Biological phosphorus removal from wastewater with a moving bed biofilm reactor process

by

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Picture on front page: The experimental setup used in the study. Photo by Rebecca Arvidsson.

Preface

This master thesis was performed at Veolia Water Technologies, Lund, in cooperation with Water and Environmental Engineering at the Department of Chemical Engineering at Lund University. It was the last step of the education Master of Science in Biotechnological Engineering. During the work, several people were involved to whom I now would like to give my sincere thanks.

I would like to express my thankfulness to my supervisors, Eva Tykesson who guided me during my work and always had something positive to say, and Karin Jönsson for valuable comments. I would also like to thank my examiner Åsa Davidsson.

I would like to sincerely thank all employees at Veolia Water Technologies, who made me feel welcome. Without your help and good advice, my work would not have been possible. I would like to express my gratitude to Steen Hemmingsen for the help with constructing the laboratory setup, programming the PLC and installing the magnetic valves, Petter Lind who introduced me to PLC programming and provided me with good advice, Kerstin Johansson who always took the time to help me, Anton Karlsson for the help with the experimental setup and the microscopy, and lastly, thank you Magnus Christensson and Thomas Welander for smart ideas and wise advice.

I would also like to thank my boyfriend Tobias and my family for the support.

Rebecca Arvidsson

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Abstract

The human population is increasing and more wastewater, rich in nutrients, is produced. Increased levels of nutrients such as phosphorus, nitrogen and organic matter in water bodies present a risk for eutrophication, toxicity to the water-living fauna and oxygen depletion. Therefore, it is important to operate the wastewater treatment plants efficiently to limit the release of nutrients to the receiving waters and decrease the energy demand and environmental impact.

Today, biological phosphorus removal (bio-P) from wastewater is achieved with activated-sludge processes. Bio-P processes with biofilm, such as moving bed biofilm reactors (MBBR) or fixed film reactors, are not yet in use in full-scale but research is ongoing. It is of interest to develop an MBBR phosphorus removal process since the process can have advantages over existing processes. MBBR processes often take less space than activated sludge processes, and less chemicals are needed compared to chemical precipitation. However, the biological phosphorus removal process requires altering of anaerobic and aerobic conditions causing challenges in the process design.

In this study, a semi-continuous moving bed biofilm reactor process for denitrifying phosphorus removal from wastewater was designed and operated. The function of the process along with the capacity of the process was evaluated in terms of aerobic phosphorus uptake rates, anaerobic phosphorus release rates, and anaerobic soluble carbon uptake rates.

No denitrifying phosphorus removal was achieved in the intended experimental setup with the existing conditions. However, after several changes in the experimental setup and in the feed composition the desired bio-P activity was achieved.

The changes that supported the development of desired bio-P activity were the following: 1) The soluble carbon was prevented from being present in the aerobic and deoxidising reactors. 2) The temperature in the reactors was decreased from 15°C to 10°C. 3) More counter ions in form of magnesium and calcium were added to the main influent.

Due to the experimental setup used when the achieved bio-P activity was obtained, no total nutrient removal could be measured. The highest achieved aerobic phosphate uptake rate was 0.13 g/(m^2·d) and it was measured after 76 days. The simultaneous anaerobic phosphate release rate was 0.2 g/(m^2·d) while the highest anaerobic phosphate release rate was 0.35 g/(m^2·d) which was achieved after 91 days. After 76 days, the anaerobic soluble carbon uptake rate was 6.4 g/(m^2·d). The microscopic study showed that some of the biomass contained polyphosphate granules at this time.

The achieved rates, except the soluble carbon uptake rate, were 6-11 times lower compared to the literature. However, the rates were still increasing at the last measurement and the process had not reached its full potential before the study was terminated. The intended process for denitrifying phosphorus removal was not tested after the temperature decrease and addition of counter ions. Therefore, more studies have to be conducted on the intended denitrifying phosphorus removal process to enable evaluation of the potential of the process, and to determine if the treatment requirements can be met.

Keywords: *biological wastewater treatment, denitrifying phosphorus removal, EBPR, MBBR*

Sammanfattning

Antalet människor på jorden ökar och därmed produceras mer näringsrikt avloppsvatten. Ökade koncentrationer av näringsämnen som fosfor, kväve och organiska ämnen i våra vatten utgör en risk för övergödning, toxicitet mot det vattenlevande djurriket och syrebrist på bottnarna. Därför är det viktigt att avloppsreningsverken drivs effektivt för minimera utsläppen av näringsämnen till våra vatten och för att minimera energiåtgången och miljöpåverkan vid reningen.

Idag sker biologisk fosforavskiljning (bio-P) från avloppsvatten med aktivt slam-processer. Bio-P processer med biofilm, som exempelvis biofilmsprocesser med rörliga bärare (MBBR) eller processer med fast biofilm, används inte ännu i fullskala men forskning pågår. Det är av intresse att undersöka om MBBR processen går att använda för biologisk fosforavskiljning från avloppsvatten eftersom det kan finnas fördelar med denna process jämfört med de i dagsläget använda processer. MBBR-processer tar ofta mindre plats än aktivt slam-processer. Dessutom används mindre kemikalier jämfört med i processer med kemisk fällning. Emellertid kräver processen för biologisk fosforavskiljning växlande anaeroba och aeroba förhållande vilket orsakar utmaningar när det gäller processdesignen.

I den här studien utvecklades och kördes en semikontinuerlig MBBR-process för kombinerad denitrifikation och biologisk fosforavskiljning från avloppsvatten. Hur väl processen fungerade samt processens kapacitet utvärderades i form av hastigheten på det aeroba fosforupptaget, hastigheten på det anaeroba fosforsläppet samt hastigheten på det anaeroba upptaget av löslig lättnedbrytbar kolkälla.

Ingen kombinerad denitrifikation och fosforavskiljning uppnåddes i processen med de då gällande förhållandena. Efter att ett flertal ändringar gjorts i laborationsuppställningen och i kompositionen av inflödena uppnådes slutligen önskvärd biologisk aktivitet.

Ändringarna som tros ligga bakom utvecklingen av önskad aktivitet var följande: 1) Den lösta lättnedbrytbara kolkällan hindrades från att komma in i de aeroba reaktorerna och den deoxiderande reaktorn. 2) Temperaturen i reaktorerna sänktes från 15°C till 10°C. 3) Mer motjoner i form av magnesium och kalcium tillsattes till huvudinflödet.

På grund av hur laborationsuppställningen var designad i slutändan, så kunde inte den totala avskiljningen av näringsämnen mätas. Den högsta uppmätta hastigheten för aerobt fosfatupptag var 0,13 g/(m^2·d) och uppmättes 76 dagar efter att försöket inleddes. Hastigheten för det anaeroba fosfatsläppet var 0,2 g/(m^2·d) vid samma tidpunkt medan den högsta hastigheten för fosfatsläppet var 0,35 g/(m^2·d) och skedde efter 91 dagar. Efter 76 dagar var hastigheten för upptag av lättnedbrytbar kolkälla 6.4 g/(m^2·d). Mikroskopistudien visade att biomassan innehöll polyfosfatgranuler i slutet på studien.

De uppmätta hastigheterna, bortsett från upptaget av kolkälla, var 7-11 gånger lägre än värden från litteraturen. Emellertid ökade hastigheterna fortfarande när försöket avbröts och processens fulla potential hann inte studeras. Processen för denitrifierande fosforavskiljning studerades inte efter det att temperaturen sänktes och mera motjoner tillsatts. Av den anledningen krävs fler experiment för att kunna dra någon slutsats om den denitrifierande fosforavskiljningsprocessen fungerar och är tillräcklig för att klara reningskraven.

Nyckelord: *biologisk avloppsvattenrening, denitrifierande fosforavskiljning, EBPR, MBBR*

List of Abbreviations

AOB	Ammonium Oxidising Bacteria
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DPB	Denitrifying Phosphorus Removing Bacteria
EBPR	Enhanced Biological Phosphorus Removal
GAO	Glycogen Accumulating Organisms
MBBR	Moving Bed Biofilm Reactor
NOB	Nitrite Oxidising Bacteria
OLR	Organic Loading Rate
PAO	Polyphosphate Accumulating Organisms
PHA	poly-β-hydroxy-alkanoates
PHB	poly-hydroxy-butyrate
PLC	Programmable Logic Controller
Poly-P	Polyphosphate
SBMBBR	Sequencing Batch Moving Bed Biofilm Reactor
SBR	Sequencing Batch Reactor
sCOD	Soluble Chemical Oxygen Demand
SRT	Sludge Retention Time
TSS	Total Soluble Solids
VFA	Volatile Fatty Acid
WWTP	Wastewater Treatment Plant

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1 Introduction

The human population is increasing and more wastewater rich in nutrients is produced. Increased levels of nutrients such as phosphorus, nitrogen and organic matter in water bodies present a risk for eutrophication, toxicity to the water-living fauna and oxygen depletion. Therefore, it is important to make the wastewater treatment plants more efficient to limit the release of nutrients to the receiving waters (Wang *et al*., 2006) and decrease the energy demand and environmental impact. In the EU, there are requirements for the minimum quality of the purified wastewater according to either a maximal concentration depending on the size of the wastewater treatment plant or a minimum percentage reduction (EU-directive 91/271/EEG, 1991). The limits also depend on the sensitivity of the receiving water (Tykesson, 2005).

Commonly, the wastewater treatment plants use biological processes for removal of organic matter and nitrogen. Phosphate removal from wastewater is achieved with chemical precipitation or with a biological process called enhanced biological phosphorus removal (EBPR) (Helness and Ødegaard, 1999) or a combination of both.

Biological treatment processes can be performed in activated sludge processes or in biofilm processes. The activated sludge is a mass of sludge consisting of living biomass which is kept moving in the water by stirring or aeration. The activated sludge is re-circled in the plant and nutrients and organic carbon are consumed by microorganisms in the sludge and are removed from the water either by conversion to atmospheric gases or with the excess sludge that is separated (Henze *et al*., 1997). In biofilm processes, the biomass is attached to a solid surface where a biofilm is formed (Henze *et al*., 1997, pp. 143). Nutrients and organic carbon are consumed by the microorganisms in the biofilm in the same way as in activated sludge processes. In this case, the produced sludge is the biofilm sloughed off from the surface.

Today, there are both activated sludge processes and biofilm processes available for biological removal of organic carbon and nitrogen from wastewater. Biological phosphorus removal from wastewater is, on the other hand, in full-scale only achieved with activated sludge processes. Processes with biofilm, such as moving bed biofilm reactors (MBBR) or fixed film reactors, are not yet in use (Falkentoft *et al*., 2001), but research is ongoing. It is of interest to see if phosphorus removal can be achieved with an MBBR process since the process can have advantages over existing processes (Falkentoft *et al*., 2001). However, the biological phosphorus removal process requires altering of anaerobic and aerobic conditions (Helness and Ødegaard, 1999). Either the water or the carriers must be moved, which both would pose technical problems if the process would be coupled to an already existing continuous wastewater treatment plant (Helness, 2007).

This study focused on biological denitrifying phosphorus removal with an MBBR process. The intention was that the process tested during this study would simulate treatment of effluent water from a wastewater treatment plant with BOD removal and nitrification. Hence the main influent to the process should contain mainly phosphate and nitrate. The influent water to the anoxic phase in the EBPR process is desired to contain phosphate and nitrate while the influent water to the anaerobic phase is desired to contain easily degradable carbon source. To address these requirements, an influent containing carbon source which corresponded to a stream from a hydrolysis reactor was used as well. To achieve altering conditions in the reactors, four reactors connected in series were operated as a combination of

continuous mode and sequencing-batch mode in the sense that the influents and effluents were continuous while the conditions in the reactors were changed like in a sequencing-batch process.

Veolia Water Technologies is prominent in wastewater treatment processes with the MBBR technology and would like to be able to offer their customers a continuous MBBR process for biological phosphorus removal combined with denitrification. The process can possible provide several benefits such as a more environmentally friendly treatment compared to precipitation processes since no chemicals are needed, and the process potentially takes less space than a corresponding activated sludge process (Ødegaard, 2006).

1.1 Aim

The aim of this master thesis work was to design and operate a laboratory-scale MBBR process for biological phosphorus removal and denitrification and to evaluate its performance and capacity, with the following questions in mind:

- Can denitrifying phosphorus removal be achieved in the process?
- What conditions are needed to achieve bio-P activity?
- What aerobic phosphate uptake rate can be achieved?
- What anaerobic phosphate release rate can be achieved?
- What anaerobic sCOD uptake rate can be achieved?

1.2 Limitations

This master thesis work was limited in time, which put limitations to for how long the practical part could be carried out. Due to the time limit, the laboratory work had to be terminated before the process had reached its full capacity.

The lack of equipment, such as peristaltic pumps in the right size, was another limitation. The process was only partly operated as desired since equipment (e.g. pumps) was unavailable. However, these problems could not be foreseen and were not due to lack of planning. Several changes in the experimental setup were made during the project and the changes had to be performed with the available equipment.

Most analyses were not performed in duplicates or by double samples since the time and available resources were limited. This gives a higher uncertainty to the results.

1.3 Outline of the work

The project started with a literature study to obtain relevant knowledge of the field. After a few weeks, the building of the experimental setup began and the practical part was started. The practical experiment was then conducted for 11 weeks while the collected data was compiled.

In this thesis the theory behind the MBBR process and diffusion limitations in biofilms is described. Wastewater treatment in form of nitrogen removal by nitrification and denitrification, phosphorus removal by chemical precipitation and biological phosphorus removal is explained as well. Sludge hydrolysis for production of carbon source is shortly described. Finally, it is explained how the experimental project was conducted and the results are presented and discussed.

2 Moving Bed Biofilm Reactor

The intention when the moving bed biofilm reactor (MBBR) process was developed to combine the best features of the activated-sludge process and biofilter process without including the disadvantages (Ødegaard, 2006). The MBBR process is based on microorganisms growing in a biofilm on carriers moving freely in the wastewater (Helness, 2007). Bacteria in biofilms are characterised by their ability to adhere to surfaces and produce a fixed film of polymers to protect themselves from sloughing off (Henze *et al*., 1997, pp. 143).

The MBBR process can be operated at aerobic, anaerobic or anoxic conditions. When aerobic conditions are used, mixing is achieved by aeration while mixing is achieved by mixers in anoxic and anaerobic conditions. The mixing is important to ensure the carriers are moving freely in the wastewater and are well distributed. To ensure that the carriers are retained in the reactor, there are sieves in the outlet (Ødegaard, 2006; Helness, 2007, pp. 48). Several types of carriers with different shapes and sizes have been developed to fit different applications. Furthermore, the filling degree can be chosen depending on the application (Ødegaard, 2006).

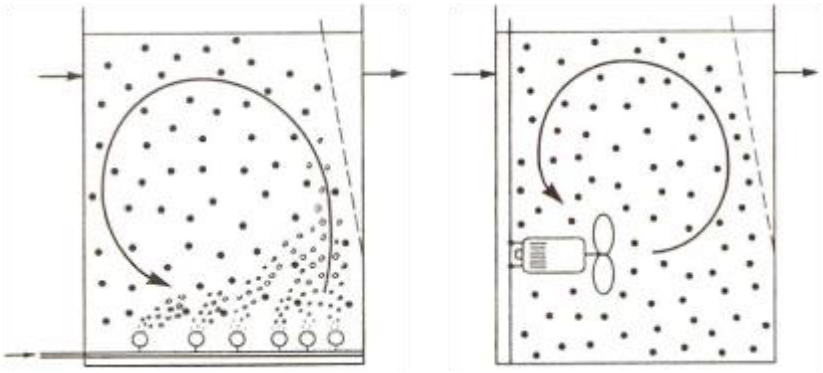

Figure 2.1. Principle of the moving bed biofilm reactor process with carriers moving freely in the water. Left. An aerobic reactor mixed by aeration. Right. An anoxic or anaerobic reactor where mixing is achieved by mixers (Rusten et al., 2006). Permission for reprint of the figure was granted by Veolia Water Technologies.

MBBRs have advantages compared to available fixed-bed biofilm reactors. To mention some, the process uses the whole reactor volume since the carriers move freely in the water (Helness, 2007, pp. 48) and there is no need for periodic backwashing (Pastorelli *et al*., 1999). In fixed filters it might be difficult to get an even distribution of the load on the filter. This problem does not occur in MBBRs since the carriers move freely in the wastewater (Rusten *et al*., 2006).

Some of the advantages of MBBRs compared to activated sludge are that the process takes less space as both the reactors and the clarifier are smaller, and the attached biomass becomes more specialised since there is no need for sludge recycle (Ødegaard, 2006). Furthermore, biofilm reactors are especially useful when slow-growing microorganisms are desired in the process, since the carriers prevent wash out (Kermani *et al*., 2008). Other advantages are that already existing overloaded activated sludge processes can be changed into MBBR processes without building new tanks (Pastorelli *et al*., 1999) and only excess sludge need to be separated (Helness, 2007, pp. 48). A disadvantage with biofilm processes is diffusion

limitations in the biofilm (Ødegaard, 2006) which are larger compared to in activated sludge since the biofilm often is thicker than flocs (Falkentoft, 2000, pp. 50). Another disadvantage with MBBR processes is the higher cost of investment.

The biofilm thickness on the carriers affects the size of the fraction of the biofilm with access to substrates. The substrates have to diffuse into the biofilm and the penetration is limited (Ødegaard, 2006). More about diffusion limitations can be read in chapter *3 Diffusion limitation*. It is desired to obtain an even distribution of substrates over the biofilm. Hence, the biofilm need to be relatively thin and evenly distributed over the carrier surface. Turbulence in the reactor increases the transport of substrates to the biofilm and prevents the biofilm from getting too thick due to shear forces (Ødegaard, 2006). The thickness of the biofilm is also regulated by abrasion, erosion, sloughing, and predator grazing.

3 Diffusion limitation

The diffusion aspect is of importance for biofilm processes, such as the intended moving bed biofilm reactor process for denitrifying phosphorus removal, since the process depends on the penetration depth which in turn depends on the concentrations of the involved substances (Falkentoft *et al*., 2001). The substances, i.e. phosphate, VFAs and oxygen, have to diffuse into the biofilm from the bulk water to enable the removal process (Henze *et al*., 1997, pp. 143). The following chapters about biofilm kinetics and hydraulic film diffusion are based on Henze *et al*. (1997).

3.1 Biofilm kinetics

The transport of substances into the biofilm occurs by molecular diffusion, see *Figure 3.1*. There is a balance over the biofilm surface, where the same amount of substance that diffuses into the film or is produced in the biofilm has to be removed or diffuse out of the film (Henze *et al*., 1997, pp. 143-144), see *Eq. 3.1*.

D	Diffusion coefficient
h	Transfer coefficient
k_{0Vf}	Zero order rate constant
k_{1Vf}	First order rate constant
L	Biofilm thickness
N	Transport through cross section
r_{Vf}	**V**olumetric **r**eaction rate of the bio**f**ilm
S	Substance concentration in bulk
S_g	Substance concentration at the biofilm surface
S_{Vf}	Substance concentration in the biofilm
β	Degree of penetration
ε	Efficiency factor
ξ´	Efficient part of the biofilm

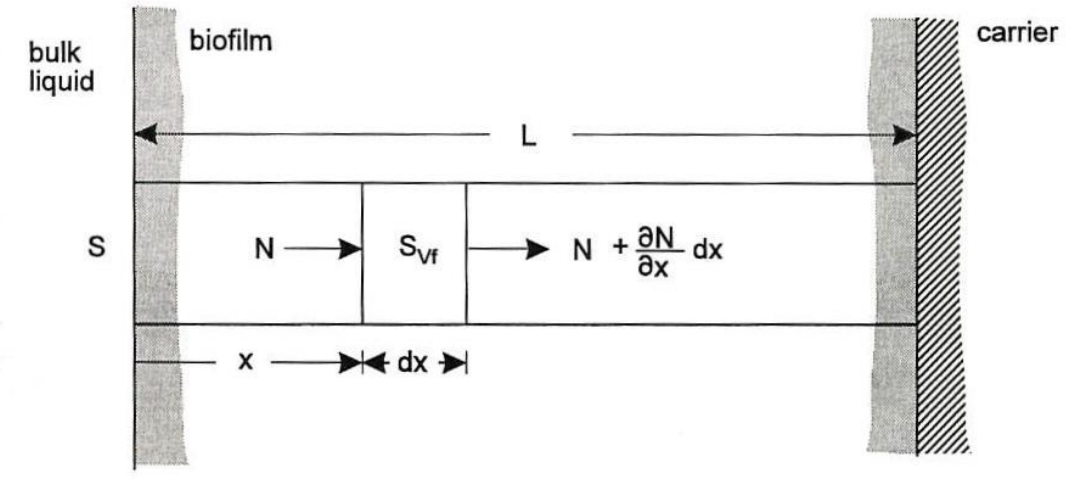

Figure 3.1. Model for an idealised biological film where the substance is moved by diffusion (Henze et al., 1997, pp. 143). Permission for reprint of the figure was granted from the copyright holder Springer Verlag.

A balance can be set up over an infinitely small biofilm under stationary conditions.

$$in = out + removed \qquad \textit{(Eq. 3.1)}$$

$$N = N + \frac{\partial N}{\partial x} \cdot dx + r_{Vf} \cdot dx$$

where N is the transport through the cross section and r_{Vf} is the volumetric reaction rate of the biofilm. The equation can be simplified, see *Eq. 3.2*.

$$\frac{\partial N}{\partial x} = -r_{Vf} \qquad \textit{(Eq.3. 2)}$$

The transport through the cross section takes place with diffusion only. The flux into the biofilm depends on the diffusion coefficient, D, and the change of the concentration distribution in the biofilm, see *Eq. 3.3*. The concentration in the bulk is assumed to be S while S_{Vf} is the concentration in the biofilm.

$$N = -D \cdot \frac{\partial S_{Vf}}{\partial x} \qquad \textit{(Eq 3.3)}$$

$$\frac{\partial N}{\partial x} = -D \cdot \frac{\partial^2 S_{Vf}}{\partial x^2} \qquad \textit{(Eq. 3.4)}$$

When equation *Eq. 3.3* and *Eq.3.4* is combined, *Eq. 3.5* is given.

$$\frac{\partial^2 S_{Vf}}{\partial x^2} = \frac{r_{Vf}}{D} \qquad \textit{(Eq. 3.5)}$$

The interpretation of *Eq. 3.5* is that the second derivative of the concentration distribution has a curving effect on the distribution. This means that if no reaction occurs in the biofilm, the concentration distribution will change linearly through the depth of the film. However, if there is production in the film, the concentration distribution curves upwards. And if there is removal the concentration distribution curves downwards (Henze *et al*., 1997, pp. 144).

Characteristic parameters were used to make *Eq. 3.5* dimensionless.

$$s_{Vf} = \frac{S_{Vf}}{S}, \xi = \frac{x}{L}$$

The dimensionless equation can be seen in *Eq. 3.6*.

$$\frac{\partial^2 s_{Vf}}{\partial \xi^2} = \frac{r_{Vf} L^2}{DS} \qquad \textit{(Eq. 3.6)}$$

In the following section, the solution of the second order differential equation with a zero order reaction and a first order reaction will be described.

In the **first order reaction**, the volumetric reaction rate in the biofilm can be written as in *Eq. 3.7*:

$$r_{Vf} = k_{1Vf} \cdot S_{Vf} \qquad \textit{(Eq. 3.7)}$$

where S_{Vf} is the concentration in the biofilm and k_{1Vf} is a first order rate constant with the dimension d^{-1}. If the reaction rate is combined with the dimensionless *Eq. 3.6*, boundary conditions are taken into account, further calculated and rewritten, the reaction per surface area can be written as in *Eq. 3.8*.

$$r_A = N = k_{1Vf} LS \cdot \varepsilon \qquad \textit{(Eq. 3.8)}$$

Where the efficiency factor, ε, can be written as:

$$\varepsilon = \frac{\tanh \alpha}{\alpha},\ \alpha = \sqrt{\frac{k_{1Vf} L^2}{D}}$$

In *Eq. 3.8*, it can be seen that the transport through the surface of the biofilm is proportional to the concentration in the bulk just outside the film (Henze *et al*. 1997, pp. 144-146).

In the **zero order reaction**, the volumetric reaction rate in the biofilm can be written as:

$$r_{Vf} = k_{0Vf} \qquad \textit{(Eq. 3.9)}$$

where k_{0Vf} is the zero order rate constant. When *Eq. 3.9* is combined with equation *Eq. 3.6* and boundary conditions are taken into account, the transport per surface area for a fully penetrable biofilm is:

$$r_A = N = k_{0Vf} \cdot L \qquad \textit{(Eq. 3.10)}$$

It requires that the biofilm is fully efficient and that removal takes place in the whole biofilm. Hence, the concentration in the biofilm closest to the carrier has to be larger than zero. Rephrased, this means that the condition $\beta>1$ has to be fulfilled.

$$\beta = \sqrt{\frac{2DS}{k_{0Vf} L^2}}$$

where β is the degree of penetration, a dimensionless parameter that expresses the relative length a substrate can penetrate the biofilm. If $\beta>1$, then the biofilm is fully penetrated.

If the biofilm is only partially penetrable, the boundary conditions are different and the transport per surface becomes:

$$r_A = N = L\xi' k_{0Vf} = \sqrt{2k_{0Vf} D} \cdot S^{1/2} \qquad \textit{(Eq. 3.11)}$$

where ξ′ is the efficient part of the biofilm.

There is a relationship between the reaction rate constants for the first order reaction and the two zero order reactions, see *Eq. 3.12*.

$$k_{0Vf} = k_{1Vf} \cdot K_S \qquad \textit{(Eq. 3.12)}$$

Where K_S is the substrate concentration at which the reaction rates would be identical (Henze *et al*. 1997, pp. 147-151).

3.1.1 Two-component diffusion

Most biological removal processes are redox-processes which require both an oxidant and a reductant. In the biofilm kinetics, it is important to find which substrate that limits the removal. This depends on the removal rate and the diffusion rate of the involved substrates. The substrate penetrating the shortest distance into the biofilm is the limiting substrate, see *Figure 3.2*.

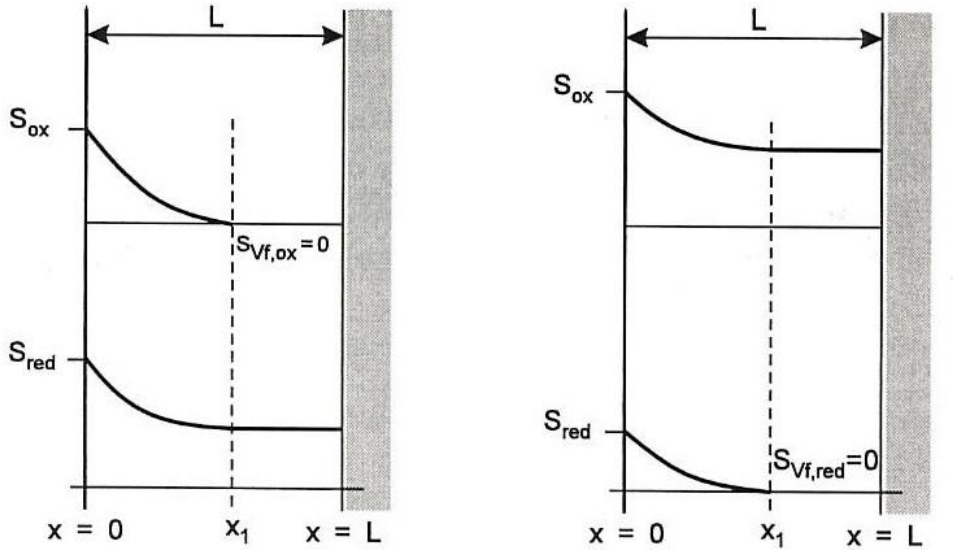

Figure 3.2. Partial penetration and limitation of two components in a biofilm (Henze et al., 1997, pp.158). Permission to reprint the figure was granted from the copyright holder Springer Verlag.

$\beta_{ox} < \beta_{red}$ The oxidant is limiting

$\beta_{red} < \beta_{ox}$ The reductant is limiting

$$\frac{\beta_{red}}{\beta_{ox}} = \frac{\sqrt{\frac{2D_{ox}S_{ox}}{k_{0,ox}}}}{\sqrt{\frac{2D_{red}S_{red}}{k_{0,red}}}} \begin{matrix} > \\ =1 \\ < \end{matrix}$$

A criteria can be based on the above statement.

$$\frac{S_{ox}}{S_{red}} \begin{matrix} > \\ = \\ < \end{matrix} \frac{D_{ox}}{D_{red}} \cdot \frac{k_{0,ox}}{k_{0,red}} \qquad \textit{(Eq. 3.13)}$$

If the ratio for substrate concentrations in *Eq. 3.13* is larger (>), the reductant is potentially limiting. The oxidant will be potentially limiting if the ratio is smaller (<) (Henze *et al*., 1997, pp. 157-159).

3.2 Hydraulic film diffusion

It is not only the diffusional limits in the biofilm that limit the reaction. There occurs a limitation in the transport from the bulk water to the biofilm as well. The transport through the hydraulic film just outside the biofilm depends on the substrate concentration in the bulk and the substrate concentration on the surface of the biofilm, see *Eq. 3.14* and *Figure 3.3*.

$$N = h(S - S_g) = -D\left(\frac{dS_{Vf}}{dx}\right)_{x=0} \qquad \textit{(Eq. 3.14)}$$

where h is the transfer coefficient for the hydraulic film diffusion and S_g is the substrate concentration on the biofilm surface.

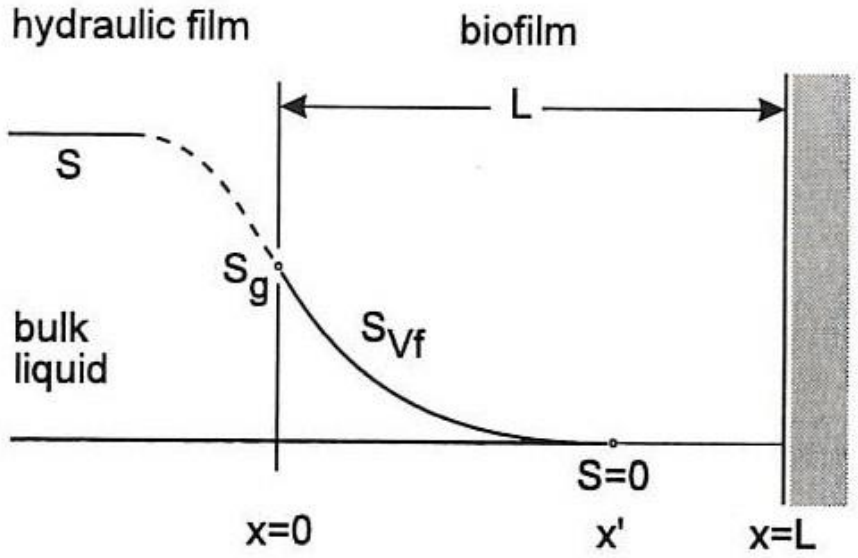

Figure 3.3. Concentration distribution in the hydraulic film and in the biofilm for a partially penetrated biofilm (Henze et al., 1997, pp. 154). Permission to reprint the figure was granted from the copyright holder Springer Verlag.

The flux across the surface of the biofilm must be the same, both in relation to the hydraulic film and the biofilm (Henze *et al*., 1997, pp. 153-156).

4 Nitrification

Ammonium oxidising bacteria (AOB) are chemolithotrophic autotrophic bacteria using ammonium as energy source and electron donor, and carbon dioxide as carbon source. The ammonium is oxidised in a two-step process, first to nitrite (NO_2^-) and then to nitrate (NO_3^-), see *Eq. 4.1 to 4.2*. These oxidations are made by different bacteria. AOB, like *Nitrosomonas*, perform the oxidation from ammonium to nitrite, while nitrite oxidising bacteria (NOB), such as *Nitrobacter*, perform the oxidation from nitrite to nitrate. The total energy release in nitrification is low. Furthermore, the first step releases approximately four times more energy than the second. This results in a low growth rate for nitrifying bacteria, and especially for NOB (Christensson, 1997; Helness, 2007).

$$NH_4^+ + 1.5O_2 \rightarrow 2H^+ + H_2O + NO_2^- \qquad \textit{(Eq. 4.1)}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad \textit{(Eq. 4.2)}$$

The total reaction of *Eq. 4.1* and *Eq. 4.2* can be written as in *Eq.4.3.*

$$NH_4^+ + 2O_2 \rightarrow 2H^+ + H_2O + NO_3^- \qquad \textit{(Eq. 4.3)}$$

Nitrifying bacteria are mesophilic and the growth rate is influenced by the temperature. The growth rate increases with the temperature up to between 35°C and 40°C, and at temperatures above that the growth rate declines (Helness, 2007).

The first step in the nitrification consumes alkalinity, which may cause a decrease in pH. The optimum pH is between 8 and 9 (Henze *et al*., 1997, pp. 79, 81), and at pH values below and above that the nitrification rates decrease (Christensson, 1997; Helness, 2007).

Nitrifying bacteria are commonly autotrophic and use carbon dioxide as carbon source (Henze *et al*., 1997, pp. 77). Therefore, organic carbon sources should be kept as low as possible since they give an advantage for competing heterotrophic bacteria (Kermani *et al*., 2008).

Nitrification in biofilm processes requires dissolved oxygen (DO) levels high enough to penetrate the outer layer of oxygen consuming heterotrophs to reach the nitrifying bacteria in the inner layers (Hem *et al*., 1994).

5 Denitrification

Denitrification is when heterotrophic bacteria convert nitrate to nitrogen gas via intermediate products, see *Eq. 5.1*. The nitrate is used as an electron acceptor by the bacteria when oxidising organic matter. However, some of these bacteria only have the ability to reduce nitrate to nitrite (Christensson, 1997; Helness, 2007).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textit{(Eq. 5.1)}$$

In addition to nitrogen gas, the intermediates nitric oxide, NO, and nitrous oxide, N_2O, are formed and can be released to the atmosphere during denitrification. Nitrous oxide is a potent greenhouse gas. The amounts formed are normally on a part per thousand level, but the levels can increase if the process is stressed (Henze *et al*., 1997, pp. 86).

Denitrifying bacteria are able to oxidise a wide range of carbon sources during denitrification, but the rate differ with carbon source. When acetic acid is used as a carbon source in denitrification, the total reaction without biomass growth can be written as in *Eq. 5.2* (Helness, 2007).

$$2NO_3^- + 2H^+ + 1.25CH_3COO^- \rightarrow N_2 + 2.25H_2O + 1.25CO_2 + 1.25HCO_3^- \qquad \textit{(Eq. 5.2)}$$

The denitrifying process increases alkalinity. However, the alkalinity increase by denitrification is smaller than the decrease in alkalinity by nitrification. The optimal pH for denitrifying bacteria is around pH 7 to 9. A pH value below 7 causes an increased production of N_2O (Henze *et al*., 1997, pp. 94).

Most denitrifying bacteria are facultative and use oxygen instead of nitrate when it is available. Aerobic conditions will therefore lead to consumption of carbon sources needed for denitrification. Denitrification is dependent on that enough easily biodegradable carbon sources are available. If acetic acid is used as a carbon source, the minimum theoretical COD consumption for denitrifying respiration, without sludge production, is 2.87 mg COD_{HAc}/mg NO_3-N (Helness, 2007).

6 Phosphorus removal by chemical precipitation

Unlike the denitrification process where nitrate is converted to nitrogen gas there is no process converting phosphate to a gas. The phosphorus removal depends on the ability to convert dissolved phosphates into suspended phosphorus which can be separated from the water (Henze *et al*., 1997, pp. 311). Phosphorus removal from wastewater is mainly achieved by chemical precipitation or by biological processes. Biological phosphorus removal can be achieved by assimilation by the microorganisms during growth, or by EBPR. Both can occur in activated sludge processes or biofilm processes.

Phosphorus removal by chemical precipitation can be divided into four steps: precipitation, coagulation, flocculation, and separation. First, metal salts of typically aluminium, iron, or calcium are added under stirring and soluble phosphate is precipitated. The precipitated phosphate forms particles during coagulation and in flocculation aggregation forms flocs which can be separated from the water (Henze *et al*., 1997, pp. 314). The separation can be performed by sedimentation, flotation, or filtration units (Christensson, 1997).

Chemical precipitation can be used to remove most of the phosphate, however, not without disadvantages. The process produces lots of sludge with increased metal content, and it is costly to add chemicals (Christensson, 1997). Additionally, the production and transportation of the chemicals consume energy.

7 Biological phosphorus removal

Municipal wastewater contains phosphorus mainly as orthophosphate and particulate organic phosphorus. When microorganisms grow, they assimilate phosphorus and hence it is removed from the water. In enhanced biological phosphorus removal (EBPR), organisms able to store a higher amount of cellular phosphorus are used. These organisms are called polyphosphate accumulating organisms (PAO) and store more phosphorus than needed for growth (Christensson, 1997).

Today, two different main types of microorganisms are known to be involved in enhanced biological phosphorus removal, these are *Candidatus Accumulibacter phosphatis* and *Tetrasphaera*. They have different physiology (Kristiansen *et al*., 2013) and their metabolic models will be explained separately.

To get a functional EBPR with *Accumulibacter*, the conditions must be altered between anaerobic and aerobic or anoxic. During anaerobic conditions, PAO take up easily biodegradable soluble organic matter, such as volatile fatty acids (VFAs), and store it intracellularly as poly-β-hydroxy-alkanoates (PHA). Depending on the easily biodegradable compounds available in the wastewater, the composition of the stored PHA varies. The energy needed for the carbon uptake is obtained from degradation of stored polyphosphate (poly-P) and degradation of stored glycogen to poly-hydroxy-butyrate (PHB). To maintain the osmotic pressure, the produced orthophosphate is released from the cell. At the same time, cations such as potassium and magnesium are released from the cell to maintain the ionic balance in the cell. PAOs are favoured, compared to other organisms, in anaerobic conditions due to their ability to take up carbon source there (Christensson, 1997).

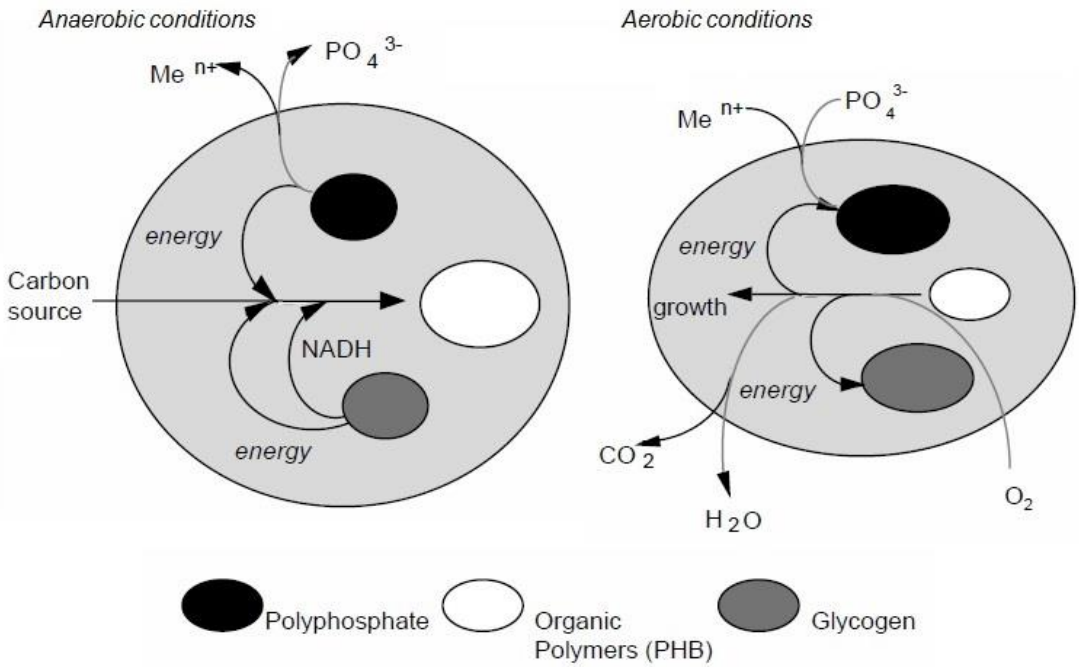

Figure 7.1. Schematic description of carbon uptake and phosphate release during anaerobic conditions (left), and phosphate uptake during aerobic conditions (right) by PAOs such as Accumulibacter (Christensson, 1997). Permission to reprint the figure was granted from the copyright holder Magnus Christensson.

When the condition is altered to aerobic or anoxic, the PAO begins to use the stored carbon for growth, synthesis of glycogen and phosphate uptake. When the phosphate uptake occurs, potassium and magnesium ions are also taken up. Phosphate, stored as polyphosphate, will later be used as energy source when the condition is altered to anaerobic again. More phosphate is taken up in the aerobic or anoxic stage than is released in the anaerobic stage due to cell growth. This gives a net uptake of phosphorus. To implement this in a wastewater treatment plant, the excess sludge should be removed after the aerobic or anoxic stage (Christensson, 1997).

During anoxic conditions, nitrate is used as an electron acceptor instead of oxygen. Similarly to when aerobic conditions prevail, degradation of PHA, accumulation of polyphosphate and synthesis take place. The nitrate used as electron acceptor is denitrified to nitrogen gas. This leads to a decreased need for carbon source since both phosphate and nitrate are removed from the wastewater at the same time, compared to when the two carbon needing processes are done separately (Kuba *et al*., 1996). Helness (2007) concluded that a majority of the PAOs, in his study, were able to use nitrate as an electron acceptor. These microorganisms are called denitrifying phosphorus removing bacteria (DPB).

To get a functional EBPR with *Tetrasphaera*, the conditions must be altered between aerobic and anaerobic, just like for *Accumulibacter*. Similarly to *Accumulibacter*, *Tetrasphaera* assimilates phosphate during the aerobic phase and stores it as polyphosphate, if organic substrates were available in the previous anaerobic phase. *Tetrasphaera* is able to form glycogen and it is suggested that glycogen is used instead of PHA as the anaerobic storage polymer. Degradation of this stored glycogen is suggested to provide carbon and energy for growth, phosphate assimilation and polyphosphate formation during aerobic conditions. The energy needed during anaerobic conditions is suggested to be obtained by fermentation and polyphosphate degradation (Kristiansen *et al*., 2013). The bacteria can potentially denitrify (Kristiansen *et al*., 2013) and therefore perform EBPR under anoxic conditions.

Traditionally, biological phosphorus removal has been based on activated sludge processes and there are several process configurations in use today. All biological bio-P processes have in common that the phosphorus removal is heavily dependent on effective separation of biomass in the clarifier after the process. The release of phosphorus from the sludge in the clarifier, secondary release, must be avoided. The problem with secondary release is expected to be smaller for biofilm processes compared to activated sludge processes, since the concentration of suspended solids in the influent to the clarifier is lower (Helness and Ødegaard, 1999).

Bacteria growing without phosphorus limitation normally contain phosphorus corresponding to 1% to 2% of their dry weight. Fast-growing bacteria have more RNA and hence have a higher phosphorus content which can be around 2% to 3%. The phosphorus content in activated sludge from EBPR plants may be up to 7% to 10% of the total soluble solids (TSS) (Lie, 1996, pp. 29). According to Henze *et al*. (2007, pp. 97) the theoretical polyphosphate content in PAOs can be up to 50%, corresponding to a phosphorus content of 15% to 20% in the bacteria.

The fractions of poly-P, PHA and glycogen in the biomass depend on the solid retention time (SRT). The SRT describes for how long the solids are in the system. A shorter SRT gives a lower fraction of poly-P in the cells in the anaerobic phase, while the fractions of PHA and glycogen are higher at lower SRTs. If the SRT is too short, the polyphosphate content in the

cells will not be sufficient for anaerobic uptake of carbon, which will limit the growth rate of the bio-P bacteria. The process may then lose its EBPR activity since fewer bio-P bacteria are formed than are washed out of the system. This happens at SRTs around 3 days (Smolders, 1995, pp. 122).

The proportionality coefficient between phosphorus uptake and phosphorus release is found to be 1.055 g P removed/g P released (Pastorelli *et al*. 1999).

Both *Accumulibacter* and *Tetrasphaera* need to take up carbon source and store as PHA and glycogen, respectively, in the anaerobic phase to be able to assimilate phosphorus in the aerobic or anoxic phase (Kristiansen *et al*., 2013). Also other microorganisms can take up carbon sources and store as glycogen under anaerobic conditions, and if they are not a part of the phosphorus removal they are called glycogen accumulating organisms (GAO). GAO counts as competitors to PAO (Tykesson, 2005).

In the competition between PAO and GAO, some environmental and operational conditions are identified as key factors and used to give PAO advantage. The key factors are pH, temperature, type of carbon source in the influent, and the ratio between phosphorus and VFA.

7.1 pH

The PAOs reduce alkalinity under anaerobic conditions when using acetic acid as carbon source due to consumption of acetic acid and release of phosphate (Henze *et al*., 1997, pp. 97).

As all other microorganisms, PAOs are influenced by pH. The acetate uptake rate is pH independent in the range from pH 6.5 to 8.0 (Smolders, 1995) while propionate uptake rate increases with an increasing pH (Oehmen *et al*., 2005a). Stoichiometry of acetate uptake with respect to glycogen consumption and PHA accumulation is also independent of pH (Filipe *et al*., 2001a). However, the phosphate release is strongly influenced by pH. The ratio between phosphate release and acetate uptake increases with increasing pH. In the pH range between 5.5 and 8.5, the ratio was increasing from 0.25 to 0.75 P-mol/C-mol with increasing pH(Smolders, 1995, pp. 25). The explanation is that more energy is required to transport acetate through the cell membrane at higher pH values and hence more energy needs to be produced by phosphate release (Smolders, 1995, pp. 43). Also Filipe *et al*. (2001a) found a correlation between pH and energy required for acetate uptake similar to Smolders (1995).

When it comes to GAOs the acetate uptake rate is faster at lower pH and the amount of glycogen consumed decreases with decreasing pH. Additionally, the stoichiometry for acetate uptake is more favourable at low pH. During anaerobic conditions, GAOs are more efficient at low pH, to a minimum of pH 6.5 (Filipe *et al*., 2001b).

Fukushima *et al*. (2010) found the PAO population to decrease when the pH was decreased from pH close to 8 to pH 6.5, and later down to pH 6. No negative effects caused by the pH reduction could be seen on the GAO population. Additionally, the phosphorus removal decreased with decreasing pH.

PAOs are suggested to be more competitive compared to GAOs in systems where the pH in the anaerobic phase is higher (Filipe *et al*., 2001a; Lopez-Vasquez *et al*., 2009b). Oehmen *et al*. (2005a) suggested an external pH around 8. Lopez-Vazquez *et al*. (2009b) simulated

bacterial populations of GAO and PAO in different conditions by changing the temperature, pH and fraction of acetate and propionate in the feed. It was concluded that depending on the temperature and ratio of propionate, an increase in pH can shift the bacterial population to PAOs. When the pH was 7, acetate was used as carbon source and the temperature was 20 °C the bacterial population consisted of coexisting PAOs and GAOs. If the pH was increased to 7.5, the PAOs became the dominant microorganism. When comparing the simulations, PAOs were the dominant microorganism in more cases at pH 7.5 than at pH 7.

7.2 Temperature

The temperature affects the efficiency of the EBPR process, but the results explaining how are contradictory. Some studies have found the EBPR process to be more efficient at higher temperatures (Mamais and Jenkins, 1992; Brdjanovic *et al*., 1997; Brdjanovic *et al*., 1998) while others have found that lower temperatures are better (Erdal *et al*., 2003.; Panswad *et al*., 2003; Lopez-Vazquez *et al*., 2009b; Ki *et al*., 2014). It is commonly known that a decrease in temperature decreases the rate of chemical and biochemical reactions. Despite that fact, Erdal *et al*. (2003) state that EBPR is more efficient at temperatures below 10°C. Stable EBPR has been achieved at temperatures as low as 5°C in both laboratory scale (Erdal *et al*., 2003; Erdal *et al*., 2006) and in a full-scale SBR (Marklund and Morling, 1994). The studied PAOs were lower range mesophilic or psychrophilic (Erdal *et al*., 2003; Panswad *et al*., 2003) and outcompeted GAO at lower temperatures which resulted in an increased PAO population and higher EBPR efficiency (Erdal *et al*., 2003). In a corresponding way, GAOs have been found to outcompete PAOs at higher temperatures (Panswad *et al*., 2003; Whang and Park, 2006; Lopez-Vazquez *et al*., 2009b; Ki *et al*., 2014). PAOs are able to adapt to lower temperatures by increasing the amount of unsaturated fatty acids in the cell membrane (Erdal *et al*., 2006). Additionally, it is suggested that the metabolic pathways are positively influenced by low temperatures (Erdal *et al*., 2003). The phosphorus uptake rate decreases with increasing temperature (Panswad *et al*., 2003).

Simulations by Lopez-Vazquez *et al*. (2009b) suggested that lower temperatures limit the GAO metabolism and are hence beneficial for EBPR. The bacterial populations of GAO and PAO in different conditions were shown by simulating changes in temperature, pH and fraction of acetate and propionate in the feed. It was concluded that, depending on the pH and ratio of propionate, a decrease in temperature can increase the PAO population compared to the GAO population. When the pH was 6 or 7, either acetate or propionate was used as sole carbon source and the temperature was 20°C, the bacterial population consisted of coexisting PAOs and GAOs. However if the temperature was decreased to 10°C, the PAOs became the dominant microorganism. When comparing the simulations, PAOs were the dominant microorganism in more cases at 20°C than at 30°C. Additionally, PAOs were the dominant microorganism in all cases at 10°C (Lopez-Vazquez *et al*., 2009b).

Whang and Park (2006) confirmed that the amount of PAO decreased with increasing temperature in the temperature range from 20°C to 30°C. However, if the SRT was decreased at the higher temperature a stable EBPR was achieved. It has been found that longer SRTs are needed at lower temperatures to prevent washout of PAOs (Erdal *et al*., 2006).

The temperature dependencies of stoichiometry and kinetics in PAOs have been studied. In the temperature range from 5°C to 30°C, the stoichiometry during anaerobic conditions was insensitive to temperature changes while the stoichiometry during aerobic conditions was affected. The kinetics were strongly influenced by temperature changes. The anaerobic

phosphate release rate (acetate uptake rate) increased with temperature and showed a maximum at around 20°C in short term. Anaerobic maintenance increased with increasing temperature in the studied temperature range. During aerobic conditions, all rates increased with temperature without an optimum in the temperature range in short term (Brdjanovic *et al*., 1997). In long term, all rates increased strongly with increasing temperature except for the phosphorus uptake, which increased moderately. The composition of the bacterial population was found to change with changing temperature and consisted of at least five to seven different bacteria (Brdjanovic *et al*., 1998). Some of the findings were supported by Baetens *et al*. (1999) who suggested that temperature affects the processes in the EBPR process differently. In the temperature range from 5°C to 20°C, the stoichiometry during anaerobic conditions was insensitive to temperature changes while the anaerobic conversion rates increased with increasing temperature. Under aerobic conditions, the rates increased with increasing temperature. Contradictory to the findings by Brdjanovic *et al*. (1998), the phosphate uptake rate under aerobic conditions was found to have a maximum at between 15°C and 20°C (Baetens *et al*., 1999).

Efficient EBPR has been achieved in laboratory scale SBRs at temperatures as high as 28°C and 32°C (Ong *et al*., 2014; Liau *et al*., 2015). The phosphate release under anaerobic conditions was lower at 32°C than at 28°C due to an increased part of GAOs (Liau *et al*., 2015). In the range from 24°C to 32°C, the amount of PAOs decreased with increasing temperature. Despite what was expected, the phosphorus removal was similar to that at the lower temperatures (Ong *et al*., 2014). The studied microbial system was thought to have unique characteristics (Liau *et al*., 2015) and the PAO population consisting of *Accumulibacter* was found to mainly consist of a special clade which is suggested to have higher tolerance to high temperatures and have a higher phosphorus accumulation at higher temperatures (Ong *et al*., 2014). The name clade comes from the Latin word for branch and a clade contains one ancestor and all its ascendants.

In conclusion, PAOs seem to be versatile microorganisms where different clades or populations thrive in different temperature ranges. Most studies have investigated the temperature dependency for *Accumulibacter* and it should be noted that *Tetrasphaera* and other suggested PAOs are not well-studied. The contradictory results for the temperature dependency of the EBPR process might get clearer if it was taken into account which microorganisms that were studied. Regardless whether PAOs prefer lower or higher temperatures, they still have to outcompete GAOs.

7.3 Carbon source

Carbon source is required for growth of microorganisms and in the case of PAOs it is also required in the anaerobic phase for synthesis of PHA and glycogen. Studies with wastewater and synthetic wastewater have shown that EBPR can be achieved with different carbon sources. Some of the carbon sources that have been tested are various volatile fatty acids (VFAs), amino acids, glucose and alcohols (Helness, 2007, pp. 17).

The anaerobic uptake of carbon source is important in the EBPR process. However, PAOs are not the only microorganism with the ability of anaerobic carbon uptake and there is a competition between these microorganisms about the available carbon source. If there is not enough carbon source for both PAOs and GAOs, the phosphate removal might be negatively affected (Tykesson, 2005).

Acetate and propionate are two common carbon sources used in literature (Tykesson, 2005) since they are two natural main products from hydrolysis of sludge. Tykesson *et al*. (2006) found the PAO propionate uptake rate to be only 30% of the acetate uptake rate. Additionally, only 20% to 30% of the PAO *Accumulibacter* was found to take up propionate while most of them took up acetate. Also Helness (2007) reported propionic acid not to be as effective as acetic acid. However, Oehmen *et al*. (2005b) found the anaerobic uptake rate for propionate and acetate by the PAO *Accumulibacter* to be similar, supporting the results found by Tykesson (2005) where the few PAOs able to take up propionate did it at the same rate as acetate uptake.

Carvalheira *et al*. (2014a) found PAOs (*Accumulibacter*) to deplete propionate before acetate and when a mixture of the carbon sources were used *Accumulibacter* had a higher anaerobic uptake rate for propionate than acetate. The uptake rate for acetate decreased with increasing propionate fraction in the feed. However, in the competition against GAOs, a mixture of both carbon sources (75% acetate and 25% propionate) was suggested to give PAOs an advantage. In another experiment, an SBR with sludge taken from a full-scale plant in Queensland, Australia, containing both PAOs and GAOs were fed with propionate. After 7 weeks, the PAO population had increased (Oehman *et al*., 2006a).

The ratio between anaerobic phosphorus release and VFA uptake is lower for propionate than for acetate (Oehmen *et al*., 2005b). Acetate has been found to have the highest ratio between anaerobic phosphorus release and COD utilised (mg/mg) when some VFAs (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid) were tested in an UCT-type pilot plant system. The other tested acids followed a pattern where the ratio increased with increasing molecular weight of the acid. The aerobic phosphorus uptake followed the same pattern as the anaerobic ratio and also here, acetate had the highest value (Abu-ghararah and Randall, 1991). In a later study, the phosphorus removal was higher in a system fed with propionate than in a system fed with acetate (Oehmen *et al*., 2005a).

The VFA uptake rates for GAOs are different from PAOs. The GAO C*ompetibacter* has been found to have similar uptake rates as *Accumulibacter* for acetate (Zeng *et al*., 2003) but much lower for propionate (Oehmen *et al*., 2005c). On the other hand, the GAO *Alphaproteobacteria* has been found to have similar uptake rates as *Accumulibacter* for propionate (Oehmen *et al*., 2006b) but much lower for acetate (Oehmen *et al*., 2005c). Despite these results, Oehmen *et al*. (2005a) found PAOs to be more competitive against GAOs *Alphaproteobacteria* when propionate was used as carbon source than against *Competibacter* when acetate was used.

Tu and Schuler (2003) found PAOs (*Accumulibacter*) to be more competitive against GAOs at low in-reactor acetate concentrations, when acetate was used as sole carbon source. An operational suggestion was to maintain a low acetate concentration in the reactor while still adding the same amount of acetate. The laboratory study was performed on a sequencing batch reactor, where the duration of the acetate influent was varied. This is however not applicable in all operational modes. Carvalheira *et al*. (2014b) found the amount of active bacteria to decrease faster for GAOs than for PAOs at low organic loading rates. Additionally, the biomass decay was concluded to be higher for GAOs than for PAOs at low organic loading rates indicating that PAOs adapt better to low carbon loading concentrations (Carvalheira *et al*., 2014b).

Lopez-Vazquez *et al*. (2009b) simulated the bacterial populations of GAOs and PAOs in different conditions by simulating changes in temperature, pH and fraction of acetate and propionate in the feed. It was concluded that depending on the pH and temperature a change in the ratio of acetate and propionate could favour the PAO population. When the temperature was 20°C, the pH was 6 or 7 and either acetate or propionate was used as sole carbon source the bacterial population consisted of coexisting PAOs and GAOs. However if the content in the feed was changed to a mixture of acetate and propionate the PAOs became the dominant microorganism. When comparing the simulations, PAOs were the dominant microorganism in more cases when a mixture of acetate and propionate was used than when acetate or propionate was used as sole carbon source (Lopez-Vazquez *et al*., 2009b).

PAO *Tetrasphaera* has genes encoding for transportation of acetate, propionate, glucose, glutamate and aspartate (Kristiansen *et al*., 2013). The absence of a certain gene used in the metabolism of acetate was suggested to explain why *Tetrasphaera* grows poorly on acetate (Kristiansen *et al*., 2013). *In situ* studies have indicated fermentative metabolism (Kong *et al*., 2008; Nguyen *et al*., 2011), which means that *Tetrasphaere* is able to form short chain fatty acids from low-molecular-weight oligomers or monomers.

Kumar and Chaudhari (2003) used glucose as sole carbon source in a laboratory scale SBR process with activated sludge. However, the phosphorus uptake and release rates were lower than rates achieved with other carbon sources (Brdjanovic *et al*., 1998). *Tetrasphaera* is able to use glucose as a carbon source but *Accumulibacter* can not (Nguyen *et al*., 2011). *Tetrasphaera* have genes encoding for a general sugar transporter (Kristiansen *et al*., 2013).

It is suggested that some *Accumulibacter* in one of the clades are able to use ethanol as carbon source (Skennerton *et al*., 2015).

Around 10 mg easily degradable COD is needed for each mg dissolved phosphorus to be removed. Furthermore, if nitrate is present in the anaerobic reactor the amount of carbon consumed by denitrification is 4 to 6 mg COD/mg nitrate (Henze *et al*., 1997, pp. 279).

7.4 Nitrate

Some PAOs are able to assimilate phosphate under anoxic conditions using nitrate as the electron acceptor. Advantages with simultaneous phosphate uptake and denitrification, except decreased need for carbon source, are lower production of sludge and energy savings since no aeration is needed (Kuba *et al*., 1996).

If the influent ratio between COD and nitrogen is higher than the optimal ratio for denitrifying phosphorus removal, while the ratio between COD and phosphorus is good, meaning that there is a nitrate shortage, the anoxic phase can with advantage be followed by an aerobic phase to remove more phosphorus (Kuba *et al*., 1996).

The presence of nitrate in the system can also cause problems. If nitrate is present in the reactor when anaerobic phosphate release is desired the performance can be affected. There are mainly two reasons. Firstly, the presence of nitrate turns the anaerobic reactor anoxic and hence anoxic uptake of phosphate might occur instead of anaerobic release of phosphate. Secondly, denitrifying bacteria and PAOs will compete for the carbon and if there is not enough carbon for the PAOs the phosphate release will be reduced (Henze *et al*., 1997, pp. 99).

7.5 Counter ions

During phosphate release and uptake, cations are simultaneously taken up and released to maintain the ionic balance in the cell (Christensson, 1997, pp. 11). Charged molecules are not able to pass through the cell membrane and each phosphate molecule (PO_4^{3-}) contains three negative charges and hence the phosphate needs to be neutralised to enable transportation into and out of the cell. The cations correlated to EBPR are potassium (K^{+1}) and magnesium (Mg^{+2}) (Schönborn *et al*., 2001; Tykesson, 2005, pp. 14). Rickard and McClintock (1992) found that the molar ratio between potassium and phosphate during anaerobic release and aerobic uptake was 0.21 mol K/mol P and the corresponding ratio for magnesium was 0.30 mole Mg/mole P during a laboratory-scale test with a synthetic water containing acetate and peptone as carbon source. Later, Tykesson (2005) found the ratios to be in a similar range with 0.28-0.33 mole K/mole P for potassium and 0.27-0.32 mole Mg/mole P for magnesium when conducting a batch test at Öresundsverket with acetate as carbon source. Both potassium and magnesium are needed at the same time and the EBPR process does not work if only one of them is available (Pattarkine and Randall, 1999).

The polyphosphate granules contain calcium (Buchan, 1983), potassium, and magnesium (Schönborn *et al*., 2001). However, calcium is not released and taken up simultaneously with phosphate (Rickard and McClintock, 1992; Christensson *et al*., 1998). Also Pattarkine and Randall (1999) found, from laboratory-scale batch tests, the impact of calcium to be limited. Schönborn *et al*. (2001) concluded that calcium alone has a limited effect on the EBPR process but that the ratio of magnesium and calcium in the influent wastewater is important to take into account if the EBPR process fails.

7.6 Microorganisms

PAOs need to take up easily degradable organic matter in the anaerobic phase and to store it intracellularly to be able to assimilate phosphorus in the succeeding aerobic or anoxic phase. Also other microorganisms can take up easily degradable organic matter under anaerobic conditions and store it as glycogen, and if they are not a part of the biological phosphorus removal system they are called glycogen accumulating organisms (GAOs). GAO counts as competitors to PAO. The names PAO and GAO describe the activity of the organisms and do not necessarily have anything to do with evolutionary development (Tykesson, 2005, pp. 8).

Today it is generally known that PAOs and GAOs are two different groups of microorganisms, however it has earlier been suggested that GAOs were PAOs with poly-P shortage. Brdjanovic *et al*. (1998) found that a culture of enriched PAOs with limited poly-P and excess glycogen content in the biomass took up almost no acetate under anaerobic conditions. This indicated that PAOs need more energy sources than conversion of glycogen to PHA, which is enough for GAOs.

At least two different kinds of phosphorus accumulating bacteria are known today, *Candidatus Accumulibacter phosphatis* and *Tetrasphaera*. Other bacteria suggested to be PAOs are *Acinetobacter* and *Candidatus Microthrix parvicella*.

7.6.1 Accumulibacter

The coccobacillus *Candidatus Accumulibacter phosphatis* (Wang *et al*., 2014) is a *betaproteobacteria* closely related to the *Rhodocyclus* genus (Hesselmann *et al*., 1999).

Based on a gene encoding for an enzyme polyphosphate kinase 1 (ppk1) *Accumulibacter* has been divided into two subgroups, with five and seven clades respectively (Kang and Noguera, 2014). Ong *et al*. (2014) suggested that one of these clades is more robust and tolerant to higher temperatures. Except the temperature tolerance, a difference between the clades is the ability to use nitrate as electron acceptor and to denitrify. Additionally, some *Accumulibacter* in one of the clades are able to use ethanol as carbon source while most *Accumulibacter* are restricted to low molecular weight substances such as VFAs (Skennerton *et al*., 2015).

Accumulibacter is both Neisser positive and Nile blue positive (Hesselmann *et al*., 1999). The cells are large and rod-shaped and often grow in microcolonies (Mielczarek *et al*., 2013).

Accumulibacter accounted for 9% to 17% of the biomass in domestic plants but below 3% in industrial plants when 10 full-scale EBPR plants with nitrogen removal in Denmark was investigated (Kong *et al*., 2005). In five plants in USA, 9% to 24% of the biomass consisted of *Accumulibacter*-related microorganisms while 40% to 69% of the PAOs in the plants where *Accumulibacter* (He *et al*., 2008)

7.6.2 Tetrasphaera

T. australiensis, T. japonica (Maszenan *et al*., 2000), *T. enlongata* (rod), *T. jenkinsii, T vanveenii* sp. nov. and *T. veronesis* sp. nov. are six known *Tetrasphaera* belonging to the family *Actinobacteria* (McKenzie *et al*., 2006). There are three known clades of *Tetrasphaera* (Nguyen *et al*., 2011).

The morphology of the cells differs between the strains. *T. australiensis* and *T. japonica* are coccoid with the diameter 0.5-1.4 µm, non-motile and appear mainly as tetrads and in clusters, but can also be found singly and in pairs (Maszenan *et al*., 2000). *T. enlongata* is rod shaped (Hanada *et al*., 2002) while *T. jenkinsii*, *T. vanveenii* and *T. veronesis* grow filamentous on one media and appear as swollen with extremely short filaments or as clusters of cocci on another media (Blackall *et al*., 2000; McKenzie *et al*., 2006). Additionally, irregular septate was common among *T. jenkinsii* and *T. veronesis* (McKenzie *et al*., 2006), *T. enlongata* shows septa as well (Hanada *et al*., 2002).

The results from Gram-staining of *T. jenkinsii, T. vanveenii* and *T. veronesis* were extremely variable. Among the cells growing on two different medias some cells were Gram-positive while others where Gram-negative, and in some cases cells were both (Blackall *et al*., 2000). *T. australiensis* and *T. japonica* were Gram-positive (Maszenan *et al*., 2000). Most of the *T. jenkinsii, T. vanveenii* and *T. veronesis* grown on one media were positively Neisser stained. Cells grown on another media were either evenly positively stained or contained positive granules indicating polyphosphate content (Blackall *et al*., 2000). Also *T. enlongata* was positively Neisser stained (Hanada *et al*., 2002). All six *Tetrasphaera* produced polyphosphate while only *T. jenkinsii, T. vanveenii* and *T. veronesis* were PHA positive (McKenzie *et al*., 2006).

T. australiensis and *T. japonica* can utilise acetate, propionate and glucose (Maszenan *et al*., 2000). *T. jenkinsii, T. vanveenii* or *T. veronensis* can utilise acetate, propionate, glucose, fructose, mannose, lactose, pyruvate, peptone and glycerol as sole carbon source (Blackall *et al*., 2000).

The temperature range supporting growth for *T. jenkinsii, T. vanveenii* or *T. veronensis* was between 15°C and 30°C. However, two of the strains were able to grow at 8°C, while none

were able to grow at 40°C (Blackall *et al*., 2000). *Tetrasphaera* is able to grow in the pH range between 6 and 8 (Masszenan *et al*., 2000).

When investigating the microflora in full-scale EBPR plants, it has been found that the number of *Tetrasphaera* exceeds the number of *Accumulibacter*, indicating the importance of *Tetrasphaera*. In well-working EBPR plants with nitrogen removal, up to 30% of the total bacterial biomass consists of *Tetrasphaera* (Nguyen *et al*., 2011). Mielczarek *et al*. (2013) investigated 28 Danish municipal wastewater treatment plants and found that on average 27% of all bacteria was *Tetrasphaera*.

7.6.3 Acinetobacter

The Gram-negative *Acinetobacter* has been isolated from EBPR activated sludge and was found able to store polyphosphate and take up acetate and store it as PHA (Fuhs and Chen, 1975). However, the ability of anaerobic simultaneous acetate uptake and phosphate release was not investigated. *Acinetobacter* was frequently found in EBPR processes and found in large numbers on aerobic agar plates inoculated with sludge from EBBR processes (Buchan, 1983; Deinema *et al*., 1985). Later on, the amount of *Acinetobacter* in the sludge was quantified and it was concluded that the amount of *Acinetobacter* was too small to explain the enhanced phosphorus removal (Cloete and Steyn, 1988; Wagner *et al*., 1994). *Acinetobacter* has also been found unable to perform the metabolism characterising EBPR organisms (Jenkins and Tandoi, 1991; Tandoi *et al*., 1998). Even if the proposed models for EBPR used on *Accumulibacter* don't fit *Acinetobacter*, the bacteria may still play a role in the phosphorus removal (Tandoi *et al*., 1998).

7.6.4 Candidatus Microthrix parvicella

Candidatus Microthrix parvicella is a filamentous bacterium in the genus *Actinobacteria* (Wang *et al*., 2014). It was first described in 1969 (Pasveer, 1969), but wrongly as *Escherichia coli*. The filaments of *Microthrix parvicella* are long and thin (diameter of 0.6-0.8 µm), have no branches and are unsheathed (Rossetti *et al*., 2005). The bacterium is Gram-positive (Andreasen and Nielsen, 1998) with possibly uneven colourisation (AnoxKaldnes, 2013, pp. 90). The filaments are Neisser-negative and contain Neisser-positive granules (AnoxKaldnes, 2013, pp. 90) which in some cases have been found to be polyphosphate granules (Wang *et al*., 2014). Wang *et al*. (2014) suggested that *Microthrix* possibly can be responsible for EBPR during sludge bulking since the phosphorus removal efficiency was not adversely affected even though there was a significant loss of *Accumulibacter*.

Microthrix parvicella can utilise long fatty acids and is able to take up nutrients during anaerobic, aerobic and anoxic conditions. The filaments are however not able to grow during anoxic conditions (AnoxKaldnes, 2013, pp. 91).

Microthrix parvicella is a common reason for bulking and foaming in municipal activated sludge wastewater treatment plants in Sweden. Additionally, around 50% of the Swedish municipal treatment plants are thought to have problems with the bacteria (AnoxKaldnes, 2013, pp. 89).

7.6.5 GAOs – Alphaproteobacteria and Candidatus Competibacter phosphatis

Alphaproteobacteria and *Competibacter* belong to the group of GAOs which are undesired competitors to the PAOs. Both GAOs have been found in increasing amounts in laboratory scale reactors with decreasing phosphorus removal activity (Panswad *et al*., 2003;

Lopez-Vazquez *et al*., 2009b; Ki *et al*., 2014). It should be noted that there is a wide range of GAOs where only a few of them are identified.

Alphaproteobacteria have tetrad morphotype (Oehmen *et al*., 2006b). *Competibacter* has large coccoidal cells which can be found both as single cells, as microcolonies, and in some cases as tetrads (Mielczarek *et al*., 2013).

Competibacter can grow in the temperature range from 10°C to 30°C. At temperatures below 10°C the metabolism of *Competibacter* is inhibited (Lopez-Vasquez *et al*., 2009a). Filipe *et al*., (2001c) found GAOs to be insensitive to a decrease in pH in the range from 6.5 to 7.5.

Competibacter can utilise both acetate and propionate. *Competibacter* enriched on only one of the carbon sources have a longer acclimation time than *Accumulibacter* when the carbon source is changed to the other (Oehmen *et al*., 2005c). *Alphaproteobacteria* is able to utilise propionate as carbon source (Oehmen *et al*., 2006b).

Saunders *et al*. (2003) found *Competibacter* in all six studied Australian full-scale plants in quantities between 1% and 12%. Also other GAOs were detected but not identified. Mielczarek *et al*. (2013) studied 28 Danish municipal wastewater treatment plants and found *Competibacter* in 11 of the plants and *Alphaproteobacteria* in 6 of the plants. However, the GAOs were only occasionally present in some of the plants and were consistent in only a few of the plants. The total population of GAOs in the plants were always lower than the population of the PAO *Accumulibacter*, which on average was found to represent 3.7% of all the bacteria.

8 Enhanced biological phosphorus removal in the MBBR process

8.1 Diffusion limitations

The diffusion is of importance for the intended moving bed biofilm reactor process for denitrifying phosphorus removal since the process depends on the penetration depth which depends on the concentrations of the involved substances (Falkentoft *et al*., 2001). The penetration depths are higher with higher concentrations in the bulk. The substances, i.e. phosphate, VFAs, ammonium, nitrate, and oxygen, have to diffuse into the biofilm from the bulk water to enable the removal process (Henze *et al*., 1997, pp. 143).

Depending on the concentrations of the substances and the thickness of the biofilm, different conditions can occur in layers in the biofilm and it is then called stratified. An example of this is when oxygen only penetrates the outer layers leaving the inner layers anaerobic (Falkentoft *et al*., 2001) or anoxic if nitrate is present (Helness, 2007, pp. 72). Nitrification and denitrifying phosphorus removal can be achieved simultaneously in a biofilm under aerobic conditions, see *Figure 8.1*. To achieve a biofilm where the inner layer consists of denitrifying phosphorus removing bacteria (DPB) and the outer layer consists of nitrifying bacteria, the conditions must be right (Helness and Ødegaard, 2001). This is possible if the oxygen only penetrates the biofilm to a certain depth and hence the deeper layers in the biofilm are anoxic (Helness and Ødegaard, 2001; Helness, 2007, pp. 72).

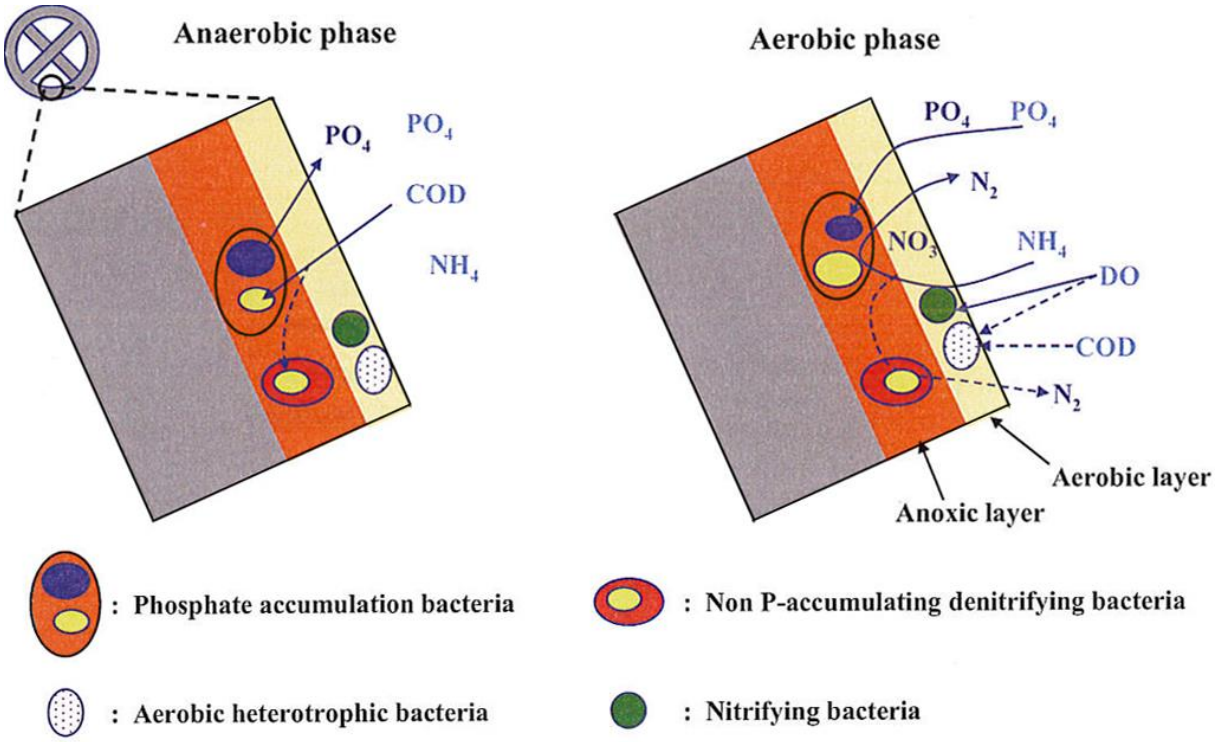

Figure 8.1. Illustration of idealised stratified biofilm with simultaneous nitrification-denitrification and phosphorus removal (Helness, 2007, pp. 72). Permission to reprint the figure was granted from the copyright holder Herman Helness.

Additionally, the DPB must out-compete other denitrifying bacteria by uptake of easily biodegradable COD in the anaerobic phase. Further, the phosphate accumulating bacteria need to grow in the deeper layers of the biofilm to ensure anoxic conditions and thereby to prevent aerobic phosphate uptake by PAO (Helness and Ødegaard, 2001).

The phosphorus removal depends on a net uptake of phosphorus due to biomass growth. It is therefore important that the biofilm can get thicker and still is not limited by diffusion of substrates in the biofilm (Falkentoft *et al*., 2001). However, Falkentoft *et al*. (2001) conducted a laboratory-scale anoxic batch experiment with phosphate concentrations up to 160 mg/l, which are very high compared to normal concentrations in municipal wastewater, and concluded that the biofilm never was fully penetrated with phosphate.

On carriers with thin biofilm, the phosphate content in the biofilm was highest close to the carrier, while carriers with thick biofilm (>100 µm) had highest phosphate content close to the biofilm surface, since the deeper layers had no access to phosphate due to diffusion limitation. The opposite result with the thinner biofilm was most probably due to competition from fast-growing non-PAO heterotrophs (Falkentoft *et al*., 2001).

8.2 The process

In an activated sludge process, the sludge is exposed to anaerobic and aerobic or anoxic conditions by circulation of the sludge to different zones in the plant. However when an MBBR process is desired, the conditions must be altered in the same reactor (Helness and Ødegaard, 1999) or the carriers must be moved between several reactors with different conditions (Helness, 2007, pp. 50).

It may be that biological phosphorus and nitrogen removal in a biofilm process is less vulnerable than the corresponding activated sludge process due to that the bacteria are stuck in the biofilm on the carriers while it can be washed out from an activated sludge process (Helness and Ødegaard, 2001). Furthermore, the problem with secondary release of phosphate in the clarifier is minor in biofilm processes compared to activated sludge processes since less sludge is separated (Falkentoft *et al*., 2001).

It is of importance that the easily degradable COD in the influent is taken up by the PAOs in the anaerobic phase to prevent and avoid competition from organisms favoured by available COD in the aerobic phase. However, the influent COD needs to be high enough to give enough PHA for phosphate uptake and biomass growth (Helness and Ødegaard, 1999). The amount of biomass in the system regulates the minimum total COD-loading rate needed (Helness and Ødegaard, 2001). To ensure that the feed contains sufficient COD in terms of getting a good COD/P ratio, carbon source can be added to the feed. Helness and Ødegaard (2001) found that the minimum organic loading rate, in form of soluble COD (sCOD), for the whole system was about 0.5 kg sCOD/(m^3·d). They also found that the anaerobic organic loading rate should be kept below 2.5 kg sCOD/(m^3·d) to maintain stable performance.

The total COD consumption for aerobic phosphate uptake by PAOs and separate denitrification by denitrifying bacteria not able to accumulate phosphate is larger than simultaneous denitrification and phosphate uptake by denitrifying phosphorus-removing bacteria in anoxic conditions. This can be turned to an advantage in a process where both nitrogen and phosphorus should be removed by executing simultaneous (nitrification-) denitrification and anoxic phosphate uptake (Helness and Ødegaard, 2001). Other advantages

with anoxic conditions instead of aerobic conditions are that the biofilm may detach easily from the carriers under high DO operation and that the process is not dependent on dissolved oxygen mass transfer (Chiou and Yang, 2008).

Out of seven studies found about EBPR in MBBR processes only one focused on denitrifying phosphate removal by altering anaerobic and anoxic conditions (Brandt *et al*., 2002).

Brandt *et al*. (2002) studied denitrifying phosphorus removal in a laboratory-scale sequencing batch moving bed biofilm reactor (SBMBBR) process with altering anaerobic and anoxic conditions. Two different synthetic wastewaters were used corresponding to a primary treated wastewater and a nitrified wastewater. The primary treated wastewater contained mainly carbon source used in the anaerobic phase and the nitrified wastewater contained mainly phosphate and nitrate used in the anoxic phase. The primary treated wastewater contained 300-500 mg acetate/l, 10-15 mg PO_4-P/l and 20-30 mg NO_3-N/l while the nitrified wastewater contained 30-50 mg PO_4-P/l and 50-80 mg NO_3-N/l. The measured rates were related to the volume of the carriers instead of the carrier surface. The anaerobic acetate uptake was 11.6 kg/($m^3_{carrier}$·d), the anoxic denitrification rate was 0.48 kg NO_3-N//($m^3_{carrier}$·d) and the phosphate removal was 0.09 kg PO_4-P/($m^3_{carrier}$·d). Later a semi-technical scale setup partially operated as a batch process was used to study nutrient removal from real municipal wastewater; no bio-P activity was achieved, however.

The other six studies about EBPR in MBBR processes focused on denitrifying phosphorus removal with simultaneous nitrification-denitrification and phosphorus removal in a stratified biofilm (Pastorelli *et al*., 1999; Helness and Ødegaard, 1999 and 2001; Helness, 2007; Restrepo *et al*., 2009; Vallet *et al*., 2009). More about stratified biofilm can be read in chapter *8.1 Diffusion limitations*. The intention with the present study was not to have simultaneous nitrification-denitrification and phosphorus removal in a stratified biofilm using altering anaerobic and aerated aerobic/anoxic conditions, but to have denitrifying phosphorus removal in altering anaerobic and anoxic conditions. However, no literature studying that subject has been found. Still, the results from the studies with stratified biofilm are of interest.

In a stratified biofilm, nitrification and denitrifying phosphorus removal is achieved simultaneously under aerobic conditions. This is possible since the oxygen only penetrates the biofilm to a certain depth and hence the deeper layers in the biofilm are anoxic (Helness and Ødegaard, 2001; Helness, 2007, pp. 72). To achieve a biofilm where the inner layer consists of denitrifying phosphorus removing bacteria (DPB) and the outer layer consists of nitrifying bacteria, the conditions must be right, see chapter *8.1 Diffusion limitations*.

Nitrification can occur in the aerobic phase since the total COD-loading rate is low. To ensure complete nitrification and avoid accumulation of nitrite, it is important that the duration of the aerobic phase is long enough (Helness and Ødegaard, 1999). The nitrate formed during the nitrification, can cause problems in the anaerobic phase (Henze *et al*. 1997, pp. 99). In a study by Helness and Ødegaard (2001) it was found that at low nitrite accumulation rates, the phosphate removal rate in the aerated phase was close to the maximum rate (<0.3 kg/(m^3·d)), while the phosphate removal rate in the aerated phase was lower (0.05 kg/(m^3·d)) when nitrite accumulation occurred. The latter was probably due to nitrogen inhibition of the phosphate uptake.

Pastorelli *et al*. (1999) studied denitrifying phosphorus removal in an SBMBBR process and concluded that the total nitrogen and phosphorus limit values set by EU, of that time, for

discharge into sensitive receiving waters could be met. To achieve this, only two stages were needed, one anaerobic and one aerobic, with addition of external carbon source in the form of acetic acid. Real wastewater was used and on average one litre contained 338 mg sCOD, 51.5 mg NH_4-N, 0.2 mg NO_3-N, and 15.6 mg PO_4-P. External carbon source in the form of about 80 mg COD/l as acetic acid was added to the water. In the aerobic phase, simultaneous nitrification and denitrification was performed in a stratified biofilm. The denitrifying phosphorus removal was performed by denitrifying PAOs growing in an inner anoxic layer on the carriers, using the carbon source stored under anaerobic conditions. The anaerobic phosphate release rate was 2.14±0.22 g P/(m^2·d) at a phosphate loading rate of 0.54±0.24 when external carbon source was used. The phosphorus content in the biomass was up to 7.5% (as g P/g TSS) or 9.0% (as g P/g VSS) (Pastorelli *et al*., 1999). The proportionality coefficient between phosphorus uptake and phosphorus release found by Patorelli *et al*., was 1.055 g P taken up/g P released supported by the ratio between aerobic uptake and anaerobic release found by Helness and Ødegaard (2001) which was 1.11 mg P/mg P released.

Helness and Ødegaard (1999) studied biological phosphorus removal in a laboratory-scale SBMBBR process. A synthetic wastewater was used and the total loading rates were varied in the range 0.3 to 1.2 kg COD/(m^3·d) as acetate and 0.012 to 0.131 kg PO_4-P/(m^3·d), the water contained ammonium as well. The highest phosphate removal rate was just above 0.2 kg P/(m^3·d) while most of the aerobic phosphate removal rates were below 0.1 kg P/(m^3·d). The phosphate removal was also presented as an average net phosphate removal of 7.7 mg PO_4-P/l (from experiments with a phosphate release higher than 30 mg PO_4-P/l). The aerobic phosphate removal efficiency was found to be strongly dependent on the operating conditions. The highest phosphate removal efficiency was 98% and was achieved at an acetate loading rate of 0.9 kg COD/(m^3·d). The COD removal efficiency was around 84% and the ammonium removal just below 80% (Helness and Ødegaard, 1999 and 2001). Helness and Ødegaard (2001) found that it was possible that nitrate was used as an electron acceptor for phosphorus removal by bacteria in the aerobic phase.

Helness (2007) studied the EBPR process in an SBMBBR process. A laboratory-scale reactor was used to study nutrient removal from synthetic wastewater. The total organic loading rate was varied in the range 1.0-4.5 g sCOD/(m^2·d) in form the of acetate, and the total phosphate loading rate was varied in the range 0.04-0.49 g PO_4-P/(m^2·d). The ammonium loading rate was in the range 0.11-1.1 g NH_4-N/(m^2·d) with an average concentration of 21.3 mg NH_4-N/l in the start of a cycle. The average net phosphate removal was 9 mg PO_4-P/l at the total organic loading rate of 2.6-2.9 g COD/(m^2·d). The ratio between removed COD and removed phosphate was 36 mg COD/mg PO_4-P. More than 85% of the sCOD was taken up in the anaerobic phase and the total nitrogen removal efficiency was 70% to 90%.

Later, a pilot-scale SBMBBR plant with altering anaerobic and aerobic conditions was studied. The treated wastewater was municipal wastewater pretreated in a coarse media filter. Additional carbon source in the form of acetate was added to the wastewater and in some experiments phosphate and ammonium were added as well. The influent after the pretreatment contained 4.2 mg PO_4-P/l, 18 mg NH_4-N/l, 104 mg sCOD/l and 205 mg COD/l. The treated water contained below 10 mg soluble N/l and below 0.3 mg PO_4-P/l, meaning that the treatment requirements were met. In both the laboratory-scale process and the pilot-scale process, simultaneous nitrification and denitrification occurred along with the phosphorus removal (Helness, 2007).

Restrepo *et al*. (2009) studied biological phosphorus removal and denitrification of a fish farm effluent in a pilot-scale SBMBBR. Each cycle involved an anaerobic phase followed by an aerated aerobic/anoxic phase. The reactor was emptied between the phases and the water used in the anaerobic phase was reused and stored in a stock tank during the aerobic/anoxic phase where effluent from the fish farm was treated. Acetate was added to the water reused in the anaerobic phase. The effluent from the fish farm used as influent to the process typically contained per one litre 24.7 mg NO_3-N, 1.6 mg PO_4-P and 27 mg sCOD, and was spiked with phosphate to receive a final concentration of 10 mg PO_4-P/l. The process removed up to 75% of the phosphate (7.5 mg P/l of 10 mg P/l) and up to 40% of the nitrate (8.5 mg N/l of 21 mg N/l) from the fish farm effluent. The aerobic/anoxic phase was aerated and the denitrification was suggested to occur in a deeper anoxic layer in the biofilm. In both the experiments by Restrepo *et al*. (2009) and by Vallet *et al*. (2009), the intention was to take advantage of how the PAOs work, to achieve phosphorus removal and gain water with increased phosphate concentration. The PAOs took up the phosphate from the wastewater in the aerobic phase and later released it in the reused water in the anaerobic phase. The phosphate from the wastewater was in this way transferred to the stock tank where it was accumulated, instead of removing biomass with high polyphosphate content like in activated sludge processes.

Vallet *et al*. (2009) studied denitrifying phosphorus removal from seawater and synthetic wastewater made from freshwater. The pilot-scale SBMBBR used was similar to the experimental setup used by Restrepo *et al*. (2009). The process was not suitable for treating seawater. The freshwater influent concentrations were 13.7±0.6 mg PO_4-P/l and 32±3 mg NO_3-N /l and after the treatment, the concentrations in the effluent varied from 9.5 to 11 mg PO_4-P/l and 24.9 to 30.6 mg NO_3-N/l. The phosphate removal rate was around 20% and was much lower than the removal rate of 75% achieved by Restrepo *et al*. (2009).

8.3 Process configurations

There are different possible configurations for biological denitrifying phosphorus removal with a moving bed biofilm process. To enable the biomass exposure to altering anaerobic and aerobic (or anoxic) conditions, the process can be either continuous or batch-wise. In a continuous process, one solution could be to pump (air-lift pump) the wastewater and carriers from one compartment to another compartment where different conditions occur. However, the water from an aerobic compartment is expected to carry over oxygen to the next compartment, causing problems if the next compartment is supposed to be anaerobic or anoxic (Helness, 2007, pp. 50). Another solution could be to move the water and carriers hydraulically in a single reactor containing different zones secluded by walls with openings (Helness, 2007, pp. 50). For this, Helness (2007) suggested three possible reactors, two different vertical loop reactors and one horizontal loop reactor, see *Figure 8.2*.

In the vertical loop reactors, the water and carriers would be moved in a loop over and under the secluding walls, see reactor A and B in side view in *Figure 8.2*. However, it is expected to be problematic to achieve in a satisfactory way.

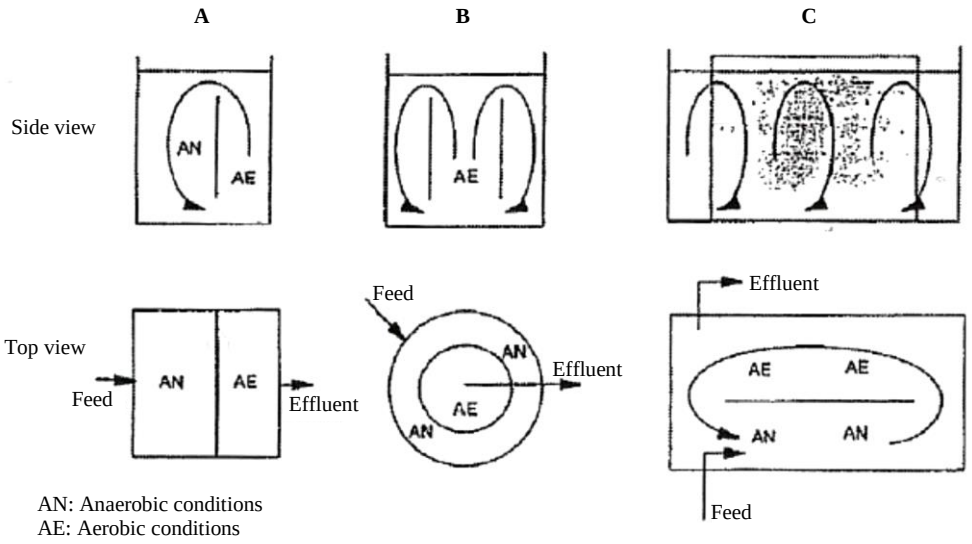

Figure 8.2. Three different suggested process configurations for continuous EBPR in the MBBR process. Reactor A and B are vertical loop reactors while C is a horizontal loop reactor. The top row shows the three different processes from the side view, while the bottom row shows the processes from the top view (Helness, 2007, pp. 51). Permission to reprint the figure was granted from the copyright holder Herman Helness.

The zones in the horizontal reactor are also secluded by walls which the water circulates around, see reactor C in *Figure 8.2*. This process could be based on a similar principle as carrousel activated sludge plants, with aerators and mixers at appropriate intervals. In this way, both good vertical mixing and horizontal movement of the water and carriers might be obtained (Helness, 2007, pp. 50).

Several studies have been conducted using laboratory-scale or pilot-scale sequencing batch reactors (SBR) (Pastorelli *et al*., 1999; Helness and Ødegaard, 1999 and 2001; Helness, 2007; Restrepo *et al*., 2009). A single reactor with carriers is used and the conditions are altered in sequences. Hence, a sequencing batch process would need a more sophisticated control strategy than a moving bed carousel (Helness, 2007, pp. 51). Initially, the reactor is filled with water and the condition in the reactor is anaerobic. After a certain time, the condition in the reactor is changed to aerobic and kept for a certain time. Thereafter, the reactor is emptied and the cycle can be run again (Helness, 2007). Another alternative is to use two different waters, one phosphorus rich wastewater and one containing carbon source. After the anaerobic phase with the carbon containing water is finished, the reactor is emptied, refilled with phosphorus rich wastewater and the aerobic or anoxic phase is initiated. When the phase is ended, the reactor is emptied and refilled with carbon containing water, and the cycle is restarted. This is recommended if the phosphorus containing wastewater also contains nitrate, to ensure no nitrate is present in the reactor during the anaerobic phase, since the condition then would be anoxic instead of anaerobic (Restrepo *et al*., 2009). However, the process becomes more technically difficult and it might not be suitable for full-scale processes.

In a wastewater treatment plant, generally, the loading rate and the influent concentrations change over time. Preferably, it would be nice to be able to control the duration of the anaerobic and aerobic or anoxic phases based on online measurements of COD and phosphate (Helness and Ødegaard, 1999).

9 Hydrolysis for production of carbon source

The hydrolysis process is important in biological treatment plants. In the process, larger organic molecules are converted to smaller easily degradable molecules (Henze *et al*., 1997, pp. 63) that bacteria are able to take up and degrade (Morgenroth *et al*., 2002). Both particulate and dissolved solids may be degraded during hydrolysis (Henze *et al*., 1997, pp. 63). According to Morgenroth *et al*. (2002), the hydrolysis process can be divided into two parts. The first part is "hydrolysis of primary substrate where organic substrate present in the original wastewater is broken down", and the second part is "hydrolysis of secondary substrates that refers to the breakdown of substrate that has been produced by the bacteria (e.g. hydrolysis of internal storage products, of substances released by the bacteria during normal metabolism, or of particles produced during decay of bacteria)". The hydrolysis process is often the rate-limiting step in biological wastewater processes since the process is slow compared to biological growth processes (Henze *et al*., 1997, pp. 63). In external hydrolysis, the hydrolysis takes place in the bulk water by extracellular enzymes released by the biomass (Henze *et al*., 1997, pp. 183).

In wastewater treatment plants with EBPR, the aim with the hydrolysis process is to improve the VFA production and the EBPR performance (Tykesson, 2005, pp. 44). The hydrolysis process can be performed in the primary clarifier with primary sludge, in the anaerobic reactor in an activated sludge process, or in a separate tank with return sludge. In the anaerobic reactor in an activated sludge process, the hydrolysis occurs at the same time as the EBPR process by having a longer sludge retention time and the produced VFA is consumed immediately (Tykesson, 2005, pp. 45). In the return sludge hydrolysis process, a part of or the whole return sludge stream can be hydrolysed in an anaerobic tank and then led back to the activated sludge process (Jönsson and Jansen, 2006).

To fit these hydrolysis processes to an MBBR process, the sludge from the hydrolysis process might need to be settled to enable that only the clear phase is introduced to the EBPR process. This is of interest since it is only the VFA from the hydrolysis that is desired and not the microorganisms in the sludge. However, it might not be problem free to settle the sludge.

9.1 Hydrolysis of MBBR sludge

In an earlier study by the author of this report, sludge from wastewater treatment plants with an MBBR process was hydrolysed to see how much VFA that could be produced. The hydrolysis process was conducted in batches for at least eight days. The intention was to investigate if the produced VFA could cover the carbon source need in biological phosphorus removal.

The initial sludge concentration was 10 g sludge/l and yielded a final VFA concentration of between 1000 mg COD/l and 1600 mg COD/l. After hydrolysing for 24 h, the VFA concentration was between 301 mg COD/l and 470 mg COD/l. It was found that the produced VFA consisted mainly of acetic acid and propionic acid. Acetic acid represented approximately 60% of the produced acetic acid and propionic acid.

The VFA production from MBBR sludges were found to be in the same range as the VFA production from activated sludge from plants with high loading rates and were higher than the VFA production from activated sludge from plants with low loading rates. However, much

less sludge is available in MBBR processes compared to activated sludge processes as the sludge is not recirculated. The amount of sludge from an MBBR process might still be enough to produce the desired amount of VFA.

10 Treatment requirements

The EU-directive (91/271/EEC) sets the limit for total phosphorus and total nitrogen in effluent wastewaters to sensitive receiving waters. The highest allowed total nitrogen (sum of organic nitrogen, nitrogen in ammonia, nitrogen in nitrate and nitrogen in nitrite) is 10 to 15 mg/l, depending on the size of the plant, or a minimum reduction between 70% and 80%. The corresponding value for total phosphorus (sum of dissolved inorganic orthophosphate, dissolved inorganic polyphosphate, dissolved organic phosphorus and suspended organic phosphorus) concentration is 1 to 2 mg P/l, depending on the size of the wastewater treatment plant, or at least 80% reduction. In Sweden, the limits are even lower, and most plants have a maximum limit between 0.3 and 0.5 mg/l total phosphorus, depending on the sensitivity of the receiving water. The value needs to be fulfilled as a monthly or quarterly average (Tykesson, 2005).

11 Materials and methods

The intention was that the studied process should simulate treatment of water from an already existing wastewater treatment plant with BOD removal and nitrification. Hence the main influent to the process contained mainly phosphate and nitrate. A feed corresponding to a stream from a hydrolysis reactor with carbon source was used as well.

The used operational mode was a combination between continuous mode and sequencing batch mode. The influents and effluents were continuous while the conditions in the reactors altered in sequences in a cycle, see *Figure 11.1*. Four reactors connected in series, where also the fourth and the first reactor were connected, were used. Two influents, the hydrolysis influent and the main influent, were moved between the four reactors against the flow direction of the wastewater. The wastewater was pumped continuously between the reactors and the minimum levels in the reactors were controlled by overflow. The reactor furthest from the reactor with the influent containing carbon source had the final effluent. The final effluent was moved along with the influents. The condition in the reactors altered between anaerobic, anoxic and aerobic according to four phases in each sequence. Each sequence lasted for 1 h.

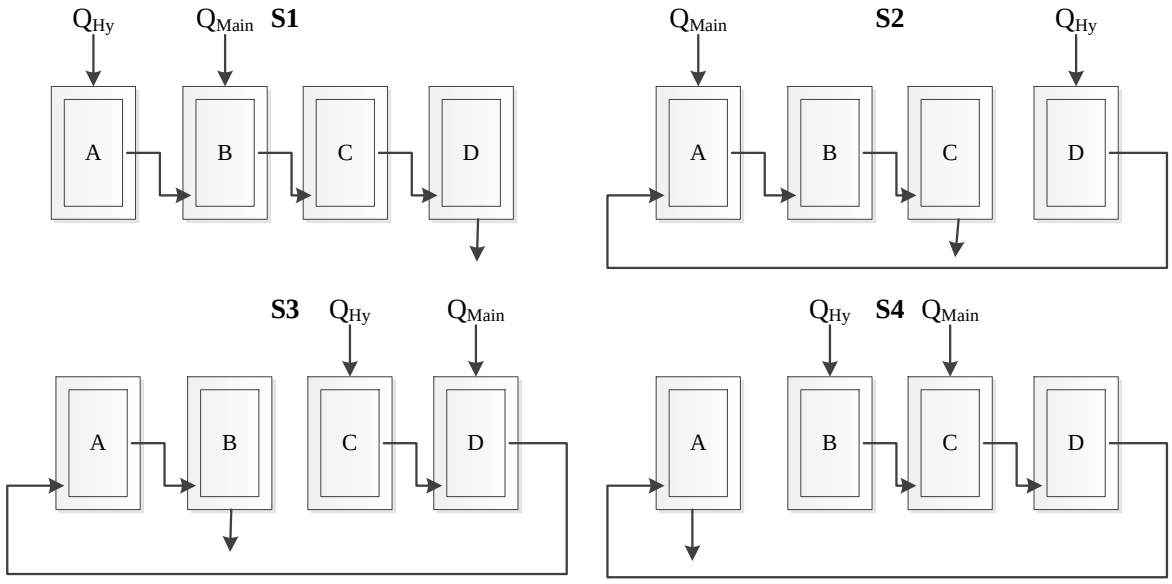

Figure 11.1. Schematic figure of the experimental setup during the four sequences (S1-S4) in the cycle from start-up to day 31. The influents and the effluent moved against the flow direction of the wastewater according to the sequences.

The cycle consisted of four sequences (S1-S4) where each reactor (R1-R4) was in a different phase (P1-P4) in the sequence, see *Table 11.1*. The conditions in the phases P1 to P4 were different.

Table 11.1. The phases in the sequences in the cycle during the whole study.

Sequence	Reactor A		Reactor B		Reactor C		Reactor D	
	Phase name	Reactor name	Phase name	Reactor name	Phase name	Reactor name	Phase name	Reactor name
S1	P1	R1	P2	R2	P3	R3	P4	R4
S2	P2	R2	P3	R3	P4	R4	P1	R1
S3	P3	R3	P4	R4	P1	R1	P2	R2
S4	P4	R4	P1	R1	P2	R2	P3	R3

In the beginning of the project, the first sequence was as following. Reactor A was in phase P1 meaning that the reactor was anaerobic and that the hydrolysis influent was pumped into the reactor during the whole phase, see *Figure 11.1* and *Table 11.2*. The effluent from reactor A was used as an influent to reactor B which was in phase P2 and had an influent of the main feed. The effluent from reactor B was influent to reactor C which was in phase P3. The effluent from reactor C was influent to reactor D, which was in phase P4, and the effluent from reactor D was the effluent from the whole system. Shortly, A was in P1, B was in P2, C was in P3 and D was in P4, where P1 means anaerobic conditions, P2 and P3 means anoxic conditions and P4 means aerobic conditions. After each sequence, the feed influents and the final effluent were moved one step against the flow direction and the reactor that previously had the final effluent now had the hydrolysis influent, see *Figure 11.1*. In sequence S2, reactor A was in P2, B was in P3, C was in P4 and D was in P1.

Table 11.2. The conditions in the different phases (P1-P4), the type of influent, and the destination for the effluent, from start-up to day 14.

Phase	Condition	Influent	Effluent
P1	Anaerobic	Hydrolysis	R2
P2	Anoxic	R1+Main	R3
P3	Anoxic	R2	R4
P4	Aerobic	R3	Final effluent

The water was supposed to flow between the reactors by gravity. To facilitate the flow, the reactor with the influent containing carbon was raised a few centimetres with a lift.

Several changes were done making the experimental setup differ from the original concept.

- Day 6 to 16: The working volumes in the reactors were increased from 0.65 l to 1 l using four external level regulators, consisting of Y-shaped tubing connectors. The regulators were also intended to remove air bubbles from the tubing and facilitate the flow between the reactors.
- Day 16 and onwards: Pumps were installed and used to pump the water instead of trying to get it to flow by gravity. The external level regulators were removed and the working volumes were again 0.65 l.

- Nitrate in the feed was partly and later fully exchanged with ammonium. Day 0 to 20: Nitrate was used as the sole nitrogen source. Day 20 to 28: Ammonium was used as the sole nitrogen source. Day 28 to 45: Both nitrate and ammonium was used as nitrogen source. Day 45 and onwards: Ammonium was used as sole nitrogen source.
- The earlier unaerated anoxic phases were changed to aerobic aerated phases, see appendix *1 Phases in the sequence*.
- Day 31: The effluent from the reactor in the anaerobic phase was pumped out of the system instead of being used as influent to the next reactor.
- Day 31: Yeast extract and peptone was added to the hydrolysis influent.
- Day 34: The hydrolysis feed was changed to be rapidly pumped in during the first ten minutes of the anaerobic phase instead of during the whole phase.
- Day 45: After the anaerobic phase was ended, the reactor was rinsed with tap water for ten minutes. The rinsing water was led to waste. Then the influent with ammonium and phosphate was pumped in for 50 minutes.
- Day 52: The temperature in the reactors was decreased from around 15°C to around 10°C.
- Day 63: The counter ions calcium and magnesium were added in higher amounts to the main influents.

After all these changes, the phases for one reactor during all four sequences were as following, see *Table 11.3* and *Figure 11.2*.

Table 11.3. The conditions in the different phases (P1-P4), the type of influent, and the destination for the effluent, from day 45 and onwards. In P2, the reactor was rinsed before the main influent is pumped in. Additionally, the effluent during the rinsing period was pumped out of the system.

Phase	Condition	Influent	Effluent
P1	Anaerobic	Hydrolysis	Waste
P2	Aerobic	Rinsing+Main	Waste+R3
P3	Aerobic	R2	R4
P4	Deox	R3	Final

The reactor R1 was unaerated and the hydrolysis influent was pumped in for ten minutes while there was no effluent. When the influent stopped, the outlet to the waste was opened. After additionally 50 minutes the first phase, P1, ended and phase P2 began with reactor R2 being rinsed with tap water for ten minutes, see *Figure 11.3*. The rinsing water was led to waste. Then the aeration started and the main influent was pumped in for 50 minutes and the effluent was pumped to the next reactor. When the second phase ended and the third phase P3 was started, the reactor remained aerated for 1h. During the last 50 minutes the effluent was pumped to the next reactor while the reactor received an influent from the prior reactor R2. In the fourth and final phase, reactor R4 was unaerated, received influent from the prior reactor, R3, and the effluent was pumped out of the system. All four reactors were operated according to the same strategy.

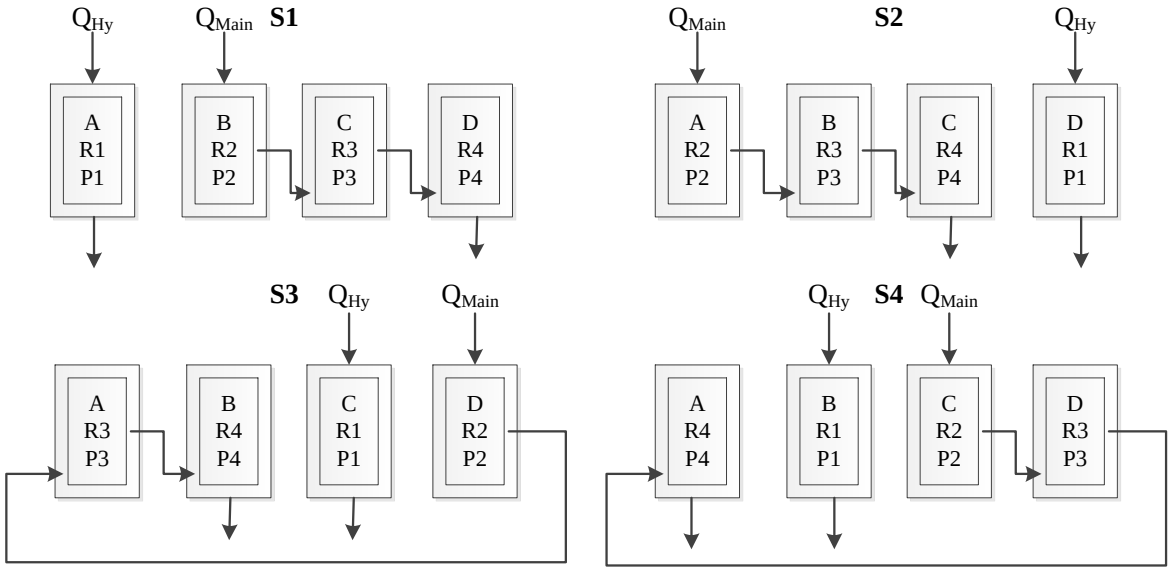

Figure 11.2. A schematic figure of the experimental setup during the four sequences (S1-S4) in the cycle, after day 45. The influents and the effluent moved against the flow direction of the wastewater according to the sequences.

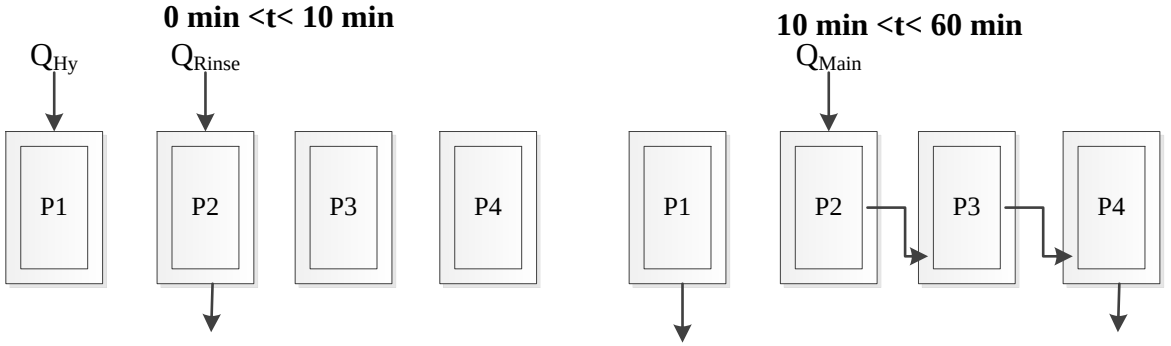

Figure 11.3. A schematic figure of the experimental setup during the first 10 minutes and last 50 minutes in one of the sequences after day 45. During the first 10 minutes in phase P1, the hydrolysis influent was rapidly pumped into the anaerobic reactor R1. Simultaneously, the aerobic reactor R2 was rinsed with tap water. When the hydrolysis influent stopped, the outlet on the anaerobic reactor R1 was opened. During the last 50 minutes, the main influent was pumped into the aerobic reactor R2.

The aerobic conditions in the reactors were maintained by aerating with air administrated via a tube submerged in the reactor. Pumps developed for aerating aquariums were used and the air rate was tuned with rotameters and kept at approximately 0.3 l/min. Air stones were used to achieve smaller air bubbles. The dissolved oxygen (DO) concentration was measured in connection with sampling and the air flow rate was adjusted if needed. Anaerobic condition was achieved by purging nitrogen gas through the water at a flow rate of approximately 0.4 l/min. Nitrogen gas was purged in all reactors, even the aerated reactors.

pH was measured but not directly controlled. During a period of two weeks, with start at day 37, the pH in the main feed was adjusted with 2 M H_2SO_4 to a final pH of just above 6.

Jacketed glass reactors with a volume of 1400 ml and a working volume of 650 ml were used, see Figure 11.4. The temperature was maintained with a water bath which pumped cool water through the reactor jackets. The jackets were connected in series and therefore the temperatures in the reactors were not exactly the same. The temperature of the water bath was controlled. The desired temperature was 15°C, but was later changed to 10°C. The temperatures in the reactors were 13.4°C to 16.4°C and later 9.9°C to 11.5°C. The temperature in reactor R2 was occasionally above 19°C during the rinsing period since the rinsing water was stored in ambient temperature.

Mechanical mixing was obtained by magnetic stirrers and the setting was close to 3, the rpm was unknown. In the beginning of the project, ordinary cylindrical magnetic stirring beads were used. These were later exchanged to magnetic stirring bars shaped as circles with magnets as crossheads on both sides.

Figure 11.4. A photo of the experimental setup as it looked from day 45 and onwards. The equipment consisted of: 1. Jacketed glass reactors. 2. Magnetic stirrers. 3. Peristaltic pumps to pump the two feeds and the rinsing water. 4. A peristaltic pump to pump the water between the reactors. 5. Magnetic valves. 6. Rotameters to control the flow rate of the nitrogen gas and the aeration rate. 7. A water bath. The programmable logic controller (PLC) used to control the setup is not shown in the figure.

The volume feed flow rates were adjusted to achieve the desired hydraulic retention times (HRTs) in the reactors. The desired HRTs in reactor R2, R3 and R4 were 2 h, giving a total volumetric flow rate of 325 ml/h for a reactor with a working volume of 0.65 L. Before day 48, when the effluent from the anaerobic reactor still was used as one of the influents to reactor 2, the hydrolysis influent was 10% of the total flow rate. This meant that the main influent flow rate was 292 ml/h and the hydrolysis stream 33 ml/h. After the change where the effluent from the anaerobic reactor was led as a final effluent instead of an influent to next

reactor, the main influent flow rate was 270 ml/50 min to keep the HRT at 2 h. The hydrolysis influent was set to 33 ml/10 min.

The influents, rinsing water and effluents were pumped with peristaltic pumps. The magnetic valves, the peristaltic pumps and the air pumps were controlled with a programmable logic controller (PLC).

11.1 Carriers

The used carrier was Z-400 which is a carrier shaped as a saddle made of recycled high-density polyethylene (HDPE), see *Figure 11.5*. It stands out from other carriers since it is designed to enable control of the thickness of the biofilm. The walls of the grid on the carriers are 400 µm high and prevent the biofilm from getting thicker. The exposed biofilm area on the carrier, which is the total bottom area of the grid cells, is 12.77 cm^2/carrier (Piculell *et al.*, 2015). The density of the carriers is 0.95 to 0.96 g/cm^3.

Figure 11.5. The saddle-shaped carrier Z-400 used in the experiment.

Different number of carriers was tried during the project. The goal was to have the highest possible number of carriers while still having satisfying mixing. Most of the time, from day 28 and onwards, 100 carriers in 0.65 l was used. Between start-up and day 6 and later between day 16 and 28, the number of carriers used in each reactor was 125 carriers in 0.65 l. For a short period of time, day 6 to 16, the working volume in the reactor was increased and the number of carriers in each reactor was 180 carriers in 1 l.

The carriers were collected at Ulricehamn municipal wastewater treatment plant (WWTP) and were already covered in biofilm. It was thought that the growth of the PAOs would be facilitated it there already was biofilm on the carriers. Ulricehamn WWTP consists of a pre-denitrification step followed by a carbon removal step, two nitrification steps and finally a deoxidation step. There is recirculation from the nitrification steps to the denitrification step. All steps are MBBR processes except for the deoxidation step which is intended to remove oxygen from the water. The carriers were taken from the first nitrification step, and had only been used in the plant for four months. Furthermore, the biomass was thought to mostly contain heterotrophs. After the carriers were withdrawn from the plant, they were stored in refrigerator for 3 days, whereafter the wastewater was replaced with tap water. While waiting

for the experimental setup to be finished, the carriers were pretreated. The carriers were moved to another container, placed in ambient temperature and stirred with a magnetic stirrer. An air pump connected to a timer was used to aerate the carriers in intervals, 1 h without aeration and then 2 h with aeration. Acetic acid and propionic acid were added a few times in the beginning of anaerobic phases to enhance the growth of PAOs. The pretreatment of the carriers lasted for 15 days before the carriers were introduced to the process.

11.2 Feed characteristics and preparation

Two different feeds were used, a main feed and a hydrolysis feed. The main feed was a synthetic wastewater containing phosphorus, and ammonium and/or nitrate to be removed along with trace elements. The hydrolysis feed represented the particle free part of hydrolysed MBBR return sludge and was synthetic. It contained VFAs in form of acetic acid and propionic acid according to the fractions found in a previously made hydrolysis project (60ww% acetic acid and 40ww% propionic acid as COD). The solutions were stored in a refrigerator after preparation and during use. The different composition of the feeds during the project can be seen in appendix *3 Feed composition*.

The hydrolysis feed was prepared by mixing $CH_3COONa \cdot 3H_2O$ (sodium acetate trihydrate), C_2H_5COOH (propionic acid), yeast extract and peptone with tap water in a 1 l container. The solution was put on a magnetic stirrer for a couple of minutes for the yeast extract and peptone to dissolve. The solution was then poured into a 5 l container and filled with tap water to 5 l.

In the end, one litre of the hydrolysis feed contained 2.54 g sodium acetate, 0.53 ml propionic acid, corresponding to 2000 mg sCOD, 0.1 g peptone and 0.1 g yeast extract.

The main feed was prepared by mixing KH_2PO_4 (potassium dihydrogen phosphate), K_2HPO_4 (dipotassium hydrogen phosphate), NH_4Cl (ammonium chloride) and during periods $NaNO_3$ (sodium nitrate) with tap water in a 1 l container. In the end $CaCl_2 \cdot 2H_2O$ (calcium chloride dihydrate) and $MgSO_4 \cdot 7H_2O$ (magnesium sulphate heptahydrate) were added as well. The solution was put on a magnetic stirrer for a couple of minutes for the salts to dissolve. The solution was then poured into a 27 l container and filled to 5 l with tap water. The trace element solution was then added and the container was filled with tap water to 27 l. On a few occasions, between day 37 and day 49, the pH was adjusted by adding 2M H_2SO_4 until the desired pH was achieved.

The composition of the main feed varied throughout the project. In the end, one litre of the main feed contained 0.044 g KH_2PO_4, 0.056 g K_2HPO_4, 0.076 g NH_4Cl, corresponding to 20 mg PO_4-P and 20 mg NH_4-N, 0.048 g $CaCl_2 \cdot 2H_2O$, 0.160 g $MgSO_4 \cdot 7H_2O$ and 2 ml of a trace element solution.

One litre of the trace element solution contained 0.36 g Mg (4.8 g $MgSO_4 \cdot 7H_2O$), 1.58 g Ca (5.8 g $CaCl_2 \cdot 2H_2O$), 0.54 g Mn (1.6 g $MnCl_2 \cdot 2H_2O$), 0.12 g Co (0.48 g $CoCl_2 \cdot 6H_2O$), 0.059 g Ni (0.24 g $NiCl_2 \cdot 6H_2O$), 0.12 g Zn (0.26 g $ZnCl_2$), 0.025 g Cu (0.1 g $CuSO_4 \cdot 5H_2O$), 0.40 g Fe (1.44 g $FeCl_2 \cdot 4H_2O$), 0.00009 g B (0.00052 g BH_3O_3), 0.00087 g Mo (0.0022 g $Na_2MoO_4 \cdot 2H_2O$), 0.00034 g Se (0.00114 g $Na_2SeO_3 \cdot 5H2O$), and 0.00076 g W (0.0014 g $Na_3WO_3 \cdot 2H_2O$).

The influent used to rinse the reactors after the anaerobic phase consisted of tap water, and was kept at ambient temperature.

11.3 Sampling and sample pretreatment

A syringe extended with a piece of tubing was used to collect samples from the reactors. The syringe and tubing were rinsed three times with water from the reactor before the sample was taken. The reactors were stirred during sampling. The sampling occurred in the beginning of a sequence, 10 minutes and 45 minutes after a sequence started. The samples were taken from all reactors during the same sequence. The sampling schedule was intended to be "moving" meaning that a sample was taken from a specific reactor in the same phase every fourth sampling. However, this was not fully followed.

Samples for analysis of sCOD, orthophosphate, nitrate and ammonium were taken daily or at least weakly from all four reactors.

The samples were filtered through a MGA glass microfibre filter directly after sampling. Samples not analysed directly after sampling were stored in a refrigerator until the analysis was executed.

Neisser staining was used to visualise polyphosphate granules in the microorganisms. To get a sample for Neisser staining, biomass from two to three carriers was scraped off and rinsed from the carriers with minimal amounts of water. The carriers were then returned to the reactor to keep the same number of carriers. The carriers were taken from the reactor in the beginning of the anaerobic phase and once in the end of the anaerobic phase. The harvested biomass was gently spread on a microscope slide and left to dry in ambient temperature until dry (around 2.5 h). The fixed smear was then stained with Neisser stain according to AnoxKalndes (2013, pp. 85-86). Samples were taken 51, 52 and 78 days after start-up.

The sample preparation before Gram staining was conducted in the same way as for Neisser staining. The Gram staining was conducted according to AnoxKaldnes (2013, pp. 84-85). Samples were taken 51 days after start-up.

Nile blue staining was used to visualise PHA inclusions. To get a sample for Nile blue staining, biomass from two to three carriers was scraped off and rinsed from the carriers with minimal amounts of water. The carriers were then returned to the reactor to keep the same number of carriers. The carriers were taken from the reactor in the end of the anaerobic phase. Further sample preparation and staining were executed according to Bengtsson *et al*. (2008). Samples were taken 51 days after start-up.

11.4 Analysis

The analyses of soluble COD (sCOD), phosphate phosphorus (PO_4-P), ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were executed using HACH Lange cuvettes and analysed in a spectrophotometer (DR 2800, HACH LANGE), see appendix *2 HACH Lange* for analysis method and measuring range.

The biomass was studied with microscope. A stereomicroscope (SZ-ET, Olympus) was used to study the structure and distribution of the biomass on the carriers. Neisser staining and a light microscope (eclipse Ni, Nikon) were used to study if the biomass was Neisser-positive and hence contained polyphosphate granules. Gram staining and a light microscope

(eclipse Ni, Nikon) was used to study if the biomass was Gram positive. Nile blue staining and fluorescence microscope (Axioskop 2 plus, Zeiss) were used to study if the biomass was PHA positive.

The feed volumetric flow rates were measured and if needed, the speed on the feed pumps was then adjusted to fit the desired volumetric flow rates. In the beginning (until a few days after day 16), the effluent flow rate was measured by collecting the final effluent for a certain time.

DO and pH were measured in the reactors directly after sampling. The pH meter (HI991001, HANNA) probe and DO meter (HQ40d Multi, HACH) probe were inserted simultaneously into the stirred reactor for a couple of minutes until the equipment showed stable values. Both the pH meter and the DO meter displayed temperature. In some cases the temperature was measured with a separate thermometer.

11.5 Calculations

11.5.1 Feed flow rates and HRTs

The volumetric feed flow rates were adjusted according to the desired hydraulic retention times (HRTs) in the aerobic reactors and deoxidising reactor (reactor R2 to R4). The HRT describes for how long time, in average, the water is in the system and was calculated using *Eq. 11.1*.

$$HRT = \frac{V_{Reactor}}{Q_{Feed}} \qquad (Eq.\ 11.1)$$

Where $V_{Reactor}$ is the working volume in the reactor and Q_{Feed} is the volumetric flow rate through the reactor.

11.5.2 Material balances over the anaerobic reactor

Carbon and phosphorus balances were formulated over the anaerobic reactor to enable calculation of the activity of the bio-P bacteria in terms of carbon uptake and phosphorus release. *Figure 11.6* was used to make the mass balances over the anaerobic reactor. The calculated amounts of consumed COD and released phosphate were later used to calculate COD uptake rates and phosphate release rates. These calculations were only carried out for the results from the measurements conducted on day 48 and later.

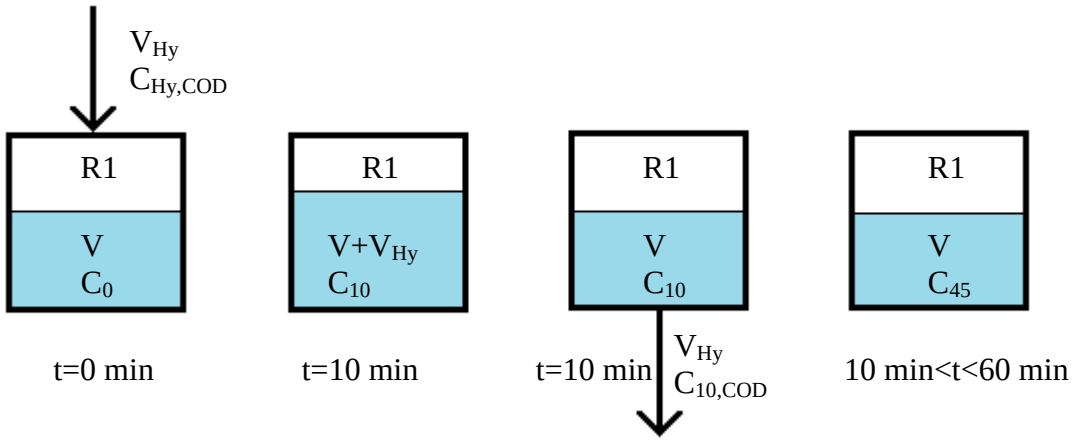

Figure 11.6. A schematic figure over the anaerobic reactor R1 during the different stages in the anaerobic phase P1.

To simplify the calculations, they were based on 10 minutes or 45 min unless something else is stated, and the volume flow, Q_{Hy}, was converted to the added volume, V_{Hy}, during the time concerned.

The following assumptions were made:

- The volume in the anaerobic reactor at time zero was 0.65 l.
- The volume in the anaerobic reactor after the hydrolysis influent was added, just before 10 minutes, was 0.65 l plus the volume of the added hydrolysis influent. At the time 10 minutes, the anaerobic reactor volume was assumed to instantly decrease to 0.65 l.
- The volume in the reactor at the end of the phase was 0.65 l, which was the same as at time zero.
- The concentrations in the influents to the anaerobic reactor were the same as the concentrations measured in the feed containers.
- The hydrolysis influent was the only influent to the anaerobic reactor. There was no leakage from the last deoxidising reactor.
- During the anaerobic phase no COD was produced and no phosphate was consumed.
- The water in the reactor was well stirred and homogenous.
- All reactors were assumed to be equal, which means that they have the same concentrations during the same phase.

When taking the assumptions into account, the COD balance from time 0 minutes until time 10 minutes can be written as in *Eq. 11.2*.

$$V \cdot C_{0,COD} + V_{Hy} \cdot C_{Hy,COD} = V \cdot C_{10,COD} + V_{Hy} \cdot C_{10,COD} + CONS_{10,COD} \qquad \textit{(Eq. 11.2)}$$

where V is the reactor volume, V_{Hy} is the added volume of the hydrolysis influent and the volume of the effluent, C is the COD concentration in the anaerobic reactor at time 0 minutes and time 10 minutes, C_{Hy} is the COD concentration in the hydrolysis influent and CONS is the consumed amount of COD during the first 10 minutes.

The COD balance was rewritten to describe the unknown COD consumption during the first 10 minutes, see *Eq. 11.3*.

$$CONS_{10,COD} = V\left(C_{0,COD} - C_{10,COD}\right) + V_{Hy}\left(C_{Hy,COD} - C_{10,COD}\right) \quad \textit{(Eq. 11.3)}$$

In a corresponding way, the COD balance from time 0 minutes until time 45 minutes can be written as in *Eq. 11.4*.

$$CONS_{45,COD} = V\left(C_{0,COD} - C_{45,COD}\right) + V_{Hy}\left(C_{Hy,COD} - C_{10,COD}\right) \quad \textit{(Eq. 11.4)}$$

where $CONS_{45,COD}$ is the consumed amount of COD during 45 minutes, V is the reactor volume, V_{Hy} is the added volume of the hydrolysis influent and the volume of the effluent, C is the COD concentration in the anaerobic reactor at time 0 minutes, 10 minutes and at 45 minutes and C_{Hy} is the COD concentration in the hydrolysis influent.

The hydrolysis feed did not contain phosphate and hence there was no term for phosphate influent in the mass balance. The phosphate balances for the first 10 minutes can be written as in *Eq. 11.5*.

$$RELEASED_{10,P} = V\left(C_{10,P} - C_{0,P}\right) + V_{Hy} \cdot C_{10,P} \quad \textit{(Eq. 11.5)}$$

where $RELEASED_{10,P}$ is the amount released phosphate during the first 10 minutes, V is the reactor volume, V_{Hy} is the added volume of the hydrolysis influent and the volume of the effluent and C is the phosphate concentration in the anaerobic reactor at time 0 minutes and time 10 minutes.

The phosphate balances until time 45 minutes can be written as shown in *Eq. 11.6*.

$$RELEASED_{45,P} = V\left(C_{45,P} - C_{0,P}\right) + V_{Hy} \cdot C_{10,P} \quad \textit{(Eq. 11.6)}$$

where $RELEASED_{45,P}$ is the amount of released phosphate during the first 45 minutes, V is the reactor volume, V_{Hy} is the added volume of the hydrolysis influent and the volume of the effluent and C is the phosphate concentration in the anaerobic reactor at time 0 minutes, 10 minutes and at time 45 minutes.

As a reminder, the calculated consumed amounts of COD and released amounts of phosphate are based on the measurements performed 0 minutes, 10 minutes and 45 minutes after the anaerobic phase started. Hence the calculated amounts are time dependent even though no time dependence can be seen in the mass balances.

11.5.3 Material balances over the aerobic and deoxidising reactors

Phosphate, nitrate and ammonium balances were formulated over the two aerobic reactors and the deoxidising reactor (R2 to R4), see *Figure 11.7*, to enable calculation of the activity of the bacteria in terms of phosphate uptake and ammonium removal. The calculated amounts of consumed phosphate and ammonium were later used to calculate uptake rates. These calculations were only carried out for the results from the measurements conducted on day 48 and later.

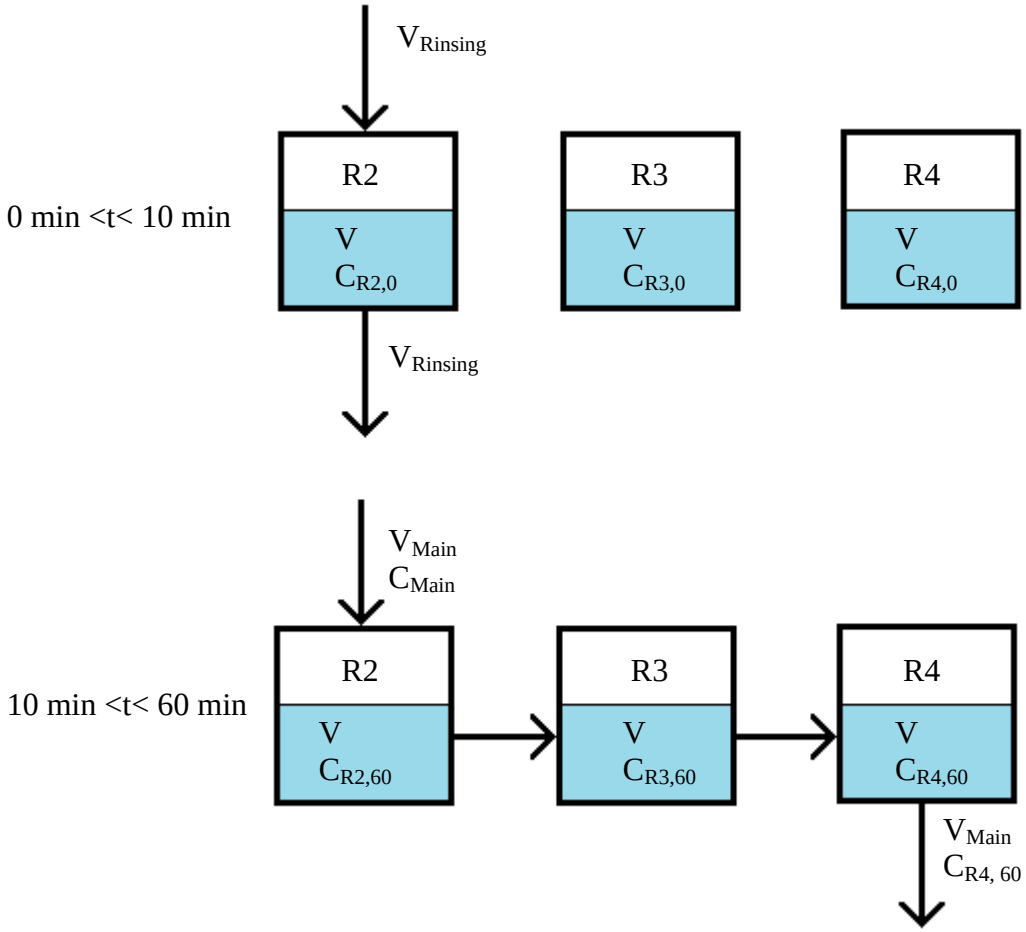

Figure 11.7. Schematic figure over reactors R2 to R4 during the different stages in a sequence.

The calculations were based on one sequence, i.e. 1 h, the reactors R2 to R4. To simplify the calculations, the volumetric flow rate, Q_{Main}, was converted to the added volume, V_{Main}, during the time concerned.

Several assumptions were needed to be able to solve the material balance over a part of the process. The following assumptions were made:

- The working volumes in the reactors (V) were constantly 0.65 l. No accumulation occurred and hence the volume of the main influent and volume of the final effluent were the same.
- The first aerobic reactor R2 was rinsed with clean tap water during the first 10 minutes and hence the initial concentrations of the substances in that reactor were those measured after 10 minutes.
- The concentrations in the main influent to the aerobic reactor R2 were the same as the concentrations measured in the feed container. When nitrate was not measured in the feed the concentration was assumed to be 0 mg/l.
- The main influent was the only influent to the aerobic reactor R2. There was no leakage from the anaerobic reactor R1.
- No reactions occurred in the tubing between the reactors.
- The water in the reactors was well stirred and homogenous.

- All reactors were assumed to be equal, which means that they have the same concentrations during the same phase. This means that the concentration in the end of the second aerobic phase in reactor 3 was assumed to be the same as in the beginning of the deoxidising phase in reactor 4.
- The consumption during the last 15 minutes in the deoxidising phase was negligible.
- The concentrations in the effluent from the whole system were assumed to be a mean value between the concentration in the beginning of the deoxidising phase and the concentration in the end of the deoxidising phase. Since the concentrations neither were measured in the deoxidising reactor in the beginning nor in the second aerobic rector in the end, the concentration in the beginning in the deoxidising reactor was assumed to be the same as in the second aerobic reactor after 45 minutes.

When the assumptions were taken into account, the phosphate balance was as seen in *Eq. 11.7*.

$$V\left(C_{R2,10,P} + C_{R3,0,P} + C_{R4,0,P}\right) + V_{Main} \cdot C_{Main,P} = \\ = V\left(C_{R2,60,P} + C_{R3,60,P} + C_{R4,60,P}\right) + V_{Main} \cdot \frac{C_{R4,0,P} + C_{R4,60,P}}{2} + CONS_{60,P} \qquad \textit{(Eq. 11.7)}$$

With the assumptions

$$C_{R2,60,P} = C_{R3,0,P}$$
$$C_{R3,60,P} = C_{R4,0,P}$$
$$C_{R4,45,P} = C_{R4,60,P}$$

and the assumption that the concentration in the beginning in the deoxidising reactor R4 was the same as the concentration in the second aerobic reactor R3 after 45 minutes,

$$C_{R4,0,P} = C_{R3,45,P}$$

the equation can be simplified to *Eq. 11.8*.

$$CONS_{60,P} = V\left(C_{R2,10,P} - C_{R4,45,P}\right) + V_{Main}\left(C_{Main,P} - \frac{C_{R3,45,P} + C_{R4,45,P}}{2}\right) \qquad \textit{(Eq. 11.8)}$$

where $CONS_{60,P}$ is the consumption, V is the working volume in the reactors, V_{Main} is the influent volume of the main feed and the volume of the effluent, $C_{R2,10,P}$ is the phosphate concentration in the first aerobic reactor (R2) after the rinsing period, $C_{R4,45,P}$ is the phosphate concentration in the deoxidising reactor (R4) in the end of the phase, $C_{Main,P}$ is the concentration in the main influent, and $C_{R3,45,P}$ is the phosphate concentration in the second aerobic reactor (R3) in the end of the phase or the concentration in the deoxidising reactor (R4) in the beginning of the phase.

The ammonium balance was calculated in a similar way as the phosphate balance and can be seen in *Eq. 11.9*.

$$CONS_{60,N} = V\left(C_{R2,10,N} - C_{R4,45,N}\right) + V_{Main}\left(C_{Main,N} - \frac{C_{R3,45,N} + C_{R4,45,N}}{2}\right) \quad \textit{(Eq. 11.9)}$$

As a reminder, the calculated consumed amounts and released amounts are based on the measurements performed 0 minutes, 10 minutes and 45 minutes after the sequence started. Hence the calculated amounts are time dependent even though no time dependence can be seen in the mass balances.

11.5.4 Loading rates

The organic loading rate (OLR) to the anaerobic reactor was calculated using the influent flow rate, the concentration in the influent and the reactor volume, see *Eq. 11.11*.

$$OLR = \frac{Q_{Feed} \cdot C_{Feed}}{V_{Reactor}} \quad \textit{(Eq. 11.11)}$$

When biofilm processes are used, the OLR can be based on the carrier surface instead of the reactor volume, see *Eq. 11.12.*

$$OLR = \frac{Q_{Feed} \cdot C_{Feed}}{N_{Carrier} \cdot A_{Carrier}} \quad \textit{(Eq. 11.12)}$$

where Q_{Feed} is the feed volumetric flow rate, C_{Feed} is the sCOD concentration in the feed, $N_{carrier}$ is the number of carriers in each reactor, and $A_{Carrier}$ is the exposed biofilm area for each carrier.

The phosphorus loading rate and ammonium loading rate, named LR in the equations, were calculated in a corresponding way as the organic loading rate, see *Eq. 11.13* and *11.14*.

$$LR = \frac{Q_{Feed} \cdot C_{Feed}}{N_{Reactor} \cdot V_{Reactor}} \quad \textit{(Eq. 11.13)}$$

$$LR = \frac{Q_{Feed} \cdot C_{Feed}}{N_{Reactor} \cdot N_{Carrier} \cdot A_{Carrier}} \quad \textit{(Eq. 11.14)}$$

where $N_{Reactor}$ is the number of reactors included, $A_{Carrier}$ is the exposed biofilm area for each carrier, and $N_{carrier}$ is the number of carriers in each reactor.

11.5.5 Uptake rates and release rates

The uptake rates, named UR in the equations, were calculated with the time dependant consumed amounts from the mass balances, the reactor volume and the number of reactors, see *Eq. 11.15*.

$$UR = \frac{CONS_{60}}{N_{Reactor} \cdot V_{Reactor}} \quad \textit{(Eq. 11.15)}$$

where $CONS_{60}$ is the time dependant consumed amount calculated with the mass balances, $N_{Reactor}$ is the number of reactors included, $V_{Reactor}$ is the reactor volume.

When biofilm processes are used, the uptake rates can be based on the carrier surface instead of the reactor volume. The uptake rates were calculated according to *Eq. 11.16*.

$$UR = \frac{CONS_{60}}{N_{Reactor} \cdot A_{Carrier} \cdot N_{Carrier}} \qquad \textit{(Eq. 11.16)}$$

where $CONS_{60}$ is the time dependant consumed amount calculated with mass balances, $N_{Reactor}$ is the number of reactors included, $A_{Carrier}$ is the exposed biofilm area for each carrier and $N_{carrier}$ is the number of carriers in each reactor. In the case of the anaerobic uptake rates, the number of reactors included was 1, while it was 3 for aerobic uptake rates. The anaerobic phosphate release rate was calculated in a similar way but then the calculated released amount was used instead.

12 Results and discussion

12.1 Explanation of changes in the experimental setup

During the project, several changes were made in the experimental setup to get EBPR activity.

In the beginning, the water was supposed to flow by gravity between the reactors. A lifting device was used to facilitate the flow between the anaerobic reactor R1 and the anoxic reactor R2 by lifting the anaerobic reactor a few centimetres while the other three reactors were at the same height. However, the flows between the reactors were extremely poor and the hydrolysis influent and main influent were accumulated in the anaerobic reactor R1 and the anoxic reactor R2 respectively. During this period, the final effluent from aerobic reactor R4 was measured. There was a great difference between the amounts of final effluent from the reactors. Gas bubbles in the tubing were identified as one of the problems. An external level regulator, consisting of a Y-shaped tubing connector, was installed on the outlet on each reactor. The height of the level regulator controlled the water levels in the reactors. The idea was to increase the water levels in the reactors and prevent gas bubbles from entering the tubing through the reactor outlet. Additionally, if any bubble went through the outlet on the reactor, the bubble was supposed to be able to exit the system through the external level regulators which also worked as a deaerating device. The flows between the reactors were not improved by the external level regulators.

In addition to mechanical problems during this period, day 0 to 16, no bio-P activity in the forms of anaerobic phosphorus release was measured. The lack of activity was not thought to be due to the mechanical problems with the process. The process during this time was batch-wise since the flows between the reactors were poor. Several studies with working EBPR have been conducted with SBMBBR processes (Pastorelli *et al*., 1999; Helness and Ødegaard, 2001; Helness, 2007). Therefore it was thought that the reasons might be that the bacteria were growing slowly and possibly that some conditions were not right.

A peristaltic pump was installed at day 16 to pump the water between the reactors and out of the system. The influents were no longer accumulated in the reactors with feed influents, R1 and R2, and the water levels in all four reactors were the same (0.65 l) regardless which phases the reactors were in. Additionally, the measured effluent volumes from the reactors were of the same size.

At day 20, it was found that nitrate was present in the anaerobic reactor R1. This showed that not all the nitrate from the main feed was denitrified during the anoxic and aerobic phases. The presence of nitrate in the anaerobic phase can have negative effects on the bio-P activity. Firstly, the presence of nitrate turns the anaerobic reactor anoxic and hence anoxic uptake of phosphate might occur instead of anaerobic release of phosphate. Secondly, denitrifying bacteria and PAOs, will compete for the carbon, and if there is not enough carbon for the PAOs the phosphate release will decrease (Henze *et al*. 1997, pp. 99).

The nitrogen source was exchanged from nitrate to ammonium for 9 days, day 20 to 28, to give the PAO more suitable conditions in the anaerobic phase. This change was intended to be temporary since the objective with the project from the beginning was to have denitrifying phosphorus removal from wastewater mainly containing nitrate and phosphate and hence it

was desired to have nitrate in the main influent. At day 28, nitrate was introduced in the main influent again but in smaller amounts and both ammonium and nitrate was used as nitrogen source. The nitrate concentration in the anaerobic reactor was analysed to ensure that the nitrate was denitrified before the reactors turned anaerobic. Although the anaerobic reactor was free from nitrate, no anaerobic phosphorus release was measured.

Another problem which did not favour the PAOs was that not all of the VFA in the hydrolysis influent was consumed during the anaerobic phase. This resulted in that most of the added VFA was available in the reactor with the main influent. This caused competition from non-phosphate accumulating organisms (Helness and Ødegaard, 1999) and a process where PAOs were not favoured. The poor anaerobic carbon uptake was thought to be due to a small population of PAOs or absence of PAOs. Additionally, no other microorganisms with ability of anaerobic carbon uptake seemed to be active.

The hydrolysis influent was pumped in during the whole anaerobic phase, which meant that the sCOD concentration in the anaerobic phase was low. Low concentrations do not promote growth as well as higher concentrations do. Additionally, systems with lower concentrations are more sensitive to disturbances. Another problem, day 0 to 37, was that the effluent from the anaerobic reactor was one of the influents to the reactor with the main influent. Hence the reactor which needed to be free from VFA to prevent competition from microorganisms unable to perform anaerobic carbon uptake, had an influent containing carbon. To solve the problem with the carbon source in reactor R2, two changes were made initially. At day 31, the effluent from the anaerobic reactor was changed to be pumped out of the system instead of being pumped to the next reactor R2. A few days later, day 34, the hydrolysis influent containing carbon source was changed to be rapidly pumped in during the first ten minutes of the anaerobic phase instead of during the whole phase. These changes were intended to give a higher sCOD concentration in the anaerobic reactor R1 and decrease the competition from microorganisms unable to perform anaerobic uptake of carbon source, by preventing the carbon source containing wastewater from entering the next reactor R2.

The purpose of the changes was to get bio-P activity. It should be noted that the change where the effluent from the anaerobic reactor was pumped out of the system instead of to the next reactor, was intended as a temporary solution to increase the chances of getting bio-P activity. This mode of operation is not really of interest since it would be difficult to utilise in a full-scale process. Additionally, the system was no longer continuous since the anaerobic reactor was operated as a batch.

Along with the discussion about competition from microorganisms unable to perform anaerobic carbon uptake, it was noticed that the biofilm on the carriers was thin. It was therefore suggested to try to increase the thickness of the biofilm. The amount of sCOD added to the anaerobic reactor R1 was hence temporarily increased for a period of time, day 31 to day 45, despite the fact that the bacteria already before the increase were unable to consume the sCOD during the anaerobic phase. The intention was to have excess sCOD in the next reactor, R2, to provide growth of microorganisms. It was thought that the growth of PAOs in the biofilm would be facilitated if there already was a sufficient biofilm. The change was conflicting with the other changes where the purpose was to prevent sCOD from entering the next reactor R2. However, the amount of added sCOD was regulated by the concentration in the hydrolysis feed which was easily changed. After 14 days with higher sCOD concentration in the hydrolysis feed, the distribution and thickness of the biofilm had increased but was still not especially thick.

Still no anaerobic phosphate release was measured and hence it could not be proved that the small potential phosphorus removal was due to EBPR and not only assimilation during normal growth of other microorganisms. At day 45 an additional change was made to prevent the carbon source from entering the second reactor R2. The change was as described below. After the anaerobic phase was ended, the reactor was rinsed with tap water for ten minutes. The rinsing water was led out of the system. Then the influent with ammonium and phosphate was pumped in for 50 minutes. It should be noted that this change was intended to be temporary and that the rinsing phase was to be removed when the bio-P activity was higher. The reason to why this operational method was of no particular interest was that it would be difficult to use in a full-scale process. Despite all changes, the anaerobic phosphate release was close to zero. However, a small uptake of sCOD was seen. Additionally, the microscopic study showed PHA-positive microorganisms with Nile blue staining, while close to none polyphosphate granules were found with Neisser staining. These results indicated that the biofilm contained the competing microorganism GAO.

The temperature was originally set at 15°C to match the climate in Swedish wastewater treatment plants. The temperature decrease to 10°C at day 52, was meant to increase the possibility of getting bio-P activity in the reactors. An earlier study has shown that a temperature decrease can shift the bacterial population from GAOs to PAOs (Lopez-Vasquez *et al*., 2009b).

At day 63, more calcium and magnesium were added to the main influent in addition to the trace element solution. Several studies have shown the importance of magnesium and possibly calcium in EBPR process (Rickard and McClintock, 1992; Pattarkine and Randall, 1999; Schönborn *et al*., 2001; Tykesson and Jansen, 2005). Potassium, another important counter ion, was expected to be present in excess since both the phosphate salts, K_2HPO_4 and KH_2PO_4, contain potassium.

12.2 Uptake and release rates

The function of the process was evaluated by calculating the loading rates, aerobic phosphate uptake rate, anaerobic phosphate release rate and anaerobic sCOD uptake rate. The rates were calculated using the mass balance in chapter *11.5.2 Material balances over the anaerobic reactor* and *11.5.3 Material balances over the aerobic and deoxidising reactors*, and the equations in chapter *11.5.4 Loading rates* and *11.5.5 Uptake rates*. The results presented below are from the period after day 45 when the anaerobic reactor was rinsed with tap water after the anaerobic phase was ended, unless something else is stated. The primary data in the form of the measured concentrations, pH and temperature can be seen in appendix *4 Primary data.*

In the material balances, several assumptions were made to facilitate the calculations. The assumptions used for the anaerobic reactor R1 were thought to be reasonable. However, some of the assumptions used for reactor R2 to R4 are less probable or not accurate. The working volumes of the reactors were not constantly 0.65 l due to accumulation of main feed in reactor R2 and R3. The assumption was used anyway since the concentrations, in the end of the sequence, almost were the same in reactors R3 and R4. This indicated that almost nothing happened in reactor R4. After a while, the walls of the tubing were covered with biomass, indicating that nutrients from the wastewater were removed not only by the microorganisms on the carriers in the reactor. The nutrient removal in the tubing was still thought to be

negligible. Since the used assumptions did not correlate with how the actual setup worked, the uncertainty of the calculated values are larger.

Another source of error regarding the calculations was that the exact volumetric flow rates of the feeds were not known for the day the analyses were made. The volumetric flow rates were measured periodically but not necessarily on the same day as the sampling and analysis. The volumetric flow rate decreased with time at the same rate as the pump tubes used in the peristaltic pumps got worn out. In the cases were the influent flow rates were unknown, the flow rates were approximated by linearly interpolating between the known flow rates.

The phosphate loading rate, phosphate uptake rate and phosphate "uptake efficiency" over the aerobic reactors and the deoxidising reactor, R2 to R4, can be seen in *Table 12.1*. The highest achieved uptake rate was 0.13 g/(m^2·d) which is 6-7 times lower than the aerobic/anoxic phosphate uptake rate of 0.85 g/(m^2·d) found by Pastorelli *et al*. (1999). However, their phosphate loading rate was 0.98 g/(m^2·d) which is almost 3 times higher than the loading rate in the present study. A higher loading rate can give a higher uptake rate if there are diffusional limitations in the biofilm, which can get lower by higher substrate concentration in the bulk. However, the biofilm in the present study was thin and it was therefore not thought to be any diffusional limitation of importance.

Table 12.1. The aerobic phosphate loading rate, aerobic uptake rates and "uptake efficiency" for the reactors R2 to R4. The uptake rates are valid during the first 45 minutes of the sequence. The uptake efficiency is for the whole sequence.

Time from start (days)	PO_4-P loading rate (kg/(m^3·d))	PO_4-P loading rate (g/(m^2·d))	PO_4-P uptake rate (kg/(m^3·d))	PO_4-P uptake rate (g/(m^2·d))	PO_4-P "uptake efficiency" (%)
48	0.036	0.18	0.013	0.07	37
49	0.063	0.32	0.010	0.05	17
51	0.059	0.30	0.012	0.06	20
59	0.069	0.35	0.019	0.10	27
65	0.067	0.34	0.024	0.12	35
76	0.067	0.34	0.026	0.13	39

The uptake efficiency was between 17 and 39%, meaning that up to 39% of the influent phosphate was taken up during the aerobic phase. This value does, however, not say anything about the total removal efficiency of the process or if the treatment requirements can be met.

The measured uptake rates were not especially impressive. However, the uptake rate was still found to increase with time when the experiment was terminated, see *Figure 12.1*. Additionally, even higher rates might be achieved if the process is optimised.

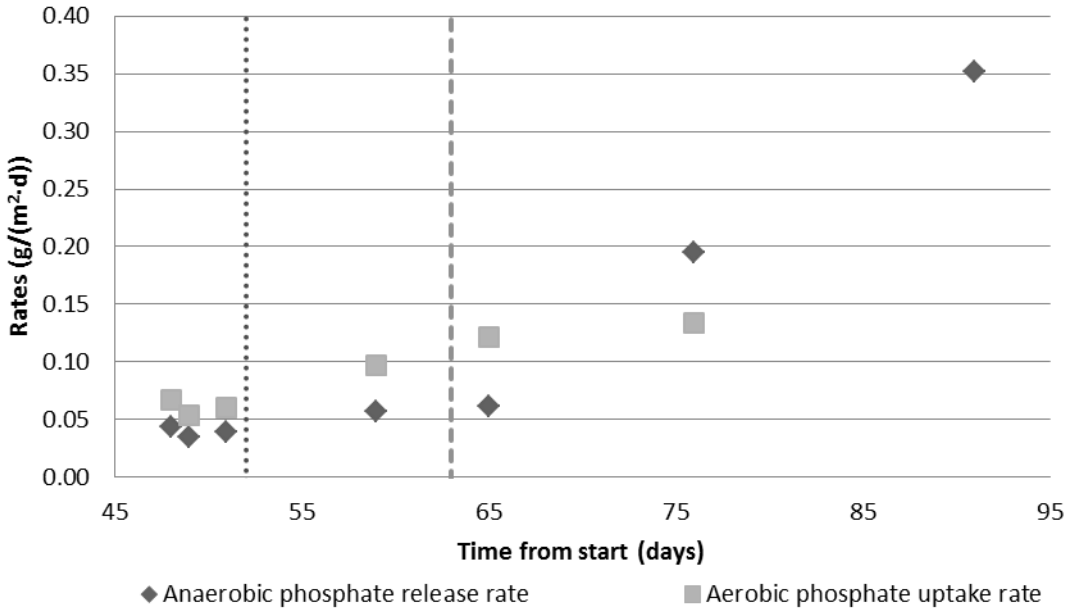

Figure 12.1. The anaerobic phosphate release rate and aerobic phosphate uptake rate during one sequence as a function of the number of days from start. The release rate is calculated for reactor R1 while the uptake rate is calculated for reactor R2-R4. The aerobic phosphate uptake rate was not measured on day 92. The dotted vertical line shows when the temperature decrease occurred and the dashed line shows when additional counter ions were added to the main influent.

Anaerobic phosphate release was achieved, indicating bio-P activity. The highest measured phosphate release rate was 0.35 g/(m^2·d), see *Table 12.2*, and was around 6 times lower than the release rate of 2.14 g/(m^2·d) obtained by Pastorelli *et al*. (1999) when they used real wastewater, indicating that calculated release rate in the present study still was poor. After day 65, there was a steep increase in the anaerobic phosphorus release rate, see *Figure 12.1*. The increase in uptake rate and release rate is somewhat consistent. However, the phosphate uptake rate did not increase as much as the anaerobic release rate to day 76. The increased rates could be caused by an increased fraction of PAOs in the biomass or that the PAOs eventually got higher rates. It is of interest to find the reason behind the increased bio-P activity. From day 45, the reactor R2 was rinsed in the beginning of the phase P2 to remove the otherwise available sCOD. Shortly thereafter, the temperature was decreased and later more counter ions were added to the main feed, see *Figure 12.1*. Biological systems are known to be slow and especially at such low temperatures as 10°C, and therefore it takes a while before the system reaches a steady state after changes are made. The experimental changes might have been conducted in a too short timespan to be able to see the individual effects of the changes. Literature supports the importance of counter ions (Pattarkine and Randall, 1999) and that a temperature around 10°C favours PAOs above GAOs (Lopez-Vazquez *et al*., 2009b). However, there are not enough measuring points to be able to conclude which of the changes that was crucial to achieve the increased activity. It may also be that it was a combination of the effects from the present unavailability of sCOD in phase P2 to P4, the temperature decrease, and the addition of more counter ions that was the reason behind the enabled growth.

The ratio between anaerobic phosphate release rate and anaerobic sCOD uptake rate was much lower than the ratio of 0.52 kg P/kg sCOD achieved in the study by Pastorelli *et al*.

(1999), see *Table 12.2*. This indicates that most of the sCOD taken up during the anaerobic phase was not used by PAOs but potentially GAOs.

Table 12.2. Anaerobic phosphate release rates, and ratio between anaerobic phosphate release rate and anaerobic sCOD uptake rate. The release rates are valid during the first 45 minutes of the sequence.

Time from start (days)	PO_4-P release rate (kg/(m^3·d))	PO_4-P release rate (g/(m^2·d)	PO_4-P/ sCOD (kg/kg)
48	0.009	0.044	0.012
49	0.007	0.035	0.011
51	0.008	0.039	0.005
59	0.011	0.057	0.010
65	0.012	0.061	0.014
76	0.038	0.195	0.030
91	0.069	0.352	-

The ratio between the amount of aerobically taken up phosphate and the amount of anaerobically released phosphate was between 2.7 to 7.9 mg/mg, but most values were between 6 and 7 mg/mg. Pastorelli *et al*. (1999) and Helness and Ødegaard (2001) found the ratio to be 1.055 and 1.11 mg PO_4-P/mg PO_4-P, respectively. Much more phosphate was taken up per released phosphate in the present study compared to what has been found in other studies. This indicates that most of the phosphate most probably was assimilated and used for growth, and not bio-P activity.

The anaerobic organic loading rate was similar during the period from which the presented results are taken, see *Table 12.3*. The anaerobic organic loading rate was just above 2.5 kg sCOD/(m^3·d), which was recommended as the highest anaerobic loading rate to maintain stable performance by Helness and Ødegaard (2001).

Table 12.3. sCOD loading rate, sCOD uptake rate and "removal efficiency" for the anaerobic reactor. The uptake rates are valid for the first 45 minutes in the phase. The "uptake efficiency" is what was expected during the whole sequence.

Time from start (days)	sCOD loading rate (kg/(m^3·d))	sCOD loading rate (g/(m^2·d))	sCOD uptake rate (kg/(m^3·d))	sCOD uptake rate (g/(m^2·d))	sCOD "uptake efficiency" (%)
48	2.7	13	0.70	3.5	26
49	2.7	13	0.65	3.3	24
51	2.7	13	1.60	8.1	59
59	2.6	13	1.11	5.6	42
65	2.5	12	0.86	4.4	34
76	2.6	13	1.26	6.4	48

The sCOD uptake rates were higher at the last points than at the earlier points, see *Table 12.3*. However, there was no correlation between time and increase in uptake rate. The anaerobic

sCOD uptake rate at the highest aerobic phosphate uptake rate was 6.4 g/(m^2·d) and was in the same area as the anaerobic uptake rates reported by Patorelli *et al*. (1999) and Helness (2007) at an anaerobic organic loading rate in the same range as in the present study. Patorelli *et al*. (1999) reported an anaerobic uptake rate of 4.15 g/(m^2·d) at an anaerobic organic loading rate of 9.23 g/(m^2·d) , while Helness (2007, pp. 67) reported anaerobic sCOD uptake rates of between 4 and 8 g/(m^2·d) at anaerobic loading rates of 10 g/(m^2·d). Overall, all anaerobic sCOD uptake rates in the present study were in the same range compared to Pastorelli *et al*. (1999) and Helness (2007). Below 60% of the added sCOD was consumed in the anaerobic phase. This means that there was no limitation in available sCOD and that there was no need for competition about the carbon source even though the GAOs took up most of the removed sCOD.

When taking into account that the uptake efficiencies were low, along with that the organic loading rates were higher than the recommended organic loading rates by Helness (2007) it can be seen that it might be that the organic loading rate in the present study was too high for the process as it was configured during the study. But if the organic loading rate was to be decreased, there might be competition about the carbon source. In that case it would be important that the conditions favour the PAOs. Anyway, a large organic loading rate did not cause any problems since the excess sCOD was rinsed from reactor R2 before phase P2 begun. Hence there was no problem with excess sCOD supporting competing microorganisms in the aerobic phases. As mentioned earlier, the rinsing of reactor R2 in the beginning of phase P2, was only intended as a temporary solution to increase the chances of getting bio-P activity and is not suitable for full-scale plants. Therefore it would be of interest to remove the rinsing period. With no rinsing period, it would be interesting to use the anaerobic sCOD uptake to regulate the organic loading rate, to ensure that the excess sCOD amount after the anaerobic phase is not too large.

The ammonium removal rate increased with time, see *Table 12.4*. The removal efficiency increased with time when looking at the results with the same loading rate (day 51 to day 76). At day, 51 and onwards, the ammonium concentration in reactor R3 decreased from 2.2 to 1.4 mg/l while the concentration in reactor R4 decreased from 2.1 to 0.7 mg/l. The ammonium is mainly removed from the wastewater by assimilation when the microorganisms grow.

Table 12.4. Ammonium loading rate, removal rates and "removal efficiency" for the aerobic reactors and the deoxidising reactor. The rates are valid for the first 45 minutes of the sequence. The removal efficiency is what was expected during the whole sequence. Ammonium was not analysed in the reactors at day 48.

Time from start (days)	NH_4-N loading rate (kg/(m^3·d)	NH_4-N loading rate (g/(m^2·d)	NH_4-N removal rate (kg/(m^3·d))	NH_4-N removal rate (g/(m^2·d)	NH_4-N "removal efficiency" (%)
48	0.016	0.08	-	-	-
49	0.029	0.15	0.024	0.120	81
51	0.060	0.31	0.038	0.194	63
59	0.068	0.35	0.043	0.218	63
65	0.062	0.31	0.050	0.257	82
76	0.062	0.32	0.055	0.277	88

No nitrate was added to the main influent during the period from day 48 to day 75. However, nitrate was still detected in the reactors and later in the container with a few days old main feed. This indicates that nitrification occurred in the main feed container. Additionally, the material balance for nitrate indicated that nitrification occurred in the aerobic reactors as well. The nitrate concentrations in reactors R3 and R4 were, from day 51 to day 76, increasing from 0.7 to 2.3 mg/l and 0.3 to 1.3 mg/l, respectively.

In the studies found about denitrifying phosphorus removal, the biofilm on the carriers was stratified and the denitrifying phosphorus removal occurred in the deeper anoxic layers in aerated reactors. The nitrate was formed in the nitrification process by bacteria in the outer aerobic layer (Pastorelli *et al*., 1999; Helness, 2007). It would be interesting to see if the phosphorus uptake in the present study was performed by PAOs or denitrifying PAOs. However, since the thickness of the biofilm was poor and the DO concentration relatively high compared to the nitrate concentration, it is unlikely that the biofilm was stratified.

12.3 Biomass characterisation

During the project, carriers were taken from the reactors to study the biofilm distribution on the carrier and the texture (fluffiness) of the biomass and the type of biomass in the biofilm. The biofilm on the carriers was studied using a stereo microscope and when the type of biomass was of interest, biomass was scraped off from the carriers and studied using a phase contrast microscope.

The biomass on the carriers was studied on the day of start-up and then 42 and 92 days after start-up, see *Figure 12.2* and *Figure 12.3*.

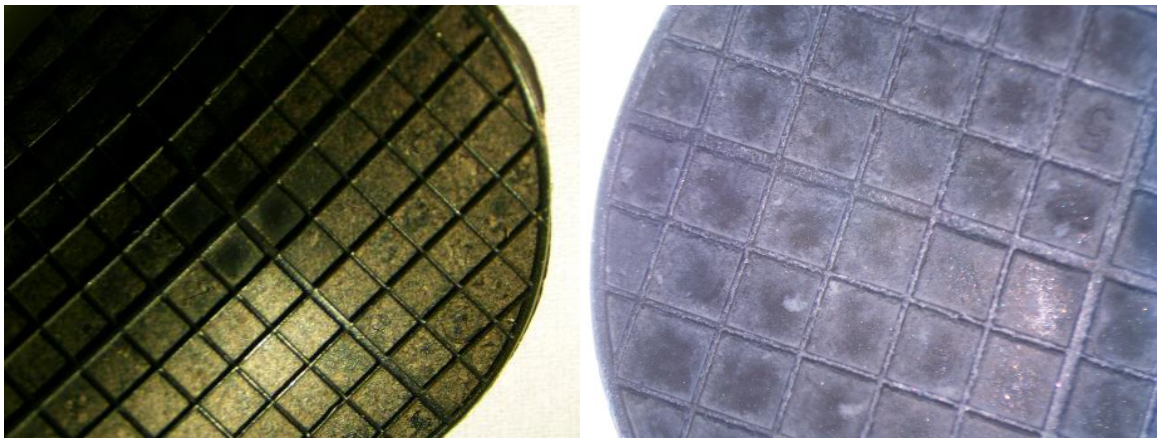

Figure 12.2. Carriers with biomass. Left: A pretreated, dried carrier from the day the experiment was started. Most of the carrier was covered in biomass, not only along the grid. Right: A carrier from the process taken 42 days after the study started. The biomass was mostly situated along the grid.

The carriers used in the study were from the beginning taken from a WWTP in Ulricehamn and had already biofilm on them when they were introduced to the process. Before the carriers were put in the reactors they were pretreated for 15 days in ambient temperature with altering aerobic and anaerobic conditions while waiting for the experimental setup to be ready. The biomass on the carriers introduced to the process was sufficient to cover the surface of the carriers, see *Figure 12.2 Left*. However, it should be noted that the studied carrier was dry and that the texture of the biomass therefore might look different.

After 42 days, the biomass did not cover the whole surface on any of the studied carriers, regardless from which reactor the sample was taken. The biomass was mostly situated along the grid, except for the more porous biomass, see *Figure 12.2 Right*. When comparing the amount of biomass covering the carriers from the day of start-up, see *Figure 12.2 Left*, with the carriers used in the process for 42 days, the amount of biomass seemed to have decreased after the carriers were introduced to the process.

If the microorganisms thrived in the process, the biomass on the carriers would be expected to grow thicker. Hence it is of interest to consider why the biomass was thin. During this period, no bio-P activity in form of anaerobic phosphorus release had been measured and the removals of nutrients from the wastewater were low. Therefore, the most probable reason to the poor biofilm is that the conditions in the reactors did not support growth of PAOs (or high growth of other microorganisms) since some environmental factors were unsuitable. A few likely reasons could be that some essential substances were missing, possibly magnesium and calcium; presence of nitrate in the anaerobic phase; and high biomass decay. Since sCOD was available in rectors R2 to R4, it would have been expected that some bacteria would grow well. However, the poor biofilm revealed the opposite. In an earlier study, the biomass was found to detach more easily from the carriers when the DO was higher than 5 mg/l (Chiou and Yang, 2008) most probably due to shear forces caused by aeration. The DO concentration in the present process was often above 5 mg/l in the aerated phases. Another explanation could be that the biofilm detached when the carriers bumped into each other and the air stone due to the mechanical mixing. However, these two causes are thought to be less likely than other environmental factors.

Several changes in the experimental setup were performed after day 42. The most important changes were the following: Reactor R2 was rinsed for 10 minutes in the beginning of phase P2 to ensure no sCOD was available in the aerobic phases. The temperature was decreased from 15°C to 10°C, and finally, more magnesium and calcium was added to the main influent.

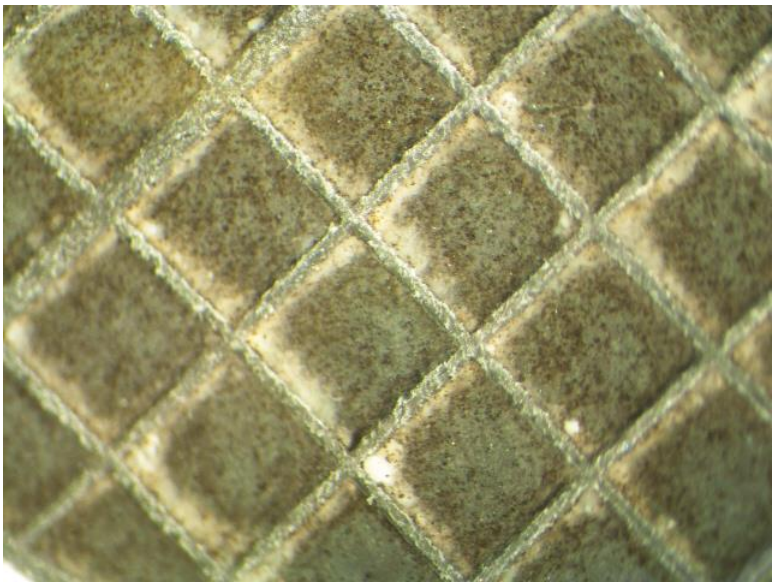

Figure 12.3. Carrier with biomass taken from one of the reactors 92 days after the experiment was started. White fluffy biomass grew along the grid while darker flatter biomass grew in the middle of the exposed surface.

The carrier taken from one of the reactors 92 days after start-up was more covered in biomass than the carrier after 42 days. There was almost no area without biomass, see *Figure 12.3*, compared to earlier when the biomass only was situated along the grid. The biomass situated along the grid was white and fluffy while the biomass situated in the middle of the exposed surface was dark and flat, see *Figure 12.3*. Since the biomass on the carriers was not studied more frequently it is unclear which of the changes in the experimental setup that enabled the biomass growth. It is also possible that all changes were needed.

Biomass was scraped off from carriers to study the type of biomass in the biofilm. The results from the phase contrast microscopic studies of the scraped off biofilm from carriers taken from the reactors after 51, 78 and 92 days can be seen in *Figure 12.4*, *Figure 12.5* and *Figure 12.6.*

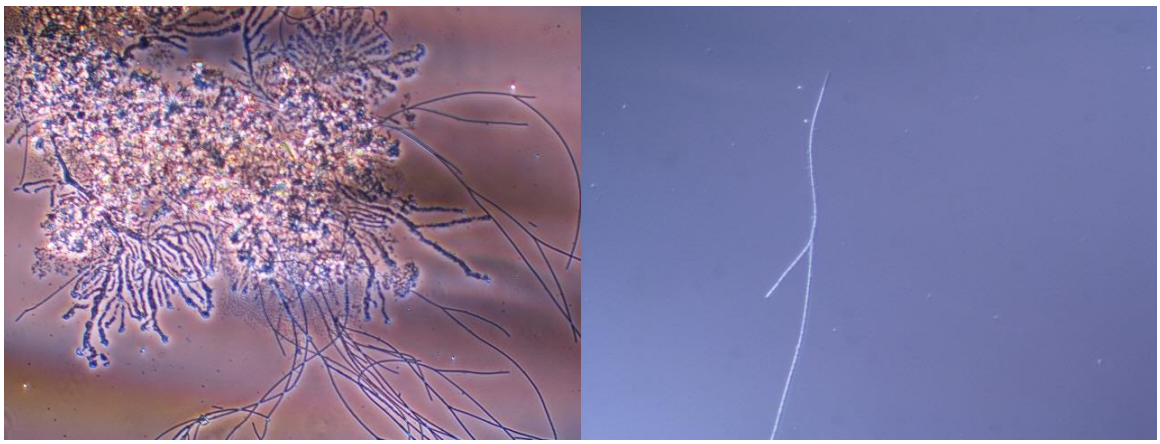

Figure 12.4. Biomass scraped off from carriers taken from the process 51 days after start-up. Left: The biofilm consisted of different kinds of microorganisms such as zoogloeal finger-shaped bacteria and filaments. Right: A filament with false branches, possibly Sphaerotilus natans.

When the biomass scraped off from the carriers taken from the process 51 days after start-up was examined with phase contrast microscope, different kinds of microorganisms in the biofilm was seen, see *Figure 12.4 Left*. There were finger-shaped zoogloea and some flocs holding it all together. The zoogloea thrives when there is lots of easily degradable carbon source or little nutrients (AnoxKaldnes, 2013, pp. 80). Filaments with false branches were also seen and these were thought to be *Sphaerotilus natans*, see *Figure 12.4 Right*. *Sphaerotilus natans* is known to thrive in plants with high loading rates and low concentrations of dissolved oxygen (AnoxKaldnes, 2013, pp. 100).

At this time, small phosphate releases in the anaerobic reactor had been measured indicating that the biomass possible contained small amounts of PAOs. However, no conclusion can be drawn about which organism that is behind the phosphate release solely from phase contrast studies.

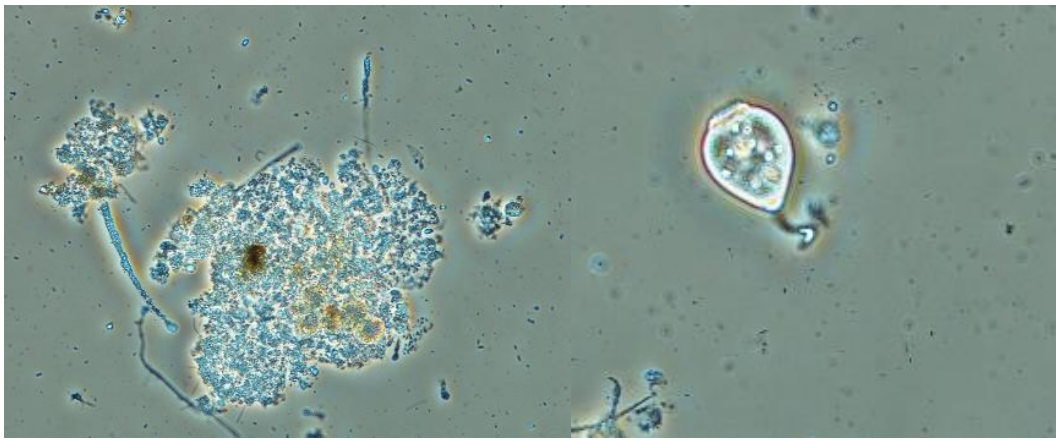

Figure 12.5. Biomass scraped off from carriers taken from the process 78 days after start-up. Left: A floc with zoogloeal bacteria. Right: A stalked ciliate.

The biomass scraped off from the carriers taken from the process 78 days after start-up looked a bit different from the biomass taken 51 days after start-up. Now, both the anaerobic phosphate release rate and aerobic phosphate uptake had increased several times. The biomass consisted of flocs which contained almost no filaments or finger-shaped zoogloeal bacteria compared to the results from day 51, see *Figure 12.5 Left*. A stalked ciliate, which is a protozoa (AnoxKaldnes, 2013, pp. 10) was found as well, see *Figure 12.5 Right*.

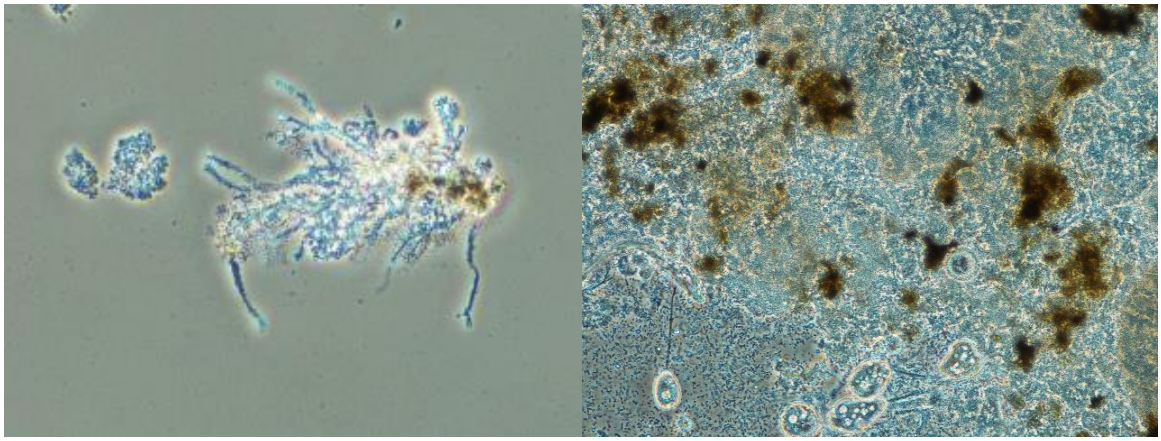

Figure 12.6. Biomass scraped off from a carrier taken 92 days after start-up. Left: A floc from the biomass from the darker areas on the carrier. Right: A compact floc with stalked ciliates from the white biomass taken from along the grid.

The biomass scraped off from the carriers taken from the process 92 days after start-up looked different from both the biomass taken 42, 51 and 78 days after start-up. The dark biomass from the carriers, see *Figure 12.3*, consisted mostly of flocs with some finger-shaped ones, see *Figure 12.6 Left*. A few stalked ciliates were found but they were not fixed to any floc. The biomass from the white areas looked different from the biomass from the dark areas. It was not concluded if the biomass was zoogloeal. The flocs were compact and there was little difference between the morphology of the microorganisms in the flocs, see *Figure 12.6 Right*. Some stalked ciliates were fixed on the surface of the flocs and lots of free-swimming ciliates were seen.

12.4 Gram staining of biomass

The biomass was Gram stained to see if the bacteria were Gram positive. Biomass was scraped off from the carriers, stained and studied using light microscope. Result from Gram stained biomass scraped off from carriers taken from the reactor in the end of an anaerobic phase after 51 days after start-up can be seen in *Figure 12.7*.

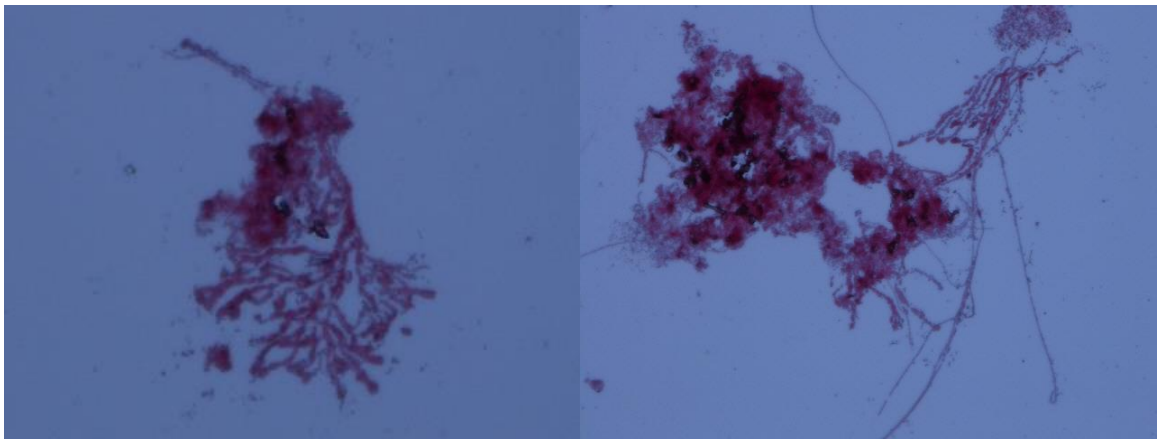

Figure 12.7. Gram-stained biomass, scraped off from the carriers taken from the process 51 days after start-up. Left: Gram-negative zoogloeal bacteria. Right: Gram-negative filaments. The darker stains are crystals.

The zoogloeal bacteria, the filaments and most of the flocs were red coloured and hence Gram negative, see *Figure 12.7*. Some darker areas can be seen in the flocs, however with higher magnification it became clear that these were crystals, see *Figure 12.7 Right*.

Most *Tetrasphaera* are known to be Gram positive (Maszenan *et al*., 2010; Blackall *et al*., 2000). The biofilm was hence not thought to contain any considerable amount of *Tetrasphaera*. No data was found about whether *Accumulibacter* is Gram positive or not and hence no conclusion can be drawn about the biomass content of *Accumulibacter*. However, the small anaerobic phosphorus release rate at day 51 indicates that the biomass contains a minor amount of PAOs.

It would have been interesting to repeat the Gram staining at day 76 or later, when the bio-P activity was a little bit higher, to see if any Gram-positive bacteria could have been seen. However, the time was limited and no analysis was made.

12.5 PHA study of the biomass

Nile blue staining of biomass scraped off from carriers was conducted to see if the biomass contained any PHA. PHA can be used to indicate the presence of the PAO *Accumulibacter* or GAOs. The result from the Nile blue-stained sample taken from reactor A in the end of the unaerated anaerobic phase taken 51 days after start-up can be seen in *Figure 12.8* and *Figure 12.9*.

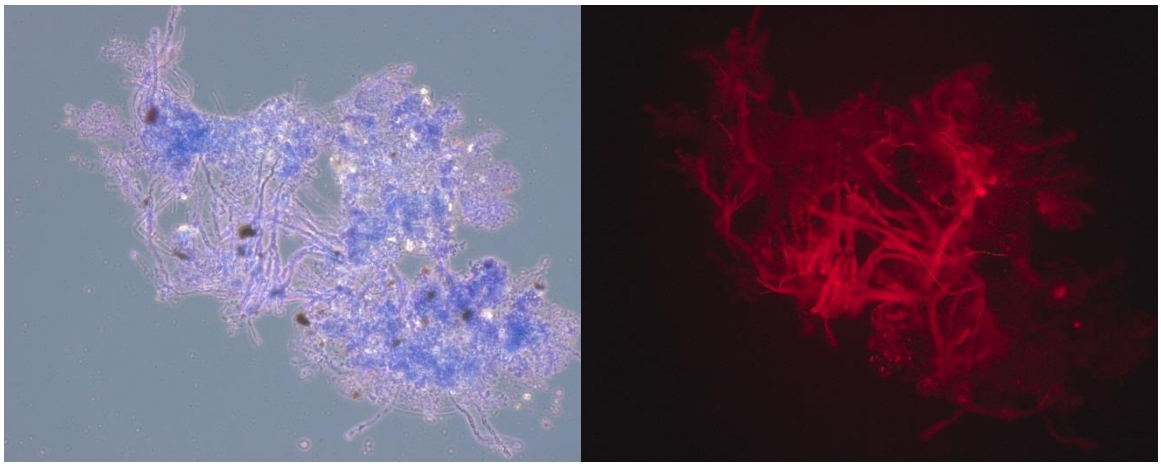

Figure 12.8. Biomass scraped off from carriers taken from the process 51 days after start-up, examined with Nile blue staining and fluorescence microscope. Right: A floc with finger-shaped zoogloeal bacteria studied with phase contrast. Right: The same floc studied with fluorescence.

It can be seen that a great part of the biomass was coloured red, it was just more or less coloured. The red colour indicates that the sample was PHA positive. The biomass with the brightest red colour, most PHA positive, was the finger-shaped zoogloeal bacteria and the filaments, see *Figure 12.8* and *Figure 12.9*.

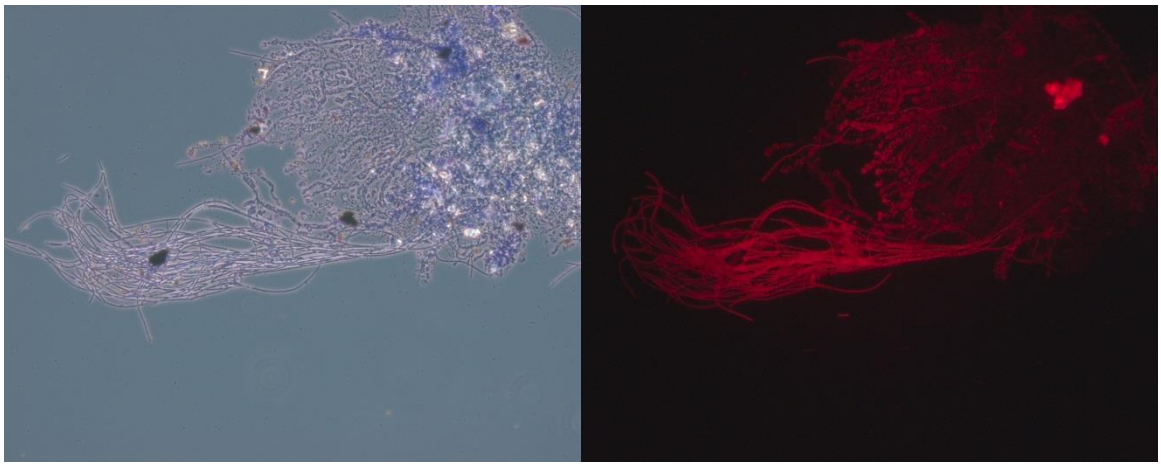

Figure 12.9. Biomass scraped off from carriers taken from the process 51 days after start-up, stained with Nile blue and examined with fluorescence microscope. Left: A floc with finger-shaped zoogloeal bacteria and filaments studied with phase contrast. Right: The same floc studied with fluorescence.

At this time, both the anaerobic phosphate release and aerobic phosphate uptake were too low to indicate bio-P activity in the process. Therefore it is most probable that the PHA-positive microorganisms were GAOs.

12.6 Polyphosphate in the biomass

PAOs store polyphosphate intracellularly as an energy supply (Christensson, 1997, pp. 11). Neisser staining of biomass scraped off from carriers was conducted to see if the biomass contained any polyphosphate. The results from the sample taken in the end of an anaerobic phase 51 days after start-up can be seen in *Figure 12.10*. The result from the samples taken in the beginning of an anaerobic phase taken 52 days and 78 days after start-up can be seen in *Figure 12.11* and *Figure 12.12*.

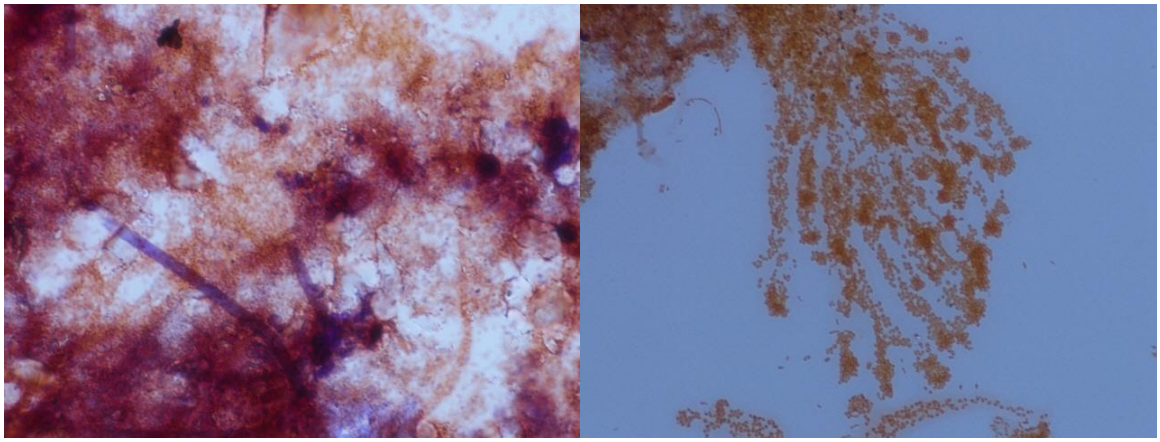

Figure 12.10. Biomass scraped off from carriers taken in the end of an anaerobic phase, Neisser stained and examined with light microscope. The sample was taken 51 days after start-up. Left: Neisser-positive filaments of type 0092. Right: Finger-shaped zoogloeal bacteria.

In the sample taken in the end of the anaerobic phase, some filaments were stained, see *Figure 12.10 Left*. The filament looking like a purple coloured tape was probably type 0092, and is often found in biological phosphorus removal plants (AnoxKaldnes, 2013, pp. 105). Few purple points was seen among the zoogloeal bacteria, see *Figure 12.10 Right*. The potential polyphosphate granules were expected to be more prominent in the end of an aerobic phase after the phosphate uptake and a new sample was therefore taken a few days later.

At day 52, only a few of the zoogloeal bacteria contained purple dots, possibly stained granules, and in one particular area the purple dots were more prominent, see *Figure 12.11 Left*. Purple dots can be seen in *Figure 12.11 Right* as well.

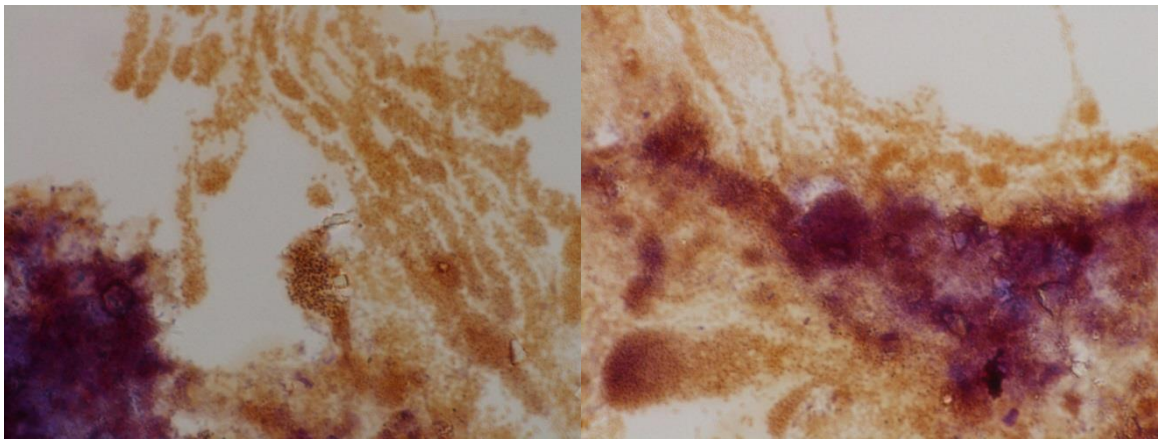

Figure 12.11. Biomass scraped off from the carriers taken in the beginning of an anaerobic phase, Neisser stained and examined with light microscope. The sample was taken 52 days after start-up. Left: A few Neisser-positive bacteria, possibly containing polyphosphate granules. Right: The floc contained a few microorganisms with stained granules. Some crystals can be seen in the floc as well.

Even though some microorganisms were stained they were very few. Additionally, the results did not look like PAOs usually do, neither the shape of the stained granules nor the morphology of the bacteria agreed with earlier findings (Tykesson, 2015).

The lower left corner in *Figure 12.11 Left,* was stained as well. The reason might be that the biofilm was thick and captured the colour even if it was not Neisser positive.

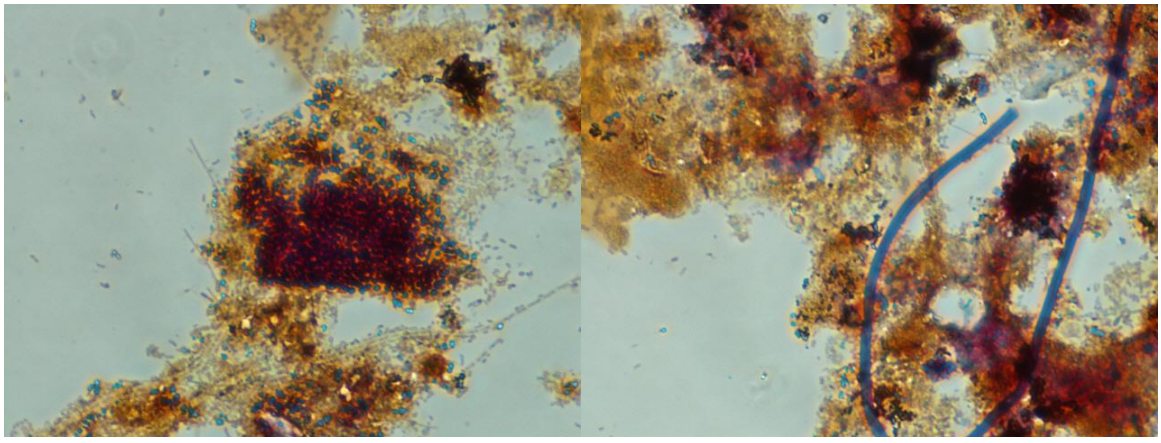

Figure 12.12. Biomass scraped off from the carriers taken in the beginning of an anaerobic phase, Neisser stained and examined with light microscope. The sample was taken 78 days after start-up. Left: Neisser-positive biomass containing polyphosphate granules. Right: Neisser-positive filaments of type 0092.

The Neisser-stained sample taken 78 days after start-up looked different from earlier samples. At this time the bio-P activity was several times higher than before, even though it still was low compared to in the literature. The biomass contained several Neisser-positive cells in the flocs, see *Figure 12.12 Left*. The stained cells were possibly PAOs. According to Tykesson (2015) the shape of the floc containing the stained cells looked like flocs with PAOs usually do. Filaments type 0092 found in the sample from day 52 was found in this sample as well, see *Figure 12.12 Right*.

When looking at the microscope pictures, small edgy square shaped crystals can be seen in most of the figures. These might be inorganic precipitants of phosphorus compounds. If the crystals are precipitated phosphate then the measured phosphate removal was not only due to biological activity. Then the already low phosphate removal rate by PAOs would be even lower.

To summarise the microscopic studies from around day 51, the finger shaped zoogloeal biomass was PHA positive. However, it contained close to no stained polyphosphate granules, except for some small areas that possible contained a little. These results, combined with morphology, suggest that the zoogloeal bacteria were neither *Tetrasphaera* nor *Accumulibacter* but potentially GAOs. This was accurate for the filaments as well. At this time, the bio-P activity in the process was very low.

The stained biomass in the flocs from the sample taken 78 days after start-up contained polyphosphate granules. The shape of the floc containing the stained cells resembled what biomass with PAOs usually looks like (Tykesson, 2015). Additionally, bio-P activity was measured in the process in the form of anaerobic phosphorus release. These results indicate that the stained cells were PAOs. However, no Nile blue staining was done to see if the cells were also PHA positive.

12.7 Summarised results

During the first 45 days, no bio-P activity in the form of anaerobic phosphate release was measured in the process. Therefore, three larger changes were implemented in the experimental setup and the way the process was operated. Day 45: The aerobic reactor with the main influent was rinsed in the beginning of the phase to ensure no sCOD was present in the reactors during the aerobic phases. Day 52: The temperature was decreased to 10°C from 15°C. Day 63: More counter ions in form of magnesium and calcium were added to the main feed.

After day 48 a very small, merely significant, anaerobic phosphate release and aerobic phosphate uptake was measured in the process. During this time, at day 51 and 52, the biomass on the carriers consisted mostly of finger-shaped zoogloeal bacteria and filaments. After microscopic studies, these microorganisms were found to contain PHA while the polyphosphate content was close to none, and therefore the finger-shaped zoogloeal bacteria and the filaments were thought to be neither *Accumulibacter* nor *Tetrasphaera*. Therefore some other microorganism, fewer in number, was behind the small bio-P activity.

After day 63, both the aerobic phosphate uptake and the anaerobic phosphate release increased. The biomass on the carriers was studied again on day 78 and 92. At day 78, the biomass looked different from earlier and was found to contain polyphosphate granules. The shape of the flocs containing the stained cells resembled to how biomass with PAOs usually looks. To the day 92, the amount of biomass on the carrier had increased, and almost all of the exposed biofilm area was covered with thin biofilm. The microscopic results combined with the increasing rates, showed that bio-P activity was achieved. However, the rates, except the anaerobic sCOD uptake rate, were lower than rates found in the literature. On the other hand, the rates were still increasing when the experiment was terminated, which indicated that higher rates could possibly be achieved.

13 Conclusions

In this master thesis work a laboratory-scale MBBR process for denitrifying phosphate removal was designed and operated. Several changes were made and evaluated during the project to achieve bio-P activity. The function of the process during the last period, day 48 and onwards, was evaluated along with the capacity of the process such as aerobic phosphate uptake rates, anaerobic phosphate release rates, and anaerobic sCOD uptake rates.

No phosphorus removal was achieved in the intended experimental setup.

The later achieved bio-P activity was supported by the following changes:

- Prevention of sCOD presence during the phosphate uptake phases
- Faster addition of the hydrolysis influent in the beginning of the anaerobic phase
- Temperature decrease from 15°C to 10°C
- Increased counter-ion addition

The temperature decrease or the addition of counter ions, or possibly a combination of both, was assumed to be the most important change for the achieved bio-P activity.

The laboratory work and analyses after the changes resulted in the following conclusions:

- The highest achieved aerobic phosphate uptake rate was 0.13 g/(m^2·d) which is 7 times lower than literature values.
- The highest achieved anaerobic phosphate release rate was 0.35 g/(m^2·d) while the release rate was 0.2 g/(m^2·d) at the highest measured aerobic phosphate uptake rate. These rates are 6 and 11 times lower than literature values, respectively.
- The anaerobic sCOD uptake rate was 6.4 g/(m^2·d) at the highest measured aerobic phosphate uptake rate, which was in the same range as literature values.
- Most of the consumed sCOD was not consumed by PAOs but potentially by GAOs.
- Most of the phosphate taken up was assimilated and used for growth and not for bio-P activity.
- Polyphosphate granules were found in the biomass.
- The rates were still increasing when the experiment was terminated and most probably higher rates can be achieved.

The final operation of the process was neither the intended nor suited for full-scale. It has not been investigated if denitrifying phosphorus removal can be achieved in the originally intended process after the temperature decrease and the counter-ion addition. Therefore, more studies have to be conducted on the intended denitrifying phosphorus removal process to enable evaluation of the potential of the process, and to determine if the treatment requirements can be met.

14 Suggestions for future work

During the project, several changes were made to achieve bio-P activity in the system. Some of these changes resulted in an experimental setup no longer comparable with a future potential full-scale process. It would be of interest to revert these changes when a stable bio-P activity is achieved to see if the original experimental setup is functional for denitrifying phosphorus removal. The most important changes that need to be reverted are the following:

- The rinsing period after the end of the anaerobic phase needs to be removed
- The effluent from the anaerobic reactor should be used as one of the influents to the next reactor
- Nitrate should be reintroduced in the main influent
- The currently aerobic phases should be unaerated and anoxic instead.

When the rinsing period is removed it is of interest to investigate the anaerobic sCOD uptake further, and use it to regulate the hydrolysis influent to ensure that the excess sCOD amount after the anaerobic phase is not too large.

It would be interesting to base the calculated rates on the amount of biomass in the reactors instead of the carrier surface. It would also be of interest to measure the phosphorus content in the biomass on the carriers and compare it with literature values.

If the project was to continue, more intricate mass balances should be made to obtain more accurate results. Differential equations could be one way to go since the process is semi-continuous. The influent volumetric flow rates need to be measured on the same day as the sampling to enable correct calculation of the incoming amounts. The sampling should be executed in all four reactors in the beginning of the sequence, 0 minutes, and in the end, close to 60 minutes.

It would be of interest to collect the final effluent and calculate the removal efficiency over the whole process. The concentrations in the final effluent should be compared to the treatment requirements, to evaluate if the process is sufficient. Furthermore, it would be interesting to use real municipal wastewater as influents to the setup instead.

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Appendices

I Phases in the sequence

During the project, the conditions in the phases P1-P4 were changed in form of which reactors that were aerated in which phases. The interval for how long each desired setting was used can be seen in *Table A.1*

Table A.1. The conditions in the phases P1-P4, in terms of aeration, were changed throughout the project.

Days from start	P1	P2	P3	P4
0	Unaerated	Unaerated	Unaerated	Aerated
14	Unaerated	Unaerated	Aerated	Unaerated
20	Unaerated	Aerated	Aerated	Unaerated
31	Unaerated	Unaerated	Aerated	Unaerated
45	Unaerated	Aerated	Aerated	Unaerated

The desired conditions in the phases, in term of anaerobic, anoxic and aerobic condition, were depending on the main feed composition and can be seen in *Table A.2*.

Table A.2. The desired conditions in the different phases depending on the composition in the main feed.

Days from start	P1	P2	P3	P4
0	Anaerobic	Anoxic	Anoxic	Aerobic
14	Anaerobic	Anoxic	Aerobic	Anoxic
20	Anaerobic	Aerobic	Aerobic	Deoxidising
27	Anaerobic	Aerobic	Aerobic	Anoxic
31	Anaerobic	Anoxic	Aerobic	Anoxic
45	Anaerobic	Aerobic	Aerobic	Deoxidising

II HACH Lange

Table A.3. List of used HACH Lange methods and the measuring range for these methods. If needed, the samples were diluted with distilled water in order to fall into the measuring range.

Analysis	HACH Lange method	Measuring range
COD	LCK 014	1,000-10,000 mg/l
	LCK 114	150-1,000 mg/l
	LCK 414	5-60 mg/l
NH_4-N	LCK 303	2-47 mg/l
	LCK 304	0.015-2 mg/l
NO_3-N	LCK 339	0.23-13.5 mg/l
PO_4-P	LCK 349	0.05-1.50 mg/l
	LCK 350	2-20 mg/l

III Feed composition

The composition in the feeds was changed several times during the project. The composition in the hydrolysis feed, see *Table A.4*, and the main feed, see *Table A.5*, during the different intervals. The ratio between acetate and propionic acid in the hydrolysis feed and the ratio between KH_2PO_4 and K_2HPO_4 in the main feed was the same throughout the whole project. The amount of trace element solution was constantly 2 ml/l and is therefore not shown in the table.

Table A.4. The desired concentration of sCOD in form of acetate and propionic acid, and the concentrations of sodium acetate, propionic acid, yeast extract, and peptone in the hydrolysis feed during the project.

Days from start	sCOD (mg/l)	Sodium acetate (g/l)	Propionic acid (ml/l)	Yeast extract (g/l)	Peptone (g/l)
0	150	0.19	0.04	0	0
6	340	0.432	0.09	0	0
13	500	0.635	0.13	0	0
14	800	1.016	0.21	0	0
15	340	0.432	0.09	0	0
16	170	0.216	0.05	0	0
18	1000	1.27	0.27	0	0
31	2000	2.54	0.53	0.45	0.45
34	2000	2.54	0.53	0.1	0.1
42	1000	1.27	0.27	0.05	0.05
45	2000	2.54	0.53	0.1	0.1

Table A.5. The desired concentration of phosphate, ammonium and nitrate in the main feed during the project. Additionally, the main feed contained 2 ml/l trace solution. After day 63 the main feed contained 0.048 g/l $CaCl_2 \cdot 2H_2O$ and 0.16 g/l $MgSO_4 \cdot 2H_2O$ as well.

Days from start	PO_4-P (mg/l)	NO_3-N (mg/l)	NH_4-N (mg/l)	KH_2PO_4 (g/l)	K_2HPO_4 (g/l)	$NaNO_3$ (g/l)	NH_4Cl (g/l)
0	10	30	0	0.024	0.028	0.182	0
13	10	5	0	0.024	0.028	0.03	0
20	10	0	3	0.024	0.028	0	0.011
22	10	0	6	0.024	0.028	0	0.023
23	10	0	10	0.024	0.028	0	0.038
27	10	3	10	0.024	0.028	0.018	0.008
31	10	6	10	0.024	0.028	0.036	0.038
34	10	10	10	0.024	0.028	0.061	0.038
37	10	15	5	0.024	0.028	0.091	0.019
43	10	8	0	0.024	0.028	0.076	0
45	10	0	5	0.024	0.028	0	0.019
48	20	0	9	0.048	0.056	0	0.034
49	20	0	20	0.048	0.056	0	0.076

IV Primary data

The measured concentrations in the main feed and hydrolysis feed can be seen in *Table A.6.*

Table A.6. The measured concentrations in the hydrolysis feed and main feed during the period day 2 to day 76. If no measurement was conducted, the cell is blank.

		Hydrolysis feed	Main feed		
Date of measurement	Time from start (days)	sCOD (mg/l)	PO_4-P (mg/l)	NO_3-N (mg/l)	NH_4-N (mg/l)
2015-02-26	2	154	11.7	32.0	
2015-03-02	6	341	11.5	31.5	
2015-03-04	8	349	11.4	31.2	
2015-03-09	13	515	11.6	32.5	
2015-03-10	14	511	11.4	5.3	
2015-03-11	15		11.4	5.4	
2015-03-12	16	799			
2015-03-13	17	341	11.3	5.3	
2015-03-16	20	176	11.4	5.3	
2015-03-17	21	970			
2015-03-18	22	966	11.2		3.1
2015-03-19	23				6.2
2015-03-20	24	960	8.2		8.4
2015-03-24	28	1000	11.2	3.3	10.2
2015-03-27	31	1000	10.9	3.2	2.0
2015-03-30	34	2688	11.9	5.9	10.4
2015-03-31	35	2292	11.3	10.2	10.5
2015-04-02	37	2284	11.0	10.0	10.1
2015-04-07	42	2192	11.9	16.2	5.4
2015-04-09	44	1079	11.4	8.3	
2015-04-13	48	2204	11.1		5.1
2015-04-14	49	2197	20.0		9.3
2015-04-16	51	2203	19.6		19.9
2015-04-24	59	2165	21.2		20.9
2015-04-30	65	2077	21.2		19.4
2015-05-11	76	2148	22.4	0.3	20.7

The raw data for concentrations for sCOD, NH_4-N, NO_3-N, and PO_4-P from all measurements during the period day 2 to day 96 are presented in Table A.7, *Table A.8*, *Table A.9* and *Table A.10*, respectively. Dissolved oxygen concentration, pH and temperature can be seen in *Table A 11*, Table A.12 and *Table A.13*, respectively.

Table A.7. The sCOD concentrations (mg/l) in the reactors 0, 10 and 45 minutes after the phase was initiated. If no measurement was conducted, the cell is blank.

		R1			R2			R3	R4
Date	Time from start (days)	0 min	10 min	45 min	0 min	10 min	45 min	45 min	45 min
2015-02-26	2			13.3					
2015-03-27	3			10.7			6.8		
2015-03-02	6								
2015-03-04	8								
2015-03-06	10								
2015-03-09	13								
2015-03-10	14			11.1			7.6	8.8	7.0
2015-03-11	15			56.6			15.2	11.3	13.3
2015-03-12	16			9.83			7.4	9.5	7.6
2015-03-13	17	6.48		11.5			6.6	7.4	6.8
2015-03-16	20			14.6			9.9	9.4	8.3
2015-03-17	21								
2015-03-18	22	9.78		40.6			8.3	9.9	9.6
2015-03-19	23								
2015-03-20	24			27.6			8.6	9.0	8.9
2015-03-24	28	8.22		31.4			8.5	9.7	8.7
2015-03-26	30								
2015-03-27	31	11.8		30.9			10.2	10.1	11.0
2015-03-30	34	39.1		84.4			62.7	33.9	34.9
2015-03-31	35	15	51.3	51.8			15.5	14.8	14.0
2015-04-02	37	18.6	63.8	63			30.4	13.2	11.9
2015-04-07	42	9.97	46.5	15.3			11.4	11.9	10.0
2015-04-09	44	15.4	53.3	52.5			16.6	15.1	12.3
2015-04-13	48	13.3	121	97.3	95.6	7.5	8.5	9.3	8.7
2015-04-14	49	16.9	122	102	84.1	7.8	9.6	9.1	8.7
2015-04-16	51	10.1	121	65.9	62.8	5.6	11.1	7.7	6.6
2015-04-24	59	9.22	114	78.8	70.6	8.0	11.9	8.2	9.1
2015-04-30	65	8.8	100	82.4	57.6	8.2	9.4	24.8	7.6
2015-05-11	76	9.77	113	73.6	74.2	8.7	10.3	6.7	9.1
2015-05-21	86		75.9	60.7					
2015-05-26	91	26.9	76.3	67.6					16.6

Table A.8. Ammonium concentrations (mg NH_4-N/l) in the reactors 0, 10 and 45 minutes after the phase was started. If no measurement was conducted, the cell is blank.

		R1			R2			R3	R4
Date	Time from start (days)	0 min	10 min	45 min	0 min	10 min	45 min	45 min	45 min
2015-02-26	2								
2015-02-27	3								
2015-03-02	6								
2015-03-04	8								
2015-03-06	10								
2015-03-09	13								
2015-03-10	14								
2015-03-11	15								
2015-03-12	16								
2015-03-13	17								
2015-03-16	20								
2015-03-17	21								
2015-03-18	22	0.0		0.0			0.1	0.0	0.0
2015-03-19	23						1.6		0.3
2015-03-20	24			1.6			3.8	2.4	2.2
2015-03-24	28	2.9		2.5			4.4	3.4	3.1
2015-03-26	30								
2015-03-27	31	2.0		1.6			2.9	2.3	2.0
2015-03-30	34	5.1		5.0			6.6	5.4	5.3
2015-03-31	35	4.7	4.5	4.4			6.2	6.3	4.9
2015-04-02	37	4.1	4.0	3.9			6.0	5.3	4.9
2015-04-07	42	0.0	0.1	0.0			1.1	0.3	0.1
2015-04-09	44								
2015-04-13	48								
2015-04-14	49	0.6	0.7	0.5	1.0	0.0	1.3	0.9	0.5
2015-04-16	51	1.9	2.0	2.0	1.9	0.1	3.2	2.2	2.1
2015-04-24	59	2.1	2.1	2.2	2.1	0.1	3.4	2.3	2.3
2015-04-30	65	1.4	1.5	1.3	1.3	0.0	2.4	1.3	1.0
2015-05-11	76	0.9	1.0	0.8	1.1	0.1	2.4	1.4	0.7
2015-05-21	86								
2015-05-26	91								

Table A.9. Nitrate concentrations (mg NO_3-N/l) in the reactors 0, 10 and 45 minutes after the phase was started. If no measurement was conducted, the cell is blank.

		R1			R2			R3	R4
Date	Days from start	0 min	10 min	45 min	0 min	10 min	45 min	45 min	45 min
2015-02-26	2								
2015-02-27	3						27.4		
2015-03-02	6								31.8
2015-03-04	8						27.5		27.0
2015-03-06	10			25.9			27.2		26.8
2015-03-09	13			24.2			22.3	23.8	24.2
2015-03-10	14			0.3			3.2	2.3	2.5
2015-03-11	15			0.1			1.4	0.4	0.1
2015-03-12	16			0.1			3.3	2.0	1.5
2015-03-13	17	3.6		2.3			4.3	3.9	3.8
2015-03-16	20			0.6			2.1	1.7	1.1
2015-03-17	21								
2015-03-18	22	0.0		0.0					
2015-03-19	23								
2015-03-20	24			0.0				0.0	
2015-03-24	28	2.1		0.0			1.4	1.2	1.5
2015-03-26	30								
2015-03-27	31	1.9		0.0			1.3	1.4	1.8
2015-03-30	34	0.0	0.0	0.0			0.0	0.3	0.0
2015-03-31	35	0.6	0.0	0.0			0.4	0.0	1.3
2015-04-02	37	0.0	0.0	0.0			0.7	0.6	0.6
2015-04-07	42	8.3	5.8	0.0			4.1	3.9	5.6
2015-04-09	44	0.0	0.0	0.0			0.4	0.0	0.0
2015-04-13	48	0.0		0.0					
2015-04-14	49	0.0	0.0	0.0					
2015-04-16	51	0.4	0.0	0.0	0.0		0.4	0.7	0.3
2015-04-24	59	0.5	0.0	0.0	0.0	0.3	0.5	1.1	0.6
2015-04-30	65	0.8	0.0	0.0	0.0	0.0	0.6	1.1	1.2
2015-05-11	76	0.3	0.0	0.0	0.0	0.3	0.8	1.2	1.3
2015-05-21	86								
2015-05-26	91								

Table A.10. Phosphate concentration (mg PO_4-P/l) in the reactors 0, 10 and 45 minutes after the phase was started. If no measurement was conducted, the cell is blank.

		R1			R2			R3	R4
Date	Time from start (days)	0 min	10 min	45 min	0 min	10 min	45 min	45 min	45 min
2015-02-26	2								
2015-02-27	3						9.4		9.8
2015-03-02	6								11.1
2015-03-04	8			8.9			9.8		9.6
2015-03-06	10			8.9			0.0		9.4
2015-03-09	13			9.5			9.2	9.6	9.6
2015-03-10	14			8.8			9.6	9.5	9.5
2015-03-11	15			9.0			9.5	9.4	9.4
2015-03-12	16			8.7			9.6	9.6	9.4
2015-03-13	17	9.5		8.7			10.0	9.5	9.5
2015-03-16	20			8.3			9.2	9.1	8.7
2015-03-17	21								
2015-03-18	22	9.0		8.6			9.3	8.9	9.2
2015-03-19	23								
2015-03-20	24			6.4			6.9	6.7	6.9
2015-03-24	28	8.8		8.2			9.5	8.8	8.7
2015-03-26	30								
2015-03-27	31	9.5		8.9			9.3	9.3	9.4
2015-03-30	34	9.0		8.8			9.1	9.2	9.2
2015-03-31	35	9.3	8.7	8.9			9.4	9.2	9.2
2015-04-02	37	8.9	8.3	8.4			9.1	9.0	8.7
2015-04-07	42	9.8	9.3	8.5			9.4	9.8	9.7
2015-04-09	44	10.0	9.8	9.7			9.9	10.1	10.0
2015-04-13	48	2.1	2.1	2.3	2.3	0.1	2.0	2.1	2.1
2015-04-14	49	4.7	4.7	4.7	5.5	0.2	4.6	5.0	4.8
2015-04-16	51	4.3	4.2	4.4	4.4	0.3	4.6	4.4	4.5
2015-04-24	59	5.0	4.7	5.1	5.0	0.2	5.0	4.7	4.6
2015-04-30	65	4.3	4.1	4.4	4.2	0.2	4.2	4.0	4.1
2015-05-11	76	3.7	3.7	4.7	4.9	0.6	4.9	4.2	4.1
2015-05-21	86		3.6	5.3					
2015-05-26	91	4.2	4.4	6.1					4.7

Table A 11. The measured concentration of dissolved oxygen (mg/l) at 0, 10 and 45 minutes after the sequence began. If no measurement was conducted, the cell is blank.

		R1			R2			R3			R4		
Date	Day	0 min	10 min	45 min	0 min	10 min	45 min	0 min	10 min	45 min	0 min	10 min	45 min
2015-03-06	10			1.3			3.8						8.8
2015-03-09	13			0.9			0.7			0.3			6.9
2015-03-10	14			0.3			2.9			1.0			6.5
2015-03-11	15			0.2			1.8			7.4			2.7
2015-03-12	16			0.4						7.4			2.0
2015-03-13	17			0.4			2.6			7.3			1.2
2015-03-16	20			0.6			4.6			6.4			5.1
2015-03-17	21			0.5			1.6			6.7			2.3
2015-03-18	22			0.3			6.8			3.4			1.3
2015-03-19	23			0.4			2.5			5.6			0.5
2015-03-20	24			0.4			2.1			3.7			0.6
2015-03-24	28	0.6		0.5	2.2		4.8	6.4		5.3	2.0		0.8
2015-03-26	30			0.2			5.2			4.4			0.3
2015-03-27	31	0.3		0.3	1.6		3.8	4.2		4.7	5.2		2.9
2015-03-30	34	0.6		0.2	0.2		0.4	0.5		2.8	1.9		0.6
2015-03-31	35	0.5		0.3	0.4		0.1	0.2		0.2	1.3		0.4
2015-04-02	37	0.4	0.3	0.3	0.2	0.2	0.6	3.2	4.8	5.7	3.2	1.2	0.4
2015-04-07	42	0.4	0.3	0.3	0.3	0.3	0.6	1.9	3.9	4.5	4.4	1.8	0.4
2015-04-09	44	0.3	0.2	0.2	0.3	0.2	0.3	1.2	1.8	3.7	3.4	2.4	0.4
2015-04-13	48	0.3	0.4	0.2	6.0	7.4	4.9	6.9	7.5	7.4	3.8	2.3	0.7
2015-04-14	49	0.5	0.2	0.4	6.5	5.9	3.3	4.2	4.3	4.6	3.9	2.1	0.3
2015-04-16	51	0.4	0.4	0.1	7.0	5.8	2.3		3.0	3.4		0.6	0.4
2015-04-24	59	0.3	0.2	0.2	5.7	5.3	3.8	7.3	7.7	7.4	4.0	1.3	0.5
2015-04-30	65	0.3	0.3	0.2	5.4	6.8	6.6	6.0	6.3	3.5	4.1	1.3	0.3
2015-05-11	76	0.4	0.3	0.3	4.0	3.7	2.6	3.7	3.8	4.0	7.1	2.4	0.4

Table A.12. The pH in the reactors at 0, 10 and 45 minutes after the sequence began. If no measurement was conducted, the cell is blank.

		R1			R2			R3			R4		
Date	Day	0 min	10 min	45 min	0 min	10 min	45 min	0 min	10 min	45 min	0 min	10 min	45 min
2015-03-06	10			7.6			7.5			7.5			7.7
2015-03-09	13			8.0			8.1			8.2			8.2
2015-03-10	14			8.7			8.0			8.0			8.1
2015-03-11	15												
2015-03-12	16			8.3			7.9			7.9			8.0
2015-03-13	17			8.0			7.6			7.7			7.9
2015-03-16	20			7.1			7.2			7.3			7.4
2015-03-17	21			7.5			7.6			7.6			7.3
2015-03-18	22			6.8			7.2			7.4			7.5
2015-03-19	23			7.4			7.5			7.6			7.0
2015-03-20	24			7.5			7.6			7.7			7.0
2015-03-24	28	7.2		7.5	7.7		7.6	7.7		7.7	7.8		7.8
2015-03-26	30			7.7			7.3			7.4			7.4
2015-03-27	31	7.8		7.8	7.8		7.2	7.7		7.3	7.7		7.5
2015-03-30	34	8.0		7.9	8.3		8.2	7.9		7.7	8.0		7.8
2015-03-31	35	8.1		8.2	8.2		8.6	8.5		8.6	7.9		8.0
2015-04-02	37	8.9	8.9	8.8	8.9	8.9	8.8	8.9	8.9	8.8	8.9	8.9	8.8
2015-04-07	42	8.2	8.3	9.0	8.9	8.9	8.4	8.2	8.3	8.2	8.2	8.2	8.1
2015-04-09	44	7.4	7.9	7.9	8.0	8.2	8.2	7.9	8.4	8.0	7.9	8.2	8.0
2015-04-13	48	7.0	7.0	7.3	7.3	7.1	7.5	7.5	7.4	7.7	7.0	7.5	7.6
2015-04-14	49	6.9	7.0	7.0	7.1	7.2	7.1	7.3	7.4	7.2	7.2	7.4	7.3
2015-04-16	51	6.6	7.1	7.5	6.8	7.2	7.4		7.3	7.4		7.3	7.4
2015-04-24	59	7.5	6.9	7.3	7.7	7.5	7.4	7.7	7.6	7.5	7.6	7.5	7.2
2015-04-30	65	7.4	7.2	7.5	7.6	7.4	7.5	7.6	7.6	7.3	7.4	7.3	7.3
2015-05-11	76	7.2	7.1	7.4	7.3	7.4	7.3	7.5	7.6	7.4	7.4	7.4	7.5

Table A.13. The temperature (°C) at 0, 10 and 45 minutes after the sequence began.

		R1			R2			R3			R4		
Date	Day	0 min	10 min	45 min	0 min	10 min	45 min	0 min	10 min	45 min	0 min	10 min	45 min
2015-03-06	10			14.8			15.4			16			15.9
2015-03-09	13			14.6			15			14.5			14.2
2015-03-10	14			14.2			14.1			15.2			14.9
2015-03-11	15			14.3			14.7			15.3			
2015-03-12	16			14.4			14.1			16.5			14.8
2015-03-13	17			15.4			15.4			15.4			15.2
2015-03-16	20			13.7			13.7			13.6			13.3
2015-03-17	21			13.4			14			14			14.1
2015-03-18	22			14.4			14.3			14.3			13.9
2015-03-20	24			13.6			13.6			13.7			15.2
2015-03-24	28	15.4		15.2	15.3		15.6	15.4		15.3	15.3		14.7
2015-03-26	30			14.4			15.9			15.4			15.2
2015-03-27	31	14.7		14.5	15.1		15.8	15.1		15.6	13.5		15.4
2015-03-30	34	15.1		14.8	14.7		15	16.4		15.7	15.7		15.4
2015-03-31	35	14.9		14.7	14.7		14.8	14.4		14.8	15.7		15.4
2015-04-02	37	15.3	14.8	14.9	14.6	14.8	15.4	14.5	14.7	15.2	14.6	14.7	14.8
2015-04-07	42	15	14.4	14.3	14.9	15	15.7	15	14.8	15.2	15	14.8	14.6
2015-04-09	44	15.1	14.6	14.5	14.8	14.8	14.9	15.1	14.9	15.4	15	15	15.3
2015-04-13	48	15.2	15.2	15	19	16.7	15.2	14.9	14.7	14.9	15.1	15.1	15.4
2015-04-14	49	15.2	15.1	15.5	18.2	16.9	15.5	15	14.8	15	14.6	14.8	15
2015-04-16	51	15.2	15.2	14.9	16.8	16.2	16.3		15.1	15.8		15.6	15.7
2015-04-24	59	10.6	10.3	10.6	12.8	10.9	10.7	9.9	9.6	10.2	10.8	10.2	11.7
2015-04-30	65	10.4	10.2	9.9	13.5	11	10.2	11.1	10.3	10.9	10.4	10.2	10.7
2015-05-11	76	11	10.8	11.5	16.9	13	11.2	10.5	10.2	10.7	10.5	9.8	10.6

V Populärvetenskaplig sammanfattning

Rening av avloppsvatten från fosfor med bakterier som växer på plastbitar

En ny metod för rening av avloppsvatten från fosfor har designats och utvärderats. Reningsprocessen utnyttjar en speciell bakteries strategi för överlevnad för att på ett miljövänligt sätt rena vattnet från fosfor. Det nya med processen var att dessa bakterier kan växa på små plastbitar.

I takt med att antalet människor ökar så produceras även mer avloppsvatten som är rikt på näringsämnen. De här näringsämnena, där fosfor ingår, kan orsaka bland annat övergödning och syrebrist på bottnarna när de släpps ut i våra vatten. För att vi ska kunna fortsätta njuta av svalkande bad i sjöar och hav utan algblomning under sommaren, krävs det att avloppsreningsverken körs effektivt för att säkerställa låga utsläpp av näringsämnena till våra vatten. Dessutom är det bra om energiåtgången och miljöpåverkan vid reningen kan minimeras.

Metoden som den här reningsprocessen är baserad på, har sin grund i att speciella bakterier tar upp mer fosfor från avloppsvattnet än vad de behöver. De använda bakterierna växer på plastbitar, så kallade bärare, som rör sig fritt i avloppsvattnet. Bakterierna bildar en tunn hinna på bärarna, som kallas för biofilm. Den här typen av process kallas därför biofilmsprocess med rörliga bärare. Avloppsvattnet renas genom att bakterierna äter upp fosforn och förökar sig. När bakterierna blir fler på bärarna kommer en del att lossna och de här bakterierna separeras från vattnet. Processen finns redan för andra typer av bakterier och för rening av andra näringsämnen och fungerar mycket bra.

Det kan finnas flera fördelar med metoden med bakterier på bärare för rening av fosfor jämfört med de i dagsläget använda metoderna där de speciella bakterier lever fritt i vattnet, i så kallade aktivt slam-processer, eller där kemikalier används för att rena vattnet från fosfor. Några av fördelarna är att en sådan anläggning skulle kunna ta mindre plats jämfört med aktivt slam-processer samt att mindre mängder kemikalier används jämfört med processer med kemisk fällning.

En försöksuppställning i laboratorieskala har byggts för att testa om det är möjligt att rena avloppsvatten från fosfor med hjälp av bakterier som växer på bärare. Vattenreningsmetoden utvärderades genom att mäta hur aktiva bakterierna var. I försöksuppställningen som testades först uppmättes inte den önskvärda aktiviteten som fås av den speciella typen av bakterier. Detta betydde att processen inte fungerade och att bakterierna saknade något. Därför ändrades styrningen av processen. Dessutom ändrades innehållet i det konstgjorda avloppsvattnet som användes för att testa processen för att mer likna riktigt avloppsvatten. Några dagar efter ändringarna började bakterierna bli aktiva. Ytterligare en tid senare var de ännu mer aktiva. Detta betyder att den nya sortens process för rening av avloppsvatten från fosfor med bakterier som växer på bärare, fungerar. Dessvärre var den uppmätta aktiviteten lägre, och därmed sämre, än vad som fåtts i andra liknande experiment. Men tiden tog slut och försöket avslutades innan processen utvärderats när den fungerade som bäst. Därmed är det högst troligt att högre aktivitet skulle kunna fås. Det krävs fler experiment för att kunna säga om reningsprocessen tar bort tillräckligt mycket fosfor för att det utgående renade vattnet ska klara kraven som finns.

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