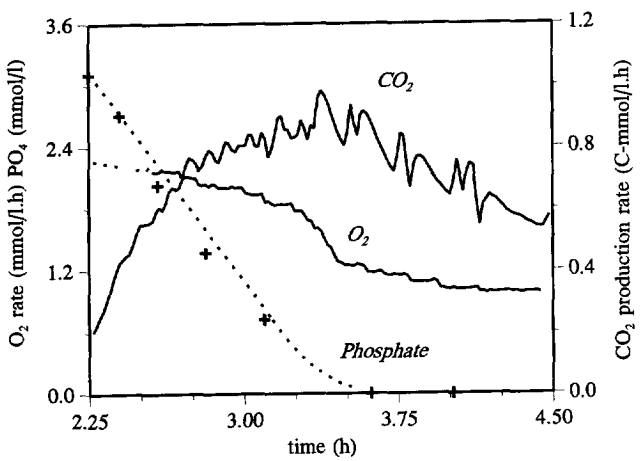


Figure 4b. CO_2 production rate, oxygen consumption rate and phosphate uptake (+) during the aerobic phase of the SBR.

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

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

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

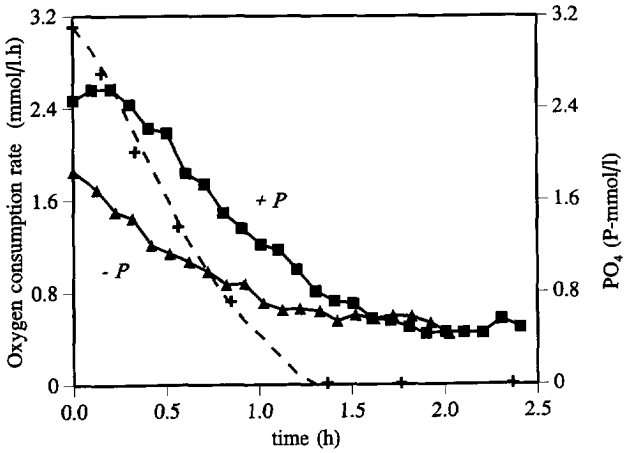
Measured compounds	flow	unit
Conversions over one cycle		
HAc consumed	6.10	C-mmol/l·cycle
CO_2 produced	3.37	mmol/l·cycle
Conversions in the aerobic phase		
PHB consumed	8.04	C-mmol/l
Ammonium consumed	0.42	mmol/l
Biomass* increase	2.10	C-mmol/l
Phosphate uptake	2.71	P-mmol/l
Glycogen produced	3.62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

* biomass excluding polyphosphate and carbon reserves

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

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

a)



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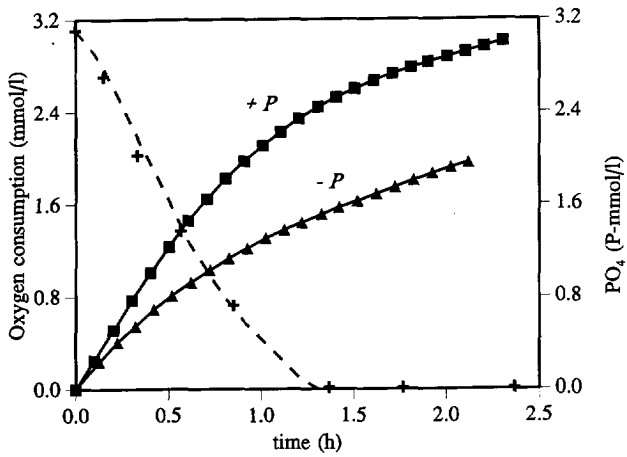


Figure 5. *Oxygen consumption rate (5a) and cumulative oxygen consumption (5b) of the biomass in the presence (■) and absence (▲) of phosphate and the phosphate concentration (+) in the liquid. The line for the phosphate uptake was calculated from the difference in oxygen consumption and a O_2/PO_4 ratio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

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

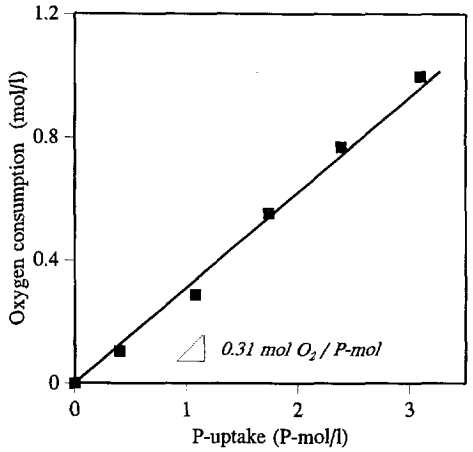


Figure 6. Stoichiometric relation between the oxygen consumption and phosphate uptake.

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

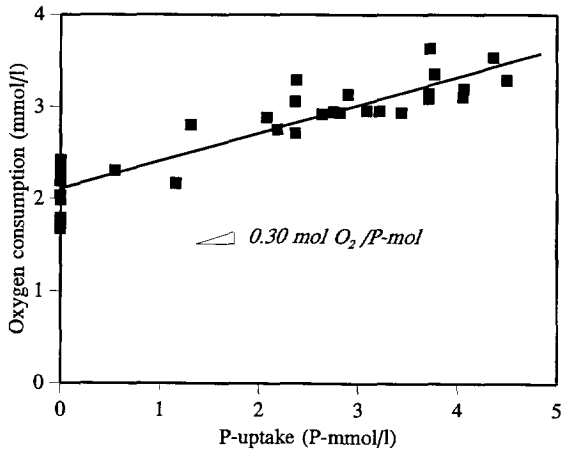


Figure 7. Effect of various amounts of accumulated polyphosphate on the oxygen consumption of the biomass.

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

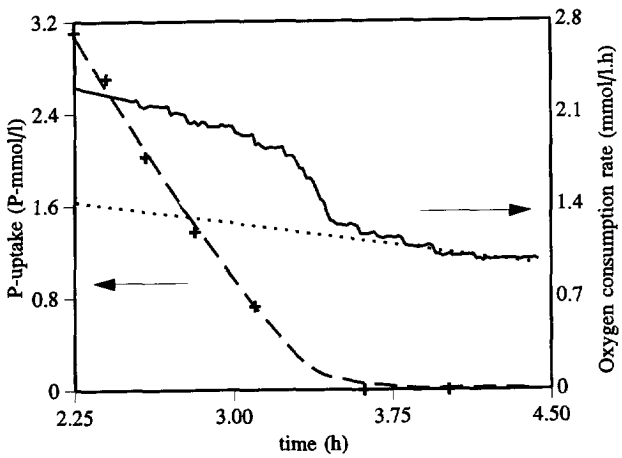


Figure 8. Total oxygen consumption rate measured in the offgas of the SBR (–) and extrapolated line (····) representing the oxygen consumption for growth and glycogen synthesis. The difference in oxygen consumption between these lines was used to calculate the P-uptake with a value of 0.3 mol/P-mol for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

Determination of the maintenance coefficient, m_{ATP}

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

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

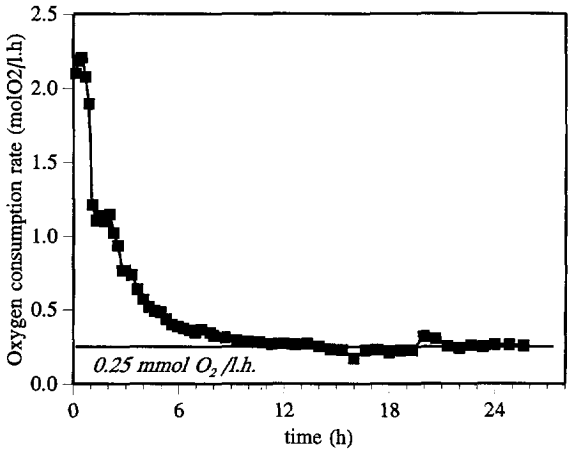


Figure 9. *Determination of the maintenance coefficient from the P-release in absence of substrate during anaerobic conditions and a biomass concentration of 55 C-mmol/l.*

Determination of the polymerisation coefficient, K

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

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

Calculation of the yield coefficients

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

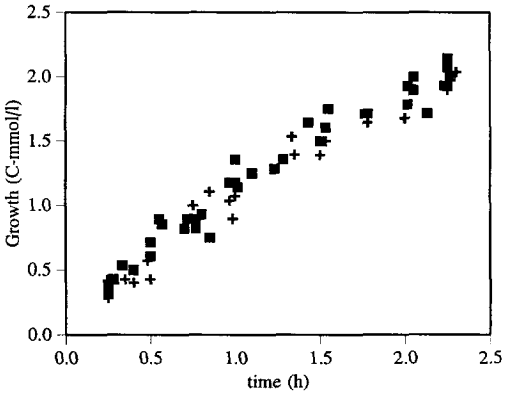
$$-r_{phb} = 1.36\ r_x + 0.27\ r_{pp} + 1.12\ r_{gl} + 4.0\ 10^{-3}\ C_x \qquad (21)$$

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

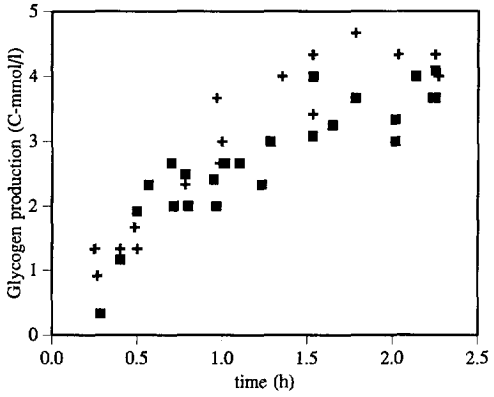
Table II. *Maximal yield values found by regression for growth, polyP and glycogen formation on PHB, acetate and oxygen in mol/mol, maintenance: mol/mol.h.*

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

a)



b)



c)

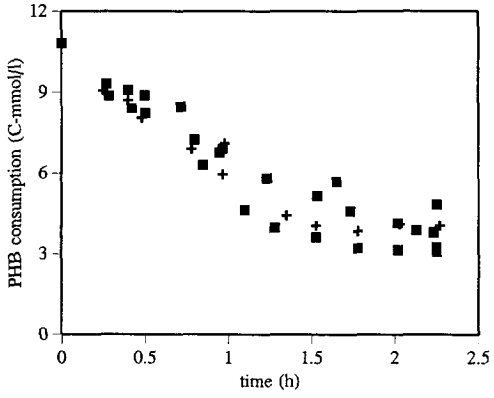
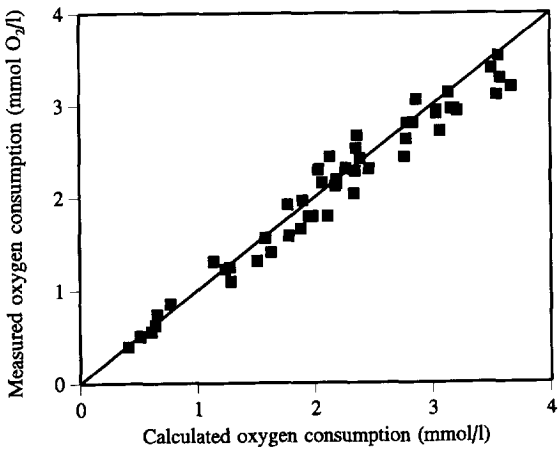


Figure 10. *Effect of phosphate accumulation on the biomass production (a), glycogen formation (b) and PHB consumption (c), in the presence (■) and absence (+) of phosphate. The data points originate from several independent experiments.*

a)



b)

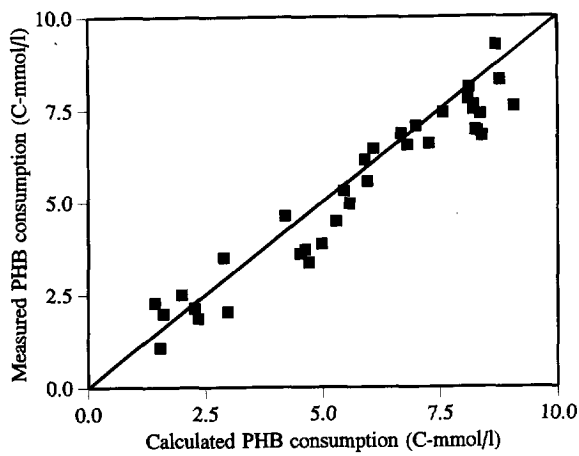


Figure 11. *Comparison of the calculated overall oxygen consumption (a) and PHB consumption (b) composed of the biomass, polyP and glycogen production and the yields of table II, and the overall measured oxygen- and PHB consumption.*

Discussion

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

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

Coefficients and overall yields

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

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

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

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

The effect of external phosphate on oxygen consumption

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

Overall conversions during anaerobic and aerobic phases

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

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

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

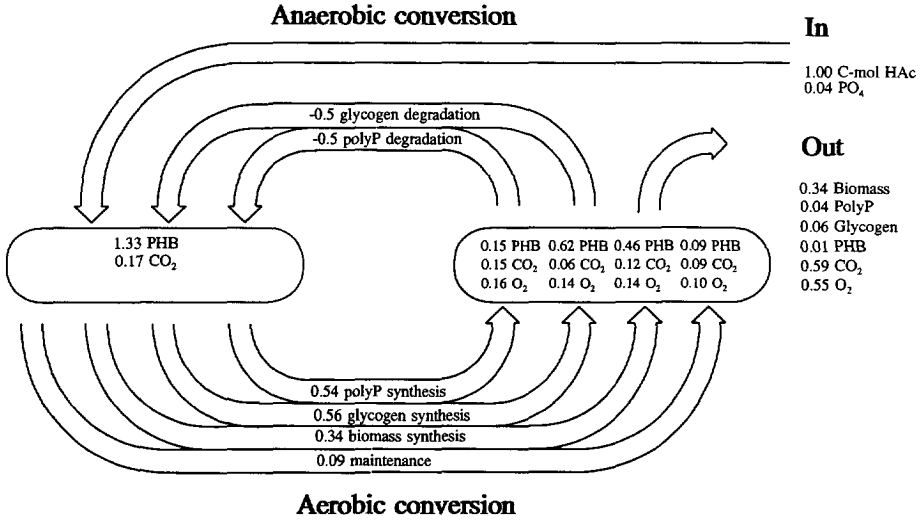


Figure 12. *Conversions, expressed in mol, during the anaerobic and aerobic phase of the biological phosphorus removal process after addition of 1 C-mol acetate, SRT 8 days.*

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

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

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

Conclusions

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

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

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

Acknowledgements *- The authors would like to thank D.C. Reuvers and G. van der Steen for the analytical work. This research was supported by Stora rwzi-2000, projectnumber 3.2.3.5/4 and the Technology Foundation of STW, project number DST92.1981.*

Nomenclature

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

subscripts

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

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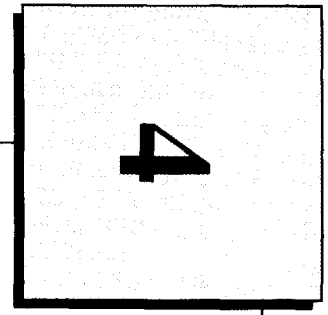
Kinetics



Kinetics of the anaerobic and aerobic metabolism

G.J.F. Smolders, J. van der Meij, M.C.M. van Loosdrecht, J.J. Heijnen

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

This chapter was accepted for publication in *Biotechnology and Bioengineering, (1995)*

Introduction

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

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

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

chapter 2 and 3 respectively, here we present the kinetic relations for the conversions in the anaerobic and aerobic zones of the process, obtained for a sludge age of 8 days.

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

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

Stoichiometry of the P-removal

Relevant components and reactions

In table I the components which play a relevant role in the P-metabolism are shown with their elemental formula. All substrates and polymeric components like biomass, PHB, polyphosphate and glycogen are expressed per mole carbon or phosphorus, which simplifies the calculation. For the same reason all components are electro-neutrally represented. The relevant reactions and their stoichiometry of the anaerobic and aerobic phase are summarized in table II; these reactions are described in chapter 2 and 3.[4,5] The anaerobic metabolism can be described by two reactions: the uptake of acetate (R_1) and the production of energy for maintenance (R_2), (table II). Phosphorus removing organisms take up acetate and store this as poly-hydroxy-butyrate (PHB), see figure 1. The energy for the transport and storage of acetate is supplied by the hydrolysis of intracellular polyphosphate and glycogen. Due to the hydrolysis of polyphosphate, phosphate is released. The energy requirement for the uptake of acetate is dependent on the pH value which is represented with the ATP coefficient α_1. The energy for maintenance purposes is generated by the hydrolysis of polyphosphate.

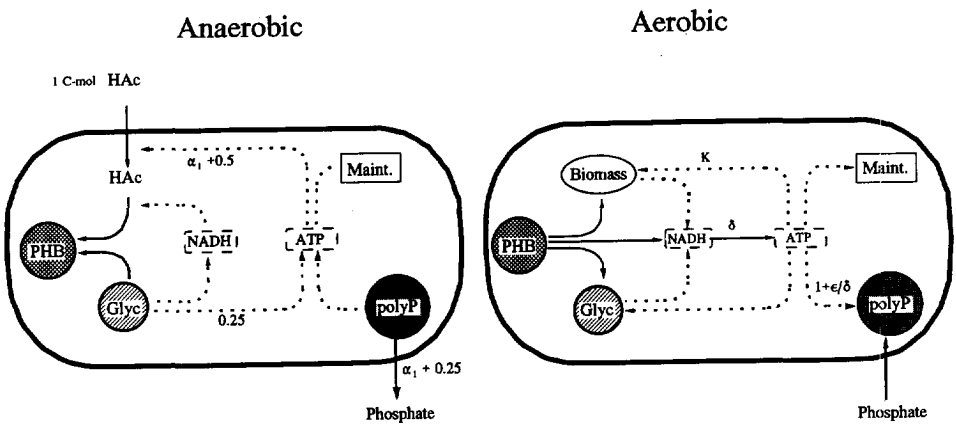


Figure 1. Metabolism of the anaerobic and aerobic phase.

The reactions in the aerobic metabolism (figure 1) are described with four reactions all of which use PHB for: the production of biomass (R_3), the synthesis of polyphosphate (R_4) and glycogen (R_5) and the energy production for maintenance (R_6). Three metabolic coefficients determine the stoichiometry of the aerobic metabolism: the P/O ratio, δ, determines the amount of ATP produced in the oxidative phosphorylation, the polymerisation coefficient for biomass synthesis, K, determines the ATP involved in biomass synthesis and the transport coefficient, ϵ, determines the energy involved in the transport of phosphate into the cell, which has only a small contribution in the overall metabolism. The metabolisms of the anaerobic and aerobic phase of the biological P-removal process are only dependent on these four coefficients (α_1, δ, K, ϵ) which were experimentally determined in chapter 2 and 3.[4,5]

Biomass fractions

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

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

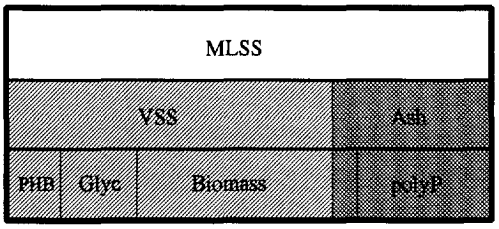


Figure 2. *Relation between MLSS, VSS and ash content and the biomass composition.*

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

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

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

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

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

Kinetics of the P-removal

Anaerobic metabolism

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

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

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

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

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

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

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

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

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

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

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

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

Aerobic metabolism

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Calculation of the concentrations in the process

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

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

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

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

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

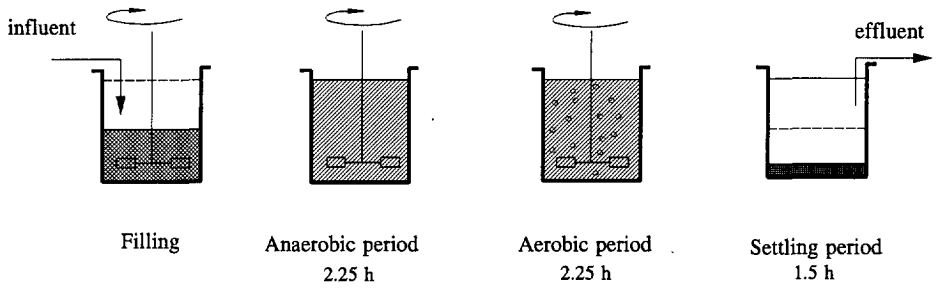


Figure 3. Operation of the sequencing batch reactor (SBR).

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

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

Media

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

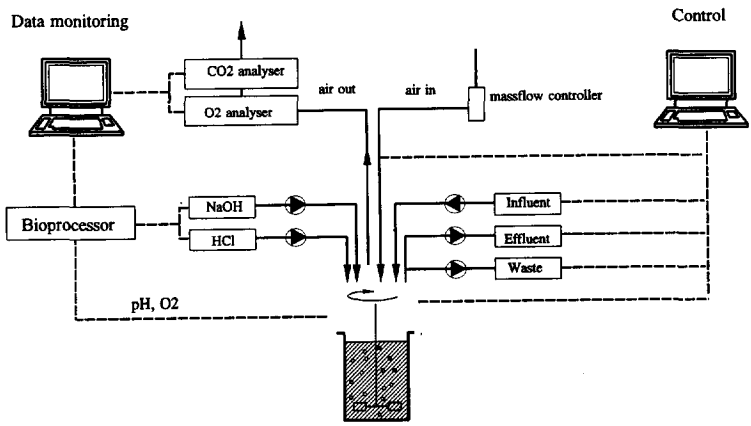


Figure 4. Experimental set-up used to monitor and control the SBR.

Measurements and analyses

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

synthesis in combination with the direct measurement of the conversion rates of these components. The percentage oxygen and carbon dioxide were measured in the offgas of the reactor. Analyses were performed as described in chapter 2 and 3. The data sets for evaluation of the kinetic model were derived by measurements over the complete cycle and in batch experiments.

Results and discussion

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

Table III. Kinetic parameters for the anaerobic and aerobic metabolism

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

Anaerobic kinetics

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

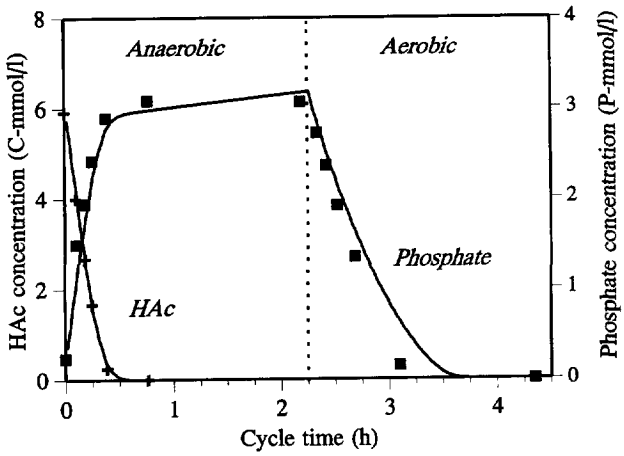


Figure 5a. Acetate (+) and phosphate (■) concentration during the anaerobic and aerobic phase. C_x = 61 C-mmol/l.

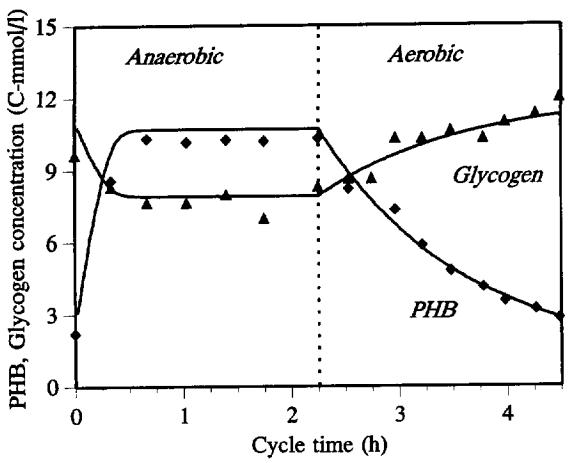


Figure 5b. PHB (♦) and glycogen (▲) concentration during the anaerobic and aerobic phase.

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

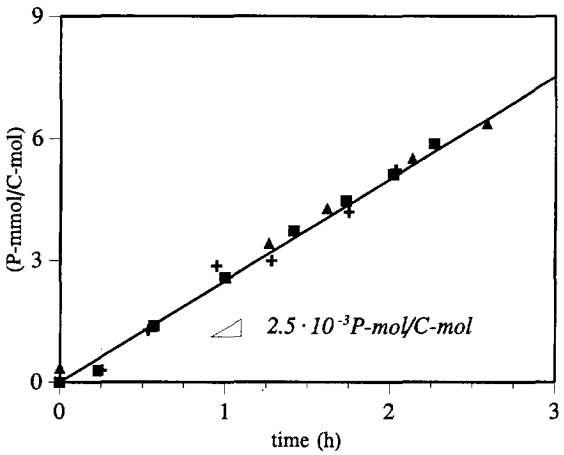


Figure 6. P-release during the anaerobic phase in absence of an external carbon source in three independent experiments.

Aerobic kinetics

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

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

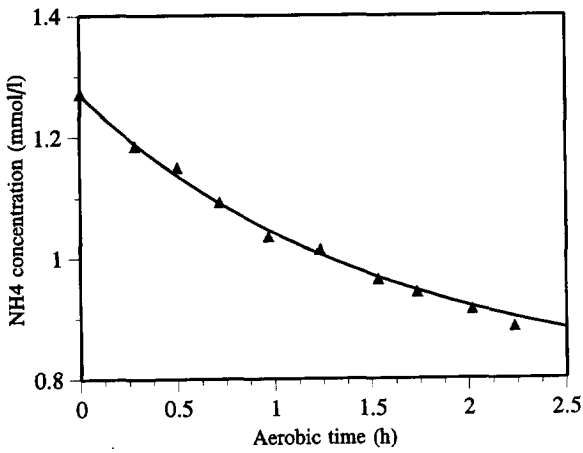
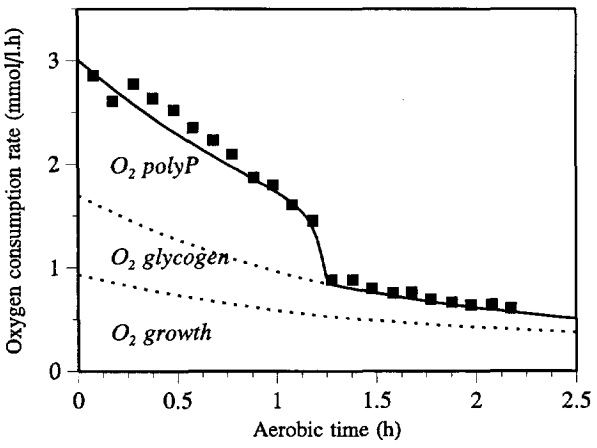


Figure 7. Ammonium consumption during the aerobic phase as a result of growth.

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

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

a)



b)

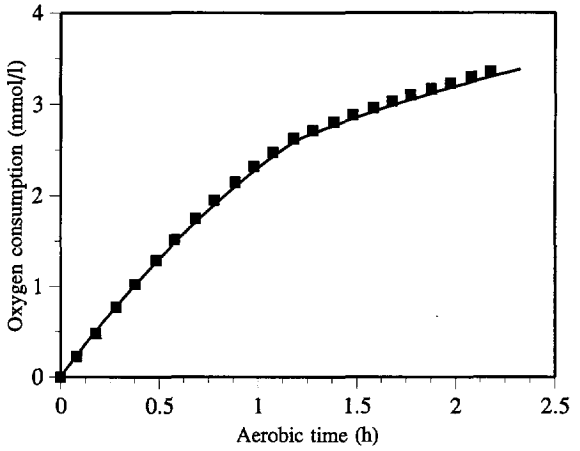


Figure 8. *Oxygen consumption rate (a) and cumulative oxygen consumption (b) during the aerobic phase. Figure 8a shows the contribution of growth, glycogen and polyP synthesis in the total oxygen consumption.*

Effect of the phosphate concentration

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

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

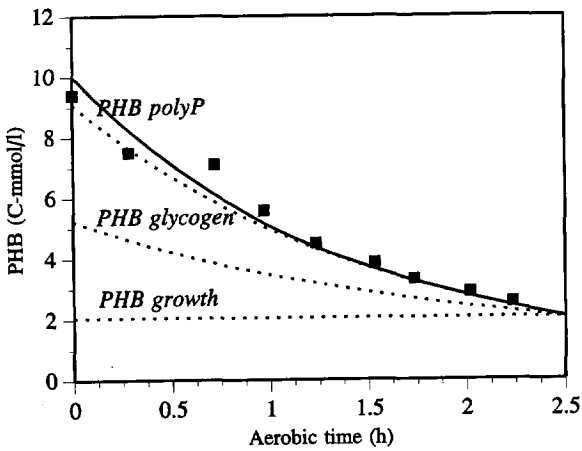


Figure 9. *PHB consumption during the aerobic phase. The contribution of growth, polyP and glycogen synthesis in the PHB consumption are shown.*

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

$$q_{pp} = k_{pp} \cdot \frac{C_p}{C_p + K_p} \cdot \left(1 - \frac{f_{pp}}{f_{pp}^{\max}}\right)^m \cdot (f_{phb})^n \qquad (13)$$

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

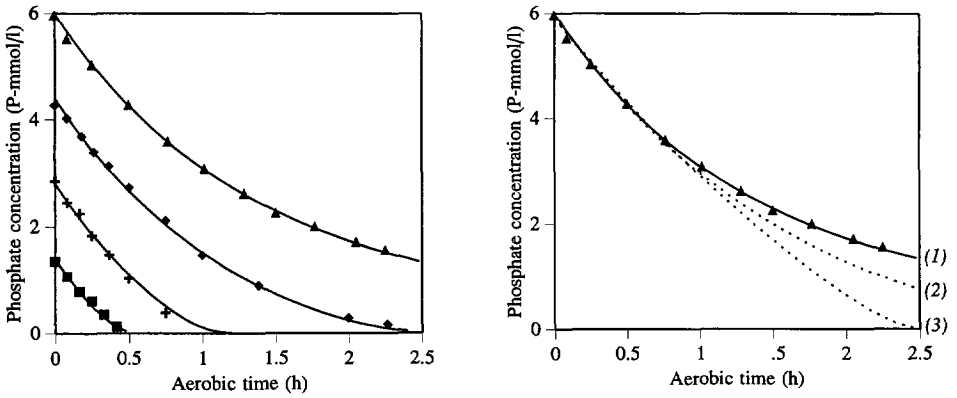


Figure 10. *Phosphate uptake during the aerobic phase in three experiments with phosphate concentrations in the range of 1.4-6 P-mmol/l (left). Comparison of three different kinetic relations to describe the polyP synthesis kinetics (right). The polyP synthesis rate is dependent on the external phosphate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

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

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

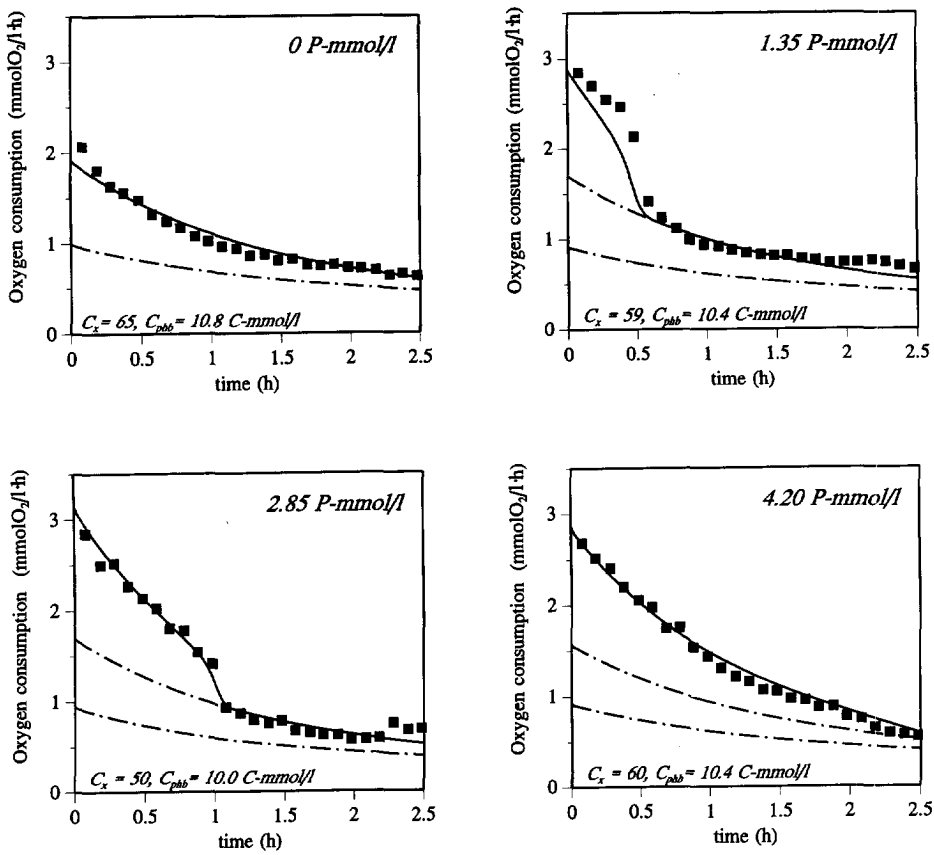


Figure 11. *Oxygen consumption rate in 4 experiments with different initial phosphate concentrations.*

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

- In the present model, an energetic evaluation is made of the biological P-removal, in which the metabolic energy involved in the production or consumption of a component was determined. The model is based on the bioenergetics of the metabolism and leads to the stoichiometry of the anaerobic and aerobic phases which is a function of the energetic restrictions expressed by 4 parameters (α_1, δ, ϵ, K). Kinetically, the production of biomass, polyP and glycogen from PHB are not coupled to each other and can all proceed independently with their own kinetics.

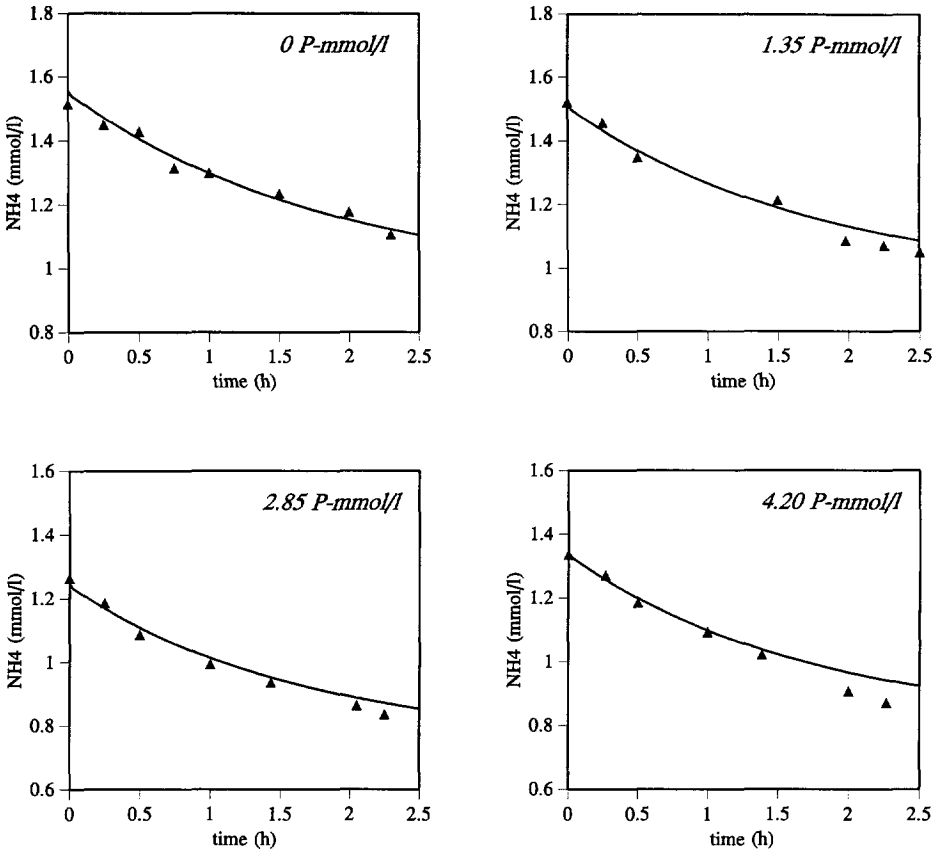


Figure 12. Ammonium consumption due to growth in 4 experiments with different initial phosphate concentrations.

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

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

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

Conclusions

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

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Nomenclature

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

subscripts

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

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Effect of the SRT

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

G.J.F. Smolders, J.M. Klop, M.C.M. van Loosdrecht, J.J. Heijnen

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

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Introduction

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

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

Biomass concentration and PHB content

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

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

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

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

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

with:

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

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

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

with:

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

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

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

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

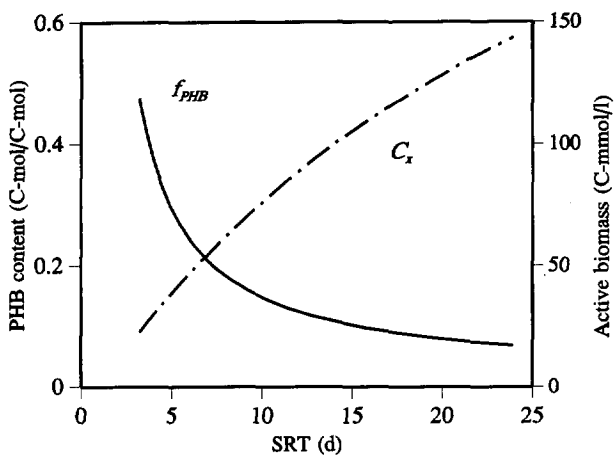


Figure 1. Effect of the sludge retention time on the PHB content and active biomass concentration in a sequencing batch reactor for an acetate loading rate of 25 C-mmol/l.d.

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

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

with

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

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

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

Model development

For the model description of the biological P-removal process in the cyclic SBR process, the mass balance over the full cycle must be made. The accumulation of a component *i* in one cycle of a sequencing batch system is a result of its conversion in the anaerobic and aerobic

phase and the transport Φ_i to or from the system. The accumulation over the total cycle, ΔC_i^{cyc}, if the conversion over a phase is represented by ΔC, becomes:

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

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

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

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

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

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

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

In equation (7), the stoichiometry of the metabolism, see table I, relating quantities of reactants consumed to quantities of products formed, is fixed and represented in the stoichiometry matrix α, which was shown in chapter 4. The development of this kind of models was described by Roels.[3] The kinetic relations for the specific rates q of the components are shown in table II. The relations for the anaerobic kinetics are as previously described. In the relations for the aerobic kinetics minor modifications can be found in comparison with previously published work.[7]

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

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

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

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

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

$$\Phi_i = \frac{\Delta V_i}{V} \cdot C_i \tag{9}$$

The change in volume during a cycle in the SBR is shown in figure 2. After the cycle is started with the addition of ΔV_i litre influent, the total volume in the SBR becomes V litre.

At the end of the aerobic phase the waste sludge is removed with a volume ΔV_w. The effluent, with volume ΔV_e, is withdrawn at the end of the settling phase.

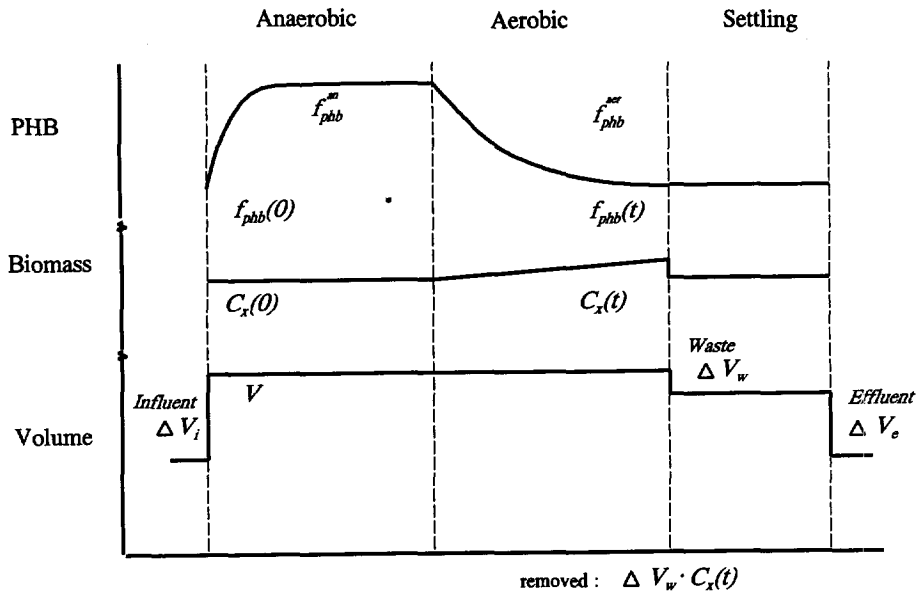


Figure 2. *Volume, biomass concentration and PHB fraction during an anaerobic/aerobic cycle in a SBR due to influent, waste and effluent flows.*

Steady state

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

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

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

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

$$V \cdot C_x(t) - \Delta V_w \cdot C_x(t) = V \cdot C_x(0) \tag{11}$$

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

$$C_x(t) = \frac{V}{V - \Delta V_w} \cdot C_x(0) \tag{12}$$

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

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

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

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

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

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

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

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

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

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

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

Materials and methods

Continuous operation of the sequencing batch reactor (SBR)

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

Media

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

Measurements and analyses

The concentrations of acetate, phosphate and ammonium were measured as well as the internally stored fractions of PHB and glycogen. Regularly the MLSS and VSS concentrations were measured by taking samples of 60 ml directly from the reactor of which a volume of 15 ml was used for the measurements, the rest was returned to the reactor. During the aerobic phase the SBR was coupled to a respirometer which measured the oxygen consumption rate with intervals of 3 minutes. This equipment was described in chapter 3. Analyses were performed as described in chapter 2 and 3.[5]

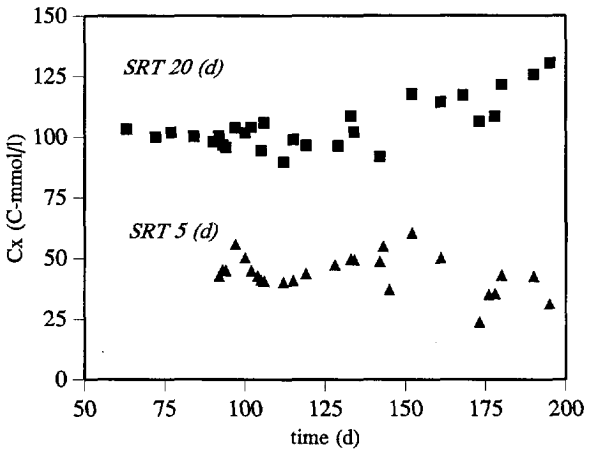


Figure 3. Active biomass concentrations in the SBR with a sludge retention time of 5 and 20 days at the end of the aerobic phase.

Experimental setup

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

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

Results

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

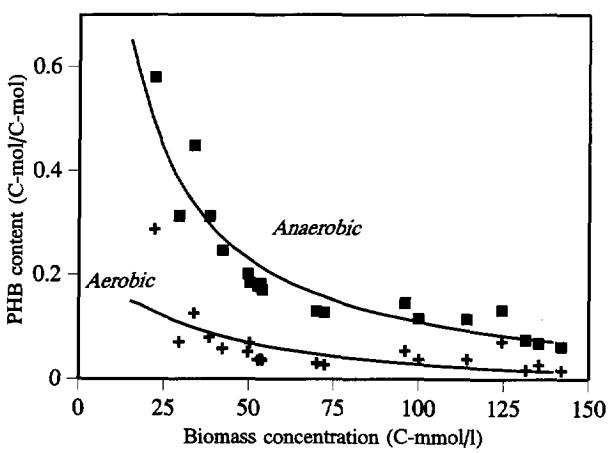
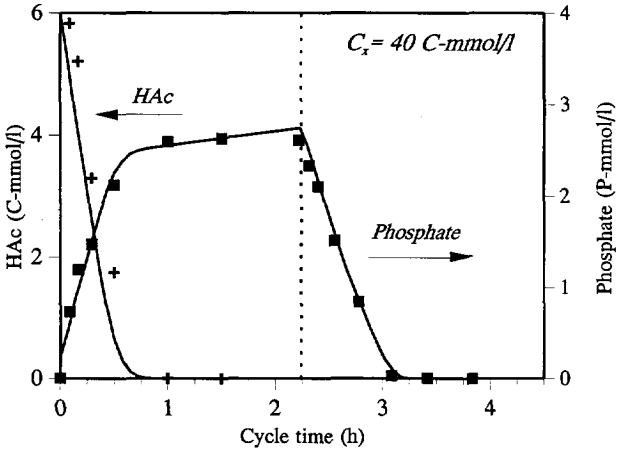


Figure 4. *PHB content at the end of the anaerobic and aerobic phase as function of the biomass concentration. The solid lines are the model predictions for the anaerobic and aerobic phase respectively.*

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

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

a)



b)

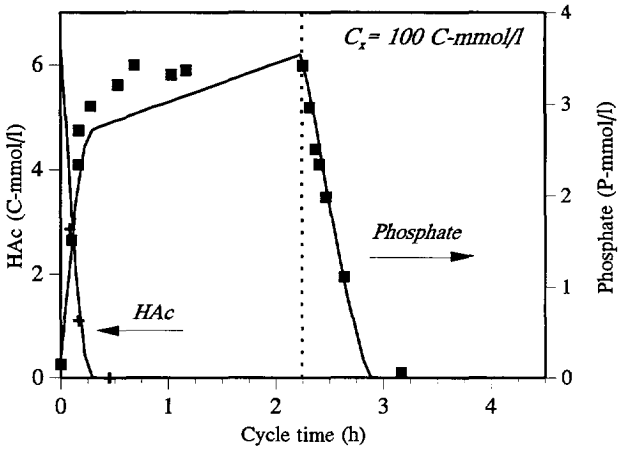


Figure 5. *Acetate and phosphate concentrations during a cycle at two different sludge waste rates resulting in a biomass concentration of a) 40 C-mmol/l and b) 100 C-mmol/l. The lines were calculated using the model.*

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

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

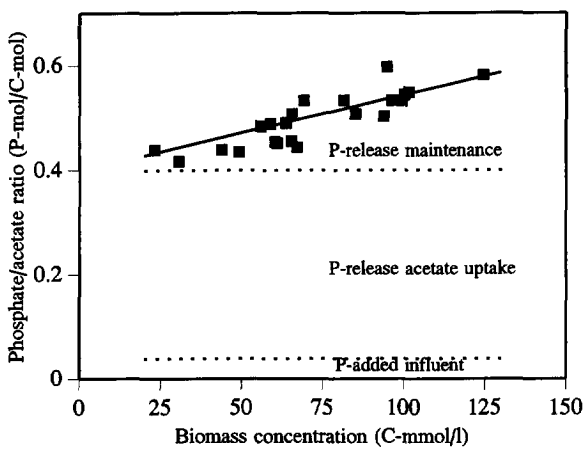
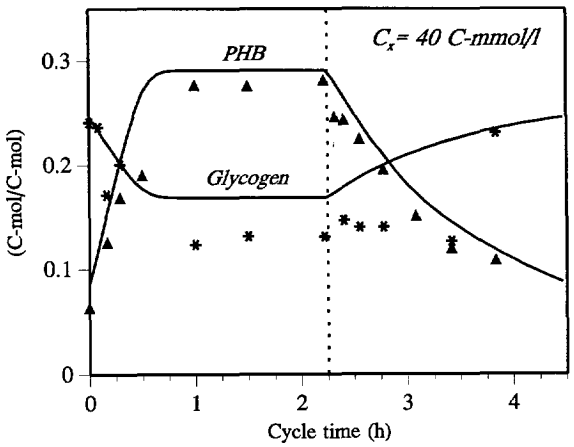


Figure 6. Effect of the biomass concentration in the reactor on the anaerobic P-release caused by maintenance. Phosphate added with the influent, released in the uptake of acetate, and resulting form maintenance contribute in the total observed phosphate/acetate ratio.

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

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

a)



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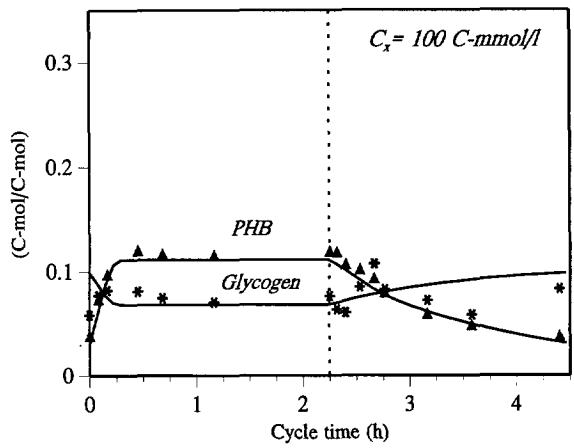


Figure 7. *PHB and glycogen content during a cycle at two different sludge retention times. a) low SRT, C_x = 40 C-mmol/l; b) high SRT, C_x = 100 C-mmol/l. The lines were calculated using the model.*

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

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

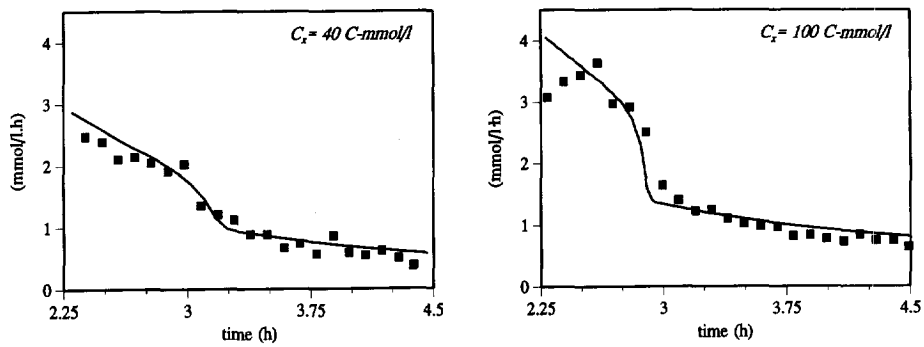


Figure 8a. *Oxygen consumption rate during the aerobic phase at a high (left) and low (right) sludge retention time.*

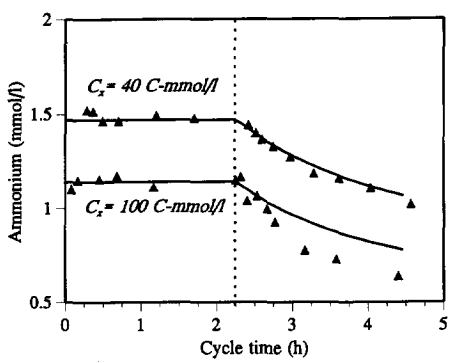


Figure 8b. *Ammonium consumption during the cycle at a high (C_x = 40 C-mmol/l) and low (C_x = 100 C-mmol/l) sludge retention time.*

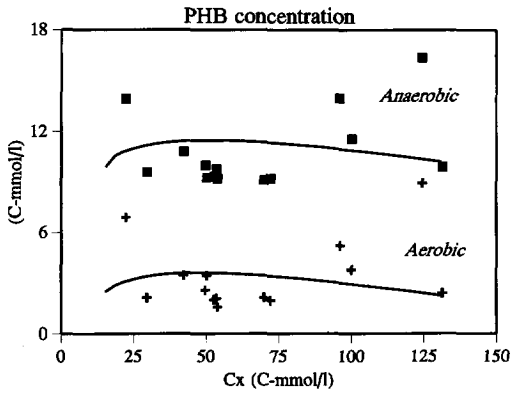
In figure 8 the oxygen consumption rate and ammonium consumption are shown. The oxygen consumption rate at the start of the aerobic phase is enhanced due to the energy requirement for the uptake of phosphate and polymerization to polyphosphate. At a high biomass concentration in the reactor, the increased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

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

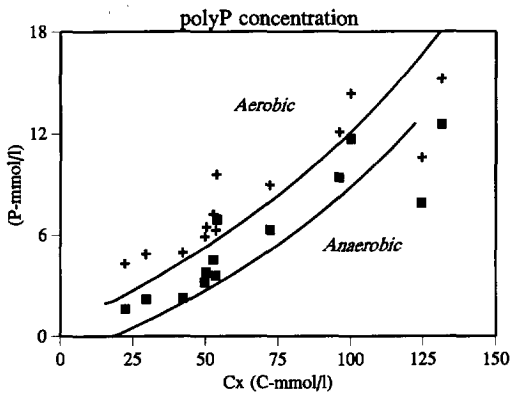
In figure 9 the predicted and measured concentrations of PHB, polyP and glycogen in the reactor at the end of the anaerobic and aerobic phase are shown as a function of the biomass concentration. The measured and calculated PHB fraction as function of the biomass concentration was shown in figure 4,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

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

a)



b)



c)

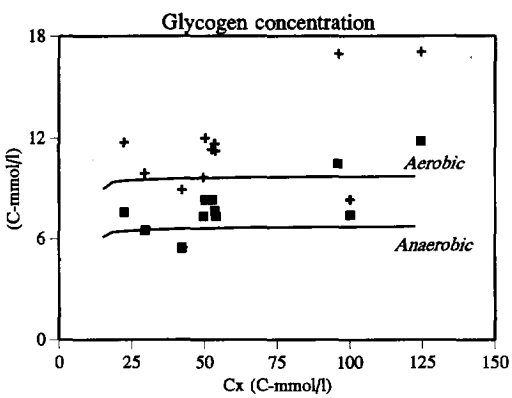
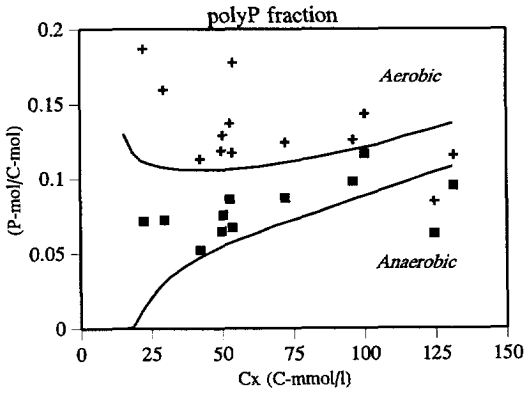


Figure 9. *Concentrations of the internally stored components as a function of the biomass concentration* C_x.

a)



b)

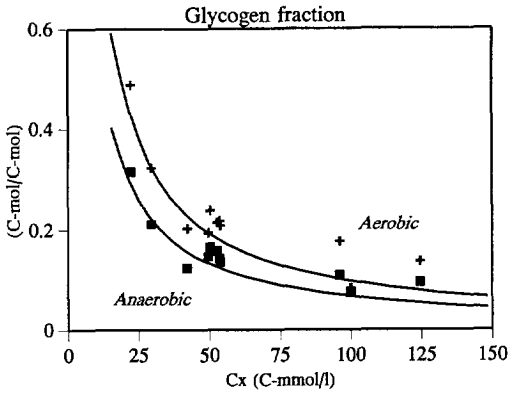


Figure 10. *Fractions of polyP (a) and glycogen (b) as a function of the biomass concentration.*

Discussion

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

constant, the biomass concentration in the reactor can be approximated, if the acetate feed and the sludge retention time in the system are known, with equation (3).

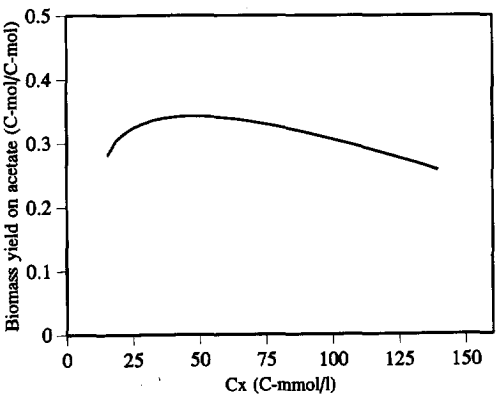


Figure 11. *Biomass yield on acetate as a function of the biomass concentration.*

From figure 5 to 10 it appeared that the metabolic model is capable to describe the experiments satisfactorily with the set of parameters shown in table II. With this set of parameters, the effect of the sludge retention time on the fractions of PHB, polyP and glycogen during the anaerobic/aerobic cycle was calculated and shown in figure 12. From these figures the importance of the storage products can be made clear.

PHB

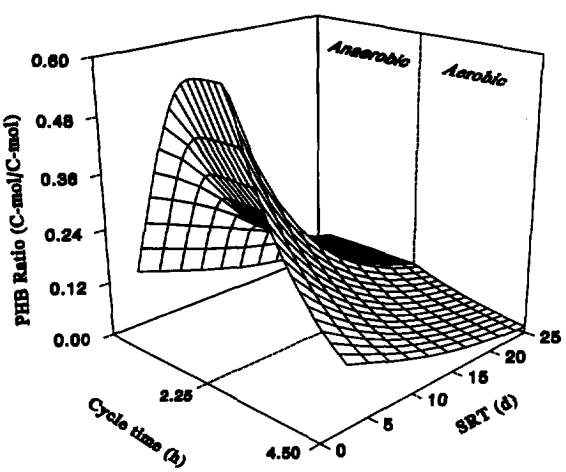
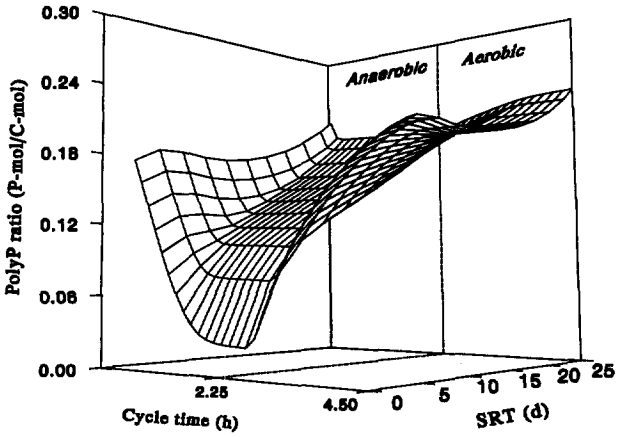


Figure 12a. *PHB content during the anaerobic phase as a function of the SRT.*

polyP



Glycogen

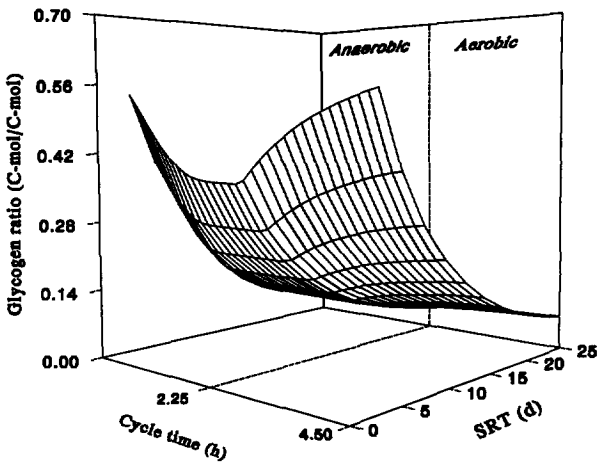


Figure 12b. *PolyP- and glycogen content during the anaerobic and aerobic phase as a function of the SRT.*

Importance of storage products in the P-removal

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

Effect of SRT

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

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

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

Effect of peak loading

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

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

Parameter estimation

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

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

In this balance, the net PHB production is very stringently bound to the removed volume ΔV_w, and the PHB content of the cells. From this balance it can be seen that kinetic parameters describing anaerobic and aerobic conversions, derived only from estimates based on measurements over one cycle are not adequate. The reason is that in one cycle it is not necessary that the steady state requirements are satisfied. If e.g. the calculated anaerobic and

aerobic PHB conversions are respectively, 8.00 and 7.90 C-mmol/l, at a SRT of 8 days a PHB content of 0.05 C-mol/C-mol is found. If a deviation of 5 % in the fit or in the measurement of the aerobic PHB consumption cycle occurs, due to a small change in the kinetic parameters, the steady state PHB content, calculated according to equation 18 will increase to 0.27 C-mol/C-mol: a 5 times higher content than measured. In the estimation of the kinetic parameters, it is therefore essential to take the steady state restictions (equation 18) into account. Similar sensitivities and restrictions apply to the glycogen content of the cells as well. These steady state restrictions were used in the parameter estimation procedure, yielding more accurate values than reported previously in chapter 4.

Conclusions

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

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

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Nomenclature

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

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Start-up dynamics

6

Validation of the metabolic model: start-up dynamics

G.J.F. Smolders, M.C.M. van Loosdrecht, J.J. Heijnen

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

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Introduction

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

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

Aim

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

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

Experimental design

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

I) Controlled acetate feed with aerobic heterotrophic biomass present

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

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

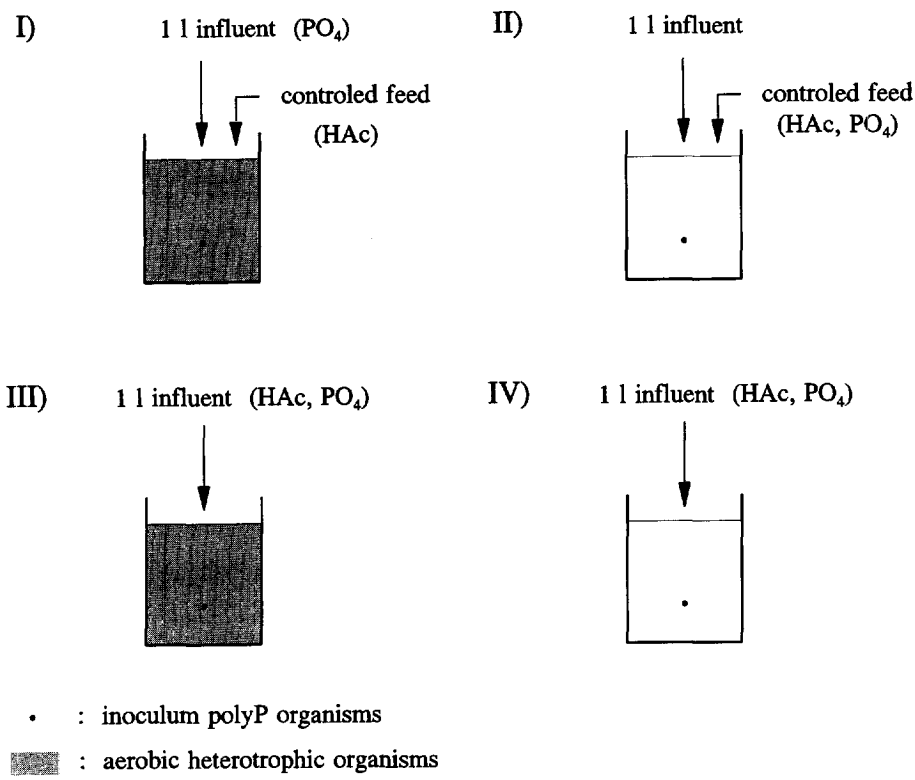

Figure 1. *Schematic presentation of the variation of the acetate and phosphate feed and presence of heterotrophic organisms in the experiments. Influent was batch wise added at the start of the cycle, the controlled feed during the entire anaerobic phase. Four experiments were performed: I) a start-up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

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

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

III) Full influent feed with heterotrophic biomass present

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

IV) Full influent feed with no heterotrophic biomass present

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

Materials and methods

HAc feed controller

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

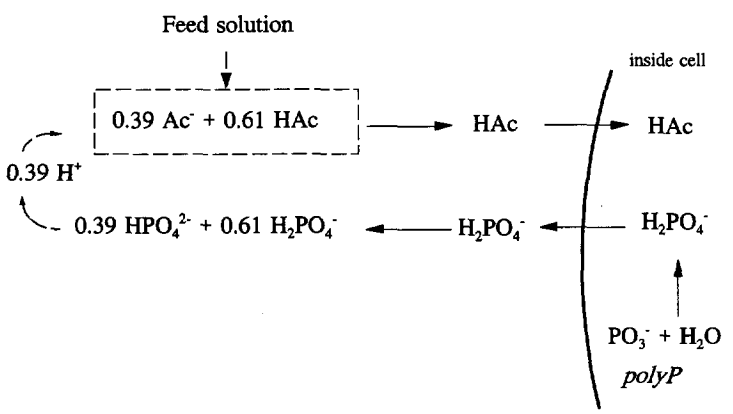

Figure 2. Required mixture of acetate and acetic acid in the feed of the reactor. During the uptake of 1 molecule acetate (2 carbons, HAc), 1 mol polyP is hydrolysed and consequently 1 mol phosphate ($H_2PO_4^-$) is released. In the subsequent dissociation of phosphate at pH 7.0, 0.39 mol protons are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

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

SBR

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

Media & analysis

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

Metabolic model

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

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

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

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

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

Results

Heterotrophic growth of non-polyP organisms

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

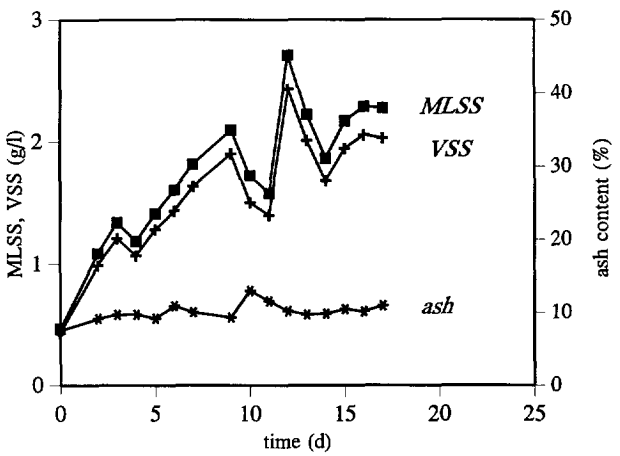

Figure 3. Start-up of a sequencing batch reactor fed with acetate in the aerobic phase.

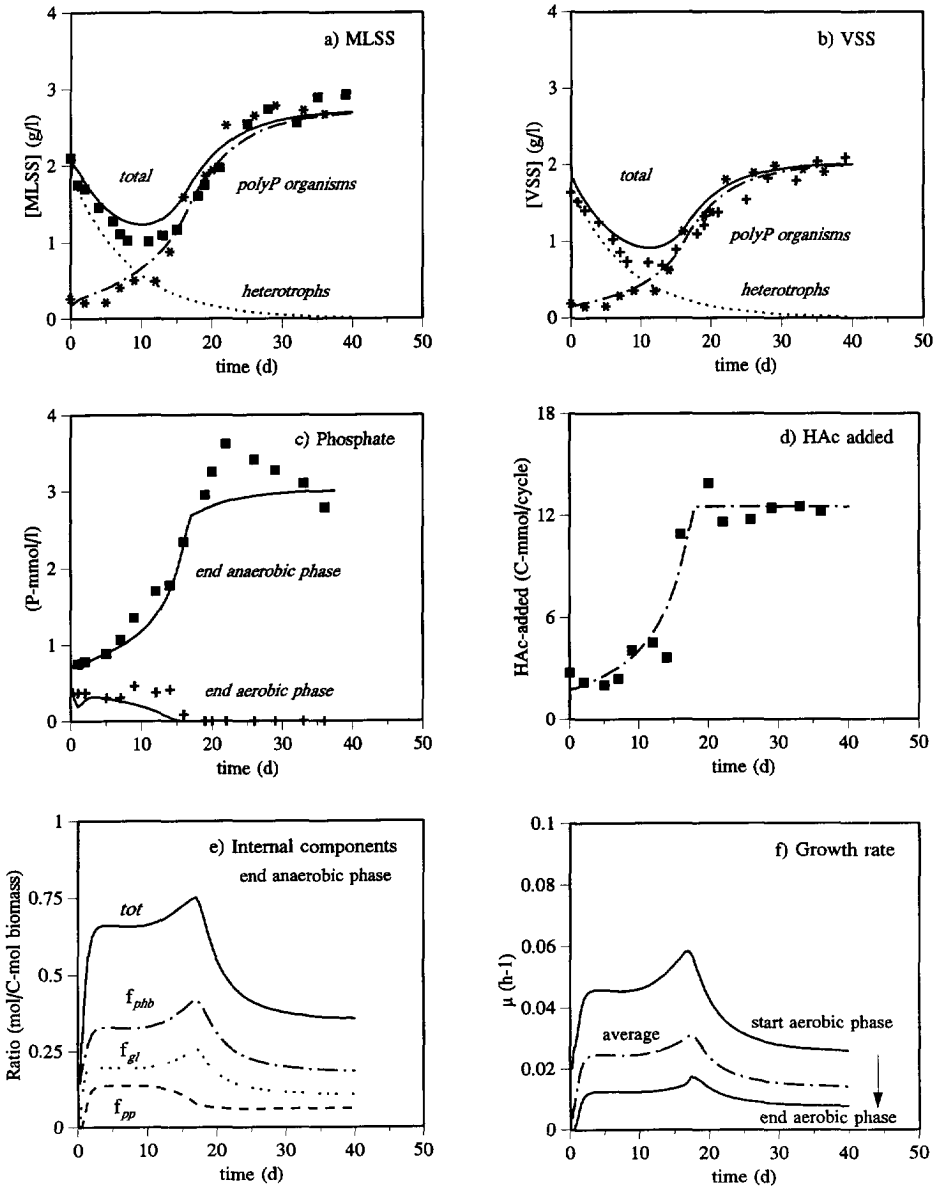

Fig. 4. Results of experiment I: heterotrophic biomass present and controlled acetate feed. a) Total MLSS (■) and polyP biomass concentration (*); b) Total VSS (□) and polyP biomass concentration (*); c) Phosphate concentration end anaerobic (■) and aerobic phase (□); d) Acetate addition during the start-up;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

Experiment I

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

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

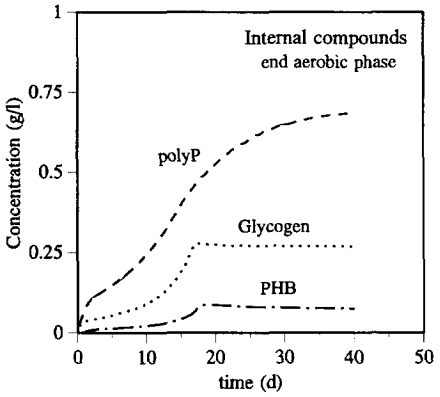

Fig. 4g. *Concentrations of the internal components during the start up*

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

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

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

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

Experiment II

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

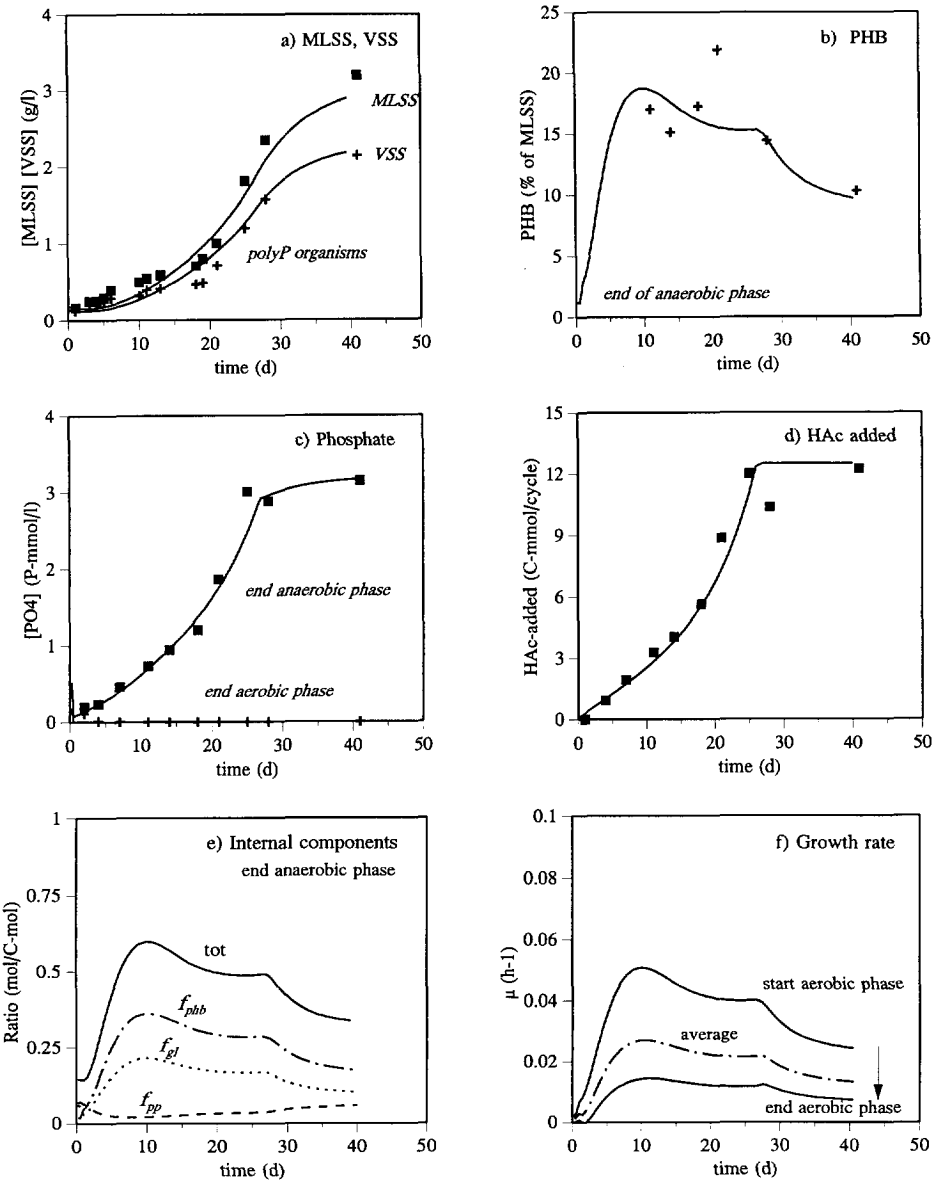

Fig. 5. Results of experiment II: no biomass present and with a controlled acetate and phosphate feed. a) MLSS (■) and VSS (+) concentrations; b) Percentage PHB of MLSS; c) Phosphate concentration end anaerobic (■) and aerobic phase (+); d) Acetate addition during the start-up; e) Internal compounds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

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

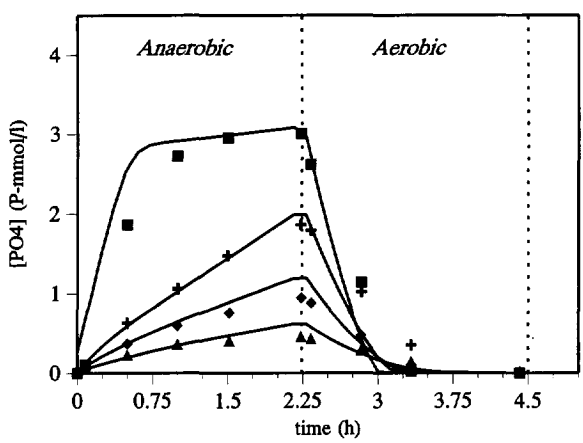

Figure 5g. Phosphate release and uptake during the cycle.

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

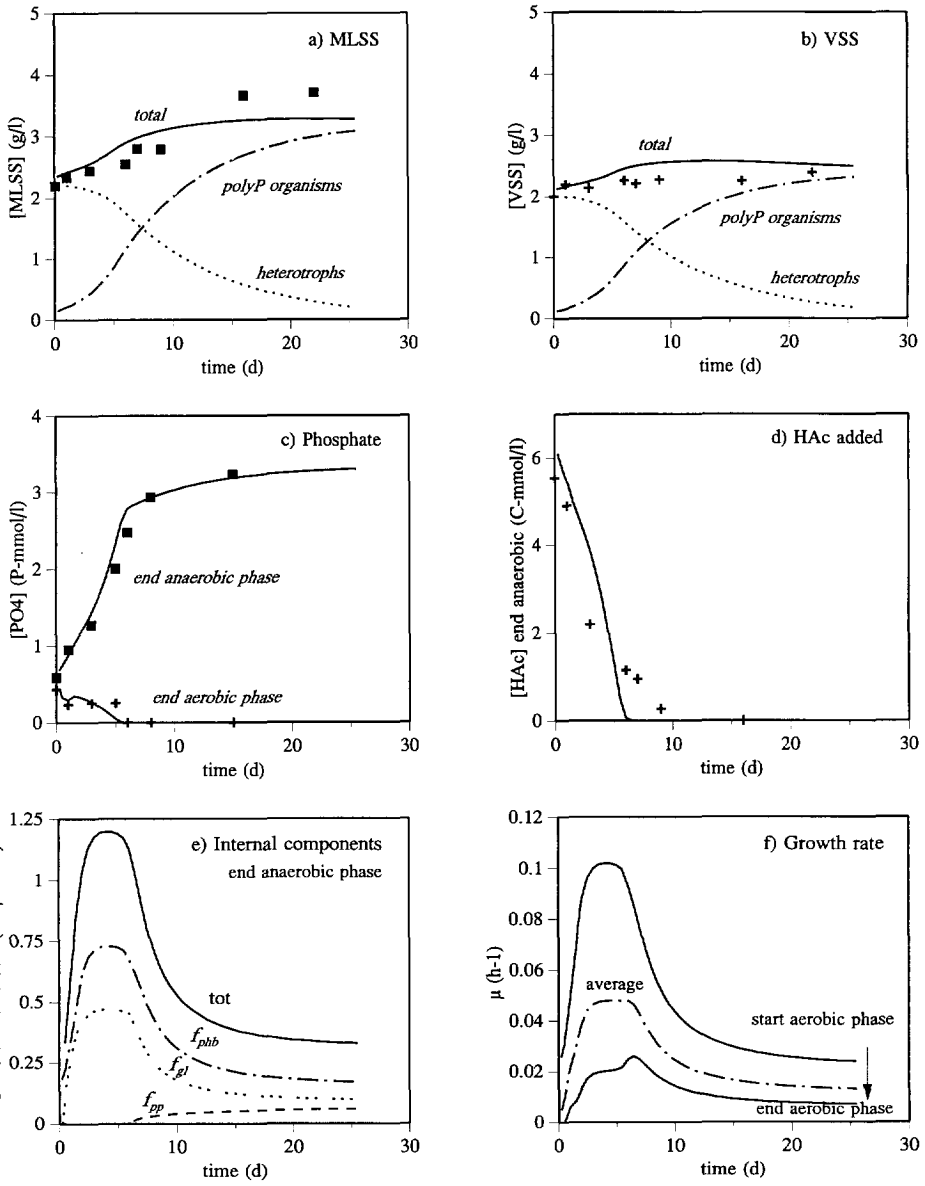

Fig. 6. Results of experiment III: biomass present at the start of the experiment and with full influent feed. a) Total MLSS concentration (■); b) Total VSS concentration (+); c) Phosphate concentration end anaerobic (■) and aerobic phase (+); d) Acetate concentration at the end of the anaerobic phase;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

Experiment III

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

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

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

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

Experiment IV

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

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

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

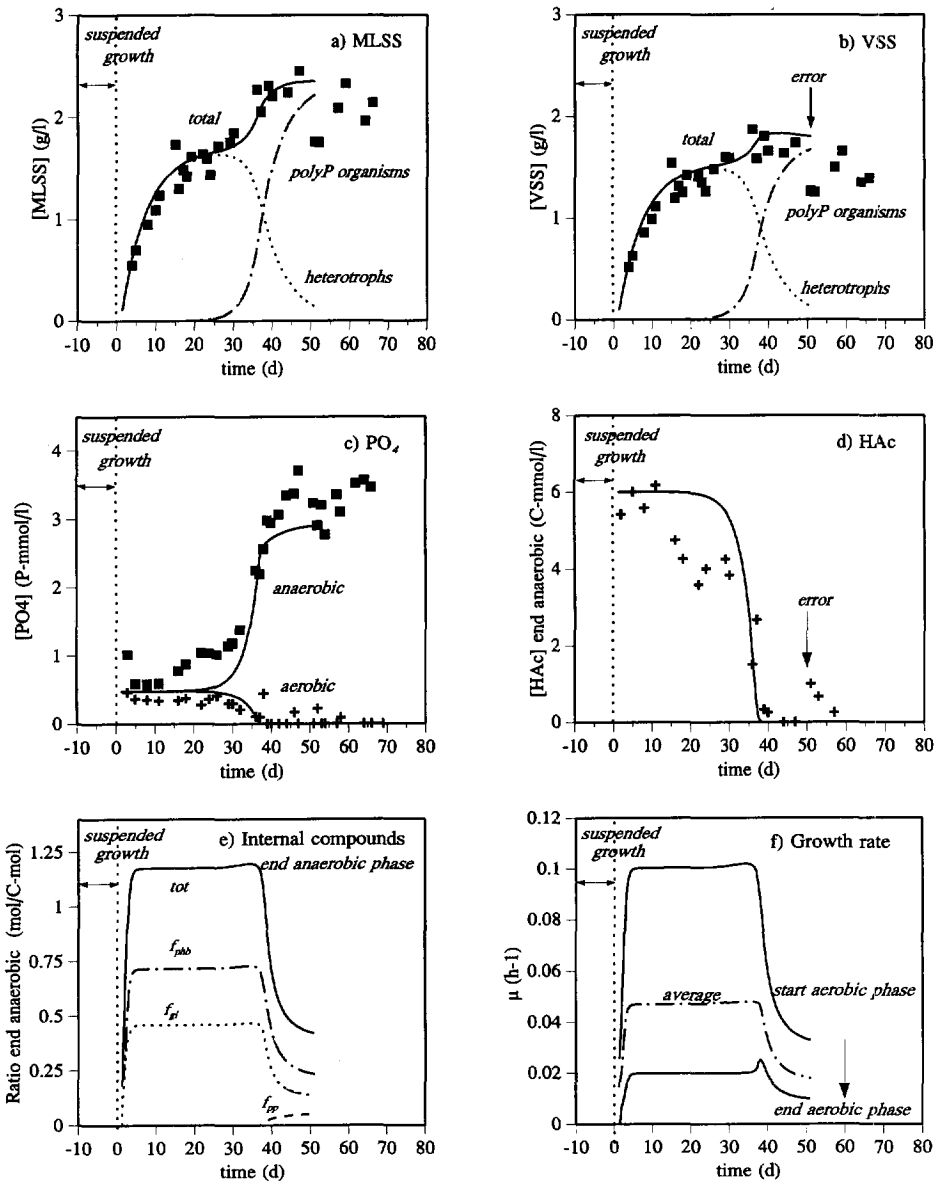

Fig. 7. Results of experiment IV: a low heterotrophic biomass concentration present at the start of the experiment and with full influent feed. a) Total MLSS concentration (■); b) Total VSS concentration (+); c) Phosphate concentration end anaerobic (■) and aerobic phase (+); d) Acetate concentration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

Discussion

Model validation

It has been shown that the metabolic model described the start-up experiments accurately. The MLSS- and VSS biomass concentrations of polyP- and heterotrophic organisms, as well as the phosphate release and -uptake dynamics predicted by the model were in good agreement with the measurements during the experiments. It is emphasized that all experiments described here, could be modelled with a single set of stoichiometric and kinetic parameters. In addition, this set of parameters is the same as the set used in chapter 5, except for the specific acetate uptake rate. The fact that the set of parameters is nearly the same as the set of chapter 5, although established independently under different circumstances, can be seen as an additional indication that the structure of the metabolic model is adequate for the description of the conversions of the biological phosphorus removal process.

Table II. Experimental setup and results

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

* Estimated from model fit (see text).

Factors effecting the start-up rate

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

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

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

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

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

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

growth for a long time. When the polyP organism finally are growing, the growth rate is comparable to experiment III.

Maximal growth rate

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

Decreased biomass yield of polyP systems

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

Conclusions

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

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

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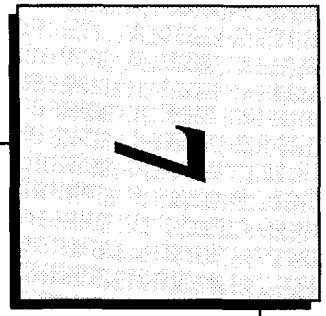
Process analysis



Steady state analysis of mainstream and side-stream process configurations

G.J.F. Smolders, M.C.M. van Loosdrecht, J.J. Heijnen

A stoichiometric analysis for the evaluation of different process configurations for the biological phosphorus removal is developed. This analysis is a steady state simplification of the general dynamic model of biological phosphorus removal developed previously. It provides a simple method to compare the phosphate uptake capacity and acetate requirement of different biological P-removing process configurations like the mainstream and side-stream process. Based on the phosphate concentration of the influent and the system configuration, the minimal required concentration of phosphorus removing organisms (polyP-organisms) in the system, and the maximal phosphate/acetate-COD ratio of the influent to obtain full P-removal can be calculated. The phosphate/acetate-COD ratio of the influent can be used as one of the main parameters in the analysis of a process configuration. The acetate requirement for the side-stream process where P-removal in a stripper tank is applied is shown to be much lower than the requirements needed in a mainstream process. The required concentration of polyP-biomass in a side-stream process is 10 times lower.

This chapter is submitted for publication in *Water research.*

Introduction

The design of biological phosphorus removing activated sludge systems is often problematic due to the wide range of possible process configurations, the high complexity of the biological phosphorus removal (BPR) process, and the comprehensive models which use a high number of parameters to simulate the process. In the last decade, a number of different process configurations has been developed for the biological phosphorus removal in activated sludge systems.[13] Clearly, the first step in the design of a BPR process is to choose an appropriate process configuration for a given waste water. A quick and clear concept to compare these different process configurations and their P-removal efficiency is useful in this phase. From the point of view of the P-removal, the configurations can be subdivided in mainstream and side-stream processes, indicating whether the anaerobic phase required for the P-removal is situated in the mainstream or the side-stream of the process. Important aspects in the evaluation are the prospective phosphate uptake capacity of the reactor and the COD requirements of the system to obtain complete phosphate removal.

Complex dynamic models for the design of BPR systems were developed, based on assumptions for the mechanism of the P-removal.[14,18] Recently, the IAWQ Task Group Activated Sludge Model No.2 was presented[2] in which the biological phosphorus removal process was also incorporated. A more mechanistic, metabolic model of the biological phosphorus removal process was developed and has been experimentally validated.[9-12] This model is based on the metabolic reactions of the organisms and describes the dynamic behaviour of the internal storage compounds.

All these dynamic models are quite complex and use a high number of stoichiometric and kinetic parameters. In the design and comparison of phosphorus removing process configurations however, not all of these parameters are required. If the assumptions are made that the process is in steady state and that the conversions will be performed completely, the analysis to evaluate the phosphate removal capacity and acetate requirement of different BPR process configurations can be simplified to a large extent. In a steady state where complete P-removal and acetate consumption is achieved, the relevance of kinetic expressions is eliminated, because all conversions are determined by their stoichiometry. Therefore, only the stoichiometric parameters of a model have to be applied.

A steady state model for the estimation of the effluent phosphate concentrations of a mainstream BPR plant was first developed by Wentzel[17] as a simplification of the dynamic model.[15,16] Maurer and Gujer[5] developed a static model for the calculation of the effluent phosphate concentrations of a P-removing process in steady state, that assumes the polyphosphate content of the polyP organisms to be constant. Only the maximal P-uptake capacity is calculated with this model. These steady state models are different from the analysis provided in this paper. Here we will not develop a model for the calculation of effluent values or the actual process values, but we want to provide a method to compare the P-removal capacity and acetate requirements of different process configurations, based on the influent characteristics.

Previously, we developed a metabolic model for the biological phosphorus removal process which was validated under dynamic conditions. One of the features of a metabolic model is that it is based on reactions in the metabolism of the organism of which the stoichiometry can be considered constant. The stoichiometry of the conversions in the process are therefore known and can be considered constant. The stoichiometric parameters following from this metabolic model will be used as basis for a steady state comparison of different BPR-process configurations.

Aim

In this paper, a straightforward stoichiometric analysis to evaluate the P-removal efficiency and capacity of 3 different BPR process configurations will be developed. Based on the phosphate concentration of the influent and the system configuration, the required concentration of phosphorus removing organisms (polyP-organisms) in the system will be calculated. The required acetate concentration for the growth of this amount of polyP-organisms can be derived. With this acetate concentration it can be determined whether enough acetate is available in the influent or that extra acetate addition is required for complete P-removal. The presence of acetate in the anaerobic zone results from: (1) acetate in the influent, (2) hydrolysis and fermentation processes, or (3) acetate added to the system. It is assumed that the readily biodegradable COD can be treated as acetate (in fermentation processes the COD is conserved). The term COD used in the text, refers therefore to HAc-COD.

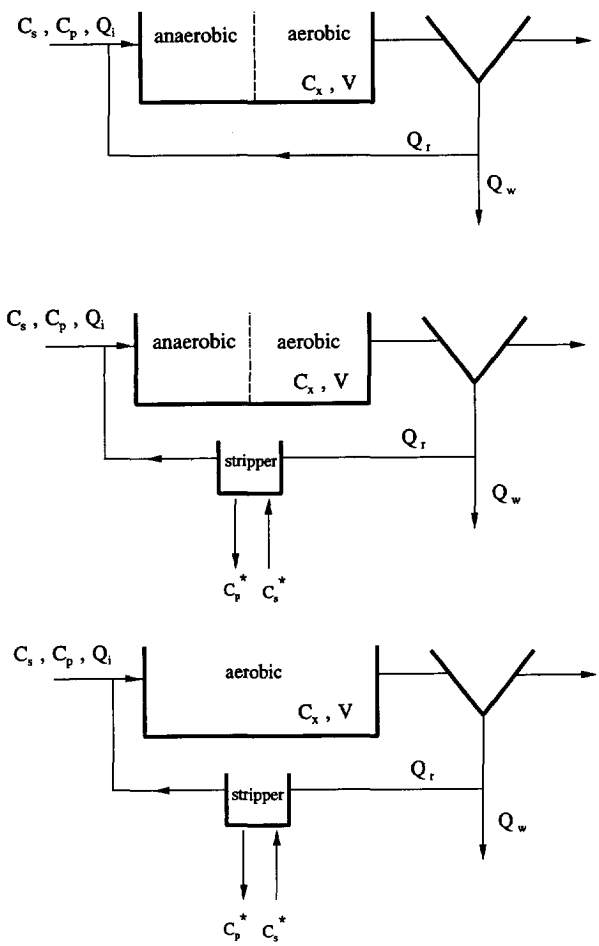


Figure 1. Schematic representation of different BPR processes. Mainstream process (top), Mainstream/stripper (middle) and Side-stream process (bottom).

Process configurations

Three types of configurations will be considered, figure 1. These are the mainstream process, the mainstream process plus phosphate stripper and the side-stream process. A main- and side-stream process are named after the place of the anaerobic phase, required for the P-removal, in the system: in the (main-) water line, or in the (side-) sludge line. In a mainstream process all phosphate is taken up inside the polyP biomass and finally removed in the surplus sludge.

If the phosphate concentration of the influent is higher than the phosphate uptake capacity of the polyP organisms, additional phosphate can be removed with a stripper tank in which acetate is added in combination with the mainstream process. The phosphate released in the stripper tank is precipitated and in contrast to the mainstream process the phosphate leaves the system both in precipitated form and as polyP in the bio-P bacteria. Because the stripper tank can also serve as the sole anaerobic phase of the biological phosphorus removal process, the anaerobic phase in the mainstream can be left out. This is called a side-stream process. An important difference between these two process alternatives is that in the mainstream process acetate from the influent is used whereas in the side-stream no influent acetate is used and acetate-COD has to be added.

In the mainstream/stripper and side-stream process, a biological phosphorus removal method is combined with a chemical precipitation method. The reason for this combination is that the biological process concentrates the phosphate inside the biomass which is subsequently released from the biomass in a stripper tank resulting in high phosphate concentrations. For precipitation methods, the chemical dosage is dependent on the desired phosphate effluent quality,[6] see figure 2. The advantage of a strippertank is therefore that the effluent demand for phosphate doesn't have to be achieved because the effluent of the stripper tank is returned to the aerobic reactor in the process. Further, a lower precipitant/P-ratio can be used because the precipitation efficiency increases at higher phosphate concentrations.

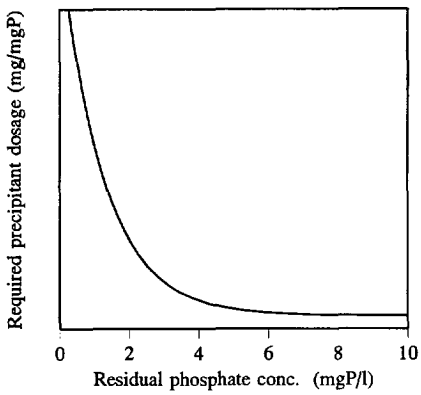


Figure 2. Required dosage of precipitant as function of the desired phosphate effluent quality.

Factors affecting the P-uptake capacity

The primary factor for the selection of P-removing bacteria in an activated sludge system is the recirculation of the sludge through an anaerobic and aerobic zone. In the anaerobic zone (no electron acceptor present) P-removing bacteria take up lower fatty acids, mainly acetate

and store these as poly-hydroxy-alkanoates (PHA, e.g. PHB or PHV). The energy for the transport and storage of acetate is supplied by the hydrolysis of the intra-cellular polyphosphate to phosphate, which is released from the cells into the liquid.[9] The reduction equivalents and part of the ATP is provided in the glycogen conversion to PHB.[1,9] In the aerobic zone PHB is used to generate energy for growth and synthesis of glycogen and polyphosphate, resulting in the uptake of phosphate from the liquid.[10]

The potential phosphate uptake capacity of a BPR system is determined by the concentration of polyP-organisms present in the system. The amount of polyP-bacteria produced in a system results predominantly from the anaerobic uptake of volatile fatty acids. These are stored as PHA and serve as internal substrate for growth in the aerobic zone. The concentration of polyP-organisms is in general only a fraction of the total biomass concentration in a system and therefore it is necessary to distinguish the concentration of polyP-organisms (C_x^{polyP}) from the total sludge concentration (C_x^{tot}). The measured P-content of the total biomass in the system is the average P-content of the polyP-organisms and the other non-polyP organisms. The observed polyP-content of the total biomass might appear to be still low, while the small fraction of polyP-organisms which contain this phosphate have already reached their maximal P-content. In that case, the system has reached the maximal P-uptake capacity although this might not appear from the observed polyP-content of the total sludge. To determine the phosphate uptake capacity of a system, it is crucial to know the maximal P-content of the P-organisms f_{PX}^{max} and the absolute concentration of polyP-organisms (C_x^{polyP}) in the system.

Stoichiometric parameters for the estimation of the P-uptake capacity

To be able to calculate the prospective P-uptake capacity of an activated sludge system, three stoichiometric parameters have to be considered:

1) The maximal phosphate content of the polyP bacteria, (f_{PX}^{max})
2) The biomass yield of polyP-bacteria on HAc (Y_{sx})
3) The phosphate release/acetate uptake ratio, in the anaerobic phase (Y_{sp})

These stoichiometric parameters, needed to calculate the polyP biomass concentration, will be discussed below.

1) Maximal P-content of the polyP-bacteria, f_{PX}^{max}

The phosphate uptake of the polyP-bacteria is found to be limited to a maximal value. Which biological limitation or energetic restriction causes this maximum is not yet clear. In the literature average P-contents of polyP bacteria of 140-200 mgP/gVSS (100-145 mgP/gCOD) are reported for enriched sludge cultures grown on acetate media,[3, 4, 10] with a maximum value of 380 mgP/gVSS (275 mgP/gCOD) reported by Wentzel.[15] The fraction P on suspended solids basis (gP/gSS) follows from recalculation of f_{PX}^{max} taking a molecular weight for one polyphosphate unit ($K_{1/3}Mg_{1/3}PO_3$) of 100 g/mol.[10] The P-content of sludge observed in activated sludge systems is in general much lower, due to the presence of other non polyP-bacterial populations in the total biomass. For the maximal P-content f_{PX}^{max} a value of 0.15 gP/gbiomass-COD was used in the calculations.

2) Biomass yield of polyP bacteria on acetate, Y_{sx}

The active biomass yield of the polyP-bacteria depends on the growth rate of the organisms in the system which is determined by the solids retention time (SRT). In figure 3a the active biomass yield on acetate is shown as function of the SRT. Also shown is the organic biomass yield in the process, which does also include storage products, like PHB and glycogen. The active biomass yield on acetate is clearly not constant as function of the SRT, because the energy demand of maintenance is taken into account. In figure 3b the active biomass yield on acetate is shown as function of the SRT for three different pH values. The decrease in active biomass yield with higher pH values is a result of the increased energy requirements for the uptake of acetate during anaerobic conditions.[9] Figure 3c shows the effect of different phosphate/acetate ratio's of the influent on the biomass yield at a pH value of 7.0. The observed differences are only minor and caused by the higher energy requirements required in the phosphate uptake at higher P/COD ratio's.

From figure 3b and 3c it can be concluded that the active biomass yield on acetate is neither strongly influenced by the pH, nor by the phosphate/acetate ratio of the influent. In theory, a lower SRT could be used to improve the concentration of polyP organisms due to an increased yield to improve the maximal P-removing capacity of an activated sludge system. However, the decrease in SRT is generally restricted by the required growth of nitrifiers and further disagrees with a policy to minimize the sludge production of an activated sludge system. An average active biomass yield of 0.34 mgCOD/mgHAc-COD can be used over a range of SRT's from 5 - 20 at pH 7.0. For exact calculations the active biomass yield going

with the actual conditions should be applied. The calculations of figure 3a-c were based on the metabolic model described previously.[9-12]

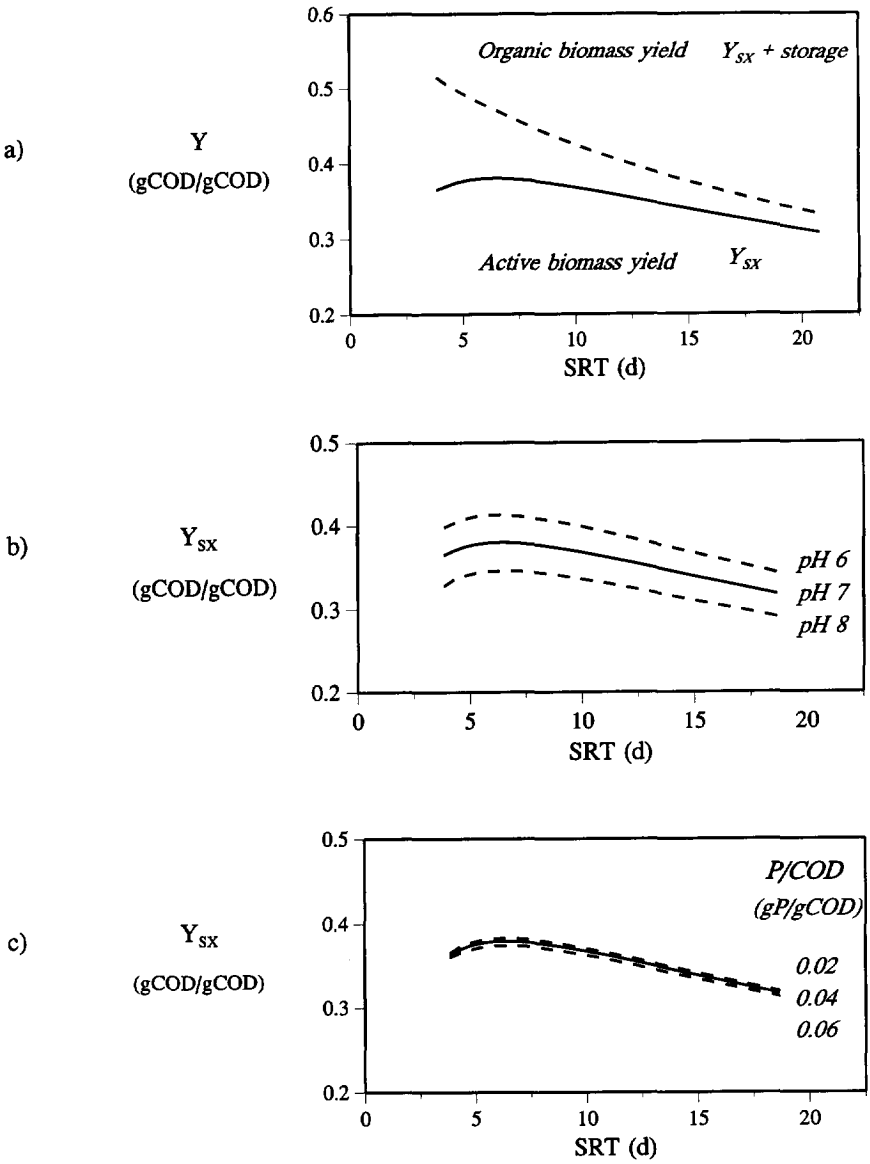


Figure 3. *Effect of the sludge retention time on the active- and organic biomass yield, a); calculated at three different pH values, b); with three different influent phosphate/acetate ratio's. c).*

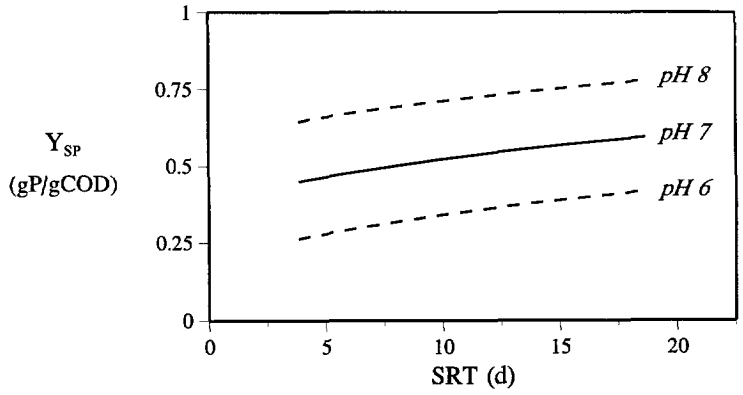


Figure 4. Effect of the sludge retention time on the acetate uptake and phosphorus release ratio in the anaerobic phase of a BPR process at three different pH values.

3) Phosphate release/acetate uptake ratio in the anaerobic phase, Y_{SP}

When the phosphate uptake capacity of the polyP organisms is not enough to take up all phosphate of the waste water, a stripper tank can be used in the process. In a stripper tank, acetate-COD is added to the polyP organisms under anaerobic conditions. The polyP organisms take up this acetate and phosphate is released, which can subsequently be removed from the water by precipitation. When a stripper is used in the process configuration, the phosphate release resulting from the addition of acetate has to be known, to be able to calculate the decrease in polyP content of the polyP organisms. The ratio between phosphate release and acetate uptake is not constant but highly influenced by the pH. As the pH increases, more energy is required for the uptake of acetate and more phosphate is released to supply this energy.[9] In figure 4 the effect of the pH on the phosphate/acetate ratio is shown as function of the sludge retention time. The increase of the Y_{SP} with the increase of the SRT is due to the increased contribution of polyP hydrolysis for anaerobic maintenance in the total released amount of phosphate as a result of the higher biomass concentration. The effect of different P/COD ratios of the influent has no significant effect on the total observed Y_{SP}. In table I the parameter values used in this paper are shown.

Calculation of the phosphate uptake capacity

For the analysis it is assumed that all reactors are designed in such a way that the substrate is completely converted and therefore the best performance of a process configuration will be calculated. The calculations are performed for steady state situations. One has to realize further, that not all phosphate in the influent needs to be removed by polyP bacteria. A fraction of the phosphate will be used for cell growth (dependent on the sludge production and therefore the SRT) including growth of non-polyP-organisms, while a fraction does not need treatment depending on the effluent standards. The phosphate used for cell growth of the non-polyP biomass is about 15 mgP/g biomass-COD, and this phosphate is fixed in the biomass and can not be released. The amount of phosphate used for growth of the "normal" biomass can be a substantial part of the phosphate load of the treatment plant and must be taken into account. See for an example Appendix 1. The remaining phosphate concentration has to be removed by polyP bacteria. In the calculations below, the phosphate concentration in the influent refers to this P-amount only.

Criteria for phosphate removal in a mainstream proces

To determine whether the phosphate uptake capacity of a mainstream process will be sufficient, two factors have to be known: the concentration of polyP organisms that can be accumulated in the process and the phosphate content of these organisms. Criteria to establish these two factors will be shown below. In figure 1 the different variables used in this approach are depicted. The volumetric substrate loading rates (acetate r_S, phosphate r_P in: g/l.h), depends on the influent flow (Q_i), the total reactor volume (V) and the concentration of substrate in the influent (C_S, C_P) according to:

$$r_S = \frac{Q_i}{V} \cdot C_S = \frac{C_S}{\tau} \qquad (1)$$

$$r_P = \frac{Q_i}{V} \cdot C_P = \frac{C_P}{\tau} \qquad (2)$$

where τ is the hydraulic residence time defined by:

$$\tau = \frac{V}{Q_i} \tag{3}$$

The production rate of the polyP-bacterial mass (r_X, in gCOD/l.h) is given by the stoichiometric relation:

$$r_X = Y_{SX} \cdot r_S \tag{4}$$

The biomass yield decreases from 0.38 - 0.31 mgCOD/mgCOD in an increasing SRT range of 5 to 20 days, as was shown in figure 3. This effect of the SRT on the biomass yield has only minor effects on the final results. The SRT is given by:

$$SRT = \frac{C_X^{polyP}}{r_X} \tag{5}$$

in which C_X^{polyP} is the mass of polyP-bacteria in gCOD/l of reactor volume.

The phosphate content of the sludge (f_{PX}, as g P/gCOD of active biomass of polyP-bacteria) can now be calculated. The loading rate of phosphorus to the system, r_p, and the production rate of polyP-bacteria, r_x, determines the phosphate content, f_{PX}, as shown in eq. (6), while the production rate of polyP bacteria was determined by the substrate loading rate to the system and the yield, according to equation (4). Therefore, only the biomass yield and the phosphate/acetate ratio of the influent determine the phosphate content of the organisms:

$$f_{PX} = \frac{r_P}{r_X} = \frac{1}{Y_{SX}} \cdot \frac{C_P}{C_S} \tag{6}$$

Equation (6) shows that the phosphate content of the organisms increases when the phosphate concentration of the influent is increased. At a certain phosphate content of the influent however, the maximal P-content of the organisms is reached, and no more phosphate can be taken up. This restriction is the maximal phosphate/acetate ratio of the influent and can be expressed as:

$$\left(\frac{C_P}{C_S}\right)^{max} = Y_{SX} \cdot f_{PX}^{max} \tag{7}$$

The maximal allowed phosphate/acetate ratio of the influent of a mainstream process is determined by the maximal P content of the polyP bacteria. The maximal P-content of the organisms can further be used to calculate the minimal required concentration of polyP-biomass to take up a certain phosphate concentration present in the influent. Combination of equation (6), (2) and (5) gives the **minimal required** concentration polyP-bacteria for complete P-removal:

$$\left(C_X^{polyP}\right)^{min} = \frac{1}{f_{PX}^{max}} \cdot \frac{C_P}{\tau} \cdot SRT \tag{8a}$$

Combination of equations (4), (5) and (1) gives the **maximal achievable** concentration of polyP-bacteria, based on the amount of acetate which can be obtained from the influent.

$$\left(C_X^{polyP}\right)^{max} = Y_{SX} \cdot \frac{C_S}{\tau} \cdot SRT \tag{8b}$$

For each situation the minimal required amount of polyP biomass, eq (8a), and the maximal achievable polyP biomass concentration, eq. (8b), must be compared. From equation (8a) and (8b) it follows that:

$$\frac{\left(C_X^{polyP}\right)^{min}}{\left(C_X^{polyP}\right)^{max}} = \frac{1}{Y_{SX} \cdot f_{PX}^{max}} \cdot \frac{C_P}{C_S} \tag{9}$$

If the minimal required polyP biomass is lower than the maximal achievable polyP biomass, then there is sufficient acetate present in the influent to remove the phosphate, see figure 5, left side, and the mainstream process will be appropriate for full P-removal. If the maximal achievable polyP biomass is lower then the minimal required biomass for the uptake of polyP, other process configurations are required like the introduction of a stripper in the mainstream process or a side-stream process:

Mainstream $$\frac{C_P}{C_S} < Y_{SX} \cdot f_{PX}^{max} \quad (10a)$$

Mainstream/stripper or side-stream $$\frac{C_P}{C_S} > Y_{SX} \cdot f_{PX}^{max} \quad (10b)$$

Phosphate stripping in a mainstream process

If the maximal achievable polyP biomass is lower than the minimal required biomass for the uptake of the phosphate, a stripper tank can be used (figure 1, middle). In this anaerobic tank acetate dosing leads to release of phosphate. The sludge is separated after this tank and returned to the process while the released phosphate is removed by precipitation. To simplify mathematics the acetate added (F_A in g/h) and the phosphate precipitated (P_P in g/h) in the stripper are defined relative to the influent flow, Q_i, (use of this definition allows for an easy comparison between the input and output of the stripper with the concentrations of HAc-COD and P in the influent):

$$C_S^* = \frac{F_A}{Q_i} \qquad C_P^* = \frac{P_P}{Q_i} \quad (11)$$

It is assumed that the stripper inflow and outflow are equal (Q_i). This is allowed because the added acetate feed and precipitated phosphate are negligible on a volumetric basis. Sometimes only a part of the return sludge is treated in the stripper tank. In the approach used here this does not lead to different mathematical expressions. The phosphate release in the stripper, C_P^*, is stoichiometrically related to the acetate addition, C_S^*, (Smolders et al, 1994a):

$$C_P^* = Y_{SP} \cdot C_S^* \quad (12)$$

In a mainstream/stripper configuration, part of the phosphate is removed by the biomass leaving the mainstream process, the rest (C_P^*) by the stripper and subsequent precipitation. The phosphate removed by the mainstream biomass follows from eq (6), realizing that $f_{PX} = f_{PX}^{max}$ and that the acetate concentration equals $C_S+C_S^*$ due to the acetate addition (C_S^*) in the stripper. The amount of phosphate removed by polyP-biomass then equals:

$$Y_{SX} \cdot f_{PX}^{max} (C_S + C_S^*) \quad (13)$$

The phosphate removed by the stripper is C_P^*, hence:

$$C_P^* = C_P - Y_{SX} \cdot f_{PX}^{max} (C_S + C_S^*) \quad (14)$$

Combination of eq (14) with eq (12) and elimination of C_P^* yields the amount of acetate addition required for the complete removal of the phosphate:

$$C_S^* = \frac{C_P - Y_{SX} \cdot f_{PX}^{max} C_S}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \quad (15)$$

Three ratios can now be defined to determine the required acetate addition and the amount of phosphate that will be removed.

1) The fraction of required acetate addition of the total acetate present in the waste water for complete phosphate removal (from equation 15) is given by:

$$\frac{C_S^*}{C_S} = \frac{C_P / C_S - Y_{SX} \cdot f_{PX}^{max}}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \quad (16)$$

2) The acetate addition to phosphate removal ratio (from eq 15) is given by:

$$\frac{C_S^*}{C_P} = \frac{1 - Y_{SX} \cdot f_{PX}^{max} \cdot C_S / C_P}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \quad (17)$$

3) The ratio of phosphate stripped and precipitated relative to the total amount of phosphate removed is:

$$\frac{C_P^*}{C_P} = \frac{Y_{SP}(1 - C_S / C_P \cdot Y_{SX} \cdot f_{PX}^{max})}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \quad (18)$$

The concentration of polyP-organisms required in the system can be calculated with equation 8b, where the acetate concentration equals (C_S+C_S^*)

$$C_X^{polyP} = \frac{Y_{sx} \cdot (C_S + C_S^*)}{\tau} \cdot SRT \qquad (19)$$

Elimination of C_S^* with eq. 15 gives:

$$C_X^{polyP} = Y_{sx} \cdot \frac{SRT}{\tau} \cdot C_S \left(\frac{C_P / C_S + Y_{SP}}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \right) \qquad (20)$$

The equations 16, 17, 18 and 20 fully describe the acetate addition, the phosphate removal and the required biomass concentration of the mainstream/stripper configuration.

Phosphate removal in a side-stream process

In a side-stream process, represented in figure 1 (bottom), there is no anaerobic zone in the water line. The stripper tank acts as an anaerobic zone for the selection of polyP-organisms. No acetate from the influent is used for growth of polyP bacteria. The required amount of acetate C_S^* now follows from eq 15 by realizing that in this case C_S equals zero. The required addition of acetate for complete removal of phosphate is now given by:

$$C_S^* = \frac{C_P}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \qquad (21)$$

The ratios for the required acetate addition, and the amount of phosphate removed in a side-stream process are different from the mainstream plus stripper:

1) The fraction of required acetate addition of the total acetate present in the waste water for complete phosphate removal can be calculated (using equation 21) by:

$$\frac{C_S^*}{C_S} = \frac{C_P / C_S}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \qquad (22)$$

2) The acetate addition to phosphate removal ratio is, using eq 21 given by:

$$\frac{C_S^*}{C_P} = \frac{1}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{23}$$

3) The fraction stripped phosphate of the total amount of removed phosphate follows by combination of eq 21 and eq 12:

$$\frac{C_P^*}{C_P} = \frac{Y_{SP}}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \tag{24}$$

In a side-stream process the acetate addition is only dictated by the phosphate concentration of the influent. If one compares eq. 16 and 22 it is clear that in the side-stream process a higher acetate dosage is required. This is obvious because in the mainstream process acetate available from the influent will also be used for the growth of polyP-bacteria, whereas in the side-stream process none of this acetate is used by the polyP bacteria.
The concentration polyP-organisms required in the system can be calculated with equation (8b) and (21), realizing that for C_S in eq 8 one must substitute C_S^* of eq 21:

$$C_X^{polyP} = Y_{sx} \cdot \frac{SRT}{\tau} \cdot C_S \left(\frac{C_P / C_S}{Y_{SX} \cdot f_{PX}^{max} + Y_{SP}} \right) \tag{25}$$

In table II an overview of the relevant equations for the three process configurations is given.

Results

Parameters

In this paper we used parameter values as shown in table I. An average biomass yield, (Y_{SX}) on acetate of 0.34 gCOD/gHAc-COD was used, see figure 3. For the maximal P-content of the biomass, (f_{PX}^{max}) a relative low value of 0.15 gP/g biomass-COD was used. The

phosphate/acetate yield parameter (Y_{SP}) is strongly dependent on the pH. If not indicated otherwise the overall observed value for pH 7 will be used (0.47 gP/gHAc-COD).

Table I. Parameters used for the calculations.

Y_{ox}	0.34	gVSS/gHAc-COD	SRT	8	d
Y_{sp}	0.47	gP/gHAcCOD	HRT, τ	12	h
f_{PX}^{max}	0.15,0.25	gP/gBiom. COD	pH	7	

Phosphate removal in a mainstream process

In figure 5a the effect of the phosphate/acetate ratio on the minimal required and maximal achievable polyP biomass concentration is shown. If the minimal required biomass concentration becomes higher than the achievable biomass concentration on the acetate in the influent, $C_x^{min}/C_x^{max} > 1$, a side-stream or mainstream/stripper configuration will be required to achieve complete P-removal.

In case the maximal polyP content of the organisms is 0.15 gP/gbiomass-COD, the maximal allowed phosphate/acetate ratio of the influent is 0.051 gP/gHAc-COD. The slope of the minimal and maximal biomass ratio is dependent on the biomass yield of the polyP organisms and the maximal P-content that can be achieved.

In figure 5b the P-content of the polyP organisms as a function of the influent composition is given. The P-content will increase until, at the critical phosphate/acetate ratio of 0.051 gP/gHAc-COD, the maximal uptake capacity of the organisms is reached. Decrease of the SRT to increase the biomass yield (from 20 to 5 days) has only minor effects on the improvement of the uptake capacity. This calculation allows to determine (i) whether a stripper tank is required (in the example when $C_P/C_S > 0.05$ gP/gHAc-COD) and (ii) the amount of COD remaining for other processes e.g. denitrification (in the example when the $C_P/C_S < 0.05$). The critical phosphate/acetate ratio is strongly dependent on the f_{PX}^{max}. Application of the highest maximal P-content for the design of a biological P-removing process is not recommended, because in that case a system is designed with no extra P-uptake capacity left.

Phosphate removal in a side-stream process

In the side-stream process no use is made of HAc-COD present in the influent. It should therefore only be used when easily biodegradable COD is limiting or absent.

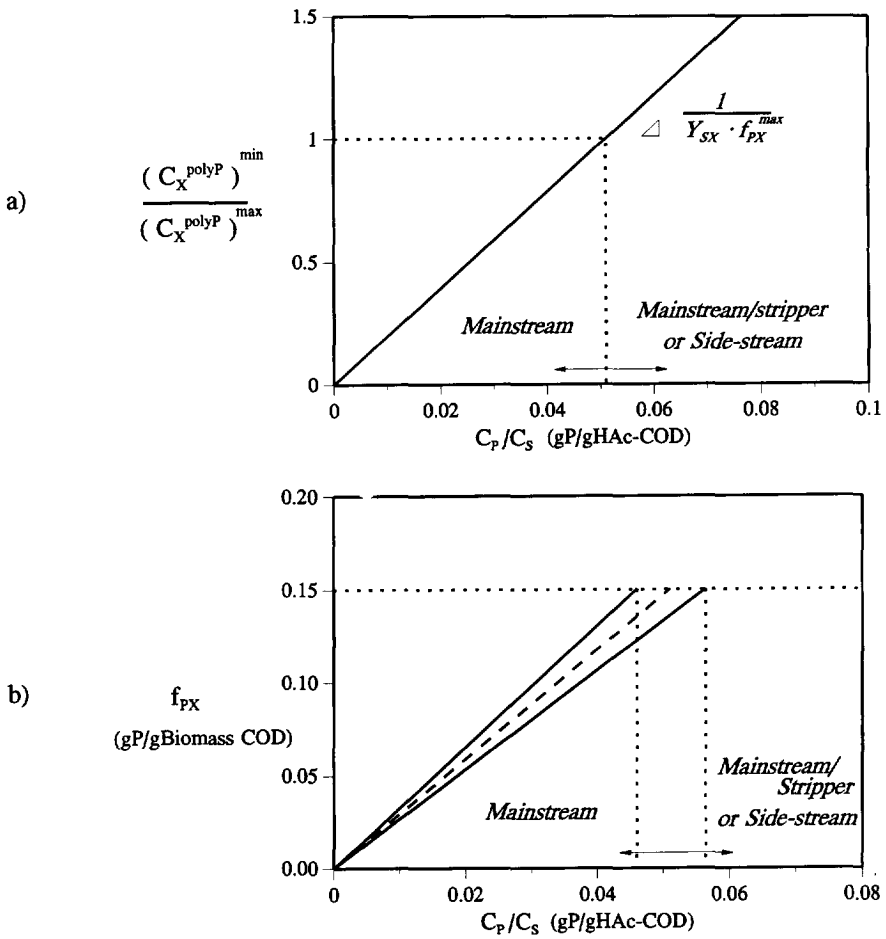
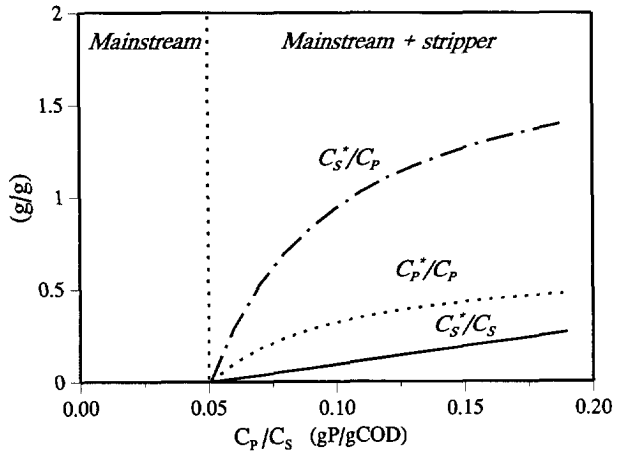


Figure 5. *a) Minimal required polyP biomass concentration for the removal of the influent phosphate concentration, and maximal achievable polyP biomass concentration on the acetate present in the influent; b) P-content of the polyP organisms as function of the phosphate/acetate ratio of the influent. If the ratio is higher than 1, the polyP organisms have reached their maximal uptake capacity.*

In figure 6 the amount of substrate that has to be added in the stripper tank of a mainstream (6a) and side-stream process (6b) relative to the influent acetate and phosphate concentration (C_S^*/C_S and C_S^*/C_P) are compared. Also shown is the amount of precipitated phosphate (C_P^*/C_P).

a)



b)

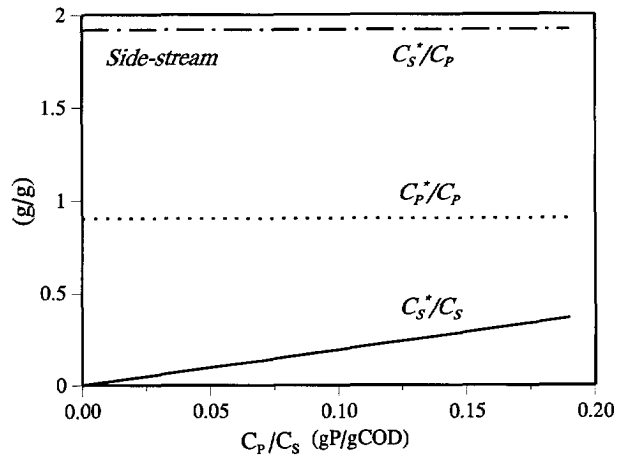


Figure 6. Required acetate addition, as a function of the phosphate/acetate ratio of the influent, to a) a stripper tank in a mainstream; and b) a side-stream process.

The most important differences between the mainstream/stripper and side-stream process are that in a mainstream/stripper process acetate has to be dosed only above a certain influent P/HAc ratio (in the example of figure 6: 0.05 mgP/mgCOD) whereas a side-stream process always requires addition of acetate.

Table II. *Calculation methods for the acetate addition and phosphate removal for different configurations of biological phosphorus removing processes. C_P,C_S : Concentrations of phosphate and acetate in the influent; C_P^*,C_S^* : Concentrations of phosphate and acetate added to the stripper per lite influent.*

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

The acetate dosage, C_S^*/C_P, in a side-stream process is about 2 mgHAc-COD/mgP in the influent while in a mainstream process substantially lower doses are required. Without HAc-COD in the influent (C_S =0) the addition of HAc to the stripper tank is identical for both processes, see table II. The amount of phosphate removed by precipitation with the stripper (C_P^*/C_P) in the side-stream process is 0.9 mgP/mgP of the influent. The other 10 % of the influent phosphate is removed inside the biomass of the polyP organisms.

Table III. *Required acetate dosage to a stripper tank and the concentration polyP-organisms required for full P-removal. SRT 8 d, HRT 12 h, pH 7.*

Configuration	Influent			Stripper		Biomass
	C_P	C_S	P/COD	C_P^*	C_S^*	C_x^{polyP}
	mgP/l	mgCOD/l	mgP/gCOD	mgP/l	mgCOD/l	gCOD
(1) Mainstream	15	500	30	-	-	2.72
(2) Mainstream	15	300	50	-	-	1.63
(3) Main/stripper	15	200	75	4.3	9.2	1.14
(4) Side-stream	15	<<1		13.5	28.8	0.16

Acetate requirement and concentration of polyP organisms

The acetate dosage to a stripper tank, the P-removal, and the required polyP biomass concentration for mainstream and stripper process configurations were calculated for four different influent phosphate/acetate ratios and shown in table III. In the first example of table III (1), the influent COD/P ratio is 0.03 mgP/mgCOD. There will be enough polyP biomass production and therefore no stripper is required for this waste water. Equation (8b) was used to calculate the concentration polyP-organisms, which was 2.72 gCOD/l. The P-content of the polyP bacteria amounts to 88 mgP/gbiomass-COD. In example (2) the COD requirements for the P-removal are calculated if the polyP organisms in the process will be operated at their maximal P-content of f_{PX}^{max} = 0.15 gP/gbiomass COD. If the phosphate/COD ratio of the influent is above this maximal value, see example (3), a strippertank has to be used to obtain full P-removal. The required amount of acetate dosage and stripped phosphate is calculated with equation (17) and (18). The polyP-biomass concentration is calculated using equation (20). For a side-stream process in which the influent COD is not used by polyP-organisms, example (4), the acetate requirement and phosphate removal is calculated according to equation (22) and (23). The biomass concentration was calculated with eq (24).

From table III it appears that the total acetate requirement and the required polyP-biomass concentration for P-removal is decreased when a stripper tank is used. This is logical because in the stripper tank the released P is not taken up again inside the biomass, but it is precipitated. Therefore much lower polyP biomass concentrations are required. In figure 7 the required polyP-biomass concentration in the system for the uptake of 1 g/l phosphate per liter of influent is shown. From this figure it appears that in the mainstream configuration always more biomass will be required. When a stripper is used, the required amount of biomass per gram phosphate is decreased.

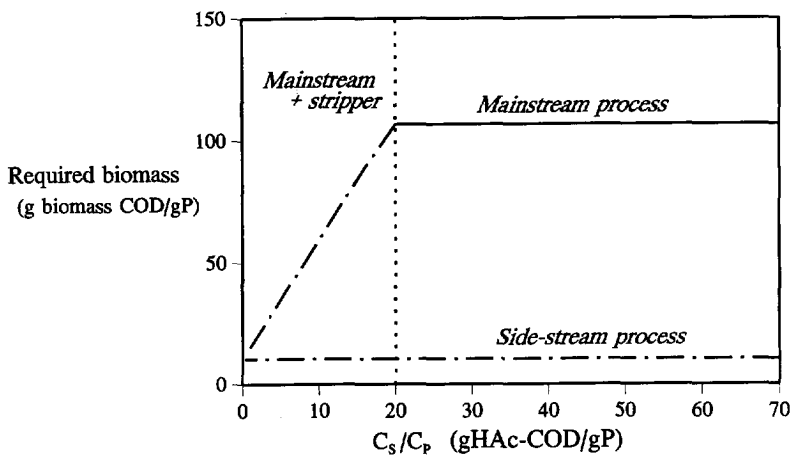


Figure 7. Required polyP-biomass production as a function of the HAc-COD/P ratio in the influent.

Effect of pH on the acetate dosage to a stripper tank

The addition of acetate in a stripper tank is dependent on the biomass yield, the maximal P-content and P-released due to acetate uptake. To optimize the acetate addition in a stripper tank, control of these parameters can be advantageous. The acetate/phosphate yield (Y_{SP}) is the only controllable parameter, by adjusting the pH in the stripper. The effect of pH control on the dosage of acetate to a stripper tank can be deduced from figure 4. The phosphate release per added amount of acetate increases at higher pH. In addition, due to the consumption of acetate the pH will also increase.

Discussion

Mainstream versus Side-stream process

Addition of chemicals to waste water treatment processes should in principle be minimized. An extra argument to avoid the addition of COD is formed by the fact that extra sludge will be produced. This will lead to a lower fraction of nitrifiers in the sludge and therefore to longer aerobic retention times. Therefore, a mainstream process will generally be the optimal process choice. When the waste water shows a shortage of acetate-COD, a stripper tank can be introduced in the mainstream process. A side-stream process, in fact a special case of the mainstream process with stripper tank, should only be used when no acetate-COD is available in the influent for biological phosphorus removal.

In table II, the calculation methods for the acetate addition and phosphate removal for different configurations of BPR processes are summarized. From these equations it can be seen that the acetate addition (C_S^*/C_P), and the stripped amount of phosphate in a mainstream/stripper process are always lower than in a side-stream process. According to these equations the required polyP biomass concentration in a mainstream process will always be higher than in a side-stream process. In a mainstream process the polyP-bacteria function as the "container" of the phosphorus, whereas in a side-stream process the function of polyP-bacteria is to temporarily concentrate the phosphate, which is subsequently released and precipitated. The phosphate concentration in the aerobic phase of a side-stream process is therefore much lower than in a mainstream process, requiring less polyP-biomass to take up the phosphate. In a mainstream process the required amount of polyP-bacteria follows from the need to store the phosphorus load to the system during one sludge residence time, whereas in a side-stream process the required storage capacity of the sludge is equivalent to the load during one hydraulic residence time.

The difference in required polyP-biomass for a mainstream process and a side-stream process can be deduced from the equation for the biomass concentration in a mainstream (20), and a side-stream (25) and equation (13):

$$\frac{C_X^{polyP}\ main}{C_X^{polyP}\ side} = 1 + \frac{Y_{sp}}{Y_{sx} \cdot f_{PX}} \qquad (26)$$

According to this equation (26), using the values of table I, a mainstream process requires 10.2 times more polyP-biomass than a side-stream process. In the side-stream process much less acetate-COD is needed for P-removal due to the additional P-removal by precipitation.

Stripper optimization

The effect of the pH on the P-release is important for the design of the stripper tank. Optimal adjustment of the pH can minimize the acetate addition (fig. 4). The growth yield and the maximal P-content of the polyP-bacteria are relatively independent of the pH. Therefore, it is not likely that there will be a large effect of the pH on the mainstream process. Only the phosphate concentration at the end of the anaerobic period will be dependent on the pH. The effect of the pH on Y_{SP} can be explained by the pH dependent energy requirement of the acetate uptake.[9] The pH effect might explain why many different Y_{SP} values have been reported in the literature. All these values are in the range covered by the pH effect. Unfortunately most authors have not reported the pH at which the experiments were performed, making it impossible to really show this relation with literature data.

Nitrogen and Phosphorus removal

In many cases in the design of a treatment plant there will be a need for both N and P removal. Often there is only a limited amount of readily available COD in the influent and therefore, nitrogen and phosphorus removing bacteria will compete for COD. To be able to obtain complete N and P-removal in that case, COD addition is required. One has the choice to add COD to increase denitrification or to add COD to increase the removal of phosphorus. For nitrogen removal approximately 3.5 mg COD is required per mgN. For phosphorus removal in a stripper tank 2 mg of HAc-COD per mgP stripped is required and P-removal in a mainstream process requires 20 mgHAc-COD/mgP. In this regard, it is clear that in case of COD shortage, a stripper tank is the most COD-efficient way for the P-removal. By using a stripper tank, more COD in the influent becomes available for nitrogen removal.

An example is given in table IV, based on a theoretical influent with an acetate concentration of 450 mgCOD/l, a nitrogen concentration of 60 mgN/l and 15 mgP/l phosphate. For the removal of 15 mgP/l in a mainstream process, 450 mgCOD will be required for the removal of phosphate when in the anaerobic phase all acetate is consumed by P-removing organisms. These organisms will not reach their maximal P-content in that case. Because all the COD is consumed no COD is available for denitrification. If the P-organisms would be operated at their maximal P-content the minimal required amount of COD is used for the removal of

the phosphate and 150 mgCOD/l would become available for nitrogen removal. For total nitrogen removal however, 210 mgCOD/l is required; still there is a shortage of 60 mgCOD/l. If a mainstream/stripper configuration is applied and only 240 mgCOD/l of the influent COD is used for the P-removal, the additional P-removal has to be performed in the stripper requiring a small amount of acetate-COD. In that case, 210 mgCOD/l of the influent would become available for the denitrification which can then be fully removed. If a side-stream process configuration was used, only 28.8 mgCOD/l would be required for the removal of phosphate and 421.2 mgCOD/l would be available for N-removal or other COD consuming processes. From the point of view of COD usage, in a combined P/N removal process clearly P-removal by a stripper tank leads to a substantially decreased requirement of total COD-addition. Whether this still holds considering other aspects (costs of chemicals, extra sludge handling) has to be considered. A full economic/environmental impact evaluation will be needed. A completely different alternative to integrate P and N removal is to utilize phosphorus removing denitrifing microorganisms as proposed by Kuba.[4]

Table IV. *Illustration of the COD requirement of several process configurations for an influent containing 450 mgCOD/l, 60 mgN/l and 15 mgP/l. Process conditions: SRT 8 d, HRT 12 h, pH 7.*

Configuration	P-removal by		COD for P-removal		COD for N-removal		Influent COD not used for N/P-removal	Shortage
	Precip. (%)	Biomass (%)	Stripper mgCOD/l	Biomass mgCOD/l	Required mgCOD/l	Available mgCOD/l	mgCOD/l	mgCOD/l
(1) Main	0	100	0	450	210	0	0	-210
(2) Main, f_{PX}^{max}	0	100	0	300	210	150	0	-60
(3) Main/stripper	20	80	6	240	210	210	0	-6
(4) Side	90	10	29	0	210	421	240	-29

Conclusions

Using the steady state analysis developed in this paper, it is possible to calculate the phosphate removal capacity of different biological phosphate removal process configurations.

The required concentration of phosphorus removing organisms (polyP-organisms) in the system, and the required acetate addition can be calculated. The phosphate concentration of the influent and the system configuration make demands upon the P/COD ratio of the influent.

The P-uptake capacity of a mainstream P-removing system is fixed with the P/COD ratio of the influent, while the acetate requirement in a side-stream process is directly dependent on the phosphate concentration of the influent. Phosphorus removal in a stripper tank has a much lower COD requirement (2 mgCOD/mgP) than the phosphorus removal in a mainstream (20 mgCOD/mgP) and requires a ten times lower polyP-biomass concentration than the mainstream process.

In case the COD concentration of the influent is too low to support both complete biological nitrogen and phosphorus removal, complete P- and N-removal can still be obtained by making a proper use of the lower COD requirement in a stripper tank. Further, control of the pH in a stripper tank gives the possibility to optimize the acetate addition. Therefore, the approach presented can also be used to evaluate different process configurations for nitrogen and phosphorus removal processes.

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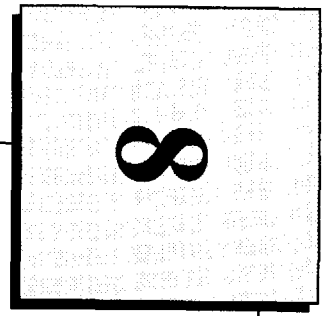
Appendix I

To obtain the phosphate uptake capacity of a biological phosphate removing process configuration, the actual phosphate concentration which has to be removed by polyP organisms must be calculated, because not all phosphate in the influent needs to be removed by biological phosphorus removal. A fraction of the phosphate will be used for cell growth (dependent on the SRT), while another fraction does not need treatment depending on the effluent standards. The remaining phosphate concentration will have to be treated. For an influent containing 450 mgCOD/l and an effluent demand of 50 mgCOD/l, 400 mgCOD has to be removed by the biomass in the system. For a typical aerobic biomass yield of 0.34 gCOD/gCOD depending on the SRT of the system (equation 3), 136 mgCOD/l biomass will be produced. For cell growth 15 mgP/gCOD is required and therefore 2.0 mgP/l phosphate of the influent P concentration is used. If the influent phosphate concentration is 8.0 mgP/l, and an effluent phosphate concentration of 1 mgP/l is demanded, only 5.0 mgP/l is required to be removed by the biological phosphorus removal. Of course this value needs to be corrected when the effluent VSS concentration contains a substantial amount of phosphate.

Outlook



Outlook

The metabolic model describing the biological P-removal process developed in this thesis was shown to describe accurately the complex conversions of the biological P-removal in labscale experiments. In this respect the model is valuable to get a better understanding of the process, the effect of modifications in the process, and the dynamic behaviour of the internal storage products and external concentrations. The model can, potentially, be used for the optimization and design of P-removing processes. The next step will therefore be the application of the model to full-scale systems. However, for the application of the model to full-scale systems, one should realize that the research was performed on labscale with an enriched culture in a sequencing batch reactor. Differences and problems when the model is applied to the full-scale process will of course arise. A brief outline of these differences is given below. Further, the differences between the metabolic model and the activated sludge model No. 2 of the IAWQ task group that was published recently, will be briefly discussed.

Extensions

In the application of the metabolic model to full-scale systems attention should be payed to three aspects. These concern the development of experimental methods to quantify the P-removal, the capacity of the model to predict dynamic influent conditions, and the relevance of aspects not examined in this thesis.

In the research described here, acetate was used as substrate. In the full-scale process, acetate is part of the COD present in the influent, and results from fermentation processes of complex COD during the anaerobic phase. Before the expected P-removal can be calculated, the available acetate fraction for the P-removal has to be known. In the metabolic model no

fermentation processes were included. The fraction of acetate as a result of fermentation during anaerobic conditions can be calculated in accordance to the activated sludge model No. 2.

The experiments described in this thesis were performed with an enriched culture with a population containing virtually only polyP organisms. In a full-scale system the P-removing population is only a fraction of the total sludge present in the system. It is therefore not possible to obtain the fraction P-removing organisms straightforwardly. Experimental methods to establish the fraction P-organisms are the measurement of the specific anaerobic P-release due to maintenance or an acetate uptake test using the specific acetate uptake rate of the organisms.

In contrast to the lab-scale situation, on full-scale a variable influent flow exists. Especially for the polyP and glycogen content of the organisms this might be important. When during a peak load during the anaerobic phase one of these compounds is exhausted, the uptake of acetate is halted. The dynamics of the influent flow and -concentration are therefore important for the P-metabolism. The model can be used to estimate when a limitation of one of these storage products will occur. In the experiments described previously these conditions have not been examined.

The amount of phosphate that can be removed by the P-removing organisms is dependent on the maximal attainable P-content of the P-removing organisms. The maximal P-content of the organisms was not determined in this research.

Furthermore, the effects of temperature on the process were not investigated in this thesis. This is important for full-scale systems because a large difference in temperature is possible between winter and summer time. Although the temperature dependency of the P-removing organisms can be modelled similar to heterotrophic organisms at first, this is not necessarily true, due to the difference in metabolism and growth on internal substrate.

In the present model the effect of the presence of an electron acceptor (oxygen or nitrate) on the acetate uptake was not evaluated. It is not clear what exactly happens when acetate is present during the aerobic phase of the P-removal. Wentzel (1989) did an experiment were acetate was added to the aerobic phase of the P-removal process, and observed that phosphate was released. Whether the acetate is taken up and converted to PHB was not measured. The investigation of this situation is worthwhile because it might occur in the full-scale process.

IAWQ model No. 2

Recently the IAWQ activated sludge model No. 2 was released by the IAWQ task group "modelling of activated sludge systems".[1] This model differs specifically from model No. 1 because the biological phosphorus removal process is incorporated in the model. The main difference between model No. 2 and the metabolic model developed in this thesis is that the latter is based on the metabolic description of the P-removal process. Although this is a more detailed approach, the overall number of parameters used in the metabolic model is smaller, while one more compound, glycogen, is considered in the model. In addition, the parameters were established experimentally, and it was shown that the model described the behaviour of the system over a wide range of sludge retention times and during start-up conditions with the same set of stoichiometry parameters and nearly the same set of kinetic parameters. An overview of the differences between both models reside in the following.

Model No. 2 does not describe the storage products glycogen and PHB separately, but considers them as one. Therefore it can be doubted whether constant parameters can be found for the kinetics. The major drawback of this approach is however that the model will not predict the system limitation as a result of a shortage of glycogen under a high acetate load.

The metabolic model uses stoichiometric parameters derived from biochemical pathways. These pathways are the same for all kind of microbial processes, reason why the stoichiometry parameters can be considered constant. In contradiction to the IAWQ model where the stoichiometry is not based on metabolism and can not be considered constant.

The metabolic model predicts a variable, pH dependent phosphate/acetate ratio, whereas this is a fixed parameter in the IAWQ model. From the experimental results it is clear that a wide range of phosphate/acetate ratios does exists which can not be neglected.

The kinetics of PHB in the metabolic model are verified with measurements while the kinetic relations in the IAWQ model are assumed to be a Monod type of relation.

The effect of SRT on the biomass yield is in the IAWQ No. 2 model described based on the biomass decay mechanism of death-regeneration. However, especially in the biological P-removal process where internal storage compounds are involved, this decay leads to release of polyP and PHB (and glycogen) into the extracellular environment. Experimental evidence for this is not available. Furthermore a large number of kinetic expressions is required to describe the degradation of these polymers to monomers. In the metabolic model the maintenance concept is shown to avoid all these problems.

Both models do not predict the effects of denitrifying phosphorus removal, the P-removal where nitrate is used as electron acceptor in stead of oxygen. The incorporation of this

process in the metabolic model is relative simple; the expectation is that only the P/O ratio (δ), the parameter for the ATP production, will be lower.
For convenience and further use of the metabolic model approach in the field of waste water treatment, appendix I contains the stoichiometric and kinetic formulation and optimal parameter set according to the IAWQ convention.

Summarizing, the metabolic model has shown to describe the phenomena on labscale adequately and the next step should be the application of the metabolic model to full-scale systems to prove its value. It has been shown that the model could describe the dynamic behaviour of internal and external compounds both during steady state as dynamic conditions, using a single set of stoichiometric parameters and nearly the same set of kinetic parameters. This is an important point, because a good indication of the adequateness of a model to describe a system is that parameters are invariant to process conditions. If the model is not correct, different parameter values will be required to compensate for model structure deficiencies. In this respect it can be concluded that the developed metabolic model has shown to be adequate and invariant to process conditions. It is expected that the model will prove its value in the design and optimization of biological P-removing systems. In the application to full-scale systems, research effort will be required to obtain a proper description of the hydrodynamics of the system. Our expectation is that the previously discussed extensions of the biokinetic model can be implemented straightforwardly in this model structure.

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Summary

A metabolic model of the biological phosphorus removal

stoichiometry, kinetics and dynamic behaviour

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The design and operation of biological waste water treatment systems is getting more and more complex due to the increase of the number of components that has to be removed by the system and the demands on the effluent quality. Beside the removal of organic compounds, the attention is now focused on the biological removal of nitrogen and phosphorus. Nutrient removal from waste water is seen as a control strategy to prevent eutrophication of surface waters. Eutrophication is the enrichment of fresh- and coastal waters with nutrients due to human activities, which severely disturb these ecosystems. To decrease eutrophication, effluent demands have to increase, as well as the knowledge of the treatment processes to realise these demands. Especially the biological phosphorus removal is a poorly understood and complex process.

Modelling

A method to increase the understanding of the complex behaviour of waste water treatment systems is the development of mathematical models. The use of these models is two-fold. Firstly, in the development of a model one has to consider the process quantitatively. A theory has to be accepted as basis for the model. Subsequently, the model is used to describe a set of data which allows two possible conclusions: the theory is falsified incase the model does not describe the data, or accepted if it does. In this way the model can be used to discriminate between different hypotheses concerning the process. Secondly, when a model is properly validated and adequately describes the experiments, the effect of changes in the process can be simulated. The model can in that case be used for the design or optimization of the process.

The aim of the research was the development of a metabolic model for the biological phosphorus removal process. In contrast to a black box model, where the system is regarded as a whole, and only the in- and out-going flows are considered, the metabolic model subdivides the system in smaller parts using the knowledge of these subsystems. In the metabolic model these subsystems are the conversions taking place on a metabolic level. The use of a metabolic model has the following advantages.
The first advantage, is that the internal subsystems which are the basis of the model are less variable than the system as a whole. Although the external conditions will fluctuate a lot, the micro organism will keep the internal conditions as constant as possible.
Second, the subsystems as defined in a metabolic model are in general easier to describe than the total system and better known. Components like ATP and $NADH_2$ can be considered, which gives the possibility to utilize all available biochemical knowledge on a metabolic level concerning these reactions.
Third, a metabolic model is based on the principle that the metabolism of micro organisms is composed of a limited number of universal metabolic pathways. It is therefore easier to base the model on these metabolic pathways than describe each separate group of micro-organisms. From a thermodynamic point of view, these pathways will require a certain amount of energy, while by selection, micro organisms will increase their efficiency and access this minimum. The amount of energy required in these pathways could therefore be considered as more or less constant, leading to a constant stoichiometry.
All relevant compounds are represented with their chemical formula. By making maximal use

of the principles of conservation of elements, mass or electrons, the minimal required number of stoichiometric parameters is defined.

Summarizing, in a metabolic model maximal use is made of the biochemical knowledge while the model is defined with a minimal number of stoichiometric parameters. The basis of the model is independent from the process conditions or concentrations, and can be considered constant. After determination of the stoichiometry, the relations describing the reactions rates in the process as a function of the relevant concentrations, the kinetics have to be established. With the stoichiometry and kinetics the complete model is defined, and the validation of the model under several conditions has to be achieved. The capability of a model to describe different conditions with the same set of parameters is an indication for the value of a model. The reason to use a metabolic model in case of the biological P-removal process is that the process is mainly based on internal storage products and reactions and therefore very appropriate for an approach on a metabolic level.

Biological phosphorus removal

Biological phosphorus removal from waste water is based on the capacity of the P-removing organisms to store large amounts of phosphate intracellularly as polyphosphate. The primary condition for the P-removal is the recirculation of the sludge through anaerobic and aerobic phases. The organisms are capable to use the stored polyphosphate for the generation of energy during anaerobic conditions. In the degradation of polyphosphate, phosphate is released to the fluid. The organisms use the energy generated from polyphosphate to store substrate intracellularly during anaerobic conditions. Afterwards, during aerobic conditions, this substrate is consumed. During the aerobic phase, the anaerobically released phosphate is taken up again as well as the phosphate of the influent and a phosphate free effluent is obtained. The phosphate leaves the process through the removal of organisms which have accumulated phosphate, in the waste sludge, or the phosphate can be released from the organisms and subsequently precipitated or regained in crystallized form. To obtain complete phosphorus removal the process must be operated such that sufficient P-removing organisms are accumulated in the process.

Stoichiometry

In chapter 2 the stoichiometry of the anaerobic metabolism of the phosphorus removal process

is described. During this phase acetate is consumed and converted to poly-ß-hydroxy butyrate (PHB). For the conversion of acetate to PHB during anaerobic conditions, both ATP and $NADH_2$ are required. The ATP is produced in the degradation of the polyphosphate, while it is unclear what compound forms the source of the reduction equivalents. In the past, two hypothesis were proposed. One of them proposes the TCA cycle as the source of reduction equivalents during the anaerobic phase (Wentzel) while the other one proposes the degradation of glycogen to PHB as the source of the reduction equivalents (Mino). In the degradation of glycogen ATP is produced also. Therefore, both theories propose different but constant phosphate/acetate ratios. Discrimination between both theories should therefore be straightforward. However, published experimentally determined values for the phosphate/-acetate ratio showed a range of values for this ratio, which made it impossible to discriminate between these theories. An explanation for this range of values was not found so far.

In chapter 2 it is shown that the phosphate/acetate ratio is not constant but strongly dependent on the pH, in the same range as reported in the literature. An explanation for this fact is that the energy costs of the transport of the acetate over the cell membrane of the organisms increases when the pH is increased. Two stoichiometric models for both hypothesis were developed both based on the pH dependent acetate transport. In both models different ratios were predicted for the phosphate/acetate, PHB/acetate and CO_2/acetate ratio. These ratios were experimentally determined in a sequenced batch reactor (SBR) in steady state. From these experiments it could be concluded that the measurements were in accordance with the model that considers the reduction equivalents to be produced from glycogen.

The stoichiometry of the aerobic phase of the biological phosphorus removal was developed in chapter 3. In the aerobic phase of the biological phosphorus removal process PHB, produced during anaerobic conditions, is used for cell growth, phosphate uptake and glycogen formation. For all these processes ATP is required which is produced in the oxidative phosphorylation, the oxidation of $NADH_2$ in which ATP is produced. All of these processes are more or less coupled to the oxygen consumption of the organisms. The yields for growth, polyphosphate and glycogen formation were quantified using the coupling of all these conversions to the oxygen consumption. By measurement of the difference in oxygen consumption in the presence and absence of phosphate, the amount of ATP produced in the oxidation of $NADH_2$ (the P/O ratio) could be established. The uptake of phosphate and storage as polyphosphate is shown to have a direct effect on the observed oxygen consumption in the aerobic phase. The overall energy requirements for the P-metabolism are

substantial: 25% of the acetate consumed during anaerobic conditions and 60% of the oxygen consumption is used for the synthesis of polyphosphate and glycogen.

Kinetics

During the aerobic phase of the P-removal, the amount of polyphosphate and glycogen consumed during the anaerobic phase have to be replenished. The overall conversions of the P-metabolism are fixed when the provided acetate during the anaerobic phase is completely consumed, the sludge age is known and the requirement is made that the initial concentrations at the start of each cycle are alike. To be able to describe the change in concentrations during the anaerobic or aerobic phase however, kinetic relations are required. The relevant conversions during both phases of the P-removal could be described with 6 reactions, and 6 kinetic relations were required to describe the reaction rates. These relations were established in chapter 4. The model was validated in experiments at a constant sludge age of 8 days, over the anaerobic and aerobic phases in which the external concentrations as well as the internal fractions of the relevant components involved in the P-removal process were monitored. These measurements included dissolved acetate, phosphate and ammonium, the oxygen consumption rate, PHB, glycogen, and biomass. The model described the dynamic behaviour of all components during the anaerobic and aerobic phases well.

Validation of the model during dynamic conditions

During aerobic conditions, phosphorus removing organisms grow on internally stored PHB. This unique feature of P-removing organisms raises the question how the growth rate is controlled by these organisms which is examined in chapter 5. In the biological P-removal process in a sequenced batch reactor, the biomass concentration is determined by the balance between the sludge removal rate at the end of an anaerobic/aerobic cycle and the biomass production during the aerobic phase. The biomass production during the aerobic phase is dependent on the PHB content of the cells, which is primarily determined by the ratio between acetate addition and biomass concentration present in the reactor, the specific acetate load. A high load will lead to a high PHB content of the cells and therefore a high growth rate. The relation between the sludge removal rate and biomass concentration affects also the fractions of polyP and glycogen to a large extent.

The metabolic model was examined by determining the capacity to describe the effect of different growth rates on the internal fractions of stored components. The metabolic model was capable to describe the changes in biomass concentration and internal fractions

satisfactorily over a wide range of sludge retention times and acetate/biomass loads, with one set of parameters. With these parameters the model described the dynamic behaviour of the components during the cycle as well. At a high acetate/biomass load, the polyP content becomes limiting in the anaerobic acetate uptake. No more acetate can be taken up if the acetate/biomass load is further increased and therefore, at this point the maximal growth rate is reached. The maximal growth rate of the biological phosphorus removal is in the range of 0.04 h^{-1}.

In chapter 6, for additional validation the metabolic model was, with the same set of parameters determined previously, applied to the dynamic conditions that occur during the start-up phase of the P-removal process in presence and absence of non-polyP heterotrophic organisms. In an SBR reactor experiments were performed to examine the enrichment of the population with polyP organisms during the start-up and the shift from non-polyP, heterotrophic organisms to polyP organisms in the sludge. The effect of different influent loading patterns for acetate and phosphate was studied. In these experiments the highest maximal growth rate of the polyP organisms at the start of the aerobic phase was 0.1 h^{-1}, while the highest average growth rate during the total aerobic phase of a cycle was 0.045 h^{-1}. During the start-up the growth rate of the polyP organisms is limited by the availability and concentration of acetate. The growth rate is further limited by the polyP content of the cells, which in turn depends on the kinetics of the aerobic phosphate uptake rate. Non-polyP heterotrophic, flocculated organisms present in the system do not disturb the growth of polyP organisms. The model describes the start-up behaviour well.

Analysis of different process configurations

A stoichiometric analysis for the evaluation of different process configurations for the biological phosphorus removal was developed in chapter 7. The considered process configurations were: the mainstream process, where the anaerobic phase is present in the mainstream of the process and the phosphate removal is entirely due to the organisms, and the mainstream process combined with a stripper tank where part of the phosphate is removed by precipitation. Furthermore, the side-stream process was considered, where the anaerobic phase is placed in the sludge line of the process and functions as a strippertank and almost all phosphorus removal takes place through precipitation. The analysis was based on the steady state simplification of the dynamic metabolic model and provides a method to easily

calculate the phosphate uptake capacity and acetate requirement in order to compare different phosphate removing process configurations. Based on the phosphate concentration of the influent and the system configuration, the minimal required concentration of phosphorus removing organisms (polyP organisms) in the system, and the maximal phosphate/acetate ratio of the influent to obtain full P-removal can be calculated. The phosphate/acetate ratio can be used as one of the main parameters in the analysis of a process configuration. The acetate requirement for P-removal in a stripper tank (2 mgCOD/mgP) is shown to be much lower than the requirements needed in a mainstream process (20 mgCOD/mgP). The required concentration of polyP biomass in a side stream process is 10 times lower. In case the COD concentration of the influent is too low to support biological nitrogen and phosphorus removal, complete P and N-removal can still be obtained by making a proper use of the much higher efficiency of a stripper tank. Further, control of the pH in a stripper tank gives the possibility to optimize the acetate addition. Therefore, the presented approach can be used to evaluate different process configurations for nitrogen and phosphorus removal processes.

It has been shown that the metabolic model of the biological phosphorus removal describes the complex conversions of the P-removal accurately with a single set of stoichiometry parameters and nearly the same set of kinetic parameters. It correctly describes the dynamics of internal and external compounds during the anaerobic/aerobic phases, over a wide range of sludge retention times and during the start-up phase of the process.

Samenvatting

Een metabool model van de biologische fosfaatverwijdering

stoichiometrie, kinetiek en dynamisch gedrag

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Het ontwerpen en bedrijven van biologische zuiveringsinstallaties voor afvalwater wordt steeds complexer door de toename van het aantal componenten dat verwijderd dient te worden en de steeds hogere eisen die aan de effluentconcentraties gesteld worden. De aandacht richt zich tegenwoordig, naast de verwijdering van organische stof, vooral op de biologische verwijdering van stikstof en fosfaat. De reden hiervoor is dat de verwijdering van deze nutriënten uit afvalwater wordt gezien als een methode om eutrofiëring van oppervlaktewater te voorkomen. Eutrofiëring is de verrijking van ecosystemen met voedingsstoffen als gevolg van menselijke activiteit, waardoor ernstige verstoringen in deze ecosystemen ontstaan. Om eutrofiëring vergaand terug te dringen worden de effluenteisen steeds verder aangescherpt waardoor een toenemend inzicht vereist is in de biologische technieken om dit te realiseren.

Modelleren

Een manier om inzicht te krijgen in het complexe gedrag van biologische zuiveringsprocessen is gebruik te maken van wiskundige modellen. Het gebruik van deze modellen is tweeledig. Ten eerste wordt men door het opstellen ervan gedwongen het proces kwantitatief te beschouwen. Er moet bij het opstellen van het model van een bepaalde theorie worden uitgaan en daardoor wordt men meestal al snel met de neus op de feiten gedrukt: het model beschrijft de dataset correct of juist niet. Het model kan dus gebruikt worden om te discrimineren tussen verschillende theorieën aangaande het proces. Ten tweede kan, wanneer een model in voldoende mate experimenteel gevalideerd is, vooraf gesimuleerd worden wat het effect van bepaalde veranderingen op het proces zal zijn. Het model kan dan gebruikt worden om inzicht te krijgen bij het ontwerp of de optimalisatie van het proces.

Het doel van het onderzoek was het ontwikkelen van een metabool model voor de biologische fosfaatverwijdering. In tegenstelling tot een blackbox model waarbij het proces als één geheel wordt beschouwd en er alleen naar de in- en uitgaande stromen van een proces wordt gekeken, wordt in een metabool model het proces onderverdeeld in deelsystemen waarbij kennis aangaande deze deelsystemen kan worden gebruikt. Het gebruik van een metabool model biedt een aantal voordelen.
Ten eerste is een voordeel van een metabool model, dat de interne deelsystemen waarop het model gebaseerd wordt, minder aan verandering onderhevig zijn dan het systeem als geheel. Hoewel de externe omstandigheden van een micro-organisme sterk kunnen veranderen, probeert het micro-organisme doorgaans zijn interne condities zo veel mogelijk constant te houden. Ten tweede zijn deelsystemen zoals gedefinieerd in een metabool model doorgaans eenvoudiger te beschrijven dan het gehele systeem en vaak al redelijk goed onderzocht in de biochemie. Componenten als ATP en $NADH_2$ kunnen ook beschouwd worden in een metabool model en dat geeft de mogelijkheid om alle beschikbare biochemische kennis van reacties op metabool nivo te benutten. Ten derde is een metabool model gebaseerd op het principe dat het metabolisme van een micro-organisme is samengesteld uit een gelimiteerd aantal universele metabole routes. Het is daardoor eenvoudiger deze routes te modelleren dan elke groep micro-organismen apart. Vanuit thermodynamisch oogpunt beschouwd zullen deze routes een bepaalde minimale energiebehoefte hebben, waarbij micro-organismen door selectie trachten hun efficiency te verhogen om zo dicht mogelijk bij dit minimum te komen. De energie die voor deze routes benodigd is, kan daardoor min of meer constant verondersteld worden, waardoor er ook constante stoichiometrische parameters mogen worden verwacht.

Alle relevante componenten in een metabool model worden voorgesteld met hun chemische formule. Door maximaal gebruik te maken van behoudsrelaties, via elementen-, massa- of electronenbalansen, wordt het minimaal benodigde aantal stoichiometrische parameters gedefinieerd. Doordat de netto conversiesnelheid van ATP en $NADH_2$ bekend is en gelijk aan nul gesteld kan worden (micro-organismen produceren netto geen ATP of $NADH_2$) kan de ingebrachte kennis gebruikt worden in de andere balansen.

Samenvattend, maakt een metabool model maximaal gebruik van kennis omtrent de metabole reacties van het organisme waardoor het model gedefinieerd wordt op basis van een minimaal aantal stoichiometrische parameters. De basis van het model is daardoor minder afhankelijk van de procescondities of concentraties en kan constant verondersteld worden. Na het definiëren van de stoichiometrie moeten de relaties die de reactiesnelheden bepalen worden vastgesteld, de kinetiek van het proces. Met de stoichiometrie en kinetiek is het totale model gedefinieerd en kan het model onder verschillende condities getest worden. Wanneer een model in staat is verschillende condities te beschrijven zonder aanpassing van de parameters, dan kan dit gezien worden als een indicatie voor de correctheid van het model. De reden om een metabool model op te stellen in het geval van de biologische fosfaatverwijdering is dat het een complex proces is dat grotendeels gebaseerd is op interne reacties en opslagprodukten en dus uitermate geschikt voor een benadering op metabool nivo.

Biologische fosfaatverwijdering

De biologische fosfaatverwijdering uit afvalwater is gebaseerd op het vermogen van fosfaatverwijderende bacteriën om fosfaat in grote hoeveelheden intracellulair als polyfosfaat op te slaan. Een belangrijke voorwaarde voor het proces is de recirculatie van het slib door een anaërobe en aërobe zone. De organismen zijn in staat om intern geaccumuleerd polyfosfaat te gebruiken als energiebron onder anaërobe omstandigheden. Polyfosfaat wordt dan afgebroken waarbij fosfaat vrijkomt in de vloeistof. De vrijkomende energie wordt gebruikt om substraat op te slaan tijdens anaërobe omstandigheden. Later, onder aërobe omstandigheden, wordt dit substraat geconsumeerd. Tijdens de aërobe fase van het proces wordt het fosfaat dat anaëroob werd afgestaan, samen met het fosfaat uit het influent opgenomen en wordt een fosfaatvrij effluent verkregen. De eigenlijke verwijdering van fosfaat uit het proces vindt plaats door de afvoer van organismen die polyfosfaat geaccumuleerd

hebben via het spuislib of door het vrijmaken van fosfaat uit de organismen dat vervolgens geprecipiteerd of teruggewonnen wordt via kristallisatie.

Stoichiometrie

In hoofdstuk 2 wordt de stoichiometrie van het anaërobe metabolisme van het proces onderzocht. Tijdens de anaërobe fase van de biologische fosfaatverwijdering wordt acetaat geconsumeerd en omgezet naar PHB. Voor de omzetting van acetaat naar PHB tijdens anaërobe omstandigheden is zowel ATP als $NADH_2$ benodigd. Het ATP wordt geproduceerd uit het opgeslagen polyfosfaat. Het is echter de vraag hoe het organisme de reduktie equivalenten produceert. In het verleden zijn hiervoor twee theorieën voorgesteld. De eerste theorie (Wentzel) stelt dat reduktie-equivalenten worden geproduceerd via de citroenzuur cyclus die tijdens anaërobie operationeel zou zijn. De andere theorie stelt dat de omzetting van intracellulair opgeslagen glycogeen naar PHB de bron zou zijn voor de reduktie equivalenten (Mino). Naast de produktie van $NADH_2$ vindt er tevens ATP-produktie plaats bij de omzetting van glycogeen. Daardoor voorspellen beide theorieën een verschillende, maar constante fosfaat/acetaat ratio. Discriminatie tussen beide theorieën zou dus eenvoudig moeten zijn. Echter, experimenteel vastgestelde waarden voor de fosfaat/acetaat ratio lieten een range aan waarden zien op grond waarvan geen onderscheid tussen de beide theorieën gemaakt kon worden. Een goede verklaring hiervoor werd nog niet gevonden.

In hoofdstuk 2 wordt aangetoond dat de fosfaat/acetaat ratio niet constant is maar sterk bepaald wordt door de pH en bovendien valt in de range van waarden die in de loop der jaren gepubliceerd werden. Een verklaring voor dit feit is dat het transport van acetaat over het celmembraan meer energie gaat kosten wanneer de pH toeneemt. Twee stoichiometrische modellen werden opgesteld voor beide theorieën, gebaseerd op dit pH-afhankelijke acetaattransport. Door de verschillende bron voor de produktie van reduktie equivalenten in beide modellen, werden verschillende ratios voorspelt voor het fosfaat/acetaat transport, de PHB/acetaat ratio en de CO_2/acetaat ratio. Deze ratios werden experimenteel bepaald in een sequencing batch reactor in steady state. Uit de metingen kon geconcludeerd worden dat metingen goed overeen kwamen met het model waarbij de reduktie equivalenten geproduceerd werden uit glycogeen.

De stoichiometrie van de aërobe fase van de biologische fosfaatverwijdering werd onderzocht en beschreven in hoofdstuk 3. In de aërobe fase wordt het PHB dat tijdens de anaërobe fase werd opgeslagen gebruikt voor de produktie van biomassa, de opname van fosfaat en vorming

tot polyfosfaat en de synthese van glycogeen. Voor al deze processen is ATP benodigd dat geproduceerd wordt in de oxydatieve fosforylering, de oxydatie van $NADH_2$ waarbij ATP gegenereerd wordt. Al deze processen zijn daardoor in meer of mindere mate gekoppeld aan het zuurstofverbruik van de organismen. Door het verschil in zuurstofverbruik van de organismen in aan- en afwezigheid van fosfaatopname te meten, kon vastgesteld worden hoeveel ATP er per geoxideerde $NADH_2$ geproduceerd werd, de P/O ratio. Met deze P/O ratio kon de yield van de produktie van biomassa, polyfosfaat en glycogeen op PHB afgeleid worden.

Aangetoond werd dat de opname van fosfaat en opslag als polyfosfaat een direct effect heeft op de waargenomen zuurstofconsumptie. De totale energiebehoefte voor het P-metabolisme is aanzienlijk: 25 % van het tijdens de anaërobe fase geconsumeerde acetaat en 60% van de zuurstofconsumptie worden gebruikt voor de synthese van polyfosfaat en glycogeen.

Kinetiek

Omdat in de aërobe fase van de fosfaatverwijdering het gedurende de anaërobe fase verbruikte polyfosfaat en glycogeen weer aangevuld moet worden, ligt het proces stoichiometrisch al voor een groot deel vast wanneer de slibleeftijd van het systeem bekend is en gesteld wordt dat de uitgangscondities aan het begin van elke cyclus gelijk moeten zijn. Om echter de verandering in concentraties tijdens de cyclus te kunnen beschrijven zijn de snelheidsrelaties van de reacties nodig. Omdat de omzettingen in anaërobe en aërobe fase met 6 onafhankelijke stoichiometrische reacties beschreven werden zijn ook 6 snelheidsrelaties benodigd. Deze relaties werden in hoofdstuk 4 afgeleid. Het model werd gevalideerd in experimenten bij een constante slibleeftijd van 8 dagen, waarbij zowel de externe concentraties als de interne fracties van de relevante componenten gemeten werden. Deze metingen betreffen acetaat, fosfaat, ammonium, de zuurstof consumptie, PHB, glycogeen en de biomassa. Het model is in staat het dynamische gedrag van al deze componenten tijdens de anaërobe en aërobe fase te beschrijven.

Validatie van het model onder dynamische condities

Fosfaatverwijderende organismen groeien tijdens aërobe omstandigheden op intern opgeslagen PHB. De vraag die in hoofdstuk 5 gesteld wordt, is hoe bij deze organismen de groeisnelheid is geregeld. Bij de biologische fosfaatverwijdering in een sequencing batch reactor wordt de biomassaconcentratie bepaald door de balans tussen de slibafvoersnelheid aan het eind van het proces en de biomassaproduktie tijdens de aërobe fase. De groeisnelheid tijdens de aërobe fase is afhankelijk van het PHB-gehalte van de cellen, dat voornamelijk bepaald wordt door

de ratio tussen acetaattoevoer en biomassa aanwezig in de reactor, de specifieke acetaatbelasting. Een hoge belasting zal leiden tot een hoog PHB-gehalte en daardoor tot een hoge groeisnelheid. De relatie tussen de slibafvoersnelheid en de biomassaconcentratie beïnvloed bovendien de fracties van polyfosfaat en glycogeen in grote mate.
Het metabole model werd getest door te onderzoeken of het in staat was de conversies te beschrijven van het proces over een range van slibleeftijden. Het bleek dat een enkele set parameters in staat was de conversies van alle reacties als functie van de slibleeftijd te beschrijven. Daarnaast beschreef het model met deze parameterset de dynamica van alle componenten tijdens de anaërobe/aërobe cyclus. Met het verhogen van de acetaat/biomassa ratio daalt de biomassaconcentratie en daarmee de hoeveelheid polyP in de reactor. Daardoor wordt op een gegeven moment het polyfosfaat limiterend voor de opname van acetaat en is de maximale gemiddelde groeisnelheid bereikt. Dit punt ligt bij een cyclusverdeling zoals gebruikt in dit onderzoek bij een groeisnelheid van 0.04 h^{-1}.
In hoofdstuk 6 werd aangetoond dat het metabole model in staat is de dynamische omstandigheden die ontstaan tijdens de opstart van het proces in aan- en afwezigheid van heterotrofe organismen te beschrijven met dezelfde set parameters die in de eerdere hoofdstukken bepaald was, op de acetateopnamesnelheid na. In een SBR werden experimenten uitgevoerd waarbij de verrijking van het slib met polyP-organismen werd gevolgd nadat een populatie met heterotrofe organismen beënt werd met een kleine hoeveelheid polyP-organismen. Het effect van verschillende influenttoevoerpatronen voor acetaat en fosfaat werd bestudeerd. De hoogste groeisnelheid van de polyP-organismen die in deze experimenten werd waargenomen was 0.1 h^{-1} terwijl de hoogste gemiddelde groeisnelheid over de gehele aërobe fase 0.045 h^{-1} was. Tijdens de opstart wordt de groeisnelheid van de polyP-organismen bepaald door de aanwezigheid en concentratie van acetaat. De groeisnelheid bleek verder gelimiteerd te worden door de aanwezigheid van het polyfosfaat, dat op zijn beurt afhankelijk is van de fosfaatopnamesnelheid tijdens aërobe omstandigheden. De aanwezigheid van heterotrofe, geflocculeerde organismen die geen polyfosfaat ophopen verstoort de ingroei van polyP-organismen niet.

Analyse van verschillende procesconfiguraties

Een stoichiometrische analyse voor de evaluatie van verschillende procesconfiguraties voor de biologische fosfaatverwijdering werd ontwikkeld en beschreven in hoofdstuk 7. Deze procesconfiguraties betroffen het hoofdstroomproces waarbij de anaërobe fase deel uitmaakt

van de hoofdstroom van het proces en de verwijdering volledig door organismen plaatsvindt, en het hoofdstroomproces maar nu uitgebreid met een strippertank waarbij een deel van het opgeslagen fosfaat wordt vrijgemaakt uit de organismen en geprecipiteerd. Daarnaast werd het zijstroom proces beschouwd waarbij de anaerobe fase in de vorm van een strippertank in de zijstroom van het proces is opgenomen en het fosfaat vrijwel volledig doormiddel van precipitatie verwijderd wordt. Deze analyse is een steady-state simplificering van het ontwikkelde dynamische model dat gebruikt kan worden om de fosfaatopnamecapaciteit en acetaatbehoefte van verschillende P-verwijderende procesconfiguraties te vergelijken. Gebaseerd op het fosfaatgehalte van het influent en de systeemconfiguratie kan de minimaal benodigde hoeveelheid polyP-biomassa berekend worden, en daarmee de maximale fosfaat/COD verhouding van het influent. De fosfaat/COD verhouding is een van de hoofdparameters in de analyse van de procesconfiguratie. Er werd aangetoond dat de acetaatbehoefte van het zijstroomproces, waar fosfaat verwijderd wordt via de strippertank, 2 mgCOD/mgP is, wat veel lager is dan de 20 mgCOD/mgP benodigd in het hoofdstroom-proces. De polyP-biomassa concentratie benodigd voor fosfaatverwijdering in een zijstroom proces is een factor 10 lager. In het geval dat de COD concentratie van het influent te laag is om zowel volledige biologische stikstof- als fosfaatverwijdering te krijgen, kan toch volledige P- en N-verwijdering verkregen worden, door gebruik te maken van de lagere COD behoefte van een strippertank. Verder kan het acetaatgebruik van een strippertank geoptimaliseerd worden doormiddel van de pH.

Er kan geconcludeerd worden dat het metabole model van de biologische fosfaatverwijdering in staat is met één set parameters de complexe conversies van de fosfaatverwijdering bevredigend te beschrijven. Het model is in staat de dynamica van zowel de interne als externe componenten tijdens een anaërobe/aërobe cyclus te beschrijven maar ook als functie van de slibleeftijd en tijdens de opstart van het proces.

Appendix I

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

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

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

Definition of the soluble and particulate components

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

Definition of values for the kinetic coefficients

Coefficient	value	units
q_{PHA}	0.36	gCOD/gCOD-PAO.h
k_X	0.14	gCOD-PAO/gCOD.h
k_{PP}	0.17	gP/gCOD.h
k_{GL}	0.8	gCOD/gCOD.h
m_{an}	$3.4 \cdot 10^{-3}$	gP/gCOD-PAO.h
m_{aer}	$4.0 \cdot 10^{-3}$	gCOD/gCOD-PAO.h
K_A	32	gCOD/m^3
K_P	3	gP/m^3
K_{PP}^{MAX}	0.34	gPP/gCOD-PAO
K_{GL}^{MAX}	1.2	gCOD/gCOD-PAO
K_{PP} switch	$1 \cdot 10^{-3}$	gPP/gCOD-PAO
K_{O2} switch	$1 \cdot 10^{-3}$	gO_2/m^3

Conversions factors

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

Nawoord

Na vier jaar promotieonderzoek komt er toch een eind aan. Ik heb me aan het begin vaak afgevraagd wat er dan bereikt zou zijn. Vier jaar is wat dat betreft een erg lange periode waaraan, als je aan het begin staat, vrijwel geen eind kan komen. Het lijkt eigenlijk op wat ik me, als zeiler, voorstel bij een oversteek van de oceaan: je moet behoorlijk gedreven zijn om er aan te beginnen. Voordat je vertrekt, bereid je je zo goed mogelijk voor, je luistert naar elk advies dat je maar krijgen kunt en je probeert de beste materialen te krijgen. En dan steek je van wal en blijkt dat ondanks alle gedegen plannen dat water toch wel heel erg groot is, en dat je werkelijk alle kanten op kunt als je dat zou willen. Dus wat wil je ? Je wilt natuurlijk toch iets moois presteren dus stel je je doel in eerste instantie ver weg, op z'n minst aan de andere kant van de oceaan, dan wel de wereld rond. En dan er op af. In de eerste tijd zie je dat je enorm vordert. Je ziet de wal achter je snel kleiner worden en dat betekent dat het lekker loopt. Maar dan ben je op open water en zijn er maar weinig referentiepunten. Je neemt uiteraard vaak via de radio contact op met je begeleiders aan de wal, die je van goede raad voorzien maar niet precies kunnen overzien wat op dat moment voor jou de problemen zijn. De beste stuurlui staan dan toch weer aan wal en jij zit daar maar midden op de oceaan. Het lijkt ook opeens allemaal wel heel erg ver. En om toch nog enige voldoening te krijgen moet er af en toe eens een leuk eilandje aangelopen worden om wat uit te rusten en te genieten van wat je al bereikt hebt. Je zit vaak in de nodige windstiltes waarbij je denkt dat je er nooit uit zult komen en stormen waarbij het ineens erg hard gaat. En plotseling als je je al enige tijd begint af te vragen waar je nou precies bent, zie je een meeuw in de lucht: LAND!. Je realiseert je ineens wat je nog moet afleggen om je doel te bereiken en dat is niets meer. Vooral op het laatste stuk slaat het ongeduld toe als je al wel dat streepje land ziet maar het maar niet dichterbij wil komen. Ongelooflijk, wat is dat laatste stuk dan nog een eind! En zelfs als je voor de haven ligt en het lijkt dat je zo naar binnen kunt, loop je nog net even voor de monding op een zandbank. Het valt niet mee het schip veilig de haven in te loodsen, maar op een gegeven moment lukt ook dat en is de oversteek gelukt. Net als zo'n reis is promoveren wat mij betreft een behoorlijke uitdaging gebleken, waarbij je jezelf leert kennen en wat uiteindelijk een veelzijdige ervaring oplevert.

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Curriculum vitae

Gerardus Johannes Franciscus Smolders werd 16 januari 1965 geboren te Arnhem. Na het volgen van het basisonderwijs te Velp, Enschede en Hengelo, werd in 1983 het VWO diploma behaald aan lyceum de Grundel te Hengelo. In datzelfde jaar werd begonnen met de studie Milieu-hygiene aan de Landbouw Hogeschool Wageningen. In 1989 studeerde hij hierin af met als afstudeerrichtingen Proceskunde en Waterzuivering. Tijdens het afstuderen werd onderzoek uitgevoerd naar de biotechnologische uitloging van zware metalen bij de vakgroep Waterzuivering. Bij de vakgroep Levensmiddelen Technologie, sectie Proceskunde werden twee afstudeeronderzoeken uitgevoerd naar de gecombineerde nitrificatie en denitrificatie met geïmmobiliseerde bacteriën en de ontwikkeling van een dynamisch model voor het beschrijven van een mengcultuur van *Nitrosomonas* en *Nitrobacter* cellen. In 1990 werd aangevangen met het in dit proefschrift beschreven onderzoek als assistent in opleiding bij de vakgroep Bioprocestechnologie van de Techische Universiteit Delft.