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Biological Phosphorous Removal in a Continuous MBBR

Daily monitoring, observations and kinetic experiments during the start-up phase

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Preface

The work carried out in this thesis is done at the Department of Hydraulic and Environmental Engineering at the Norwegian University of Science and Technology in the spring of 2018.

I would like to send a great deal of gratitude to my supervisor Professor Stein Wold Østerhus for all his guidance and help throughout the semester, and his patience when helping me understand and discuss. I have been lucky to be able to write my master thesis in an incredibly interesting field and have enjoyed the process thoroughly. The Department of Hydraulic and Environmental Engineering here at NTNU has been helpful and always helped fix any administrative questions efficiently.

The most exciting and enjoyable part of this process has been to be able to work so closely with my co-supervisor postdoc. Blanca Magdalena Gonzales Silva. She has so much knowledge and valuable viewpoints on everything I have done, and I have learned a lot from interesting discussions with her. She is very positive and gives great guidance and advice, and I hope we get the chance to work with each other again.

This semester has thought me much about what it means to work in a lab and how entertaining, demanding and fun this could be. There is no doubt that this has been one of my most enjoyable semesters throughout my time at NTNU due to all the practical experiences I have garnered.

A huge thanks to Chunbo He, Abaynesh Belay Fanta and Sina Shaddel; doctoral candidates at NTNU for always answering questions about both theory and practical experimental conditions. But mostly thanks for being kind and helpful and making the time spent in the lab social.

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Abstract

My master thesis is a small part of a larger project called RECOVER. The overall aim of RECOVER is to research the sustainability and reusability of wastewater resources. This master thesis investigates biological removal of phosphorous from wastewater, which is only a small part of the RECOVER researching carbon, phosphorous and nitrogen Recovery in wastewater.

There is an increasing concern for the sustainable delivery of adequate phosphorous resources in the future. Depleting storages of phosphate rock has created a necessity for establishing innovative technology that can help deliver phosphorous in adequate measures. With increasing populations, we need to find sustainable ways to produce phosphorous to meet requirements of food-security. Increasing concentrations of wastewater and industrial water, especially in urban areas, also makes up risks for the sustainability of aquatic habitats and ecosystems. Phosphorous loading into these systems that exceeds their bearing capacity make up a risk for eutrophication and toxicity in these waters. There is already unmistakable evidence of this many places today. Therefore, we need to run our wastewater treatment efficiently and sustainably. Innovative solutions and research into sustainable ways for phosphorous removal will maybe help us solve both beforementioned problems.

Phosphorous can be removed both chemically, physically and biologically. Many WWTP in the world removes phosphorous biologically, and current standard practice for bio-P removal is to use activated sludge processes. Bio-P processes using biofilm with suspended carriers in an MBBR-process is currently not used in full scale, however research on this field is ongoing. There are advantages to the process of Bio-P removal in MBBR some being the reduced need for space and easier handling of sludge. To utilize biological processes for phosphorous removal has many advantages to chemical precipitation like reduced costs for chemicals and an end-product of phosphorous that is not chemically bound.

The Bio-P process in a MBBR does however have some challenges regarding its process as alternating anaerobic and aerobic conditions are required. This necessitates high quality demands for the process design and a better understanding of the operational parameters affecting the process then what we currently have. As the EBPR process is environmentally friendly, sustainable and cost-effective research into possibilities for the implementation of this process in a controlled environment is needed.

The daily monitoring of DO, Temp, pH, was useful tool to observe the changes in the quality of the influent wastewater and use this information to understand process performance better. The main results from evaluation of the daily influent and effluent concentrations of phosphorus, ammonium and sCOD showed signs of the process being affected by dilution in the water. A potential potential shift in the microbial population before and after dilution due to a sudden shift in parameters of substrate feeding, DO, T and pH may have been experienced.

Based on results from kinetic experiments in beaker with wastewater and acetate it was almost zero net consumption of sCOD. Removal rate within the anaerobic chambers were on average is 0.00047 mg sCOD/m^2*hr, yet EBPR performance was observed. This is potentially due to a different strain of bacteria inhibiting the biomass than what was previously expected. It was

observed that PAOs present in the cMBBR instead potentially ferment other more complex organic molecules.

PAOs survived the low organic loading, thou the anaerobic activity and net uptake decreased. Potentially due to focusing all their energy on survival and growth, outcompeting GAOs. This is positive results regarding initiating this process in the average Norwegian treatment plants as low organic carbon loading is a recurring difficulty to overcome, but it seems PAOs can be competitive.

The biomass has shown the ability to utilize glucose in the anaerobic zone with consequently increased P-removal, however net uptake was not improved when glucose was used at the sole carbon source.

Results from beaker experiments show P release rates in range of 0.6 – 4.7 mg PO4-P/m^2*hr and P uptake rates between 1 and 8.2 mg PO4-P/m^2*hr which is significantly lower than comparable literature.

The results also show a strong correlation between temperature and increased rate of release and uptake with a temperature coefficient of 1.077 for anaerobe release of acetate and 1.031 for glucose.

The fact that there is documented P-removal occurring in the pilot shows signs that the biomass has been able to establish itself in a way where it is able to thrive and grow despite conditions being far from what literature states as optimal for EBPR.

Sammendrag

Min masteroppgave er en liten del av et større prosjekt kalt RECOVER. Målet med RECOVER er å bedre utnyttelsen av avløpsressurser. Denne masteroppgaven omhandler biologisk fjerning av fosfor i avløpsvann, som bare er en liten del av prosjektet som overordnet ser på mulighet for karbon, fosfor og nitrogen-gjenvinning i avløpsvann.

Det er en økende bekymring rundt tilgangen på tilstrekkelige fosforressurser i fremtiden. De reduserte lagrene, og det reduserte minedriftspotensialet av denne ressursen har skapt et behov for å etablere innovativ teknologi som kan bidra til å levere tilstrekkelige mengder fosfor i fremtiden. Befolkningen øker, og vi må ha bærekraftige måter å produsere fosfor på for å tilfredsstille kravene til økt matproduksjon. Økende konsentrasjoner av avløpsvann, særlig i byområder, utgjør også risiko for bærekraften i akvatiske habitat og økosystemer. Fosforbelastning inn i akvatiske system, som overskrider deres bæreevne, utgjør en risiko for eutrofiering og toksisitet i disse akvatiske miljøene. Det er allerede umiskjennelig bevis på dette mange steder i dag. Nødvendigheten av å rense avløpsvannet vårt effektivt og bærekraftig er derfor på dagsorden. Innovative løsninger og forskning knyttet mot bærekraftige måter for fosfor fjerning vil kanskje hjelpe oss med å løse begge de ovennevnte problemene.

Fosfor kan fjernes både kjemisk, fysisk og biologisk. Mange steder i verden fjernes fosfor biologisk i dag. Dagens praksis for biologisk fosforfjerning er å bruke aktivslamprosesser. Bio-P prosesser som bruker biofilm med suspenderte bærere i en MBBR-prosess brukes for tiden ikke i fullskala, men forskning på dette feltet pågår. Noen fordeler med Bio-P fjerning i MBBR er det reduserte behovet for plass samt enklere håndtering av slam. Benyttelse av biologiske prosesser for fjerning av fosfor har mange fordeler framfor kjemisk utfelling, som reduserte kostnader for kjemikalier og et sluttprodukt av fosfor som ikke er kjemisk bundet.

Bio-P-prosessen i en MBBR har imidlertid noen utfordringer da alternerende anaerobe og aerobe forhold kreves. Dette setter høye krav til prosessdesign og en bedre forståelse av driftsparametre som påvirker prosessen enn hva vi for øyeblikket har.

Ettersom EBPR-prosessen er miljøvennlig, er det nødvendig med en bærekraftig og kostnadseffektiv undersøkelse av mulighetene for gjennomføring av denne prosessen i et kontrollert miljø.

Den daglige overvåking av DO, Temp, pH var nyttig verktøy for å observere endringene i kvaliteten på avløpsvannet og bruke denne informasjonen til å forstå prosessytelsen bedre. Hovedresultatene fra evaluering av de daglige inn og ut konsentrasjonene av fosfor, ammonium og sCOD viste tegn på at prosessen ble påvirket av fortynning i vannet. Et potensielt skiftte i mikrobiell populasjon før og etter fortynning på grunn av et plutselig skifte i parametere av substrat, DO, T og pH kan ha blitt opplevd.

Basert på resultater fra kinetikk eksperimenter med avløpsvann og acetat var nettoforbruk av sCOD nesten lik 0. Fjerningsgrad i de anaerobe kamrene var i gjennomsnitt 0,00047 mg sCOD/m^2*hr, men EBPR-ytelse ble observert. Dette skyldes potensielt en annen streng av bakterier som opptar biomassen enn det som tidligere var forventet. I stedet ble det observert at PAOer tilstede i cMBBR og potensielt fermenterer andre mer komplekse organiske molekyler.

Observasjoner tilsier at PAO overlevde lav organisk belastning, men anaerob aktivitet og netto opptak ble redusert. Dette er potensielt grunnet at PAOene måtte fokusere all energi på

overlevelse og vekst, samt utkonkurrering av GAOer. Dette er positive resultater når det gjelder igangsetting av denne prosessen i gjennomsnittlige norske behandlingsanlegg, da lav organisk karbonbelastning er et gjentakende fenomen og det ser ut til at PAOer kan være konkurransedyktige under disse forholdene.

Biomassen viste evnen til å utnytte glukose i den anaerobe sonen og dermed økt P-fjerning, men netto opptaket ble ikke forbedret når glukose ble brukt som den eneste karbonkilden.

Resultatene fra kinetikk eksperimenter viser at rate for P slipp ligger i området 0,6 til 4,7 mg PO4-P/m2*hr og P opptak mellom 1 og 8,2 mg PO4-P/m2*hr. Dette er signifikant lavere enn sammenlignbar litteratur.

Resultatene viser også en sterk korrelasjon mellom temperatur og økte slipp- og opptaksrater i eksperimenter. En temperaturkoeffisient på 1,077 for anaerobe slipprater ved dosering av acetat ble funnet, og 1,031 for glukose.

Det faktum at det er dokumentert P-fjerning i piloten, viser tegn på at biomassen har vært i stand til å etablere seg på en måte der den kan trives og vokse til tross for at forholdene ligger langt fra hva litteraturen tilsier som optimale.

List of Abbreviations

BOD	Biological Oxygen Demand
bsCOD	Biodegredable Soluble Chemical Oxygen demand
rbCOD	Readily Biodegradable Chemical Oxygen Demand
(s)COD	(Soluble) Chemical Oxygen Demand
DNPAOs	Denitrifying PAOs
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
GAOs	Glycogen Accumulating Organisms
MBBR	Moving Bed Biofilm Reactor
N2	Nitrogen gas
NH4-N	Ammonium
NO3-N	Nitrate
OHOs	Ordinary Heterotrophic Organisms
Ortho-P	Orthophosphate
PAOs	Polyphosphate Accumulating Organisms
PHA	Poly-Hydroxy-Alkanoate
PHB	Poly-Hydroxy-Butyrate
PO4-P	Phosphate
Poly-P	Polyphosphate
tCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved Solids
TP	Total Phoosphorous
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

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1 Introduction

This is a project report based of the work I have done during my master thesis in the spring of 2018 contributing to 30 ECTS. This report is a review of relevant literature regarding the work I have done in the wastewater laboratory, at the Department of Hydraulic and Environmental Engineering, during my master thesis as well as a documentation of methods used for experiments, the results from these experiments and discussion surrounding the results and future work. This document will serve as a tool when an article about the continuous MBBR, situated in the wastewater laboratory here at NTNU, is no longer in its start-up phase can be published.

My aim for my master thesis has been to document the start-up phase of the Continuous MBBR set up here at NTNU and help with delivering results that can lead to optimizing the process of removing phosphorous in a continuous MBBR. This report documents, to a certain degree, the state of the art in many of the relevant processes for the work on the Continuous MBBR as well as the status of the pilot throughout the period of 25.01.2018 to 31.05.2018. that can be of relevance for all who are engaged and working in this project.

Some of the literature review presented here was part of the preliminary work for my master thesis and handed in as a report before I started my laboratory work.

1.1 Background for phosphorous removal

Phosphorous and nitrogen stems from many sources such as detergents, fertilizers and human and animal waste (Al-rekabi, 2015). Phosphorous is present in all organisms, plants and all biological materials. Agricultural and urban activities are however the leading causes for aquatic ecosystems being excessively loaded with nutrients. The excessive loading of recipient waters with phosphorous did for many decades clearly deteriorate receiving freshwater bodies. The awareness to reduce this loading has in the last decades been put on the agenda and affected legislation has been set in place to reduce the impacts nutrient loading has on ecosystems. Implementation of laws and regulations regarding the usage of especially phosphorous in products has however helped decrease the excessive nutrient loading in lakes (Carpenter et al., 1998; Allan et al., 1997).

One of the most important nutrient for growth is phosphorous. It stimulates growth of algae and other microorganisms that are photosynthetic. One of these algae is the cyanobacteria (blue-green algae) (Carpenter et al., 1998).

Some organisms being able to proliferate at the expense of others in a waterbody can stem from the levels of nitrogen (N) and phosphorous (P) being excessive. Nutrients act as a pollutant in freshwater ecosystems when their levels are evaluated to be excessive, meaning they exceed the natural levels the aquatic ecosystem is able to handle (Pease et al., 2010). Excessive levels of nutrient in aquatic ecosystems can in effect causes rapid algae growth. This in turn demands a higher amount of dissolved oxygen because the microbial community utilizes this in the decomposition process of algae (Faucette et al., 2014; Carpenter et al., 1998). The process of

increased algae growth and the effect this has on aquatic ecosystems is what is called eutrophication (Carpenter et al., 1998; Vymazal, 2007).

To avoid the negative effect on ecosystem, as the one eutrophication creates, our excess input of phosphorous must in effect be removed from wastewater so that the load into aquatic ecosystems is not above the ecosystems bearing capacity. There are different ways to remove phosphorous from the wastewater one being the removal through biological methods and another being chemical precipitation. However, within both methods there are several process configurations that operators can use. These differing configurations can all remove phosphorous with different input parameters, operating conditions and at vastly differing costs.

In wastewater we find phosphorous as soluble, particulate or organically bound phosphorous. The orthophosphate fraction is soluble. Depending on what is the solution pH we can find this phosphorous in different forms like phosphate ions or phosphoric acid. Polyphosphate is particulate, but can be converted to phosphate through hydrolysis or by biological activity. Polyphosphate will not be precipitated out of the wastewater by chemical precipitation (Al-rekabi, 2015). Organic P can be converted though microbial decomposition (Minnesota Pollution Control Agency, 2007).

1.2 Chemical phosphorous removal

There is little doubt that the dominant removal process for phosphorous in wastewater has been through chemical precipitation, at least in Norway. The efficiency of the method and the easily controllable and well-known technology involved is some of the drivers for this process being commonly used for phosphorous removal. A high removal degree within a controlled environment is a large advantage. Especially in regions where the outlet requirement of phosphorous to receiving aquatic systems is so low that it is hard to accurately achieve without reliability in the process. During a conversation with operators at NRV/NRA during Karriæredagene here at NTNU they informed me that because of the instability of the biological process, the necessity to use chemical removal methods is completely necessary as their outlet requirements are too low to use biological methods of removal with the current technology (NRV/NRA, 2017). The large sludge production, that mainly stems from the fact that a large quantity of chemicals need to be added, the alleviated cost of chemicals and transportation of these, and lastly the high metal content in the sludge are considered as the main reasons for looking into the developing of new and better technology (Herman Helness, 2007). Chemicals used are either lime or metal-salts (US-EPA, 2010, ch. 3.2). The chemical precipitation only removes the phosphate fraction, which is normally between 50-80 percent of the Total Phosphorous (TP) in the wastewater (US-EPA, 2010, ch. 3.3.1)

Through the addition of either metal-salts as iron and aluminium, which are the two most commonly used, the precipitation mechanism for removal of phosphate is as follows:

$$Al^{3+} + PO4^{3-} <-> AlPO4 \qquad \text{(Eq. 1.1)}$$

$$Fe^{3+} + PO4^{3-} <-> FePO4 \quad \text{(Eq. 1.2)}$$

Phosphorous is removed from the wastewater though the addition of chemicals and the subsequent precipitation which in turn leads to a high degree of sludge to dispose of (US-EPA, 2010, ch. 3.5). Another disadvantage of the chemical precipitation of phosphorous is that in this process the phosphorous will be chemically bound and be a part of the sewage sludge. The sewage sludge is handled many ways throughout the world today, however in Norway it is commonly disposed on agricultural land after hygenization and stabilization. Treatment facilities currently using biological removal of phosphorous does however, to improve sludge separation properties and comply with regulations for outlet phosphorous requirements, use metal salts in the process today. This will decrease the potential for phosphorous recovery as a fertilizer (Saltnes et al., 2016).

One issue with the phosphorous being chemically removed in the sludge and then disposed of on agricultural land is that it will not be available for plant uptake as it is metal bound, or it will affect the uptake rate in plants, and this will most probably be slower. The rate of uptake in plants is also dependant on the metal-salts used (Minnesota Pollution Control Agency, 2007; Abdi, 2012). There are also discussions concerning the dosage of metal-salts, and if we reduce the dosages, which was discussed on the half-year seminar in the Recovery project, the plant-uptake could be substantially higher without diminishing the removal rate. Phosphorous that will not be available for plant uptake, or to a smaller extent then soluble phosphorous, can when disposed of on agricultural land be transported to an aquatic environment during precipitation events. This is of course also a possibility for soluble P, however considering the increased plant availability, the probability it will be taken up by plants leads to a decreased risk. When transported to an aquatic environment, sedimentation of particulate bound phosphorous can lead to it changing form in response to the change in environmental conditions (Minnesota Pollution Control Agency, 2007). The decomposition of the particle bound phosphorous can make it available for uptake by algae and the process of eutrophication will be enabled (Carpenter et al., 1998; Allan et al., 1997). The uncertainty considering the nutrient transport with runoff and sediment transportations during precipitation events makes it difficult to assess how much of the phosphorous that will be transported away from the non-point source to aquatic ecosystems nearby (Pease et al., 2010). However, studies show that there is severe loading of nutrients from agricultural and non-point sources that negatively affect the receiving waters. Pollutant loading must be dependent on factors surrounding the delivery of water over some large area. (Allan et al., 1997; Carpenter et al., 1998; Ye et al., 2016). By removing and recovering phosphorous through the biological process removal some of the highly negative effects of the disposal of sewage sludge on the surrounding environment can be reduced (Ye et al., 2016).

This way removing phosphorous biologically can not only minimize the production of sludge needed to be disposed of, the phosphorous will hopefully also be able to be taken out as a separate and more usable product. This phosphorous can then be sold as a product by and within itself and may in the future be able to work as a substitute for phosphate rock. (Helness, 2007; Ye et al., 2016; Mayer et al., 2016).

Because of the newfound interest associated with recovery of phosphorous as a resource, and an important one at that methods for it to be biologically removed is needed. Enhanced biological phosphorus removal (EBPR) promotes the removal of P from wastewater without the need for chemical precipitant (Oehmen et al., 2007)

1.3 Lack of phosphorous as a resource

There are many reasons for why researchers now have chosen to look into not only the removal of phosphorous but also now has started valuing it as a crucial resource to recover. The depleting mining resources of phosphorous has been of great concern for a long time. Different estimates made based of off the worlds current mining potential and easily available phosphorous as phosphate rock has been made in various forums for a long time. However, many different sources refers to phosphorous to be almost depleted as a mining-resource by the end of the century (Cordell et al., 2011).

Since the global market for phosphorous today mainly is a product of the mining production of phosphorous, phosphorous is considered a non-renewable resource. Growing populations leads to a need for increased global food-production and the need to establish secure development of agricultural products (Ye et al., 2016). Yuan et al., (2012) states that the potential in recovering phosphorous from municipal wastewater in theory can accounts for 15 to 20% of the global phosphorus demand.

One of the large issues regarding phosphorous as a depleting resource, while the demand for the product increases is that this affects the global price of the good. This can and will most probably lead to an even more unjust redistribution of a highly important product between countries in the world than it is today (Cordell et al., 2011). Many countries can suffer from a skewed distribution and availability of phosphorous. By stimulating markets through subsidies, taxes and/or investment grants there is a possibility to shift the markets to look for more renewable phosphorous resources and facilitate in a transitions (Cordell et al., 2011). By researching innovative solutions for recovering phosphorous from sewage we can create sustainable solutions and take more advantage of the possibilities and benefits of a circular economy (Cordell et al., 2011; Genovese et al., 2017; Mayer et al., 2016) as well assisting developing countries with highly important technology. This can contribute to developing countries being more self-sufficient in regard to phosphorous production and not be so highly dependent on the global, as the price of this good is expected to increase over time. We must work towards a more equitable distribution of phosphorous across the world (Cordell et al., 2011).

1.4 Scope of the study and outline for the work

The scope of this study is to do experiments on the continuous MBBR pilot at NTNU, document the methods used as well as findings and discuss results and future work. The experiments includes documenting the progress of the reactor as well as performing experiments to see how the process could be optimized. The scope also includes reviewing literature and to understand our current state of knowledge and technology surrounding EBPR and what operational

parameters that affect this process. There are many current process solutions capable of performing EBPR. The current literature reviewed on topics that is affecting the drivers of EBPR has in turn been used to understand results and findings from the lab work, as well as to adapt and create an optimized process for recovery of phosphorous in the continuous MBBR pilot at NTNU. In total 30 kinetic experiments has been performed in addition to the daily measurements of parameter within the pilot and of its influent and effluent concentrations.

2 Enhanced Biological Phosphorous Removal (EBPR) - Literature Review

The Enhanced Biological Phosphorous Removal (EBPR) process refers to the removal of phosphorous from wastewater through a biological process utilizing microorganisms that goes under the collective name of Polyphosphate Accumulating Organisms (PAO). These organisms has the capability of assimilating phosphorous to a much larger degree than Ordinary Heterotrophic Bacteria (OHO) (Yuan et al., 2012; Mino et al., 1998). Their ability to take up carbon sources in the anaerobic stage without the presence of an external electron acceptor makes them favourable for this process, although some other organisms such as Glycogen Accumulating Organisms (GAO) also inhibits this ability (Mino et al., 1998). Biological Nutrient Removal (BNR) is normally a collective name that refers to the combination of biological nitrogen removal and the EBPR process under one, and not the removal of phosphorous through chemical precipitation (Oehmen et al., 2007).

Municipal Wastewater can have many distinct characteristics and different influent loads, however the ranges of phosphorous in wastewater are typically between 4 and 8 mg P/l (Al-rekabi, 2015; US-EPA, 2010, Ch.5.2). In wastewater we will find phosphorous either as Phosphate/Orthophosphate (PO_4^{3-}), Polyphosphate (Poly-P) or organically bound phosphorous. Differences in amount of industry connected to the treatment facility, regulations regarding detergent usage and production and if the sewer system is separate or not are some factors that affects the loading of phosphorous in the treatment plants. In municipal wastewater the 50-80% phosphorous is mainly in soluble form as orthophosphate (US-EPA, 2010).

Mihelcic et al., (2011) sates that phosphorous produced pr. capita in Norway annually is between 0.55 and 0.62 kg. This stems from both urine and feces but will vary depending on the type of food and it origin.

Microorganisms affects the removal in the liquid buld through assimilation and other biological processes (Ødegaard et al., 2001). During growth periods phosphorous is assimilated in the organisms removing it from the water bulk. Some phosphorous will therefore be removed by OHO in a regular biological COD and N-removal system through assimilation (Seviour et al., 2003). The EBPR process is however especially useful for removing phosphorous as it utilises bacteria that enhances this process under alternating conditions (Kristiansen et al., 2013). These alternating conditions are essential for the EBPR to work.

The enhanced biological phosphorus removal (EBPR) process has been implemented in many wastewater treatment plants worldwide. It has shown satisfactory results regarding phosphorous removal from wastewater streams, so there is little doubt that the EBPR process indeed can be capable of efficient phosphorus removal (Al-rekabi, 2015). However, disturbances in the process which can lead to periods of insufficient P removal have been observed at full-scale plants on numerous occasions. Also under conditions seemingly favourable for EBPR (Oehmen et al., 2007; Zuthi et al., 2013). More research is needed to understand these disturbances.

In the EBPR process the bacteria that is referred to as PAO, or if nitrification and denitrification is performed, DPAO, is a collective name given for a string of different bacteria (Wong et al., 2013). The dominance of the PAO population in EBPR communities is achieved by the cycling

of the biomass through alternating anaerobic and aerobic phases where they experience feast and famine situations (Kristiansen et al., 2013). See Fig. 1 for an overview for their microbial pathways and see chapter 2.1.1 for explanation into their microbial pathways.

Because the amount of phosphate excreted during the anaerobic phase is less than the amount taken up during the aerobic phase, the net phosphorus taken up into the organisms is higher than initial values. The phosphorus is so readily removed from the wastewater by separating the phosphorus-rich sludge in the sludge separation step (Mino et al., 1998; Oehmen et al., 2007; Zuthi et al., 2013).

To achieve satisfactory results for phosphorous removal in the EBPR-process one important aspect for how well the removal process develop is the concentration of biomass that can assimilate phosphorous above Ordinary Heterotrophic Organisms bearing capacity. However, there is another mechanism that is also essential for the effectiveness of the removal which is how efficient the separation of biomass is. This is because phosphorous is removed from the process through the excess sludge being withdrawn. (Helness, 2007).

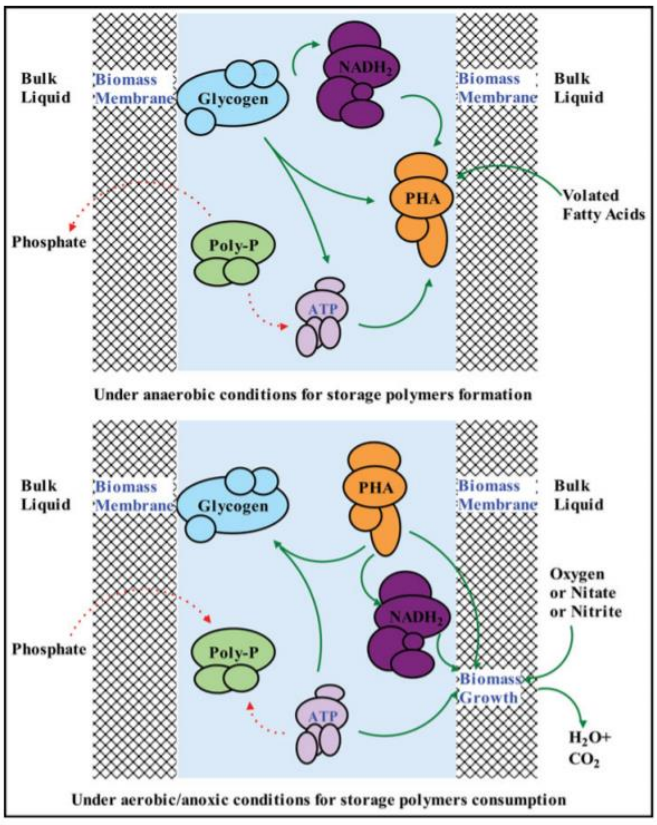


Figure 1 - The differing metabolic pathways for microbial storage. It shows the electron donors and acceptors. The red dottet line shows the specific pathway only relevant for PAOs, otherwise the same representation is relevant for GAOs (Ye et al., 2016)

When looking at the whole production cycle EBPR is a more environmentally friendly and sustainable alternative than chemical precipitation of phosphorous from wastewater (Oehmen et al., 2007).

2.1 Microbiology

Mino et al., (1998) stated that the microorganisms responsible for EBPR never had been isolated or identified. It has in later literature been stated that the common recognition is that the bacterial process is difficult and complex and involves what seems to be numerous bacteria. (Seviour et al., 2003). There are however two microorganisms that was defined by Kristiansen et al., (2013) to make up some of the organism structure known as PAO and these are, Candidatus Accumulibacter Phosphatis and Tetrasphaera. According to Kristiansen et al., (2013) they have markedly different physiology.

Accumulibacter will function in much of the same way as described in these chapters by taking up VFA and storing it as PHA, while Tetrasphaera seems to be more versatile then Accumulibacter (Kristiansen et al., 2013).

A key difference between these two bacteria types is that the Tetrasphaera can take up glucose and then ferment this to create succianate and other components, the Tetrasphaera will then use the stored glycogen to provide energy for replenishment of the poly-P reserves and provide energy for growth instead of PHA (Kristiansen et al., 2013). Kristiansen et al., (2013) suggest that glycogen and not PHA is used as the anaerobic storage polymer. The differing bacterial physiology shows that the PAOs carrying out polyphosphate accumulation are versatile (Kristiansen et al., 2013).

Nielsen et al., (2010) concluded that the microbial communities in different EBPR plants were rather similar and not as diverse as previously suggested. In their study of microbial communities in 25 Danish EBPR plants they found many identical or closely related core species which made up the majority of the communities in the plants (Nielsen et al., 2010).

Barnard et al., (2017) found that Tetrasphaera is a broad strain of bacteria we yet have to learn a lot about. Their ability to ferment complex organic molecules sets them apart from Accumulibacter. These complex organics include amino acids and produce carbon for storage. VFA is not the preferred substrate source. Some Tetrasphaera can produce VFV due to their ability to ferment, this can again be utilized as substrate by other strains of PAOs (Barnard et al., 2017). Tetrasphaera has many benefits linked to EBPR and therefore they can have a great impact on the EBPR process.

2.1.1 Polyphosphate Accumulating Organisms (PAO)

Polyphosphate accumulating organisms (PAOs) is the group of microorganisms that in essence is responsible for the removal of phosphate and is a community of different strings of bacteria. PAOs can store phosphate above what is required for their growth. Pastorelli et al (1999) found a direct proportionality between the P release and uptake of 1.055 g P(removed)/ g P(released). Fig. 1 and 2 shows the Accumulibacter PAOs schematic for microbial pathways in the aerobic

and anaerobic conditions. In Fig 2. it is also possible to see release and uptake of metal-salts in the process.

PAOs have strict requirements in the cycling though the anaerobic, aerobic and/or anoxic stages and it is for this reason this process is more complex compared to the more ordinary processes of biological N and COD removal (Zuthi et al., 2013). EBPR process needs to be facilitated by preferable conditions for the bacteria to proliferate (Helness, 2007). To facilitate this process understanding of PAOs replenishment and growth above other microorganisms must be understood (Kristiansen et al., 2013). Fig. 3 shows the observations made in full-pilot and laboratory scale work and shows how various substances responsible for EBPR normally behaves in the process.

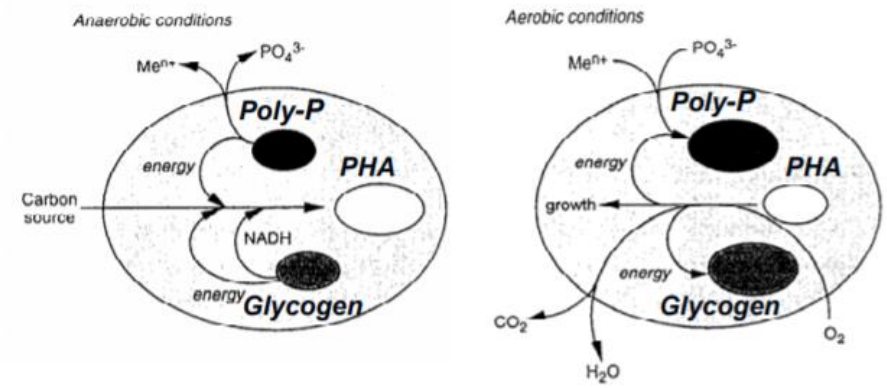


Figure 2 - The different uptake and releases that happen within the anaerobic and the aerobic stage in the EBPR process by PAOs – more precisely Accumulibacter (Helness, 2007 originalt fra Christensen et al., 1997).

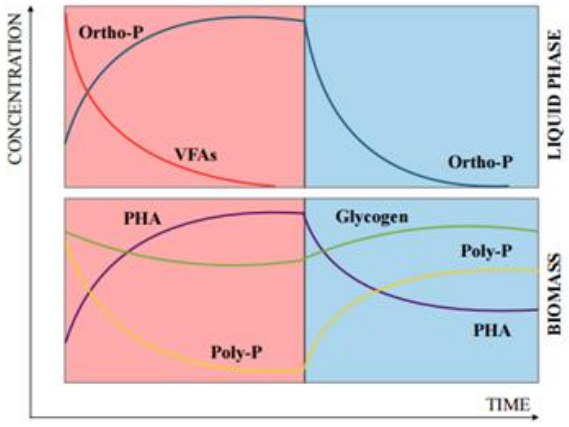


Figure 3 -These observations have been seen and full-, pilot and laboratory scale work and shows what is normally observed in EBPR of the concentrations of VFA, phosphate, Poly-P, PHA and Glycogen in the liquid phase and the biomass throughout the anaerobic and aerobic sequencing. Henze et al., (Ch. 7, 2008)

The Tetrasphaera encompasses different traits from the Accumulibacter such as fermenting more complex organic molecules. Fig. 4 shows some differences in the metabolic pathways between Accumulibacter and Tetrasphaera.

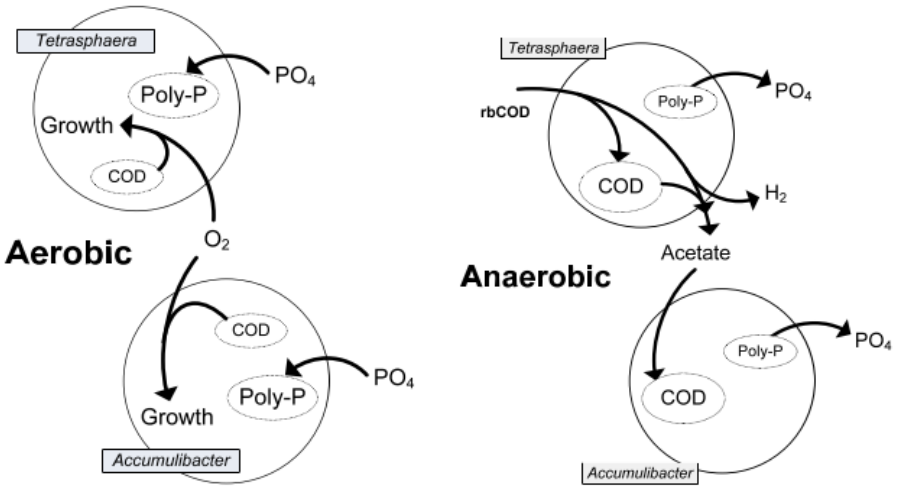


Figure 4 - The difference between microbial pathway of a Tetrasphaera and an Accumulibacter

2.1.1.1 Anaerobic conditions for PAO

PAOs are able to store P by accumulation in the bulk. They then store it as intracellular polyphosphate under alternating aerobic and anaerobic conditions. If there is substrate available in the form of Readily Biodegradable COD (RBCOD) primarily VFA, like acetate or propionate the Polyphosphate Accumulating Organisms (PAO) will take this up and store this intracellularly as poly-β-hydroxy-alkanoates (PHA) of which poly-β-hydroxy-butyrate (PHB) is the most common. The PAOs will then release P (orthophosphate) which they have stored intracellularly as poly-P. This happen through decomposition of the polyphosphate. As this process requires energy this is supplied by the intracellular poly-P being hydrolysed and degradation of intracellular glycogen which has previously been assimilated. The poly-P is released as phosphate from the cell which causes a significant increase in the concentrations of soluble phosphorous in the liquid bulk. In this process to maintain the ionic balance relevant metal ions such as magnesium (Mg^{2+} and K^{+} is released (Helness, 2007; Kristiansen et al., 2013; Mino et al., 1998; Seviour et al., 2003;Ye et al., 2016).

There has been evidence of some strains of PAO being able to utilize different sources of carbon, such as Tetrasphaera described in chapter 2.1.

2.1.1.2 Aerobic conditions for PAO

PHAs are oxidized as RBCOD carbon is no longer available in the liquid bulk. The PAOs stored PHA from the anaerobic step serves as energy for the uptake and assimilation of phosphate from the water to store this intracellularly though poly-P synthesis. Glycogen reserves can also be restored through synthesis if there is enough energy sources available. Oxygen (or nitrate) present in the reactor is the external electron acceptor, and is reduced in this stage. As phosphate is taken up so is magnesium and potassium ions so that the ionic balance is preserved (Helness,

2007; Kristiansen et al., 2013; Henze et al., 2008, ch.7; Ye et al., 2016). See Fig. 1 and 2 for the different microbial pathways during the anaerobic and aerobic stages.

Mino et al., (1998) defined the biomass growth as; *the result of the difference between the PHA oxidized minus the PHA utilized for poly-P and glycogen formation purposes, favouring the replenishment and formation of intracellular stored compounds over growth.* The phosphate uptake can be four times higher than the released value under anaerobic conditions (Mannina et al., 2017; Mino et al., 1998).

In comparison to the ordinary heterotrophic organisms (OHOs) the PAOs have an enormous advantage when it comes to their ability to metabolize carbon and release phosphorous in the absence of external electron acceptors. OHOs are not able to utilize carbon without any external electron acceptors (Yuan et al., 2012). However if there is a transfer of oxygen or nitrate (or both) back to the anaerobic zone OHOs can utilize the rbCOD present and not only leave a decreased amount of carbon available for the PAOs, but this also gives OHOs a chance to grow in the biomass and take up valuable space on the carriers. This can lead to a reduced efficiency of the process on two accounts.

2.1.2 Glycogen Accumulating Organisms (GAO)

Glycogen accumulating organisms also has the ability of taking up carbon sources under alternating anaerobic and aerobic/anoxic for their proliferation. Unlike PAOs GAOs rely on energy from glycoses of intracellular glycogen as energy source for the storage of PHA from the VFA uptake. Therefore, the release and uptake effect of phosphorous will not be experienced (Filipe et al., 2001). This is highly unwanted in an EBPR. Therefore the existence of GAOs and the competitions they subject the PAOs to may inhibit the phosphate release and uptake by PAOs which in turn will reduce the efficiency of the process (Ye et al., 2016; Erdal., 2003)..

In Nielsen et al., (2010) they found that GAOs were far from a large part of the bacteria-culture in Danish plants. Less than 1% of the bacteria culture in the Danish plants they did their studies on was made up of the GAO community.

GAOs ability to compete with PAO is affected by a whole variety of parameters some being; temperature (T), pH, cations, dissolved oxygen and carbon sources.

2.1.3 Denitrifying PAOs (DNPAOs)

Henze et al., (2008) states that anoxic P uptake can decrease the efficiency of the P-removal in the system, so if it through a design point of view is mostly valuable to maximize P-removal the anoxic P-uptake can seem to have a negative effect on this. However, there is unmistakable evidence that the DPAOs have the ability to take up P under anoxic conditions although it seems with a decreased overall yield from PAOs, because of the less efficient energy generation in the process (Zeng et al., 2003).

An important advantage of DNPAOs is the high efficiency use of substrate in the bulk. Substrate is one of the limited resources available in Norwegian wastewaters and phosphorous and nitrogen removal happens simultaneously this reduces some of the stress on substrate sources when both nutrients are necessary to remove. As research of Norwegian wastewater show is that this is typically diluted with low values of rbCOD (Ødegaard, 1999). This is a disadvantage of estimating the potential for EBPR in Norway. Any process solution that maximizes the use of the limiting resources is therefore highly advantageous.

2.2 Differing parameters and their effect on EBPR

There is a necessity to understand what the disturbances in the BNR stems from and how we can control and understand the process better. For the implementation of the EBPR process in places where outlet restrictions are strict reliable processes are of imminent importance (Zuthi et al., 2013).

Zuthi et al., (2013) stated that frequent constraints to the operational and system parameters in Activated Sludge (AS) plants affects its overall efficiency, especially when looking at nutrient removal. This same conclusion is also relevant for biological nutrient removal in biofilm reactors as these systems experiences disturbances and constraints. Not in all cases will constraints experienced in Activated Sludge plants be transferable to biofilm reactors, however many operational parameters that affect AS plant will also affect biofilm reactors in a similar way.

2.2.1 Temperature-effect on EBPR

Table 1 - Brief overview of some of the authors discussed in this chapter and their view on temperature effect on EBPR

EBPR process	Author	Effect
Low temperature effects being beneficial (<20°C)	Lopez-Vazquez et al. (2009) Erdal et al,. (2003) Ye et al., (2016)	Reduced competition from GAOs
High temperature being beneficial (>20°C)	Baetens et al., (1999) Mulkerrins et al., (2004)	Reduced kinetic rate Stochiometric rate unchanged

The temperature influences the EBPR process, however exactly how is difficult to assess as studies explaining the effects shows contradictory results. There are many differing studies that have showed that both high temperatures (Baetens et al., 1999) and low temperatures (Leyva-Díaz et al., 2016; Lopez-Vazques et al., 2009) are beneficial. It has overall been established that at elevated temperatures the biochemical and chemical reactions happens more quickly, which increases the kinetic rate of reactions more quickly than at lower temperatures. Mulkerrins et al., (2004) stated that the biochemical and chemical reactions double with an increase in every 10 °C up to the optimum point of the substrate. However some studies show that PAOs have a competitive advantage over GAOs at low temperatures (Lopez-Vazquez et al., 2009) which may be a reason for EBPR showing promising results for removal efficiency at lower

temperatures regardless of the negative effect this has on the reaction process. Others have shown the opposite; Baetens et al., (1999) found that the aerobic and anaerobic kinetics increased with increasing temperatures up to 15-20°C, and that the stoichiometry was uninfluenced by temperature in the anaerobic process. The author looked at temperature affect as a whole of the process, and not comparingly between PAOs and GAOs.

The temperatures effect on removal efficiency can be described through the Arrhenius equation. Erdal et al., (2003) states that many studies report the decrease of EBPR with a decrease in temperature in accordance with the Arrhenius relationship. The Arrhenius Equation can be written as

$$k = k(0)^{\left(\frac{E}{RT}\right)} \qquad \text{or}$$

$$k(T) = k(20) * \beta^{T-20} \qquad \text{(Eq. 2.1, Crittenden et al., 2012, ch.5)}$$

Where k(20) is the rate of the process at a standard temperature, and k(T) is the rate affected by the temperature coefficient at a the actual temperature of the liquid.
Erdal et al., (2003) showed that although there was a decrease in kinetic rates in their study with a temperature shift from 20 to 5 °C, the systems still had better performance at 5 degrees. The PAOs outcompeted GAOs, and this can be because the GAOs rely on the glycolysis, however since glycolysis is negatively affected by low temperatures they might have been outcompeted because of this.

Lopez-Vazquez et al., (2009) did in their study of the modeling of the PAO-GAO competition collect various temperature coefficients of different metabolic pathways for PAO and GAO at various temperatures in the system under anaerobic and aerobic conditions, see Fig. 3

Lopez-Vazquez et al., (2009) also found results showing the relationship between PAO and GAO competition regarding their maximum substrate uptake with two different substrates; acetate, graph (a) and propionate, graph (b). These graphs show the combined temperature and pH effect on different carbon sources, see Fig. 4.

From the graph it is possible to read of that both bacteria communities react to the change in temperature, however GAOs sensitivity to it is much higher than PAOs.

Table 2 – Temperature coefficients for the anaerobic and aerobic metabolic processes of PAO and GAO.

Process	Organism	
	PAO (*Accumulibacter*)	GAO (*Competibacter*/ *Alphaproteobacteria*-GAO)
Anaerobic acetate uptake rate	1.054[a]	1.054[b]
Anaerobic maintenance	1.096[c]	1.028[d]
PHA degradation	1.129[e]	1.141[f]
Glycogen production	1.125[g]	1.090[h]
Poly-P formation	1.031 [e]	–
Aerobic maintenance	1.064[c]	1.054[d]

a Lopez-Vazquez et al. (2007b), valid from 10 to 20 °C.
b Lopez-Vazquez et al. (2007b). Valid from 10 to 40 °C in combination with the inactivation expression.
c Brdjanovic et al. (1997), valid from 5 to 30 °C.
d Lopez-Vazquez et al. (2007b), valid from 10 to 40 °C.
e Adjusted in this study based on the observations of Brdjanovic et al. (1998), valid from 5 to 20 °C.
f Lopez-Vazquez et al. (submitted for publication), valid from 10 to 30 °C.
g Meijer et al. (2002), valid from 10 to 30 °C.
h Lopez-Vazquez et al. (2008b), valid from 10 to 40 °C in combination with its inactivation expression.

Figure 5 – Temperature coefficients for PAO and GAO for various metabolic pathways (Lopez-Vazquez et al., 2009)

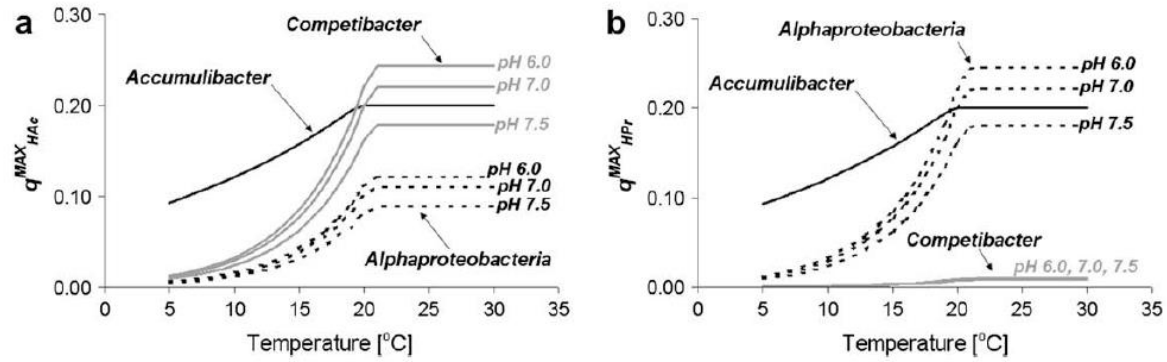


Figure 6 - The temperature and pH effect on substrate uptake by PAO and GAO with usage of acetate and propionate as carbon source (Lopez-Vazquez et al., 2009)

Lopez-Vazquez et al., (2009) found that the effects in the temperature scale of 5-35°C on the competition between PAOs and GAOs in anaerobic and aerobic environment revealed that GAOs is in favour of carbon source uptake at temperatures above 20°C. This is also in accordance with Erdal et al., (2003) results. Above this temperature range Ye et al., (2016) also states that biological phosphorous removal is difficult to achieve as the EBPR process is inhibited. The possible reason for this is related to the increased competition for substrate in the anaerobic zone for PAOs which affects the efficiency of the process negatively (Erdal et al., 2003; Ye et al., 2016). Several studies have shown that PAO, at low temperatures, is the dominant microorganisms regardless of the used influent carbon source or pH. (Ye et al., 2016; Lopez-Vazquez et al., 2009). However, from Fig. 4 it is possible to see that PAOs also experiences reduced substrate uptake at temperatures below 20°C, so their kinetic process is not unaffected, and it seems the increased EBPR efficiency is a function of PAOs reduced uptake rate vs. the reduced competition from GAOs.

Ye et al., (2016) stated that PAOs and GAOs are psychrophilic and mesophilic, respectively which is not to different from Mulkerrins et al., (2003) which stated in their review that PAOs were lower range mesophilic or psychrophilic, while GAOs is thought to be mid-range mesophilic. Erdal et al., (2003) also found that the PAO were psychrophilic.

Baetens et al., (1999) on the other hand found that at that at 5°C a breakthrough of acetate was found to the aerobic zone was experienced. Baetens et al., (1999) then found that is was not the stoichiometry of the anaerobic zone that was affected, but the kinetics of both the aerobic and anaerobic zones, and that all conversion rates in both zones increased with increasing temperature.

The studies referred to above are all from activated sludge based systems.

Baetens et al., (1999) stated that the prediction on the effect temperature has on biological nutrient removal is far from straight forward as the different influences of temperature has influence on many sub-processes. It is therefore necessary to account for these influences on the overall system.

There are numerous review looking at the temperature effect on EBPR at high temperatures (>20 °C). However, as this is a review looking into relevant literature for my work on the

continuous MBBR pilot here at NTNU I have not looked more into these as temperatures in municipal wastewater in Norway seldom climbs above 15°C.

2.2.2 pH-effect on EBPR

Table 2 Brief overview of some of the authors discussed in this chapter and their remarks on the pH effect

pH-range	Author	Effect
pH-ranges: 5.5 to 8.5	Smolders et al., (1994) Oehmen et al., (2007)	GAOs thriving in lower pHs and PAOs increasing efficiency up to 8.5
pH-range: 6 to 7.5	Filipe et al., (2001) Lopez-Vazquez et al., (2009) Wang et al., (2013) Mulkerrins et al., (2004)	Aerobic stage: decreased PAO efficiency with low pH, GAO largely unaffected Anaerobic stage: decreased GAO efficiency with high pH. PAO largely unaffected Overall biomass yield was the same in the two reactors Increased removal with pH increase from 6.6 to 7.8 Increased removal with pH increase from 6.3 to 7.2
pH-range: >8	Wang et al., (2013) Oehmen et al., (2007)	Decreased removal efficiencies above 8.2 Decreased

Microorganisms are influenced by pH, and this also includes PAOs and GAOs. At higher pHs the energy required for substrate uptake increases as the pH of the internal cell stays constant and the pH in the external environment changes. Because of this energy gradient for substrate uptake through the membrane increases (Smolders et al., 1994). Smolder et al., (1994) found that the PAO uptake varied in the pH ranges from 5.5-8.5 increasing with a range from 0.25 to 0.75 mol P/mol C within this pH-range. This increase was explained by the pH increase as more energy for phosphate degradation was needed (Oehmen et al., 2007; Smolders et al., 1994). Filipe et al., (2001) also found that in tests done in the pH range of 6-7.5 the accumulation range of phosphate increased for PAOs in the aerobic stage, while the uptake of GAO was independent in the same pH range, and more likely to thrive in a low pH. Filipe et al. (2001) therefore states that the phosphorous removal process is dependent on pH in the aerobic stage, if this below 6.5. However the overall biomass yield between GAO and PAO were essentially the same in this pH range (Filipe et al., 2001).

In the anaerobic zone Filipe et al., (2001) also stated that with an increase in the pH PAOs were largely unaffected while GAOs showed decreased acetate uptake and a higher degree of glycogen consummation within the cell. Filipe et al (2001) then found that above the pH of 7.25 the GAOs did not grow in the anaerobic zone. Filipe et al., (2001) also found that the uptake at

pH 6.5 was around 40% less efficient then at 7.0 which causes ripple effect such as reduced degradation of PHA and reduced growth.

Lopez-Vazquez et al., (2009) showed in Fig. 4 the combined effect of pH and temperature on the anaerobic uptake rate of the two different substrates acetate (a) and propionate (b). From this Lopez-Vazquez concludes that Accumulibacter is largely unaffected by pH in the anaerobic stage, while GAO is largely affected with decreased uptake of substrate with an increase in pH.

Oehmen et al., (2007) stated that with an increase in pH to a very high level >8 a decrease in the performance of EBPR has been noticed. This is also in accordance with Wang et al. (2013) who found that between the pH of 6.6 to 7.8 the removal increased, while with a further increase from 7.8 to 8.2 the removal decreased. The biomass was found to contain more PAOs than GAOs at this pH.

Mulkerrins et al., (2004) stated that one study saw that when the pH decreased from 7.2 to 6.3 in the influent the efficiency of the phosphorous removal decreased, and 15 days were necessary to establish the same steady-state again.

Baetens et al., (1999) found that the anaerobic phosphate release vs acetate uptake in their study was higher than previously reported studies. Baetens et al., (1999) stated that this was because of the high pH in the experiments.

Ye et al., (2016) concludes that the pH effect on the competition between PAO and GAO have not been definitely established, so there is a need for more studies on the area. However, this conclusion was based of a study on the EBPR process on the pH-ranges from 7.2 to 8.3, and that there in this range were no relationship between pH and phosphorous removal. This conclusion does not state anything about pH above or below this level, and if there then is an effect on EBPR, which based on the literature review there seems to be.

Zhang et al., (2005) found that when the pH was decreased from 7.0 to 6.5 this effected the process with a reduction of the efficiency of 99.9% to 17% two weeks after the pH was lowered.

2.2.3 Organic substrate effect on EBPR

Table 3 Brief overview of some of the authors discussed in the chapter and their remarks on the effect of organic substrate

Input	**Author**	**Effect**
COD/TP ratio	Ohemen et al., (2007) Sriwiriyarat et al., (2005) Zuthi et al., (2013) Ye et al., (2016) Mulkerrins et al (2004)	Low ratio (10-20 mg/l) more favorable for PAO Ratio of 18-20 had best effect on EBPR Stable feed composition of COD/P Ratio of 18-20 had best effect on EBPR Suggested COD/TP ratio > 40

rbCOD or soluble COD rbCOD/TP>15	US-EPA (2010) Saltnes et al., (2016a) US-EPA (2010) Helness (2007)	A better measure to evaluate process efficiency than COD/TP Minimum design value approx. 10 mg RBCOD/ mg$P0_4^{3-}$ Recommended first gen. design value 20 mg RBCOD/ mg$P0_4^{3}$ rbCOD/TP>15
bsCOD/PO4-P=>40 BOD/P>15-20	Saltnes et al., (2016)	Found that they have a bsCOD/PO4-P ratio of >40 with good results Stated that BOD/P ratio should be more than 15-20
Unutilized carbon in anaerobic stage	Lopez-Vazquez et al.,(2009)	OHO can proliferate on the unutilized RBCOD from the anaerobic step
Carbon source	Oehmen et al., (2007) Mulkerrins et al., (2004) Lopez-Vazquez et al,. (2009)	GAO cannot compete as well for propionate as acetate Conflicting between usage of acetate vs. propionate Simultaneous presence of acetate and propionate
Glucose	Nielsen et al., (2010) Kristiansen et al., (2013) Oehmen et al., (2007) Mulkerrins et al., (2004)	Could enhance the proliferation of PAOs, however not if used as the sole carbon source Glucose has shown to increase growth of GAO

It has been shown that Norwegian wastewater is normally dilutes and has low influent values of rbCOD (Ødegaard, 1999). This is, on a general basis, what seems to be one main disadvantage for introducing this process for the potential of EBPR.

Helness (2007, pp.17) states that many various sources of carbon can work in EBPR. This can be VFA, amino acids, glucose and alcohols. Which type of carbon we have and how available it is has consequences for the effectiveness of the process as PAOs use the carbon for synthesizing, accumulating and storing products (Kristiansen et al., 2013). Through the fermentation of sludge two of the main products are acetate and propionate. These are therefore commonly used in experiments and referred to in literature. Mulkerrins et al., (2003) stated that 7–9 mg of VFA are needed to remove 1 mg of phosphorus, while Jonsson et al., (1997) found the average value to be 14 mg VFA pr. 1 mg P, however with large fluctuations. The necessary theoretical ratio will vary as acetate, propionate and butyrate all are various sources of VFA, and varying levels of presence of these can have an effect on the system (Helness, 2007).

EBPR performance is affected by the length of the anaerobic period so that the PAOs have the possibility of completely removing the Readily Biodegradable COD (RBCOD), however the loading rate of RBCOD must also be high enough for growth in the reactor (Sriwiriyarat et al., 2005). Their results in a study in EBPR in a IFAS system operated at 10°C found significant differences in removal with differing loading rates see Fig. 5. Zuthi et al., (2013) also states that

it is important to maintain a stable feed composition into the reactor as the COD/P ratio is highly important for maintaining a successful operation of EBPR. Oehmen et al., (2007) stated a high COD/P ratio, around 50mg COD/mg P, was found in many studies to favor GAOs instead of PAOs. A low COD/P ratio of about 10-20 mg COD/mg P is therefore more favorable for PAO growth. Oehmen et al., (2007) concluded that this of course this should not compromise the sufficient amount of VFA (Oehmen et al., 2007). It must be highlighted that neither Oehmen et al., (2007) nor Zuthi et al (2013) has stated anywhere in the article whether the P is orthophosphate or Total P (TP), which makes the correspondence with the results below difficult. Henze et al., (2008) stated that properly designed EPBR could consist of a biomass of 40% PAOs as the active organism, which will remove around 10-12 mg PO4-P/L pr. 500 mg COD, giving a rate of about 50 PO4-P/COD.

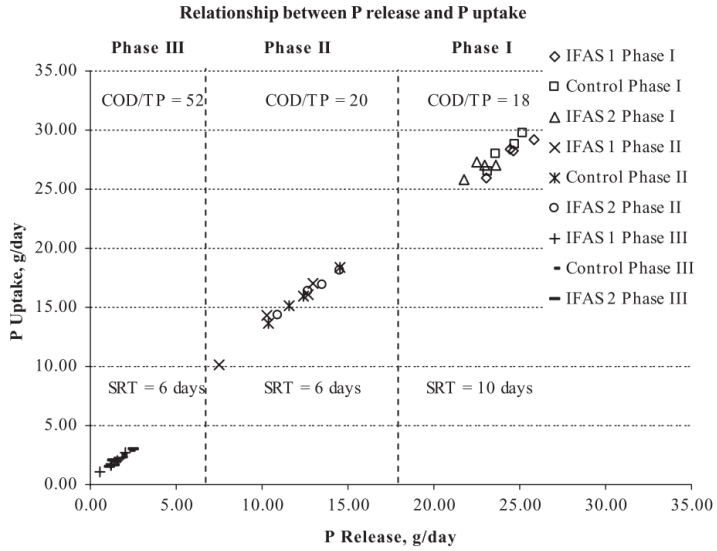


Figure 7 - COD/TP ratio affects the efficiency of the EBPR process (Sriwiriyarat & Randall, 2005)

At the COD/TP ratio of 18-20 Ye et al., (2016) states, from their review of various studies, that 90% of the phosphate is removed in the EBPR process, which corresponds well with the results from Sriwiriyarat et al., (2005), see Fig 5 above. Sriwiriyarat et al., (2005) found that in their study with EBPR in an IFAS system the change in COD/TP ratio had a much larger effect on the EBPR process than the change in mean cell residence time. They found that when the COD/TP ratio changes from 20 to 52 this decreased the phosphorous removal with 50 %, while a change in mean residence cell time from 10 to 6 days only decreased with 10 %. This experiment was done with an experimental temperature of 10 +/- 1°C, and with municipal wastewater making some of the results relevant to look at for this review (although the mean residence cell time is not as interesting to look at as that operational parameter is highly different from an AS to an MBBR). This is opposite of what Mulkerrins et al., (2004) wrote in their review were a suggested a COD/P ratio > 40 was given based of off literature.

Saltnes et al., (2016) measured the bsCOD/PO4-P ratio and found this to be around 40 and concluded they should have good potential for EBPR as their experience from other plants was that a relationship of BOD/P>15-20 was necessary ratio for an efficient process.

Helness (2007) pp. 113 stated that the recommended first-generation design criteria should be a ratio of 20:1 mg RBCOD/mg $P0_4^{3-}$. In chapter 5.2 in US-EPA Nutrient Control and Design Manual the minimum ratio for RBCOD:TP is presented to be 10-16 mg/l. However, as orthophosphate makes up approximately 50-80 percent of the TP means this value is about 5-8 to 8-13 mg RBCOD/ mg $P0_4^{3-}$ for effluent concentrations less than 1 mg/l.

Lopez-Vazquez et al., (2009) states that type of carbon source in has direct effect on the anaerobic metabolism of PAO and GAO and indirect effect on their aerobic metabolism because the maximum aerobic yield is affected by the uptake pf PHA in the anaerobic zone. Therefore, if the PAOs are not able to utilize the carbon coming in, or is outcompeted by other bacteria in the anaerobic stage this will have multiple effects some being; in the aerobic stage, OHO proliferating can be experienced because of unutilized carbon from the anaerobic stage, or having had much smaller growth during the anaerobic stage than preferable.

Oehmen et al., (2007) in their review states that in studies where acetate has been the sole source of carbon, PAO has outcompeted GAO, while other studies with the same operational conditions show the opposite results; that GAO has proliferated. Oehmen et al., (2007) states that overall it seems that results so far strongly suggest that some strands of GAO cannot compete as well with PAOs for propionate uptake as compared to acetate uptake, while other strands of the bacteria have the opposite effect, and can compete with PAOs for propionate. Oehmen et al. (2007) concludes that since more strands of the GAO bacteria can compete with the PAOs for acetate than for propionate, this could be a reason for having a higher degree of propionate in the stream, as it seems that the simultaneous presence of acetate and propionate has a positive effect on PAO. For eliminating GAOs in lab-scale studies Oehmen et al., (2007) states that this is feasible by alternating the carbon sources over a period. Mulkerrins et al., (2004) concluded that the differing mechanisms of the carbon in the anaerobic environment could make it act either in favour or not for EBPR.

Lopez-Vazquez et al., (2009) also found that the efficiency of phosphate removal was not satisfactory when acetate or propionate was used as the only carbon source, although Lopez-Vazquez et al., (2009) states that PAO could take up acetate and propionate equally well. This leads to the understanding that other parameters are affecting the uptake. Lopez-Vazquez et al., (2009) found that the simultaneous presence of acetate and propionate as carbon sources with a relationship of 75:25 or 50:50 ratios favoured PAOs over to GAOs no matter the pH. If however there only were presence of acetate or propionate as the carbon source PAOs were only favoured over GAOs at a high pH (Lopez-Vazquez et al., 2009).

The highest rates of phosphate release in the anaerobic stage has been achieved with acetate and propionate as carbon sources (Mulkerrins et al., 2004). However, it is also stated in their review that although this is the most effective carbon sources, of the various VFAs studies propionate was the least efficient.

It is necessary to ferment glucose and ethanol to VFA before they can be utilized in the EBPR-metabolism as only acetate and propionate can be used directly (Helness, 2007, pp. 19). This seem however to be mostly relevant for the Accumulibacter, as this does seem to contradict the results by Nielsen et al., (2010) which states that some strands of the PAO can utilize glucose and amino acids for growth, although some might utilize acetate as well. This is also found by Kristiansen et al., (2013) which states that the same PAO strand Nielsen found is able to utilize acetate but grows more poorly on this. Mulkerrins et al., (2004) stated that changes in the influent organics from VFA to sugar like glucose could enhance growth of the GAO. Oehmen et al., (2007) on the other hand stated that the effects of adding glucose to phosphate removal in EBPR system can have a positive effect, however not if it is added as the sole carbon source as deterioration of EBPR systems has been observed with glucose as the only source (Oehmen et al., 2007). Mulkerrins et al., (2004) stated that with the prefermentation of glucose there was reported a greater removal efficiency.

Jonsson et al., (1996) stated that in their study the VFA-potential in the influent to their treatment plant was not adequate for the outlet demand for phosphorous, and a hydrolysis of the sludge was necessary before the EBPR step.

2.2.4 Presence of ions – Effect on EBPR

Pattarkine et al., (1999) states that the role of metal cations in the EBPR process is highly important, and that the availability of both potassium (K^+) and magnesium (Mg^{2+}) are essential to establish a well-functioning EBPR, and that both are required not only one or the other. When phosphorous is released and taken up these metal cations are also released and taken up. See Fig. 2. It does seem that calcium play a limited role in this process as it was not taken up in the anaerobic stage shown by Pattarkine et al., (1999).

In an experiment done by Aguado et al., (2006) in a SBR operated for EBPR the conductivity variations during a cycle was significant. The variations in conductivity showed similar trend to phosphorus concentration in both the anaerobic and the aerobic environment. Aguado et al., (2006) therefore states that the ionic conductivity could be used to get information on the EBPR performance and its stability. This information can then be used when adjustments to the cycles of length in the anaerobic and aerobic stages are to be decided (Aguado et al., (2006). An online process control like this, which is based on sensors which are inexpensive and easy for operators to use could then be applied, as the measurement of ionic conductivity is a relatively reliable and simple (Aguado et al., 2006). However, these measurements cannot be fitted to consider only the phosphorus concentration variations as pH values and dissociation species must be considered. Aguado et al., (2006) states that to be able to gain a good relationship between the experimental data all biological processes which takes place in the AS SRB needs to be considered, as every ion variation in the system will affect the measurements.

The experiment by Aguado et al., (2005) showed that through statistical analysis of the experimental data there were a strong correlation between the phosphorous and cations. This was 0.28 mol K / mol P and 0.36 mol Mg / mol P. This numbers are similar to the results from another experiment were 0.27-0.36 mol K/mol P and 0.29-0.32 mol Mg / mol P was found

(Mulkerrins et al., 2004). During the uptake in the anaerobic zone and the degradation of poly-P the composition of poly-P based on the release of phosphorous was 1/3 K: 1/3 Mg: P03- (Smolders et al., 1994).

Although the influent levels of potassium and magnesium in municipal wastewater is high enough to eliminate deficiency as something that is likely to occur regularly, short term deficiencies of potassium can occur (Mulkerrins et al., 2004). When these periods of limited potassium availability was simulated by Mulkerrins et al., (2004) they found this to negatively affect the EBPR.

2.2.6 Dissolved Oxygen (DO)

Table 4 Brief overview of some of the authors discussed in this chapter and their remarks on the effect DO

Dissolved Oxygen	Author	Effect
Oxygen level	Mulkerrins et al., (2004)	Anaerobic zone: 0-0.2 mg/l for efficient process Aerobic zone: >2 mg/l
GAO vs PAO	Ye et al., (2016) Carvalheira et al., (2014)	Low levels for the proliferation of PAOs over GAOs PAO higher affinity for DO at low levels outcompeting GAO

In a EBPR system the anaerobic zone must not contain oxygen levels above 0.2 mg/l for the process to be efficient (Mulkerrins et al., 2004). Jonsson et al (1996) stated that oxygen levels as high as 3 mg/l was measured entering the biological treatment step in their study, and a lot of effort had to be put into hindering this oxygen from entering the anaerobic zone.

Mulkerrins et al (2004) states that numerous studies show that dissolved oxygen (DO) levels should be around 2 mg/L for successful EBPR. In this review it is also stated that if nitrification is desired the concentrations a DO concentration needs to be 3 to 4 mg/l, and DO-concentration above 4 mg/l does not seems to further stimulate BNR.

Ye et al., (2016) stated that the efficiency of phosphorous removal can be improved with low DO concentrations since GAO is reduced at low DO levels. Therefore, PAO will be able to proliferate.

Carvalheira et al., (2014) found that the DO level had an effect on the competition between PAOs and GAOs as Accumulibacter, a string of PAOs had a higher affinity for DO than GAOs at low levels, and they therefore were able to maintain their activity level, while the GAOs decreased. In their study they wanted to test the how the DO levels affected the microbial community, especially the competition between PAO and GAO. Carvalheira et al., (2014) therefore stated that low aeration could be beneficial for the EBPR process with levels around 2 mg/l being Favorable for PAOs. The study was done on two SBR-reactors with AS. They stated that previously research has given variations in DO levels from 0.5 – 3 mg/l all being

beneficial for EBPR. Carvalheira et al., (2014) also reported previous studies were DO levels were between 0.15 and 0-45 mg/l with variable removal efficiencies (61 to 99%), but not the effect in had on the microbial community.

Chen et al., (2014) also found that that lower DO levels benefited EBPR, as there was a shift in the population of PAOs. The PAO population increased with decreasing DO-levels. This study was done in an aerobic/extended idle regime, and showed better results than the anaerobic/oxic process it was compared to.

3 State of the art – Literature Review

In conventional biological treatment of COD and/or nitrogen only a small amount of phosphorous is removed through cell synthesis in the microbial community, mainly from ordinary heterotrophic organisms (Seviour et al., 2003). This amount is 0,02 mg P/mg VSS (Henze et al., 2008, ch. 7). Therefore, the P-removal needs either chemical precipitation or enhancement for it to be greatly removed from the wastewater (Zuthi et al., 2013). Through the EBPR process as much as 0,06-0,15 mg P/mg VSS can be removed (Henze et al., 2008, ch. 7).

There are many processes configuration in which phosphorous removal can be enhanced. The most common is EBPR in an activated sludge plant (AS). Another commonly used process configuration is the IFAS system which combines AS and MBBR. Sequencing batch reactors (SBR) is not a common or regularly used technology for EBPR in biofilm systems (Helness, 2007; Saltnes et al., 2016). Currently there is research considering the possibility of utilizing a continuous MBBR system for phosphorous removal.

To encourage the development and growth of PAOs all biological phosphorous removal is designed to reassure a cycle of bacteria through anaerobic and aerobic environments. (US-EPA, 2010, ch. 6.3.2)

3.1 Activated sludge phosphorous removal (ASP)

Activated sludge (AS), also commonly referred to as suspended solids culture has become a well-established removal mechanism for Biological Nutrient Removal (BNR) through nitrification, denitrification and phosphorous removal (Al-rekabi, 2015). This is also the most common way of biological removal of nutrients. Currently all full-scale practice is that remove both phosphorous and nitrogen biologically are based on activated sludge processes, as this is a well-established practice for phosphorous removal, and that high removal efficiencies has been achieved through this removal process (Al-rekabi, 2015). Sludge is recirculated through the different reactors, with differing conditions and as mentioned in chapter 2. Operationally BNR removal is relatively easy to manage in a AS-plant as each process is separated within its own reactor (Al-rekabi, 2015). Because of the recirculation of sludge this process is continuous. See Fig. 6 for flow schematic. Settled sludge is pumped to the anaerobic zone where, if there are advantageous conditions surrounding parameters discussed in chapter 2-2.2.6 PAOs accumulate organic substrate and release phosphorous. The liquid bulk is then transported to the aerobic zone where PAOs utilize the stored PHA to take up phosphorous and restore glycogen storages in its cell, see Fig. 7 for a schematic of how various substances behave in the two chambers.

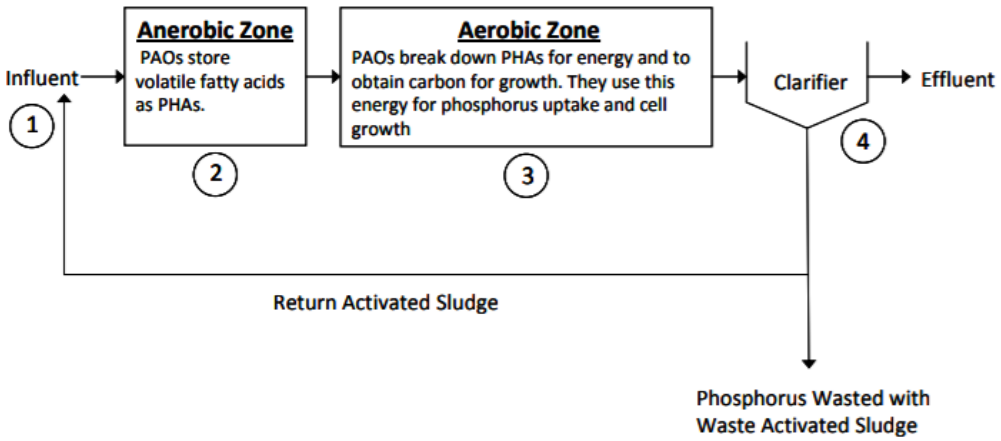


Figure 8 Flow schematic for an AS system with EBPR. The RAS is the Return Activated Sludge which is the settled sludge from the clarifier being returned to the anaerobic step. (US-EPA, 2010, ch. 5-1)

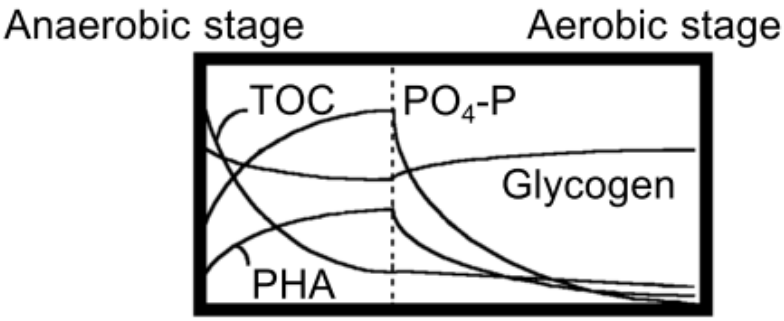


Figure 9 A schematic of how various substances behave in the reactors under differing anaerobic and aerobic environments (Mino, 2000)

This conventional process of removing nutrients, although well-established has its major drawbacks. The suspended solids load is more than ten times higher than that of MBBR (Ødegaard, 2006), which makes the process expensive for investments and resource demanding in terms of space (Al-rekabi, 2015). Because of necessity of returning sludge there will be high sludge loads in the reactor, and the sludge load in to the separation unit will be high. The separation step is an important efficiency factor in the process of as the solid-liquid fractions is separated here, and the AS process may suffer from separation problems (Mannina et al., 2017). There has been experienced problems with sludge settle ability in many AS plants for various reasons (Al-rekabi, 2015). For the sludge separation step it is also important to avoid secondary P-release in the sludge in the clarifiers as anaerobic zones can develop there (Al-rekabi, 2015)

The various key challenges mentioned above is a part of the reason why several studies have been performed considering new innovative technology in regard to Biological Nutrient Removal (BNR). Some of these technologies are Membrane Bioreactors (MBR), MBBRs and Fixed Film Activated Sludge (IFAS) (Mannina et al., 2017).

By including an anoxic chamber, the EBPR and nitrogen removal can easily be complimented in the AS process. There have been many full-scale processes set up where the combined

removal of nitrogen and phosphorous happen through AS processes with various configurations for enhancing removal of both substances. Configurations can differ in the placement of the anoxic chambers for either pre- or post-denitrification among others. Fig. 8 shows a flow schematic of an AS process for BNR with pre-denitrification. All the removal processes need dedicated tank space for the process to happen. In the tanks that are not aerated it is necessary with mixers to be assured that the reactors are completely mixed. It is also necessary with two different return flows for both the denitrification and the return of sludge to the anaerobic step (US-EPA, 2010).

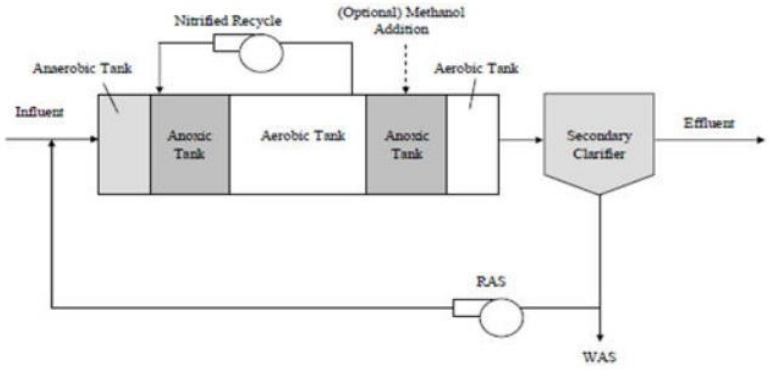


Figure 10 Flow schematic for an AS system with EBPR and nitrification and pre-denitrification. The RAS is the Return Activated Sludge which is the settled sludge being returned to the anaerobic step.

3.1.1 Membrane Bio Reactor (MBR) with AS

An AS system with MBR is a modified version of the Conventional AS (CAS). Through the combination of a biological and membrane process with either ultra- or microfiltration the treatment efficiency and process control increases (Leyva-Díaz et al., 2016). However, the MBR processes will tend to suffer from some of the same system constraints as AS plants Zuthi et al., (2013), as well as membrane fouling which increases operational costs (Leyva-Diaz et al., 2016). Zuthi et al., (2013) also states that an overall better removal efficiency of phosphorous has been reported in the AS MBRs over the AS plants. One reason for this may be that the membrane, which is used in the separation step, may completely be able to retain the PAOs. The particle size of PAO is typically larger than the pores in a microfiltration membrane, which is about the size of 0.2 µm (Radjenovic et al., 2008, pp. 60). See Fix. 9 for a schematic of the process.

Zuthi et al., (2013) also concluded that various configurations of ASPs/MBRs may affect the removal kinetics of other nutrients. Therefore, an optimized system that does not compromise the treatment quality target of other nutrients is desired (Zuthi et al., 2013). This is relevant for all process configurations for EBPR removal, and also the continues MBBR process which I will look into, as an overall well-functioning process configuration is of importance.

In the conventional ASPs a wash-out effect of PAOs might affect the efficiency of the system, as it is not easy to maintain a full recirculation of PAOs without any being lost in the sludge.

Temperature also affects this process (Sriwiriyarat et al., 2005). This is a problem, based on the MBR configuration, that can be eliminated or greatly reduced with AS MBRs.

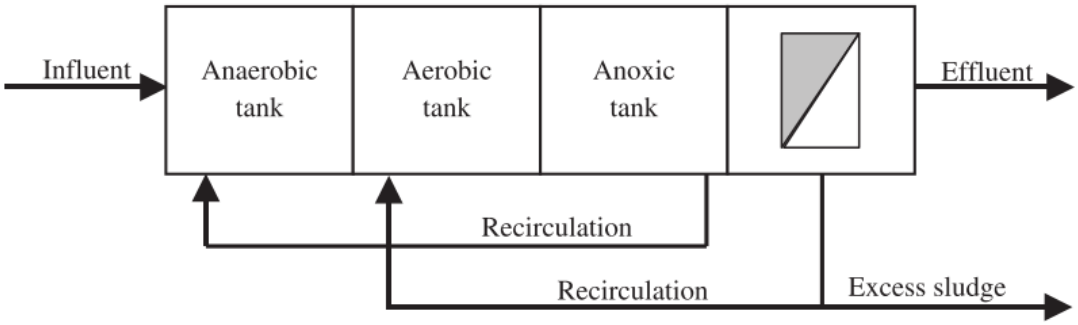


Figure 11 - Flow schematic for an AS plant with MBR (Zuthi et al., 2013)

Radjenovic et al., (2008) states that a lot of research show promising results using MBR for removal of nutrients including phosphorous. As MBR AS systems are set up and function well, it seems that incorporating a MBR in with the MBBR also could be a solution. However, for the EBPR process, in terms of increasing its efficiency for recirculation of sludge with an active biomass, the separation step is not relevant.

Leyva-Díaz et al., (2016) stated that the reduced fouling with MBBR-MBR is a great advantage over AS MBR, as well as increased biological reaction rates through the accumulation of a highly concentrated biomass.

3.1.2 Sequencing Batch Reactors with AS (SBRAS)

Sequencing Batch Reactors (SBRs) is one of several process configurations that can be used to achieve EBPR (Aguado et al., 2006). A SBR works through a fill and draw batch system. All treatment steps are performed sequentially for a specific volume of water in a single set of basins. The steps normally go as follows; fill, react, settle and decant. The control system is a key in SBRs and it can have configurations set up with sensors, timers and so on to meet the necessary demands for a specific plant (US-EPA, 2010). See Fig. 10 for a schematic of an SBR. During the clarifying period none of the liquid-bulk should enter or leave the tank so that turbulence in the supernatant is avoided.

An SBR process may be more complex and require more piping than the CAS process. Compared to the continuous AS process the SBR process may also require a higher volume because of the time of filling and drawing the reactors. However because of all the processes that can happen within one batch an efficient utilization of the volume can be achieved (Al-rekabi, 2015)

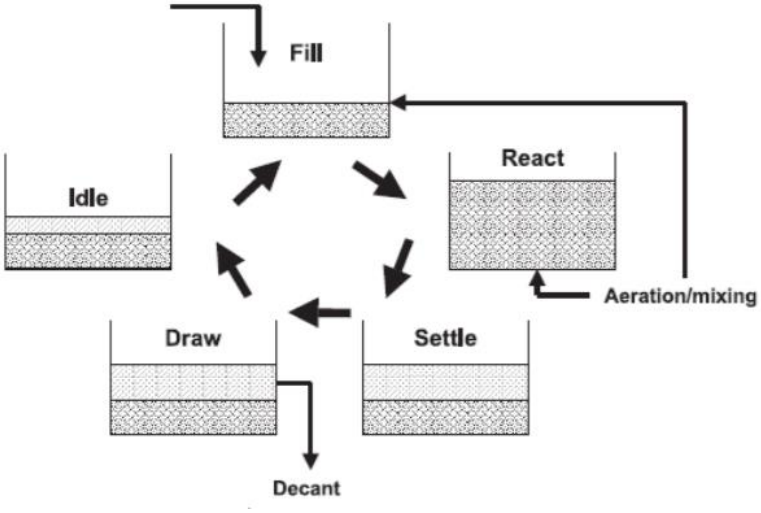


Figure 12 - Operation schematic of a SBR (US-EPA, 2010, ch. 6.2.1.4)

Zhang et al., (2006) stated in their study that a sequencing batch membrane bioreactor (SBMBR) operated much better considering nutrient removal than a conventional MBR. In the SBMBR system the removal of total phosphorous was as high as 90 % during most of the experiment. This experiment also included removal of nitrogen.

3.2 Moving Bed Biofilm Reactor (MBBR)

The basic principle of the process is plastic carriers moving freely around in the reactor experiencing growth of biomass on them where microbial processes happen because of diffusion of substrate from the liquid bulk.

The MBBR has incorporated some of the positive features of the activated sludge process and biofilter processes to create a hybrid of these (Al-rekabi, 2015). Carriers moving freely in the wastewater establish microbial film on which microorganisms can grow and develop. The biomass is attaching to certain area of a carrier, and it is at these areas the microbial turnover process is happening while the liquid bulk contains the substances that will participate in the turnover (Ødegaard et al., 2014, pp. 492-494). This biofilm can include differing layers and differing microbial communities that incites removal of substances in the water based on the conditions (Al-rekabi, 2015). Within the reactors the operation can be both aerobic, anaerobic and anoxic. Within the MBBR process the whole reactor volume is utilized and there is no need for sludge recycle. To utilize the whole reactor volume, mixing of the carriers is important. In the aerobic reactor this happens because of aeration while in the anaerobic (or anoxic) chambers,| mixers are used (Ødegaard et al., 2014, pp. 500). The completely mixed reactors allows the reactor volume to be fully active, which is an advantage of the MBBR process (Ødegaard et al., 2001). The sludge loading to the sludge treatment process in greatly reduced from AS (Helness, 2007; Ødegaard et al., 2014, pp 501; Simonsen, 2008). See Fig. 11 for an overview of how the reactor volume is utilized.

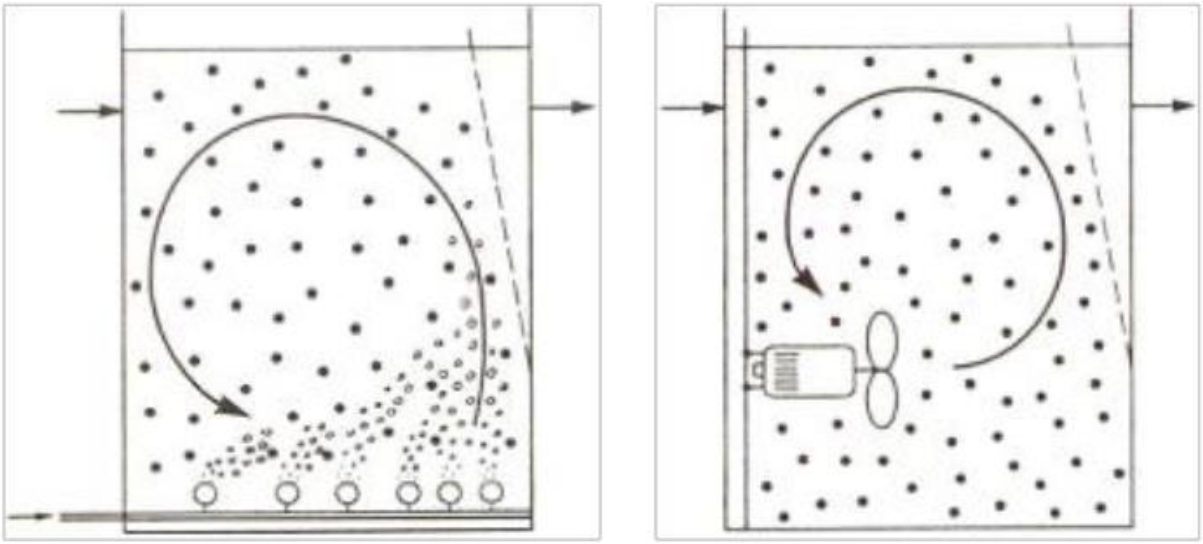


Figure 1 - The principles of a MBBR with two reactors; aerobic (left) with mixing by aerating and anaerobic (right) with mixing by rotation. The dots is a representation of the carriers, however not in scale (Ødegaard et al., 2014, pp. 500).

One of the most important advantages of the MBBR is that the biomass is more specialized and active (Ødegaard, 2006). Inactive biomass is continuously washed out of the reactor as it erodes off carriers. In comparison with an activated sludge system with recycled biomass wash-out effect, as previously stated can be experienced, and one will never be able to develop the same specialized biomass as in a MBBR (Ødegaard et al., 2014, pp. 501-502). Helness & Ødegaard, (2001) stated that the vulnerability of sludge loss was a main reason that make the biofilm process more favourable above AS. The specialized biomass is a product of having carriers fitted into a single environment full-time making the biomass experts that thrive in the conditions within their reactor (Ødegaard et al,. 2006). As PAOs are quite slow growing organisms this can be very useful, (US-EPA, 2010, ch. 5.4.4). As the thickness of the biofilm increases biofilm will erode off and the process of separating the sludge from the water is easier in this process than with activated sludge since it is not pertinent with thickening of the sludge before return to the reactor, as no sludge is returned. (Ødegaard et al., 2014, pp. 501). As biofilm is eroded off the, the inactive biomass including PAO bacteria will erode off and be lead to the separation stage, which is how phosphorous is removed.

Commonly MBBR reactors has not been used in removal of phosphorous because of the necessity to exploit the PAO to alternating anaerobic and aerobic conditions. In traditional MBBRs EBPR is not possible as the carriers are within each batch and not moved into varying operating conditions.

There are also disadvantages to the MBBR process one being diffusion limitations. For the removal of phosphorous through the EBPR process in an MBBR diffusion limitations can be experienced both in the aerobic and the anaerobic reactor. Therefore, although the process works well within one reactor, for the process to work it must not experience constraints in any of the reactors. As the biofilm is thicker in an MBBR than flocks in the activated sludge process the diffusion constraints are of upmost importance (Falkentoft et al., 2001).

Falkentoft et al., (2001) also stated that whether biofilters contains more active biomass than AS systems needs more research. This study was done on a biofilter and not a MBBR but the relevance of the question still remains as the active biomass present when using biofilm has large implications on the EBPR process.

Activated sludge plants that are overloaded can be transformed though hybrid processes also becoming MBBR plant (Sriwiriyarat et al., 2005), which I will come back to.

3.2.1 Biofilm and Diffusion

Falkentoft et al., (2001) highlighted the diffusion as one of the most important parameters in the MBBR process with EBPR. With higher concentrations in the bulk the penetration depth for which VFA, phosphate and oxygen has to travel increases, and the penetrations depth is a function of the involved substances (Falkentoft et al., 1997; Ødegaard et al., 2001).

Generally, we wish to have a thin biofilm, so substrate can diffuse into the biofilm and reactant products can diffuse out. It is beneficial for the efficiency of the EBPR process if it is distributed evenly over the surface of the carrier (Ødegaard, 2006; Ødegaard et al., 2014, pp. 501). In Fig. 11 different layers in a biofilm is shown, as well as the boundary layer. From Fig. 11 one can see different processes happening within the film such as, hydrolysis, diffusion, absorption and so on.

The transport of substance into the biofilm happens through molecular diffusion. Therefore, the driving force of the process is the kinetic energy of the solution molecules or diffusion only (Henze et al., 2008, ch.18).

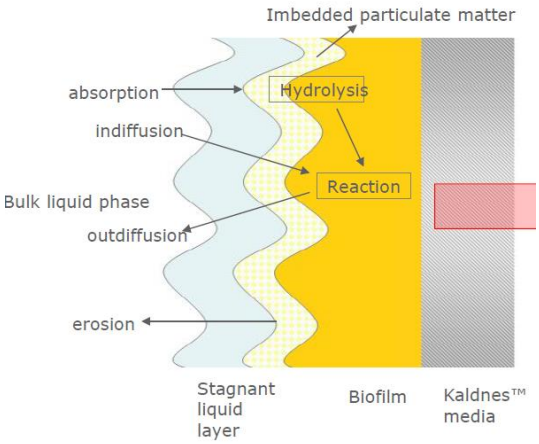


Figure 13 The way a biofilm works by differing processes. Within the biofilm biochemical reactions happen (Ødegaard et al., 2014, pp. 493)

Development of new suspended carriers for MBBRs needs to look into not only the total increase in available protected surface area of the carrier, which directly is a step to increase biofilm, but another important point is to allow for good conditions for transport of substrates into the biofilm (Mašić et al., 2010). For full substrate penetration of oxygen and other substrates a thickness of 100 µm is usually preferred (Al-rekabi, 2015). If it is preferable to have both aerobic and anoxic and or anaerbic layers on the biofilm and increase in the thickness could be beneficial (Piculell et al., 2016)

For the EBPR process a crucial factor is the biofilm thickness. It must be optimized for the well-functioning of PAO, meaning it must be of an optimal thickness that enhances the PAO ability to take up substrate like VFA, phosphate and oxygen and release substances within relevant stages of the process. With changes in the penetration depth comes changes in the effectiveness of the process. Falkentoft et al., (2001) stated that need of relatively thin biofilms to avoid diffusion limitation is wanted, but this again might challenge the concentration of

active biomass, which again can reduce the volumetric removal rate to a level like that in activated sludge systems.

Transport of oxygen through diffusion is more limited in a biofilm process in comparison to what is found in a suspended biomass as convection is higher in an AS than in the biofilm-process. Many previous studies have shown that transport of dissolved oxygen (DO) is close to a first order kinetic rate in a biofilm-process (Piculell et al., 2016).

The diffusion processes are driven by the changes in concentration which leads to transport into the biofilm of substances in the bulk. In the biofilm the substances are either removed or there is a production of a new substance (Crittenden et al., 2012, ch.7). There must be a balance over the biofilm surface so that the same amount diffused into the biofilm is either removed, produced within the film or diffused out of the film (Henze et al., 2008, pp. 460) see Eq. 2.2. The diffusion coefficient (D) is an important parameter in molecular diffusion as the flux is dependent on this. The flux into the biofilm is also dependant of the concentration in the bulk (C0) and in the biofilm (Cb). The mass flux because of molecular diffusion was described by Fick's law. The dC(a)/dz shows the change in concentration with the change in length per unit, see Eq. 2.1.

$$J(A) = -D * \frac{dC(a)}{dz} \qquad \text{(Eq. 3.1, Henze et al., 2008, pp. 461)}$$

J(A): Mass flux of component in solvent due to diffusion

D: Diffusion coefficient of component in solvent. Since components flow from high concentration to low concentration this constant has a negative sign in from of it.

C(a): Concentration of component

Z: distance in the concentration gradient direction

If there are no reactions occurring in the biofilm the concentration gradient will be constant and the distribution in concentration in the film will change linearly. If there is removal within the biofilm the concentration gradient will no longer be constant and the distribution of concentration in the biofilm will curve down. However, Fick's second law states that is there is a reaction in the biofilm the concentration gradient will also not be constant anymore, as with removal, and the concentration distribution will curve up. See Eg.2.2.

$$\frac{\partial C}{\partial t} = -D * \frac{\partial^2 C(a)}{\partial z^2} - r \qquad \text{(Eq. 3.2, Henze et al, pp. 460)}$$

r = Rate of conversion in the biofilm pr.volume

If the process under is looked at as an idealized version of the biological film, we can say that the bulk is where C=0. The entire length of the Fig. 12 is the film layer while one can see the removal mechanisms happening along the film. It is within this layer the mass transfer is happening with substrates fluxes through the layer. At point x there has been a transport through the film of length x giving a concentration of C_x. Through the change in Δx there will be a change in the concentration which gives a concentration in the biofilm of $C_x+\Delta x$ at point $x+\Delta x$ (Crittenden, 2012, ch.7).

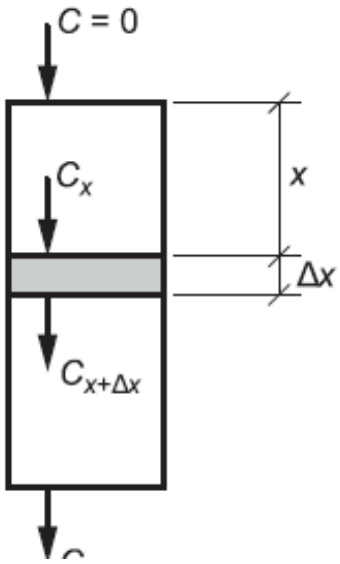


Figure 14 - Mass balance for a biofilm

The overall mass-balance of what diffuse in is equal to what diffuses out minus the removal that happens in the film.

The most probable outcome for this process is that it should have quite a small layer of biofilm so that the PAO can find an optimum point to establish themselves in to enhance the P-recovery. If the biofilm layer is too thick this process can be greatly reduced. This can happen if the substrate concentration is to great in the liquid bulk (Ødegaard et al., 2000). The net uptake of phosphorous due to biomass growth is the main driver for the phosphorous removal process which will increase the biofilm thickness (Helness, 2007). This can also create obstacles as the need for a relatively thin biofilm also is seen as a necessity for diffusion optimization (Falkentoft et al., 2001).

An issue with too thick biofilm is not only diffusion limitations but also detachment. If the biofilm is too thick the detachment can be very damaging for the biofilm and detachment of active biomass beneficial for the process can be experienced (Al-rekabi, 2015).

Assuming steady-state for Eq. 2.2 analytical solutions for first and zero rate expressions can be calculated. For more complex rate expressions numerical solutions are required.

The zero-order rate expression

$$r(vf) = k(0) * X(F)$$ (Eq. 3.3, Henze et al., 2008, pp. 461

r=(vf): The rate of conversion within the biofilm is dependent on the zero-order constant and the active biomass X(F) within the biofilm

The first-order rate expression

$$r(vf) = k(1) * X(F) * C(F)$$ (Eq. 3.4, Henze et al., 2008, pp. 461)

r=(vf): The rate of conversion within the biofilm is dependent on the first-order constant, the active biomass X(F) within the biofilm and the substrate concentration in the biofilm

The EBPR process is a redox process, so it requires both an oxidant and a reductant. For the process of removing phosphorous though the EBPR in the aerobic stage the electron acceptor is the oxygen and the electron donor is the substrate in the liquid bulk. Weather one of these is the limiting substrate is dependent on the operating conditions within the reactor, the differing inlet concentrations and how these affect removal rates and diffusion rates of the substrates. It is important to find the limiting substrate to evaluate how the process can be improved. Fig. 13 shows two-component diffusion, where limitations are met regarding amount of substrate.

There needs to be enough electron acceptors in the form of dissolved oxygen (or nitrate or nitrite in an anoxic process). However, with supplying oxygen the negative effect that can stem from this is the shear stresses caused by the aeration. Li et al. (2003) found that the DO concentration

in the liquid phase that supplied enough DO and avoided shear stresses that eroded the biofilm before it was able to grow too thick was about 5 mg/l.

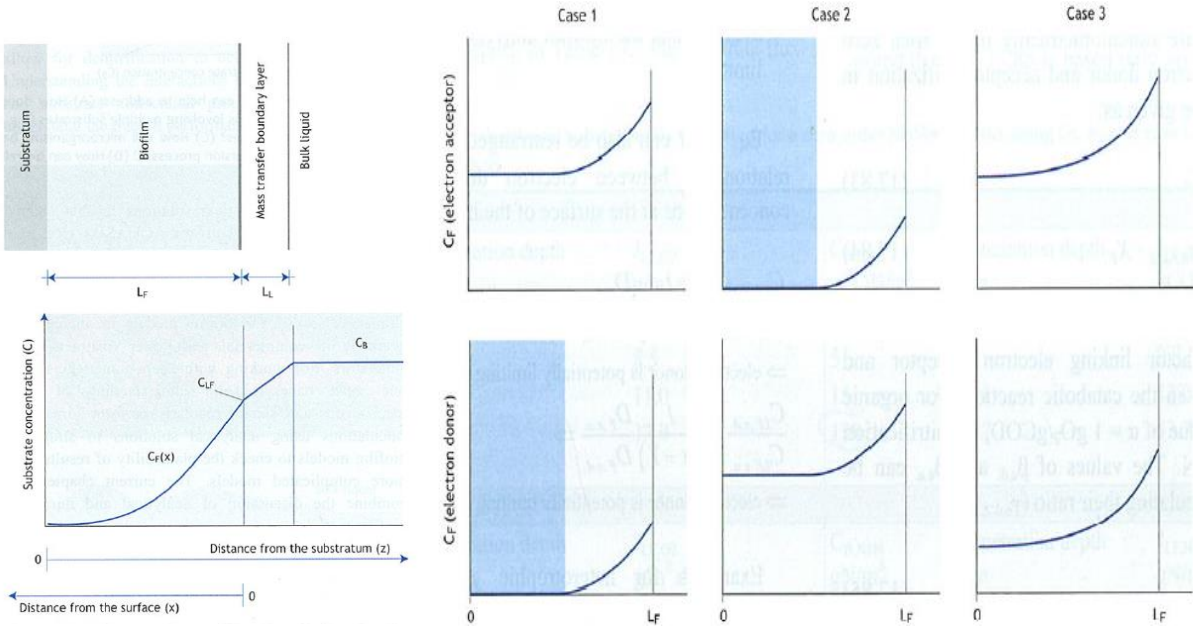


Figure 15 The diffusion process depends on the bulk concentration as well as the biofilm thickness shown in figure to the left (Henze et al., 2008, pp. 460), while in the figure to the right there is two-component diffusion showing which component, the electron donor or the electron acceptor is the limiting substrate. Case 1 – Donor limiting. Case 2 – Acceptor limiting. Case 3 – Neither limiting (Henze et al. 2008, pp. 479)

Masic et al., (2010) stated that the faster the bulk flow, the thinner the biofilm becomes, as well as an increased oxygen supply decrease the thickness of the biofilm. Masic et al., (2010) therefore concluded that increasing flow rates has a stronger effect on biofilm erosion than on biofilm growth.

Al-rekabi, (2015) also discuss how flow and turbulence affect the nature of the carrier media. As the performance in the reactor is dependent on adequate flow and mixing, this in turn affects the turbulence. Thick and/or fluffy biomasses are not desired, and adequate turbulence erodes excess biomass to maintain a smooth and even thickness. If the turbulence becomes too high active biomass can detach.

Masic et al., (2010) also discussed the importance of the thickness of the boundary level to induce diffusion. Mašić et al., (2010) results show that there is a relationship between the boundary layer thickness and the utilization of oxygen, and the results shows that by decreasing the boundary layer thickness it is possible to achieve a more efficient utilization of oxygen (Mašić et al., 2010). This experiment is done with the aim of nitrifying in the MBBR with nitrate as the electron acceptor. It is therefore not directly applicable to the continuous MBBR process

with phosphorous removal, however it still shows that the boundary layer is of importance. See Fig. 14 for the oxygen profile through the biofilm and the boundary layer.

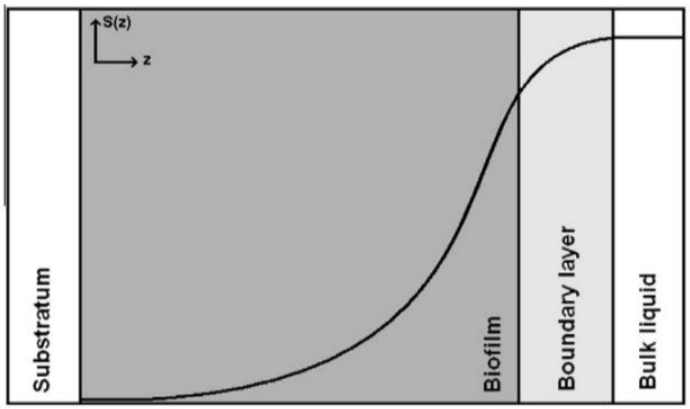


Fig. 9. The oxygen profile through different phases, as shown in [23].

Figure 16 - Oxygen profile through a biofilm, showing the amount of oxygen at the different phases (Mašić et al., 2010)

3.2.2 Different type of carriers

There are several types of carriers that is developed and they all have different properties relating to; surface area, loading as a fraction of total reactor volume, size, cost, shape and so on. The main point of the carriers is to have a very high surface area and easily enable complete mixing so that the distribution in the reactor is homogeneous with regards to substrate and biofilm (Helness, 2007; Simonsen 2008). The properties of the carriers can influence the diffusion processes between the carriers and the substrate in the reactor. If the carriers have areas that are difficult to reach this can lead to problems with diffusion. See Fig. 15 for an overview over carriers produced by both extrusion and molding.

Total surface area consists of the carriers inner and outer surfaces. The effective surface is the area on the carrier where biofilm is attaching (Ødegaard et al., 2000).

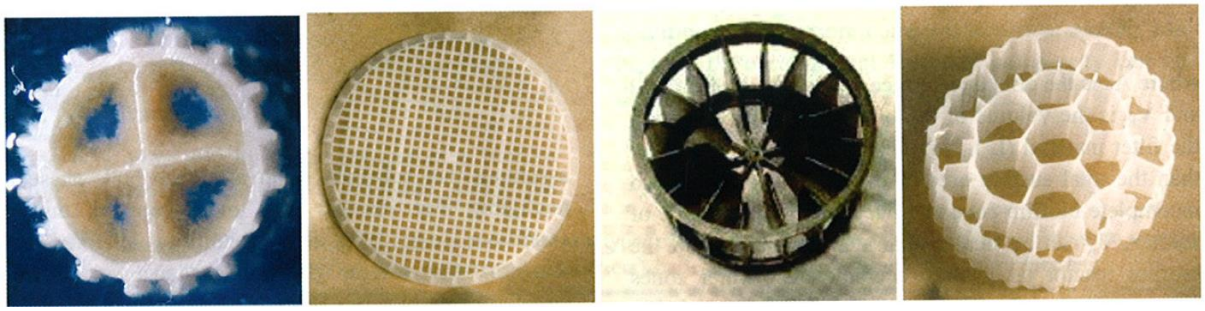


Figure 17 Showcasing different carrier types. From the left; AnoxKaldnes K1, BiofilmChip, Natrix Type, AnoxKaldnes K3 (Henze et al., 2008, pp 499)

3.2.2.1 Classical Kaldnes carriers

Ødegaard et al., (2001) did a study on carriers of various sizes to study whether the size or the shape of the carrier affected the removal rate of the substrate. See Fig. 16 for an overview of the carriers used. Ødegaard et al., (2001) presented results using three different carriers under the same substrate loading. In this study Ødegaard et al,. (2001) state that it is difficult to assess the kinetic effect of wastewater COD as there are so many variations in the inlet quality and differences in spesific compunds in this water. When for example doing lab scale testing and using substrates as acetic acid, ammonia or nitrate its relatively easy to establish a rate curve for the compound (Ødegaard et al., 2000). The kinetics between carriers and COD in the wastewater and determining removal rate is much more difficult to assess as the influent quality of wastewater is continiously changing, and averages from one area can not be easily transmitted to another area.

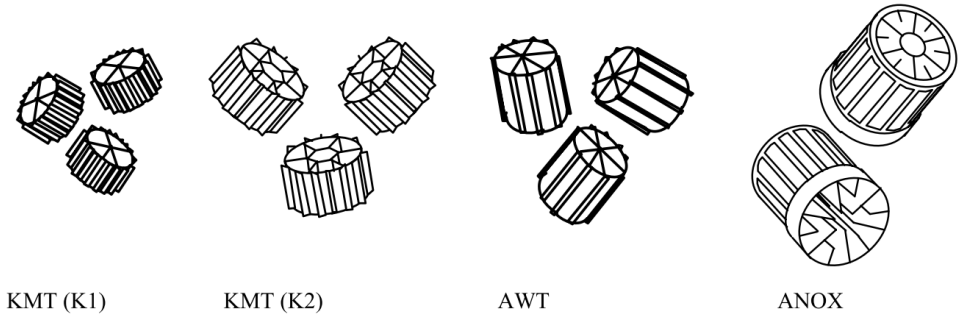


Fig. 2 The four biofilm carriers used

Figure 18 The biofilm carriers used by Ødegaard et al., (2001) to check how size, shape and effective area affected the removal process of COD

Substrate removal rate can be considered first order at very low bulk concentrations, while it is reduced at higher bulk concentrations, meaning when the substrate concentration increases the kinetic rate decreases (Ødegaard et al., 2000). This is because the transport of substrate through the biofilm is being limited at very high bulk concentrations.

Removal rate vs loading rate equation given in Ødegaard et al (2000).

$$r = r(maks) * \frac{L}{L+K} \qquad \text{(Eq. 3.5)}$$

r(max): maximum substrate removal rate

L: Substrate loading rate

K: Constant, will vary for various substrates and operational conditions

Ødegaard et al., (2000) found that with low substrate loading rates carriers with varied sizes gave almost the same rate of removal with a given volumetric substrate loading rate, however when the substrate loading rate increases the removal rate in terms of volumetric loading started to move away from each other. For dimensioning of MBBR it is more beneficial to look at the area loading rate. When Ødegaard et al., (2001) looked at the removal rate with regards to the effective surface area the removal from all carriers fell along the same curve (Ødegaard et al.,

2000), see Fig 17. Ødegaard et al., (2001) therefore concluded that the shape and the size of the carrier seems to be of minimal importance as it is the effective surface area amongst the carriers in the reactor that is of importance for removal.

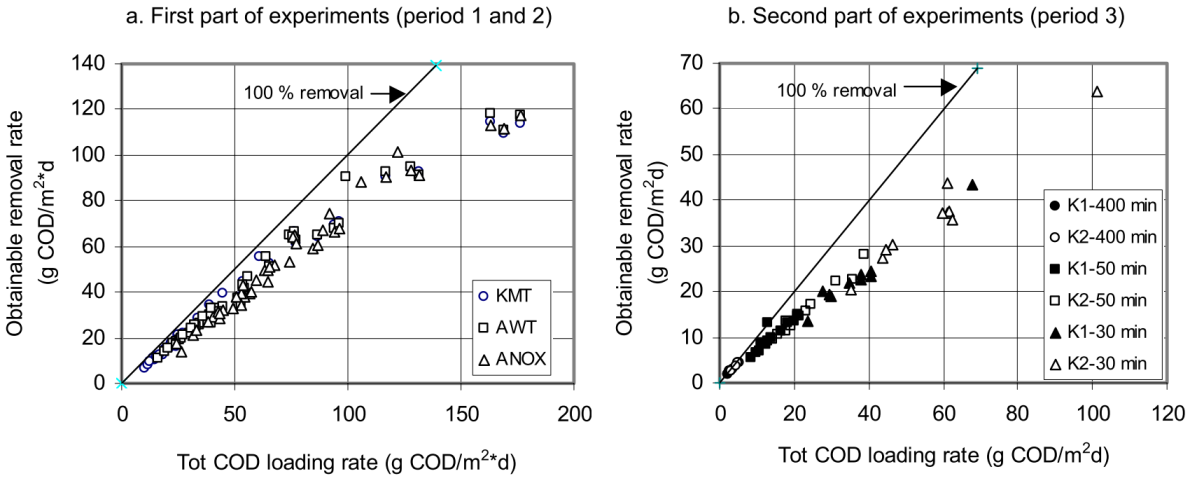


Figure 19 Results by Ødegaard et al., (2001); effective area being the deciding parameter of removal, not shape and size of the three carriers tested. If effective area is the same amongst different carriers in different reactors removal will be similar.

From Ødegård et al., (2000) experiment the results show removal rate vs loading rate with respect to soluble COD. Because the process of removal of PAO is dependent upon the RBCOD with respect to VFA it is important to look at the removal process of soluble COD for the carriers. Although soluble COD and RBCOD are not one and the same, they are linked and can help serve as a rule of thumb. Ødegaard et al., (2001) results indicated that very high organic load rates could be used in a MBBR.

As Ødegaard et al., (2001) evaluated the size and shape of the carriers in Fig. 16 and concluded that their effective area was of importance not the shape, I have looked into reviews on other type of carriers than Ødegaard et al., (2001) studies, mainly biochip carriers.

3.2.2.2 Other type of carriers

Mašić et al., (2010) experiment to determine the oxygen profile on suspended carriers with biofilm growth they decided to do the experiment on a BiofilmChip P as this was developed to allow good conditions for transport of substrate. One negative part of the BioifilmChip P is that it is more expensive due to it having to be molded, while the other carriers like K1, K3 and K5 all are produced by extrusion and chopping (Ødegaard, 2014, pp. 503). Several places in literature and online is the carrier chips specifically mentioned in regards to be able to control the thickness in the biofilm in a better way. See Fig. 18 for information on BiofilmChip P.

Fig. 1. Images of BiofilmChip P.

Table 1
Characteristics of BiofilmChip P.

Length	3 mm
Diameter	45 mm
Protected surface per carrier	6.818×10^{-3} m^2
Protected surface per m^3 carriers	900 m^2

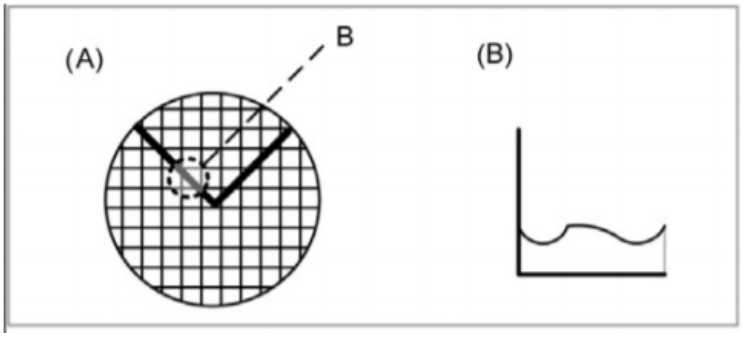


Figure 20 - Characteristics of a biofilm Chip (left), showing a biofilm chip and how the biofilm can look across the surface (right) (Mašić et al., 2010)

Deng et al., (2016) has found a promising alternative media, that is a development of the Kaldnes carrier with sponge attached. Deng et al., (2016) states that this has gained much interest for reasons such as its low-cost and high porosity. The high porosity helps leads to a quick and stable attachment and growth of microorganisms on the carrier. It is also stated that the effectiveness of usages of sponges in MBBRs for organic and nutrient removal has been highlighted in recent studies.

Deng et al., (2016) used a plastic carrier, modifies using a sponge to investigate the functionality of this new media in enhancing and increasing the treatment efficiency in a MBBR system. See Fig. 19 for the experimental setup as well as a highlighted picture of the plastic media with the sponge.

The aim of using the modified plastic carriers is to improve the pollutant removal efficiency of MBBR by increasing attachment areas for biomass, while the usage of these carriers also minimized the membrane fouling in the membrane bioreactor (MBR) separation unit.

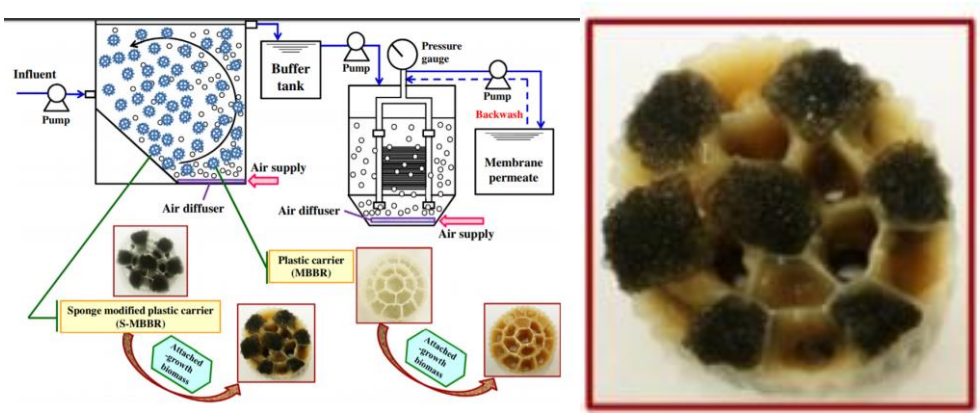


Figure 21 Experimental setup of the sponge-MBBR-MBR and the MBBR-MBR (Deng et al., 2016). The sponge carriers are the modified plastic carriers in the bottom left corner being modified versions of the classic plastic carriers.

Deng et al., (2016) evaluated the performance of modified sponge carriers in both MBBR and MBBR–MBR systems. When comparing the results in the modifies sponge MBBR to the classic MBBR, the sponge carriers could improve nutrient removal and organic efficiencies overall. Because the sponge modifies carriers experienced more attached growth, and therefore

had a more active biomass, the modifies carriers facilitated phosphorous removal to a higher degree than the unmodified version. The sponge MBBR obtained a higher phosphorous removal efficiency of about 70%, while the classic MBBR experienced a removal of about 64%. There was also an efficient fouling reduction in the MBR.

Piculell et al., (2016) decided in their study; the evaluation of biofilm thickness on nitrification in a MBBR to use the AnoxKaldnes Z-carriers, see Fig. 20, because it with its design enables control of the biofilm thickness. The walls on the chip is 400 µm, which prevents growth above this.

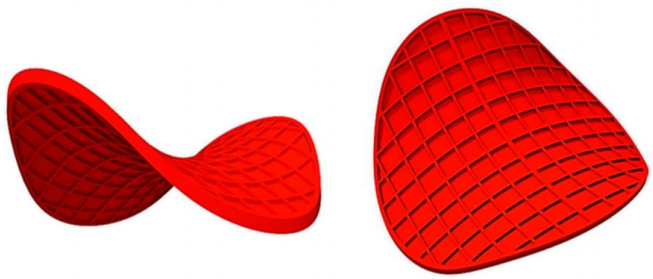


Figure 22 AnoxKaldnes Z-carrier (Piculell et al., 2016)

Ecologix Systems have developed a carrier called BioChip25, which they describe as a chip where the inactive biomass can more easily be discharged from the chip, and therefore there will be less inefficiency caused by a lack of substrate supply in the anaerobic chamber and in lack of oxygen supply in the aerobic. This is because no dead biomass blocks the active biomass. This biofilm carrier has the average thickness of approximately 1.0 mm and the substrate and oxygen can diffuse from both sides into the carrier to a depth of 0.5 mm (Ecologix-systems, 2017). See Fig. 21 for picture of the biochip.

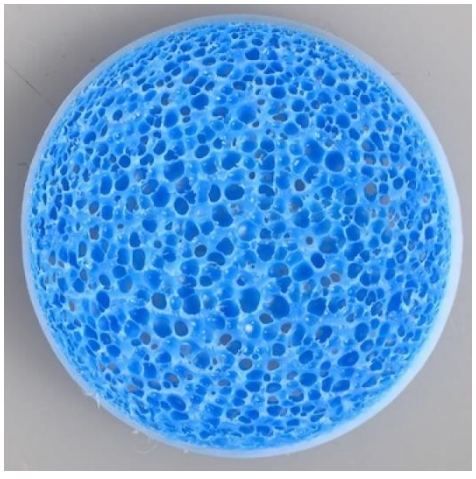


Figure 23 BioChip 25 from Ecologix Systems (Börner & Trübenbach, 2017)

3.3 Integrated Fixed Film Activated Sludge (IFAS)

As mentioned in part 3.1 activated sludge, there was a need for two return flows for BNR removal. This can be space and cost demanding. Therefore, other solutions have been looked into.

The Integrated Fixed Film Activated Sludge (IFAS), a variation of the AS and MBBR process, gets its name from the integration of biofilm carrier technology within conventional activated sludge, and this integration of both fixed film and activated sludge makes it a hybrid process. What makes the process so special is that it enables activated sludge systems to, within the same volume, experience increased productivity without increasing RAS, by adding biofilm carriers. It therefore does not become necessary to increase the reactor size to increase efficiency (Sriwiriyarat et al., 2005). The increased efficiency is in terms of space; by adding biofilm carriers, and in terms of time and resources; by inducing a more compact and quick process, because of the reduced hydraulic retention time (HRT) (Helness, 2007). Because of the increase in volumetric productivity the IFAS systems deliver improved performance while reducing the solids impact on clarification processes. The return activated sludge flow is what enables the phosphorous removal. See Fig. 22 and 23 for different variations of process configuration. In Fig. 23 EBPR is enabled.

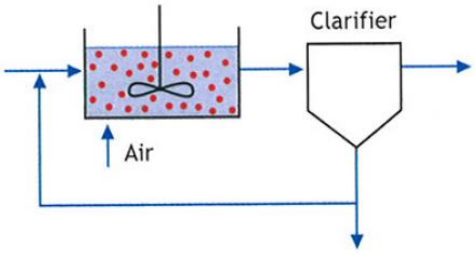


Figure 24 - Showing a conventional IFAS process; both MBBR and activated sludge within the same ractor (Henze et al., 2008)

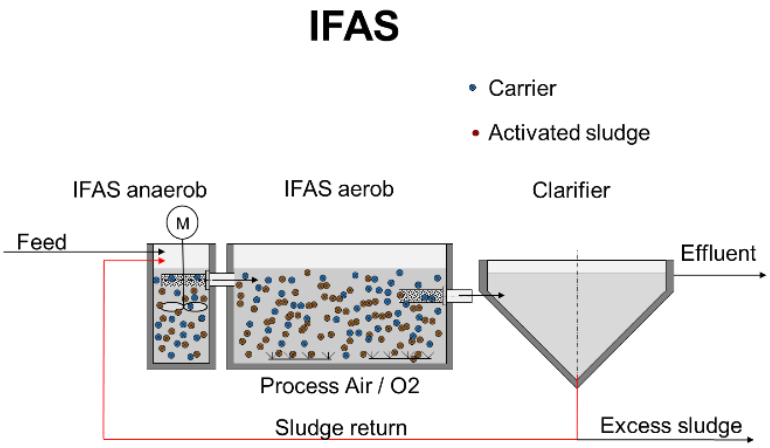


Figure 25 - The IFAS process with anaerobic and aerobic zones where we can have EBPR (Börner & Trübenbach, 2017)

One problem that stems from the AS process of biological nutrient removal is the separation of sludge and it seems like the separation step actually benefit from implementing IFAS technology as the solids load decrease in the sludge handling step (Mannina et al., 2017).

Sriwiriyarat et al., (2005) stated that although it is unlikely that EBPR would be possible by biofilms growing in continuous-flow systems with fixed operational zones, it is reasonable that EBPR could be maintained in IFAS systems where the biomass responsible for phosphorous removal is in suspended form and circulates throughout the system.

Mannina et al., (2017) found that in their study on IFAS system with an MBR that there were significant fluctuations concerning the attached biomasses activity level. This study looked at the effect of influent C/N-ratio. Here they found that the heterotrophic activity observed in the attached biomass generally was lower compared with the suspended biomass while the autotrophic activity of the attached biomass was higher compared to the suspended biomass. They therefore suggest that the results show the specialization of the biomass. Suspended biomass showing a greater organics removal capability and the attached biomass showing a greater nitrification capability, likely due to the high retention time of biofilm on the carriers (Mannina et al., 2017). In a continuous MBBR the retention time of the carriers will be indefinite as they continuously are moved around.

Considering the discussion in chapter 3.3 it is likely to believe that the carriers in a continuous MBBR will not establish this specialized biofilm in the same way as MBBRs or IFAS systems. One key difference is the movement of carriers into various operating conditions in the CMBBR in comparison to the IFAS MBR. How this affects the development of a specialized biomass and the timeframe for this to develop is probably quite different from a batch system.

3.4 Sequence Batch Reactor with MBBR (SBBR)

Helness et al., (2007) pp. 51 states that the Biofilm SBR (SBBR) probably will resemble the activated sludge SBR, however as previously noted with MBBR in comparison to AS this process will be able to utilize the volume more efficiently, especially for both EBPR and nitrogen removal. This can be done since it is possible to have the simultaneous denitrification and phosphate release, and nitrification and phosphate uptake which is not possible in a AS SBR, as well as a reduced hydraulic retention time (HRT) in comparison to AS SBR. This process happens through the biofilm on the carriers having various anaerobic and aerobic zones which permits the simultaneous removal of nitrogen and phosphorous. The HRT is reduced as the necessity for a separate anoxic phase is removed (Al-rekabi, 2015; Henze et al., 2008, ch 7).

Pastorelli et al., (1999) also stated that SBR concept for EBPR could be beneficial as one could operate SBRs with differing temporal scales, however not spatial scales.

Helness (2007) considered the possibility of removing phosphorous in a SBBR. The results showed that biological removal of phosphorous and nitrogen was possible. With filters Helness (2007) stated that a final clarifier after the biological step probably would not be necessary, only a separation unit. There is a higher likelihood of establishing the specialized biomass discussed in chapter 3.2 and 3.3 than there is for a continuous MBBR.

A process where SBBR is used would likely need more demanding and sophisticated control strategies than a continuous MBBR moving in some form of carousel mode. SBBR will also most probably also be more costly because of higher investments costs due to piping mechanisms and so forth (Al-rekabi, 2015).

3.5 Continuous Moving Bed Biofilm Reactor (Continuous MBBR)

Historically MBBR has not been a process in which EBPR has been relevant, or possible to use. As previously shown Helness (2007) looked at the use of MBBR in a SBR, however now there has become a bigger interest into looking at the MBBR as a continuous process where carriers follow the flow of the water. In this process the Sludge Retention Time (SRT) will be the same as the Hydraulic Retention Time (HRT) as the suspended mass will follow the flow of the water through the process (at least if the configuration is set up to function well). This process must also be set up so that the carriers follow the liquid bulk in the same speed, if not either carriers or the liquid bulk can have been transported into new chambers before the microbial processes have had time to happen, reducing the processes efficiency and enabling OHOs to proliferate.

In this system as with the SBR the microorganisms will experience alternating anaerobic and aerobic conditions, however differing from the SBBR is that the carriers are what will be moved around to different conditions and not differing conditions within the same reactor. The first reactors will be anaerobic, with mixers, while the aeration is controlled in the latter reactors. The inactive biomass/surplus sludge detach from the biofilm in the end chamber. Here the carriers will be held back with a strainer and continue in the reactor, while the surplus sludge will go to the separation stage. The surplus sludge contains the inactive biomass. Phosphorus that has been taken up from the water in a greater amount than at the beginning of the process can therefore be discharged from the system periodically. See Fig. 24 and Fig. 25 for a process schematic of how a continuous MBBR can look.

Because the carriers are moved into the different zones this requires even more thought as to which carriers to use. The biofilm that establishes for the carriers are no longer specialized to one condition but need to function well under differing conditions. This is also the case for SBBR, although movement from differing reactors is not an issue. Because of the movement in what can remind of a carousel mode, this can lead to increased sheer stresses on the carriers, which again can affect the erosion process of the biofilm.

With the CMBBR one loses the ability to operate it with very differing temporal scales in the different chambers as the sequence of events in no way happens as it does in a SBR (Pastorelli et al., 1999). In a CMBBR one will have a spatial sequence of events where processes does not happen temporally spaced from each other, but where differing biological processes occur simultaneously.

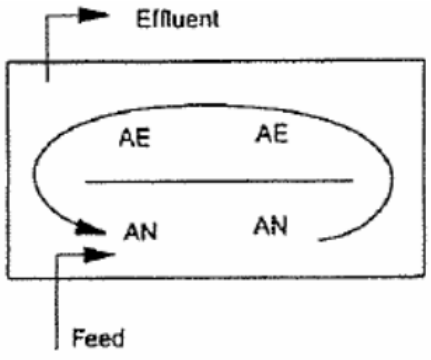


Figure 26 - One example of how a process schematic for a continuous MBBR could look (Helness, 2007, pp 51)

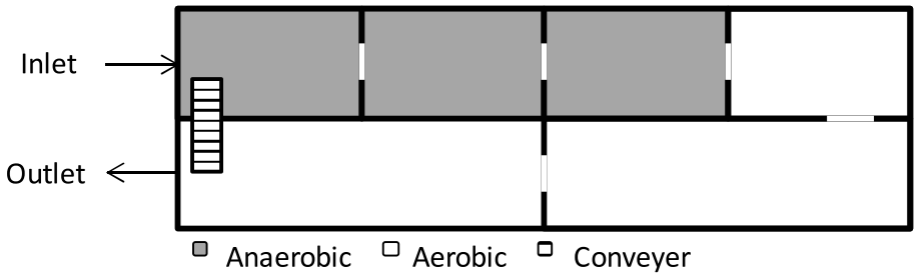


Figure 27 - A similar process schematic to the one above, operating in a carousel mode. The carriers are transported through openings in the walls, and the conveyer transports the carriers for a new loop, while excess biomass is transported out of the reactor (Saltnes et al., 2016).

4 Materials and Methods

Materials and methods used during experiments are described in this chapter as well as tests that have been performed to assess the pilot's parameters and performance.

There are several types of experiments performed. Firstly, experiments on the daily conditions in the pilot as well as the daily influent- and effluent relationship of concentrations of PO4-P, NH4-N and sCOD in the pilot. These measurements and tests have been performed to assess the daily performance of the pilot.

Secondly kinetic experiments performed in beakers under varying conditions with both influent wastewater, synthetic wastewater and a combination of these for investigating the bio-P uptake and removal and development of this throughout the spring semester.

Thirdly experiments to assess the development of biomass has been performed.

4.1 Pilot Performance

The pilot was set up with running wastewater only 75 days before my work on the master thesis started, and there was a lot of work needed in terms of calibrating the pilot and finding efficient and good methods for running it. See appendix F for information of occurrences that can have influenced pilot performance. Some will be discussed later.

The air-flow has been kept constant to make sure that changes DO-concentrations do not stem from changes in air-flow in to the chambers.

The speed of the conveyor belt has also been kept constant, except for short periods of time when accumulation of carriers has occurred in the last chambers in the aerobic zone, see appendix F for overview of these occurrences.

4.1.1 Optimization of the Pilot

Fig. 31 shows various pictures of the pilot set-up in the lab. Fig. 30 shows a picture of the two holding tanks, where the capacity is 3.5 m3 in each. The Salsnes filter soon to be installed is visible in front of the holding tanks.

The influent wastewater to the holding tank is collected from an apartment complex located near the lab, more precisely Klæbuveien 14 at Lerkendal. The raw municipal wastewater originating from these apartments are transported to a pumping pit where hourly pumping's transport the raw wastewater into the two 3.5 m3 holding tanks located at the lab. In the holding tank the sludge is settled and no stirring is performed. Sludge has been drawn out of the holding tank daily throughout the semester to avoid sludge build up above the placement of pumps to the influent in the pilot and the 24-hour composite sampler. Emptying of sludge in the holding tank has been done through closing the inlet valves to the holding tank before opening the outlet valve of each holding tank for approximately 30 seconds each. This has been done daily to avoid that larger particles will not be pumped in to the pilot or the composite sampler.

Figure 28 - The two holding tanks where wastewater is pumped in. In the front the Salsnes filter which is in the process of being installed is visible.

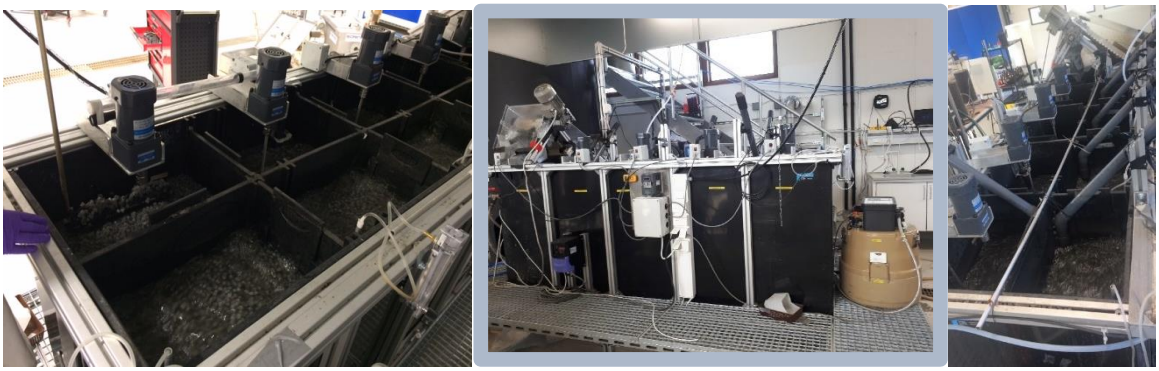


Figure 29 - Pictures of the pilot set up before the and after installation of static DO-sensors

The pilot has a total volume of 1,06 m3, appendix B, divided into 1 anaerobic zone with 4 anaerobic chambers and 1 aerobic zone with 6 aerobic chambers. The inlet wastewater entering the first anaerobic zone of the pilot plant is taken from the holding tank, see Fig. 30.

The carriers used in the pilot is the K1 Kaldnes, with specific surface area of 500 m2/m3, and is produced by Krüger Kaldnes of Veolia Water Solutions & Technologies. see appendix B, Tab. B1 for specifications of the carriers used in the pilot. In the pilot the filling degree is 60%.

The conveyor placed in chamber number 10 leads carriers from the last aerobic chambers to the first anaerobic chamber. The speed of the conveyor belt is constant, however during large build up periods in the last chambers the speed has been shortly increased.

The HRT in the pilot is currently 8 hours, while the effective HRT in empty bed without carriers is 6 hours and 21 minutes, see appendix B.

4.2 General Measuring Procedures

4.2.1 Sampling Method

Hourly variations were measured to track the hourly concentrations. See Fig.32 for plotted results. These results showed that the average PO4-P concentration throughout the 24 hours was 5.4 mg/L. Based on these results all samplings for kinetic experiments in beaker was therefore collected at around 09.00 to avoid sampling at concentration peaks. Hourly variations could impact the results through the sampling as grab samples indicates the concentration at that set time. Grab samples are highly uncertain. In order to get representative results, it was decided to use composite samples for the daily measurements. Before samples were collected the pipe were always left to drain for one to two minutes so that any residue in the pipe was drained out and not collected in the sample. The automatic sampler for measurements of daily concentrations in- and out accounted for any discrepancies in hourly variations.

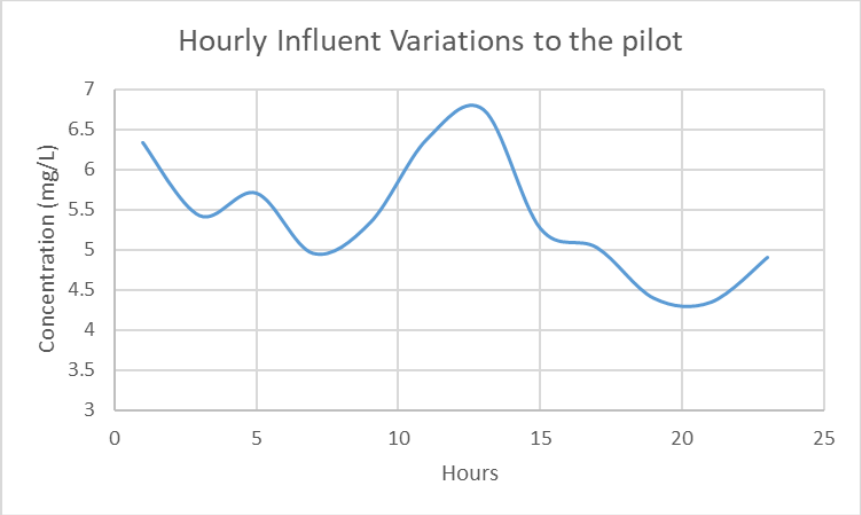


Figure 30 - Hourly variations in the pilot over 24 hours

4.2.1.1 Composite Sampling Technique

The In-and Out relationship have been measured from a 24 hours composite sample from the pilot. The autosamplers (Teledyne Isco - Model 3700 - Full-Size Portable Water Sampler) was placed by the holding tank for influent composite samples and by the pilot for effluent composite samples. Each of the autosamplers collect influent and effluent samples respectively, see Fig. 33 and Fig. 34 for placement and overview of the autosamplers.

The autosampler takes a 100 mL sample every hour, at a set time, and collects them in a plastic sampling bottle. One plastic sampling bottle contains 6 hourly samples. The 24-hour composite sample is in total made up of 4 plastic sampling bottles each containing 6 100 mL hourly samples. Each day when the in-and out relationship is measured the 4 bottles placed inside the autosampler which contains the samples from the last 24 hours is extracted. The 4 bottles containing the 6 hours composite samples for influent and effluent were taken out. 100 mL was extracted from each bottle and filled in a beaker, making a 400 mL composite sample of the 4 bottles for both influent and effluent water.

For more information of calibration and handling of the autosampler see appendix G for more details.

Figure 31 The automatic composite sampler for effluent samples placed by the outlet of the reactor

Figure 32 - The automatic composite sampler for influent water placed next to the top of the holding tank

4.2.1.2 Grab Samples delayed for retention time

Grab samples taken to track pilot performance was extracted from the middle of the chambers at 38.5 minute intervals; the effective HRT within each chamber in the pilot, see appendix B for data on the pilot HRT.

4.2.2 Cuvette tests

In accordance with Hach Dr. Lange cuvette test and the manual given for usage of these; PO4-P, TP, sCOD, NH4-N, NO3-N, Mg2+, has been measured. See Tab methods given by Hach Dr. Lange range. The daily In-and out samples of NH4-N and PO4-P have been diluted 1:2, while sCOD have been performed 1:1. During kinetic experiments dilution of cuvettes have differed throughout the kinetic because of changes to the concentration of substances. For more information on the description of cuvette tests and how they are performed see appendix A.

Table 5 -- Hach Dr. Lange analytical methods and ranges

Analysis performed	Method prescribed by Hach Dr. Lange	Range (mg/l)
NH4-N	LCK303	2.0-47.0
NO3-N	LCK340	0.23-13.5
TN	LCK138	1.0-16.0
PO4-P	LCK348	0.5-5.0
TP	LCK348	1.5-15.0
sCOD	LCK400	1.0-1000.0
sCOD	LCK114	15.0-150.0
Mg2+	LCK326	0.50-10.0

After the Hach Dr. Lange cuvettes have been used by method given in Tab. 5, the results were found analysing the cuvettes in the Hach Dr. Lange DR1900 Portable Spectrophotometer. The DR1900 uses a wavelength range of 340 to 800 nm to read of results.

The digestion of samples were performed in a DRB200 thermostat which can fit up to 12 samples at a time. It was necessary to digest samples for measurement of TP and sCOD.

4.2.3 Filters

All samples of PO4-P, NH4-N, NO3-N and sCOD have been filtered through a 0.45 µm cellulose and nitrate filter. All filters, according to standard procedure, need to be rinsed in distilled water at least three times. Sartorius is the producer of the specific filter used here. The filters were rinsed in distilled water, produced in the lab, and filtrated with distilled water before filtration of the samples were conducted. The choice to use a filter with a pore size of 0.45 µm was taken so that only the truly soluble fraction of the water analyzed. The use of a centrifugal machine was therefore essential since the process of filtration consumed far less filters from when filtration directly followed the mixing.

4.2.4 DO

Dissolved Oxygen (DO) has been measured with a portable DO-sensors stabilized for some time within each chamber. The DO-sensors used is WTW Oxi 3315 portable DO meter and has been calibrated throughout the semester. It has an accuracy down to a thousandth of a mg. In March static sensors were installed in 5 of the aerobic chambers. DO and temperature values within these chambers were read of from a logging screen attached to the pilot and then written in the log-book. For the remaining chambers DO continued to be read of by the WTW Oxi 3315 portable meter.

The kinetics has been monitored with WTW Oxi 3315 portable meter continuously throughout the whole experiment.

4.2.5 Temperature

Temperature has been measured with WTW Oxi 3315 and read of at the same time as the DO for both kinetics and daily measurements

4.2.6 pH

The pH has been measured through a portable pH-sensor; sensIONTM PH3. The pH meter has been placed in each chamber daily and after stabilization the pH has been read off. The pH-sensor has been calibrated throughout the semester. The same portable pH meter was used for monitoring kinetics and was monitoring the continuously throughout the experiments.

4.2.7 Weighting

The Sartorius digital lab scale balance analytical A 120 S 0.1 mg delta range has been used for weighting of substances for all experiments.

4.3 Monitoring of the Pilot

4.3.1 Procedure for measurement of DO, T, pH, Flow and Transportation of carriers pr. second

Daily the parameters in the pilot of Dissolved Oxygen (DO), pH, Temperature (T), mixer speed and air demand has been measured. These parameters have been measured within each chamber before it has been logged.

Placement of the sensors, used to read of pH, DO and T, was of some importance. The DO was not placed directly above the air-diffusors nor directly above the holes for movement between chambers.

The effluent flow was in January and February measured daily and over time as the pilot became more stable it was measured once or twice a week. As effluent flow is, when the pilot is operated steadily and under functioning conditions, equal to influent flow, the HRT has been calculated using the effluent flow.

Effluent flow has been measured using a 5-litre bucket. The time for the bucket to fill until the 5-litre mark was recorded. The measurement has been done consecutively three times in a row to account for any discrepancies. The average value has then been found. The flow has been found using Eq. 4.1.

$$\mathrm{Q(efl)(l/min)} = \frac{V}{t} \qquad \text{(Eq. 4.1)}$$

V = Volume (L)

t = Time (min)

Checks have also been done on the transportation of carriers in the pilot. See appendix B Tab.B4 for target values for transportation of carriers between chambers for various HRTs. To analyse the speed of movement of carriers a basket has been placed under the conveyor belt in chamber

1 for a set number of rotations. Carrier transportation pr. second has been calculated using Eq. 4.2

$$\text{Carriers/c} = \frac{236\ \text{carriers}}{200\ mL} * \frac{V(c)}{t} \qquad \text{(Eq. 4.2)}$$

V(c) = Volume of carriers collected over a set number of rotations (mL)

t = time of collection of carriers

A relationship of 236 carriers pr. 200 mL has been established, see appendix B.

4.3.2 Procedure for measurement of daily influent and effluent concentrations; PO4-P, NH4-N and sCOD

Daily the In-and out relationship between orthophosphate, ammonium and soluble COD has been measured. Sampling is based on composite sampling described in Ch. 4.2.1.1

The beakers where, after sampling, placed on a magnetic mixer for complete mixing, before a 10 mL sample was taken out using syringes for both in- and out. These sample was then placed in a centrifugal machine for 10 minutes before filtration.

After filtration the ammonium and phosphorous sample was diluted 1:2 while the soluble COD remained 1:1. Dilution mixture consisted of 1 part sample and 1 part distilled water produced at the lab. The parameters NH4-N, PO4-P, sCOD were determined using Hach Dr. Lange cuvette tests and results found using Spectrophotometer, see Ch. 4.2.2.

4.4 Method for measuring attached biomass on the carriers

Biomass presence has implications of the EBPR process, and is therefore a key parameter designing and operating the process (Henze et al., 2008).

Since the beginning of January until the beginning of May I have performed a weekly measurement of the amount of biomass on each carrier. During some periods measurements has been performed twice a week.

The method used for assessing biomass growth on the carriers was through the method explained in Rusten et al. (2016). As described in the article the method consists of collecting 15 biofilm carriers. As a standard, the collection was done by collecting one or two carriers from all ten chambers. The carriers were then placed so that excess water could drain of, before they were placed in a porcelain bowl in the oven for 24 hours at 105 °C.

After drying, the carriers with biomass were taken out of the oven and placed in a desiccator for cooling off to balance temperatures. The carriers were then weighted in The Sartorius digital lab scale balance analytical A 120 S 0.1 mg delta range with the attached biomass. After weighting the carriers were soaked for 30 minutes in full strength domestic sodium

hypochlorite. During soaking the carriers were stirred in the solution to make sure all traces of biomass was removed. After soaking the carriers were placed in a strainer and dosed in warm water for a while before they were scrubbed. Any remaining biomass was thoroughly scrubbed and washed of. After biomass removal the carriers were put in to the oven for drying at 105°C for 24 hours. The sodium hypochlorite solution was collected and deposited in a secure container.

After drying the carriers were cooled off in the desiccator and the weight of the clean carriers were recorded. The biomass on the carriers was calculated with Eq. 4.3.

$$TS(c) = \frac{\text{W (sample)} - \text{W(clean)}}{N} \qquad \text{(Eq. 4.3)}$$

TS(c) = Weight of TS on 1 carrier in the reactor (mg TS/carrier)

W(sample) = Weight of carriers dried at 105 °C for 24 h (mg)

W(clean) = Weight of carriers washed and scrubbed in chlorine (mg)

N = Number of carriers

Eq. 4.4 was used to calculate biomass pr. beaker kinetic at the given time when the kinetic was performed. This value can of course also be used to calculate the amount of biomass in the reactor over time. See Tab. B2 in appendix B for characterisation of the carriers used for calculating how much biomass was present for each kinetic experiment.

$$TS(beaker\ kinetic) = TS(c) * 708 \qquad \text{(Eq. 4.4)}$$

TS = Total biomass present with a filling degree of 60% or percent (g TS)

TS(c) = Biomass pr. carrier (mg/carrier)

708 = Number of carriers pr 200 mL, see appendix B

TS present in the reactor, or in a beaker pr. m^2, is calculated using Eq. 4.5. See appendix B for specifications on the SA for K1 carriers.

$$TS/m2 = \frac{TS}{SA} \qquad \text{(Eq. 4.5)}$$

TS/m2 = Total biomass pr.m^2 surface area (g TS/m2)

TS = Total biomass present with a filling degree of 60% or percent (g TS)

SA = Specific Surface Area (m^2)

To establish the biomass influence on the kinetic experiments the biomass presence have been sometimes been evaluated with the rate of uptake and the rate of release in bio-P experiments to find the specific uptake and specific release of the reactor at that point in time with regards to biomass.

To evaluate the TP content pr. m^2 measured TP/TSS content, see Ch. 4.5 for method has been calculated using Eq. 4.6. Biomass on the carriers is measured as TP/TSS and TP/VSS, see Ch. 4.5. The TSS content on the carriers will however be equal to the TS content in the attached biomass.

$$TP/m2 = TS/m2 * TP/TSS \quad \text{(Eq. 4.6)}$$

TP/m2 = Total P in the biomass pr.m^2 surface area (g TS/m2)

TS/m2 = Total biomass pr.m^2 surface area (g TS/m2)

TP/TSS = gTP content in the biomass pr. gTSS, see Ch. 4.5 for calculations

4.5 Method for measuring Total Phosphorous (TP) content in attached biomass

The filling degree of carriers in the pilot is 60%. This same filling degree was used for evaluation of TP/TSS-and VSS in each measurement to make sure it was done equal each time. A 100 mL sample was used with a filling degree of 60%. With a 100 mL sample necessary number of carriers is 71, see appendix B.

Distilled water, produced in the lab, was added into the sample of carriers into a glass beaker before it was put into the Ultrasonic cleaner (Elma Transsonic T 460/H Ultrasonic Cleaner), see Fig. 35 to, detach the biomass from the carriers for approximately 15 minutes.

After detachment the carriers were removed from the beaker and the distilled water and biomass were mixed with a magnetic mixer. After mixing there was an immediate partitioning of the sample in to three 25 mL samples so that there were triplicate samples to reduce errors.

Total suspended solids (TSS) was measured by filtering the three samples through Whatman GF/C filters of 1.2 µm. The clean filters had first been dried in an oven for 2 hours in an aluminum cup before they had been placed in a desiccator for cooling off. The samples were weighted before filtration vacuum pump, see Fig. 36 for picture of the set up with the filter and vacuum pump. When the filters was properly soaked in distilled water, the three samples were filtered. After filtration they were put in the oven for drying at 105 °C for 2 hours. When taken out of the oven the dry filters were put in the desiccator for cooling off before the weight of the filters with biomass was recorded. TSS was calculated using Eq. 4.7.

After weighting of the TSS the filters were placed in the incinerator for 30 min. at 550 °C to determine the share of VSS in the biomass. After incineration the filters were placed in the desiccator for cooling down, before the ash residue on the filters were measured. VSS was calculated using Eq. 4.8

The average weight of the residue on the filters before and after incineration was established.

$$TSS = \frac{W(f) - W(b)}{V(s)} \qquad \text{(Eq. 4.7)}$$

W(f) = Weight of filter with biomass after drying in oven for 2 hours at 105 C (g)
W(b) = Weight of blanc filter after drying in oven for 2 hours at 105 C (g)
V(s) = Volume Sample (L)

$$VSS = \frac{W(f) - W(i)}{V(s)} \qquad \text{(Eq. 48)}$$

W(f) = Weight of filter with biomass after drying in oven for 2 hours at 105 C (g)
W(i) = Weight of filter after incineration in oven for 30 min550 C (g)
V(s) = Volume Sample (L)

Figure 33 - Elma Transsonic T 460/H Ultrasonic Cleaner used for detaching biomass on carriers so that the TP content on the biomass could be established.

Figure 34 - The vacuum pump used for measurements of TSS, VSS and TDS. Sample was filled, after a pre-dried and weighted filter GC/F filter was placed in the set-up.

After taking out 3x25 mL samples for measurement of TSS and VSS the remaining water sample in the beaker was mixed further, before a sample was taken out to measure the TP. The TP content was measured using Hach Dr. Lange cuvettes and in accordance with the method given, see Ch. 4.2.2 X. The TP sample had to be diluted 1:2. Some measurements were done where the detached biomass was so large in the water phase that TP had to be diluted 1:4. These measurements were not included as it was necessary that all measurements for measuring TP in the biomass was done similarly.

The value of TP/TSS and TP/VSS was calculated using Eq. 4.9 and 4.10

$$TP/TSS\ (gTP/gTSS) = \frac{TP\ (mg/L)}{TSS\ (g/L)} \qquad \text{(Eq. 4.9)}$$

$$TP/TSS\ (gTP/gVSS) = \frac{TP\ (mg/L)}{VSS\ (g/L)} \qquad \text{(Eq. 4.10)}$$

4.6 Method for sampling carriers for microbial community analysis

Sampling of carriers has been done approximately once a week since the pilot started running with real wastewater. The collection of carriers has been done in duplicates. Ten carriers each have been collected in to two centrifugal tubes, where the date has been recorded. The collection of the 2x10 carriers has been done by taking 1 carrier from each chamber. The collection of 1 carrier from each chamber was chosen to reduce sampling errors by choosing only carriers from 1 chamber. Excess water on the carriers has been let to run off. The carriers were then placed into the centrifugal tubes and put in the freezer. The analysis of the carriers will be carried out at the Department of Biotechnology and Food Science at NTNU where Illumina Sequencing can help determine the microbiology presence on the carriers. Documentation of the development of the microbial community established on the carriers throughout the spring can be established. Some samples will also be sent to Nano-technology lab of the Faculty of Sciences at NTNU to perform scanning electron microscopy of the carriers to evaluate development of biofilm thickness. For analysing the biofilm thickness the carriers will be sent for Variable Pressure Electron Scanning Microscope (VPSEM) at the NANO laboratory.

Due to the 20-week time constrain of my master thesis it has not been possible to include these results in my master thesis.

Tab. J1 in appendix J depicts a timeline of when samples have been taken.

4.7 General procedure for all Beaker Experiments

Kinetic experiment in beakers have been performed mainly according to protocol "Experimental Methods in Wastewater Treatment" by Loosdrecht et al., (2016). These methods are based on EBPR in activated sludge systems.

See appendix C, Tab. C1 for information and name of all the kinetic experiments performed.

4.7.1 Temperature Control

When performing kinetic experiments to assess the status of the pilot at said time conditions were kept as similar to the conditions in the pilot as possible. Temperature was controlled to be in the range temperatures observed in the pilot. Temperatures between 10 and 14 degrees was most commonly measured in the pilot in January, with temperatures starting between 10-11 in

the first chambers and ending between 13-15. Temperature control between 10-14 degrees was therefore established. The temperature control was made up of a bucket placed outside the beaker. Ice was filled in the bucket throughout the experiment, see Fig 35 and 36.

For experiments performed in room-temperature no temperature control during the experiment has been performed.

For experiments at temperatures between 28-32 °C a waterproof heater was put into a waterfilled container to heat up the water. The beaker with sample was placed inside the container.

During all kinetic experiments the same temperature control method has been executed.

4.7.2 Time steps for sampling

The first couple of kinetic experiments were performed with a HRT of 480 minutes equalling 48 minutes retention time in each chamber, see appendix B. After revaluating the kinetic experiments and the retention time used it was decided to reduce the HRT during kinetic experiments to the effective water retention time in the pilot. This retention time is set to approximately 6 hours and 21 minutes, see appendix B.

As to establish equal methods of performing kinetics it was decided that the WW kinetics would continue performed with an HRT of 480 and that S, S+W and P kinetics would be performed with a HRT of 380. See Fig. appendix C of an overview of the various kinetic experiments performed.

Samples were collected at t = 24, 48, 72, 96, 144, 192, 240, 336, 384, 432, 480 minutes, or t = 18, 38, 57, 76, 114, 152, 190, 228, 304, 342, 380 minutes. All samples were analyzed for PO4-P and sCOD and some for NH4-N and NO3-N. Later in the semester during some experiments samples were only collected and not analyzed on every interval to minimize over usage of cuvettes, as it was not always critical to analyses all the sampling points.

At each sampling point the temperature, pH and DO levels have been recorded.

4.7.3 Sample Volume

Samples extracted were kept to a minimum as the volume of the mixture will decrease. The volume of the sample should not decrease by more than 10% of the original volume as this can give results that are unreliable and not comparable with other experiments. At the 12 sampling times 5 mL was extracted. The total reduction volume was 7.5 % for each kinetic.

4.7.4 Start of Experiment

Influent sample of 794 mL was collected from the influent waterpipe after running of this for one to two minutes. 600 mL of carriers were collected from chamber 10 in the reactor. The carriers collected were left to drain of excess water before the experiment began.

Influent sample was dosed with nitrogen gas to remove oxygen and achieve anaerobic conditions in the wastewater before the start of the experiment. DO levels below 0.018 mg

DO/L, was the limit set for anaerobic conditions. When these conditions were reached in the sample and the carriers drained off the experiment could start.

The sample was placed on the magnetic mixer. The mixer speed was set to 200 rpm to achieve complete mixing. Mixing was in all experiments established through a magnetic mixer and/or aeration for complete mixing. See Fig. 35 for a set-up of the kinetic experiment Sample t= 0 was taken after the sample was mixed for 1 minute. The carriers were added after sample t=0 was taken.

4.7.7. Aerobic Phase

Oxygen was pumped into the reactors with a small electric pump.

During kinetic experiments where several experiments were run in parallel, the exact same set-up as explained above has been completed. An oxygen pump with more air diffusors has then been used.

The aerobic phase has not been kept constant throughout all experiments. During some experiments the anaerobic phase has been prolonged to evaluate the possibility for further release.

During kinetic experiments the aeration has been kept above 5 mg DO/L to account for results not being influences by too low aeration.

4.8 Beaker Experiments with Wastewater

Laboratory kinetic experiments to evaluate P-release and P-uptake status within the pilot have been carried out 7 times throughout the semester.

Results obtained from performing experiments to establish the release and uptake rates give information into the actual bio-P performance in the reactor at a given time and is not necessarily a relation to the efficiency of the process. However, the results can be used for indicating what can be done to optimize the process.

Table 6 - Experiments Performed mimicking the reactor behavior, see appendix C and D for all beaker experiments performed

Date	Experiment	Sample	Experiment Conditions	Conditions in pilot at time	Substrate dosage
30.01.2018	WW1	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control		None
06.02.2018	WW2	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control		None
21.02.2018	WW3	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control		None

01.03.2018	WW4	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control		None
09.03.2018	WW5	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control		None
19.03.2018	WW7	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control	Very high dillution - Experiment unsuccessful	None
10.04.2018	WW7	794 mL influent wastewater + 600 mL carriers	anaerobe/aerobe, 8 HRT, Temperature control	PO4-P(in) approximately 40% lower than average influent levels due to dillution	None

Temperature was controlled between 10-14 °C and influent wastewater with no substrate addition was used.

All samples were filtered through 0.45 µm cellulose nitrate filter and analysed for PO4-P, sCOD, NH4-N, NO3-N, see Ch. 4.2 and Ch. 4.7 for procedures.

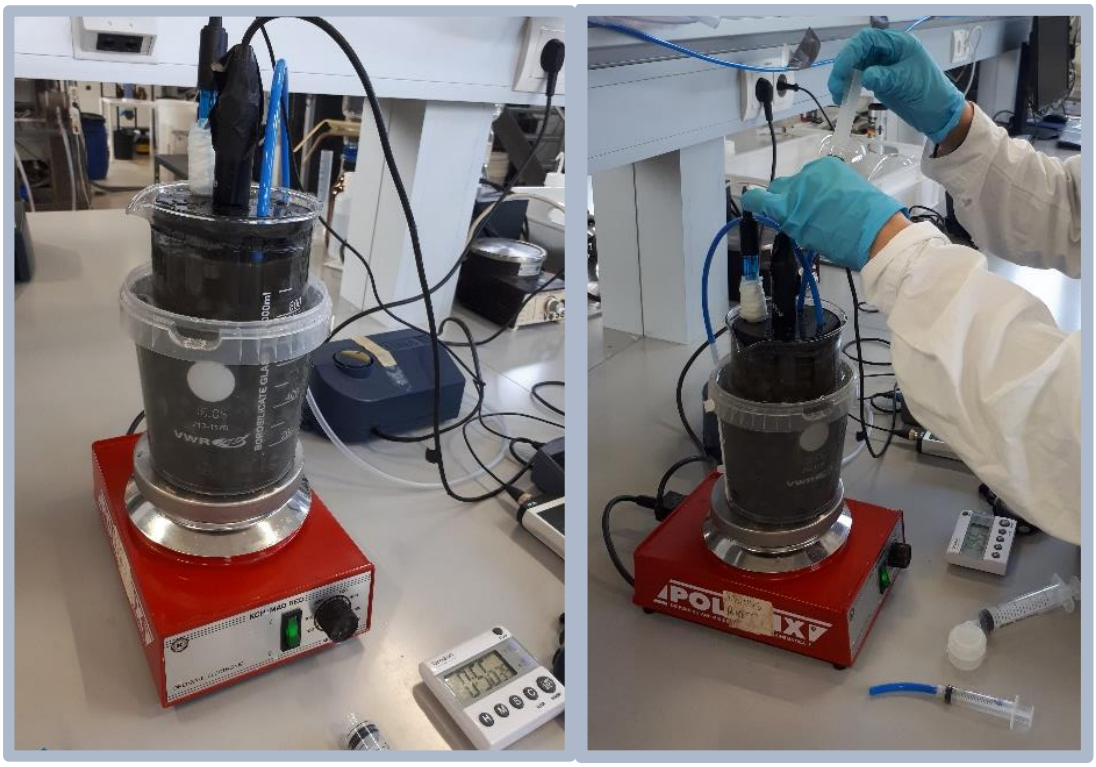


Figure 35 - Set-up of Kinetic Experiments. When no control of temperature was needed the container loaded with ice, in which the beaker was placed in, was removed

4.9 Beaker Experiment with Synthetic Wastewater

Beaker experiments with synthetic wastewater har been performed to analyse the biomass' reaction under various conditions. The nutrient concentration has been constant while there have been variations in substrate source. Substrate was dosed in excess to determine the biomasses maximum P release capacity. See calculations in appendix E.

Table 7 - Experiments Performed with synthetic wastewater, see appendix C and D for all beaker experiments performed

Date	Experiment	Sample	Experiment Conditions	Substrate dosage
20.03.2018	S1	Synthethic wastewater (Smolders et al., 1994)	anaerobe/aerobe, 6.35 HRT, T=10-14	300 mg acetate/L
21.03.2018	S2	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=10-14	NO3 instead of O2 in aerobic phase
22.03.2018	S3	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=28-32	300 mg acetate/L
22.03.2018	S4	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=Room	300 mg acetate/L
09.04.2018	S5	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=10-14	No substrate addition (experiment without carbon)
09.04.2018	S6	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=Room	No substrate addition (experiment without carbon)
12.04.2018	S7	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=10-14	300 mg glucose/L

A synthetic wastewater mixture of 794 mL distilled water and additions of phosphate, ammonium, sCOD, magnesium, calcium and micronutrients were added according to instructions in Smolders et al., (1994). See Tab. 8 for target concentration throughout all experiments. PO4-P and NH4-N dosage were added based on the higher range of influent values experiences through In-and out measurements. Remaining additions were done in excess so results were not determined by lack of substances, method given in Smolders et al., (1994)

Table 8 - Target concentration of nutrients and salt added, see appendix E for calculations

Substance	Concentration (mg/L)	Weight (g)
PO4-P	7	0.0255
NH4-N	50	0.151
sCOD	80/300	-
Mg2+	90	0.071
Ca2+	14	0.0084
Micronutrients	0.3 mL/1L	0.23 (mL/0.794L)

Each addition of carbon has been added as a concentration correlating to sCOD. Experiment set-up follows descriptions given in Ch. 4.2 and 4.7.

Each salt was weighted in a plastic measuring cup. A couple of deciliters distilled water was first added to a volumetric flask before each salt was added directly after weighting. The remaining sample of distilled water was then added through a funnel, completely dosing each plastic measuring cup in water so that no remaining salt was left on the cup. After addition of water and salt a thorough mixing on a magnetic mixer and shaking of the sample was performed for the salts to dissolve completely.

After the sample was completely mixed nitrogen gas was added to reach anaerobic conditions; below 0.018 mg DO/L. When anaerobic conditions were reached the first sample of t=0 was taken and pH and DO levels were recorded. The carriers, drained for water, was added.

4.10 Beaker Experiments with Influent Wastewater and Substrate Additions

The experiment was performed by dosing an excess of substrate source as approximately 80 or 300 mg sCOD/L to the wastewater. Calculations can be found in appendix E. The experiments were performed for evaluation of the biomasses possible release and uptake with varying conditions.

Experiment conditions and start up follow descriptions given in Ch. 4.2 and 4.7. Additions of substrate was performed as described in Ch. 4.9.

Table 9 Experiments Performed with influent wastewater and substrate additions, see appendix C and D for all beaker experiments performed

Date	Experiment	Sample	Experiment Conditions	Conditions in pilot at time	Substrate dosage
14.02.2018	W+S1	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=10-14		80 mg Acetate/L
12.03.2018	W+S2	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=Room		80 mg Acetate/L
14.03.2018	W+S3	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=Room		300 mg Acetate/L
15.03.2018	W+S4	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=10-14		300 mg Acetate/L
17.04.2018	W+S5	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=10-14	Dilution due to snowmelt	300 mg Glucose/L
19.04.2018	W+S6	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=Room	Dilution due to snowmelt	No substrate addition
19.04.2018	W+S7	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=Room	Dilution due to snowmelt	300 mg Glucose/L
19.04.2018	W+S8	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=Room	Dilution due to snowmelt	300 mg Acetate/L

26.04.2018	W+S9	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=Room	300 mg Glycerine/L
26.04.2018	W+S10	794 mL influent wastewater + substrate addition	anaerobe/aerobe, 6.35 HRT, T=10-14	300 mg Glycerine/L

4.11 Beaker Experiment with Varying Oxygen Input

Two kinetics were run with the same influent wastewater and two different oxygen pumps was used. Both were held at a temperature level between 10-14°C.

The pump-efficiency of the two pumps used was set at two different levels throughout the experiment. DO-levels was constantly measured, and the pump efficiency was altered throughout the experiment. As utilization of oxygen decreased during the experiment input airflow changed to keep the DO at the same level throughout.

Table 10 – Experiments analyzing DO effect on EBPR

Date	Experiment	Sample	Experiment Conditions	Substrate dosage
28.02.2018	DO1	Synthethic wastewater (Smolders et al., 1994)	anaerobe/aerobe, 6.35 HRT, T=10-14	300 mg acetate/L
28.02.2018	DO2	Synthethic wastewater	anaerobe/aerobe, 6.35 HRT, T=10-14	300 mg acetate/L

For synthetic beaker experiment with addition of NO3-N instead of oxygen, to survey uptake under anoxic conditions addition of 15 mg NO3-N/L was added to synthetic wastewater. See appendix E for calculations. NO3-N was added for the first anoxic time-step and re-added when measurements showed that concentrations were low.

4.12 Batch tests following the performance of the pilot

Batches of 200 mL were taken from each chamber with 38.5-minute intervals to follow the effective water retention time in each chamber.

They were placed on a magnetic mixer before samples were taken out and analyzed following procedures presented in Ch. 4.2 and 4.7.

Table 11 - Batch test with HRT of 38 minutes in each chamber, see appendix C and D for graph of full kinetic

Date	Experiment	Sample	Experiment Conditions	Conditions in pilot at time	Substrate dosage
08.03.2018	P1	200 mL grab sample from chambers	anaerobe/aerobe, 38 HRT, T=10-14		None
05.04.2018	P2	200 mL grab sample from chambers	anaerobe/aerobe, 6.35 HRT, T=Room	Dilution due to snowmelt	None

4.13 Method for Determining Solids (TS, TSS, TDS and VSS).

TS can vary widely based if there is a period with heavy rain- or snowmelt or drought. Its value can have high effect on the process with both increased and reduced efficiency.

Procedures for determining the solids load in the water was based on the Method 1684 given by the EPA for Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids (EPA, 2001).

In January samples for analyzing the influent TS, TSS, TDS and VSS load was taken. TS, TDS, TSS and VSS were measured.

TSS and VSS loads were analyzed in triplicates the same way as described in Ch. 4.5. An influent sample of wastewater was divided into triplicate of 25 mL sample after mixing before being filtered through 1.2 µm GF/C filter.

TDS was measured as the TDS value was of relevance for the Titra-5 Program for analyzing VFA levels. TDS is one of the parameters needed to be implemented in input parameters in the tool. See appendix H.

TDS samples were collected after filtration of the 25 mL samples for TSS. The remnant water after filtration were poured into a pre-weighted porcelain bowl. Distilled water was used to wash out the flask used for filtration so that all remnants of particles was collected. The sample was then put in the oven at for 24 hours at 105 °C. The dished were taken out, put in the desiccator for cooling off and then immediately after cooling weighted with biomass.

$$TDS = \frac{\mathrm{W(TDS)} - \mathrm{W(pb)}}{V(s)} \qquad \text{(Eq. 4.11)}$$

TDS = Total Dissolved Solids (g/L)
W(TDS) = Weight of porcelain bowl with residue after evaporation at 105 C for 24 hours (g)
W(pb) = Weight of clean porcelain bowl (g)
V(s) = Volume Sample (L)

TS was measured collecting triplicate 80 mL samples from an influent wastewater sample mixed on a magnetic mixer for 1 minute. The samples were poured into pre-weighted porcelain dishes and put in the oven for 24 hours at 105 °C. The dished were taken out, put in the desiccator for cooling off and then immediately after cooling weighted with biomass.

$$TS = \frac{\mathrm{W(TS)} - \mathrm{W(pb)}}{V(s)} \qquad \text{(Eq. 4.12)}$$

TS = Total Solids (g/L)
W(TS) = Weight of porcelain bowl with residue after evaporation at 105 C for 24 hours (g)
W(pb) = Weight of clean porcelain bowl (g)
V(s) = Volume Sample (L)

4.14 VFA-analysis

At the beginning of the semester filtered samples for VFA analysis' were taken to the analytical water lab at the Department of Hydraulic and Environmental Engineering for Ion Chromatography (IC) analysis. Through IC-analysis the VFA levels in the water could be established. These results were then to be analysed by the technical staff at the lab to establish the VFA concentration at different timesteps during the kinetic. It was not possible to acquire accurate results from the IC-test. One problem occurring was how thorough the cleaning of the IC-machine had to be after running wastewater samples as the same machine also was used for analysing many other samples requiring a completely clean environment

Performing VFA estimation with the IC-analytical tool is still a possibility and something that will be investigated further in the upcoming months. Because of the time 20-week time-constraint on my master thesis it has not been possible to achieve.

After the difficulties using the IC-tool decisions were made to analyse the VFA levels in the wastewater using the 5-point Titration method and the analytical computer programme for this method used at IVAR. See appendix H for how the 5-point Titra-Method.T his method is straight forward and simple to follow and based on the method given in Moosbrugger et al., (1993).

The results from the titration were put into the Titra-5 program and the program calculated the VFA level.

As the VFA loads in wastewater is highly inconsistent and varying it is necessary to perform the 5-point titration method on duplicate or triplicate samples to trust the results. There is also reason to believe that the VFA levels in are low, which increases the inaccuracy of the method. When triplicate samples are measured its highly important to measure then within 15 minutes due to the volatility of the sample.

4.14 Sources of error in the experimental set-up

Several sources of error are present when performing the experiments

4.14.1 Instruments

The sampling volume in each Hach Dr. Lange cuvette is only a couple of mL can lead to inaccuracies, as the small sample for measurement volume can be unrepresentative. To avoid this all samples have been thoroughly mixed in a magnetic mixer. This does not however remove all uncertainties.

4.14.2 Sampling Variations

The largest error probably stems from sampling of grab samples. By running batch tests in the pilot as there are large uncertainties to if a representative sample is collected. Homogeneous samples are difficult to collect; HRT, variations in flow can all give deviations in results.

Kinetic experiments performed in beaker should, had time permitted it, been performed multiple times. Ideally as experiments always should be performed more than once to see what trends is and what is deviations departing from this. Due to sampling challenges of wastewater it is impossible to establish the same experiment under the exact same conditions later on, as it is not possible to retrieve the same samples again. This makes it difficult to know if results show the trend or the deviations at a given time. This will however be discussed, but overall samples represent the point in time when they were taken, and lines towards observed trends can be discussed, but no definitive conclusions can be taken. When several parallel tests are performed over longer periods is when trustworthy results is most normally found. As time went on during the semester a better understanding of which kinetics should be performed and why developed.

Sampling deviations can be present in all samples. One can never be completely sure of having a homogenous sample. For all samples of biomass collected variations will occur, although the sampling process has tried to reduce the error as much as possible.

4.14.3 Variations in how to perform experiments

By performing experiments differently variations in results based of this can occur. Different measuring tools used can have deviations. To account for this all measuring tools used for analysis has been the same. Good routines on how to collect samples has been established so that the collection process has been identical each time. This reduced the error a little bit, as variations are then caused by parameters beyond the measurers control.

4.14.4 Uncertainties with Methods

Some experimental performance has been difficult to perform exactly alike for all experiments. During control of temperature between 10-14 °C minor changes to the loading of ice lead to large fluctuations in temperature which were not instantly possible to read of. Therefore, temperature correction by taking out ice, or filling in more was delayed. However, temperature continuously fluctuates in the pilot throughout the day and in between chambers. It is difficult to establish a more functional way to control it than what has been done without attaining more advanced equipment .

5 Results and Discussion

5.1 Pilot

The pilot had been running with wastewater for approximately 75 days when I started performing laboratory work. It seems relevant to conclude that when I started my work with the project it was in effect very early in the pilot's start-up phase.

The pilots start-up-phase has been filled with some technical- and operational constraints. When discussing and evaluating results it is important to bear in mind that operational difficulties may at times have had effect on the outcome. Tab. F1 in appendix F presents occurrences happened in the pilot from 25.01.2018 – 31.05.2018.

Some samples were after filtration, either refrigerator or freezed so that new measurements could be performed if necessary. Values clearly showing large deviances from expected values have been removed from the data set if new measurements were not a possibility. Due to some operational problems in the pilot at times leading it to not receive influent water, affected parameters measured (DO, pH and T). These values have been removed from the data sheet at appropriate times.

5.2 Rate through linear regression and R^2-values

Through performing P-release and uptake tests in beaker experiments the goal is to present the results in the type of graph presented in Fig. 36. This is of course a theoretical description of rates, and as none of the experiments performed have as clear tendencies. It has been very difficult to standardize a calculation method for the rates for all the results.

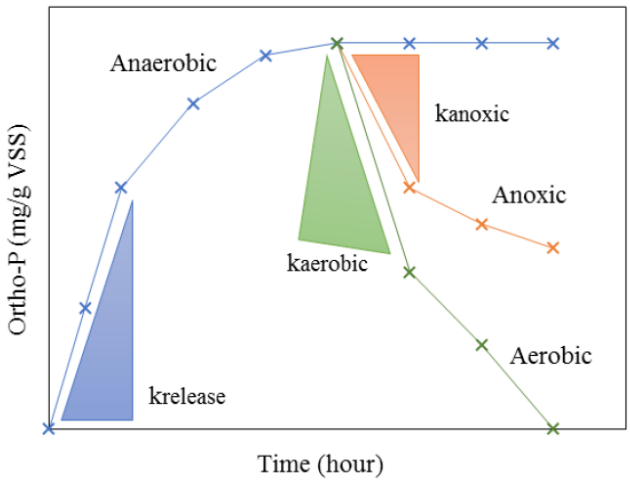


Figure 36 -PO4-P development in P-uptake and release test. (Loosdrecht et al., 2016)

All the results from kinetic experiments are normally calculated using the linear part of the release after the second timestep. A sharp reduction in PO4-P and sCOD concentrations can normally be seen here, despite anaerobic conditions. This is most probably due to some oxygen being present on the carriers and when they are added to the sample this oxygen is immediately utilized. For some cases when the linear part of the curve does not correlate to this, at least three

points of the most linear part of the curve has been used, in both the anaerobic and aerobic zone. All the results from the kinetic experiments are presented as hourly concentrations. A pre-requisite for all the results from kinetic experiments are that they are presented with the rate of uptake and rate of release given in units mg PO4-P/hr*m^2 so that the results are more easily comparable to literature, while the specific release and uptake is given in mg PO4-P/hr*gTS*L to compare with biomass development at the time. The rates have been calculated by using the concentrations changes over the linear part of the curve of at least three points divided by the timestep in which measurements have been taken and the specific surface area of the sample.

In Excel the regression line for the beforementioned timestep used for calculating the release and uptake rates was found within the defined linear segment. R^2 values was determined using the linear regression tool.

The length of the aerobic and anaerobic zones was not always kept constant. This choice was discussed during kinetics and most often made in instances to see if there was more potential for release.

From Tab. 12, 13, 14, 15 the R^2 value for the release and uptake rates from the experiments. Most rates will probably be underestimated due to the curvature of the release and uptake.

Table 12 - R2 value for the release and uptake rates from the experiments. In Ch. 4.8 experiment conditions are given

Experiment	P release rate (mg PO4-P/m^2*hr)	R^2	P uptake rate (mg PO4-P/m^2*hr)	R^2
WW1	0.56	0.08	0.44	0.33
WW2	1.35	0.76	1.08	0.85
WW3	1.60	0.8884	2.58	0.96
WW4	1.14	0.956	2.03	0.941
WW5	1.07	0.94	2.75	0.93
WW6		N/A		
WW7	1.76	0.97	1.93	0.98

Table 13 - R2 value for the release and uptake rates from the experiments. In Ch. 4.10 experiment conditions are given

Experiment	P release rate (mg PO4-P/m^2*hr)	R^2	P uptake rate (mg PO4-P/m^2*hr)	R^2
W+S1	3.77	0.80	8.33	0.77
W+S2	2.23	0.87	8.19	0.96
W+S3	3.32	0.99	7.22	0.95
W+S4	2.01	0.97	7.22	0.97
W+S5	3.74	0.99	4.11	0.92
W+S6	2.53	1.00	3.75	0.98
W+S7	2.42	1.00	3.69	0.99
W+S8	4.77	0.99	7.89	1.00
W+S9	3.97	0.98	1.82	0.87
W+S10	2.33	0.97	0.85	0.95

Table 14 - R2 value for the release and uptake rates from the experiments. In Ch. 4.9 experiment conditions are given

Experiment	P release rate (mg PO4-P/m^2*hr)	R^2	P uptake rate (mg PO4-P/m^2*hr)	R^2
S1	1.15	0.93	4.18	0.97
S2				
S3	0.77	0.93	1.57	0.89
S4	3.77	0.98	5.30	0.91
S5	1.59	0.85	N/A	
S6	2.76	0.94		
S7	2.09	0.95	2.76	0.88

Table 15 - R2 value for the release and uptake rates from the experiments. In Ch. 4.12 experiment conditions are given

Experiment	P release rate (mg PO4-P/m^2*hr)	R^2	P uptake rate (mg PO4-P/m^2*hr)	R^2
P1	1.56	0.91	1.98	0.96
P2	0.73	0.86	2.57	0.80

Table 16 - R2 value for the release and uptake rates from the experiments. In Ch. 4.11 experiment conditions are given

Experiment	P release rate (mg PO4-P/m^2*hr)	R^2	P uptake rate (mg PO4-P/m^2*hr)	R^2
DO1	0.69	0.93	1.39	0.972
DO2	1.05	0.91	1.92	0.991

5.3 Biomass development

The development of the biomass has been analyzed weekly according to method shown in Ch. 4.4, to document the development of amount of biomass on the carriers. The results are presented as g TS/m² present in the pilot at a given time. As Shown in Fig. 37 the increase in biomass pr. carrier was substantial. In February the total biomass in the reactor was approximately 2.23 kg, while in late April biomass had increased to approximately 3.567 kg.

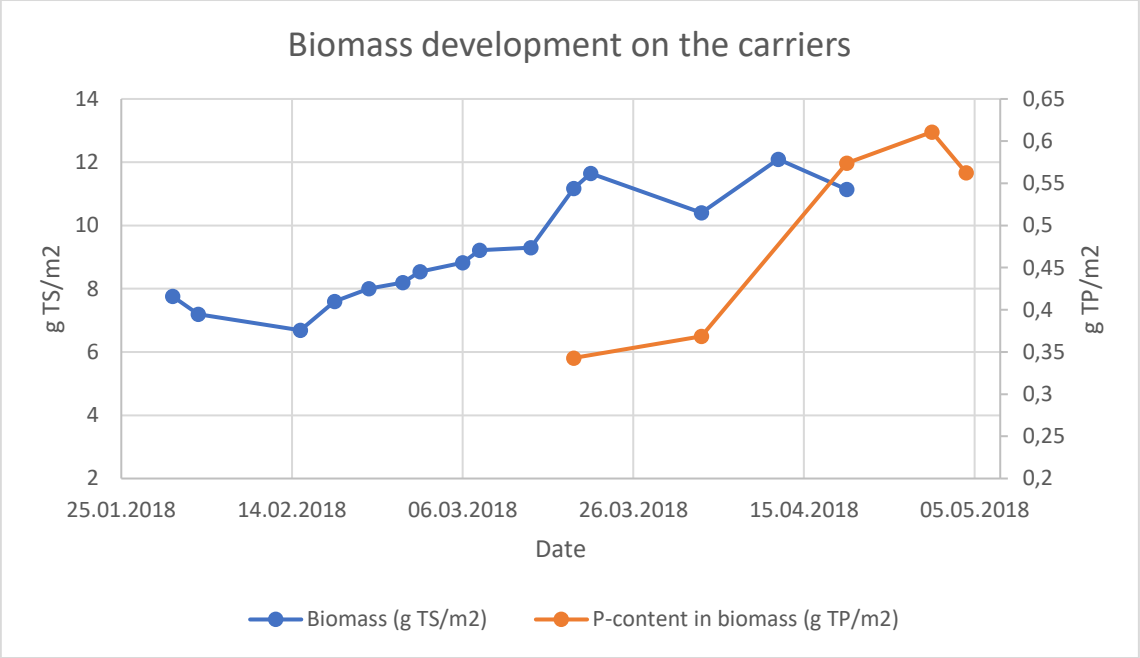


Figure 37 - Biomass development on carriers from January to May

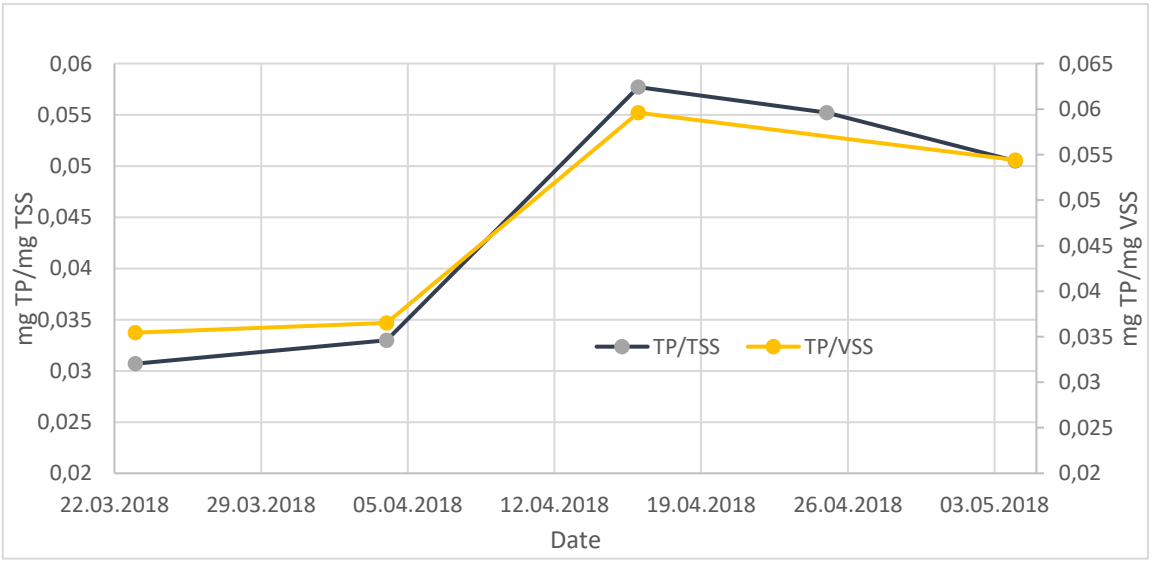


Figure 38 - Development of TP/TSS and TP/VSS

Typical active PAO can incorporate up to 0.38 mgP/mgVSS or 0.17 mgP/mgTSS (Henze et al., 2008, Ch. 7.2). This is the theoretical value, and in practice it is difficult to achieve as high P-content as this. Based on the results presented in Fig. 38 the biomass contains well above the bearing capacity of OHOs of 0.015 mgP/mgTSS. However, the ratio is still significantly lower

than the theoretical value presented by Henze. Currently the TP/TSS is 70% lower than the theoretical achievable value in regard to TSS, and 85% lower in regard to TP/VSS. However, these values are given for AS systems. As discussed in Ch. 3.2.2 there are several other factors to evaluate for biofilm systems. Control of biofilm thickness to hinder diffusion limitations as well as allowing for a high degree of active biomass is important in an MBBR. The value of TP/TSS in the reactor will most probably never give results as high as literature states possible, and for an MBBR values may differ from AS.

As the biomass has grown in sheer size, the P-content has also increased. Increased P-content of the biomass over such a time-period is not surprising in general. However, this increase is a little surprising in the pilot, as increased removal was not detected during the increase of P-content. Through literature it is stated that shifts in conditions such as pH or low substrate loading can lead to changes in the microbial community (Carvalheira et al., 2014; Wang et al., 2013). Due to snowmelt and the subsequent dilution the system experiences many sharp and sudden changes. Snowmelt started around March 20th and ended approximately April 26th. During the period of low substrate loading the system has had to adapt to new demanding circumstances as well as pH, DO and temperature changes, see Ch. 5.5.1,-2,-3 for development of DO and T, and appendix F for occurrences in the pilot, and snowmelt.

Carvalheira et al., (2014) found that during periods of low organic loading the GAO and PAO metabolism acted differently. GAOs biomass decayed 4 times faster than that of PAO. PAOs were therefore better adapted for survival during low substrate loading. This can have led to PAOs taking up a larger part of the biomass and outcompeted GAOs during the low substrate loading phase, where P-content in the biomass increased.

The microbial population have shown little ability in utilizing acetate in the anaerobic zone. Therefore a potential explanation is that PAOs present before dilution had enough influent sources of substrate to sustain themselves. That's probably why they contributed to removal, even with a little developed biomass. While during dilution they have been forced to survive on low substrate loads and utilized all substrate sources for survival, growth and proliferation, outcompeting the GAO, however not contributing to the efficiency of the EBPR-process with increased %P-removal.

Carvalheira et al., (2014) also found that although the PAOs survived the low organic loading the anaerobic activity and net uptake was very low, focusing all their energy on survival and growth outcompeting GAOs. This is positive results regarding initiating this process in the average Norwegian treatment plants as low organic carbon loading is a recurring difficulty to overcome and shows that PAOs can be competitive during these conditions.

Tu & Schuler (2013) found that lower substrate loading over time lead to the consumption of the PAOs internal carbon and energy sources. Experiment S5 and S6 shows the biomass release in synthetic water with no additions of substrate. Through this experiment it is possible to see how the biomass utilizes its internal carbon sources for release and how due to this utilization the biomass is producing sCOD throughout the anaerobic zone, see Fig. 39

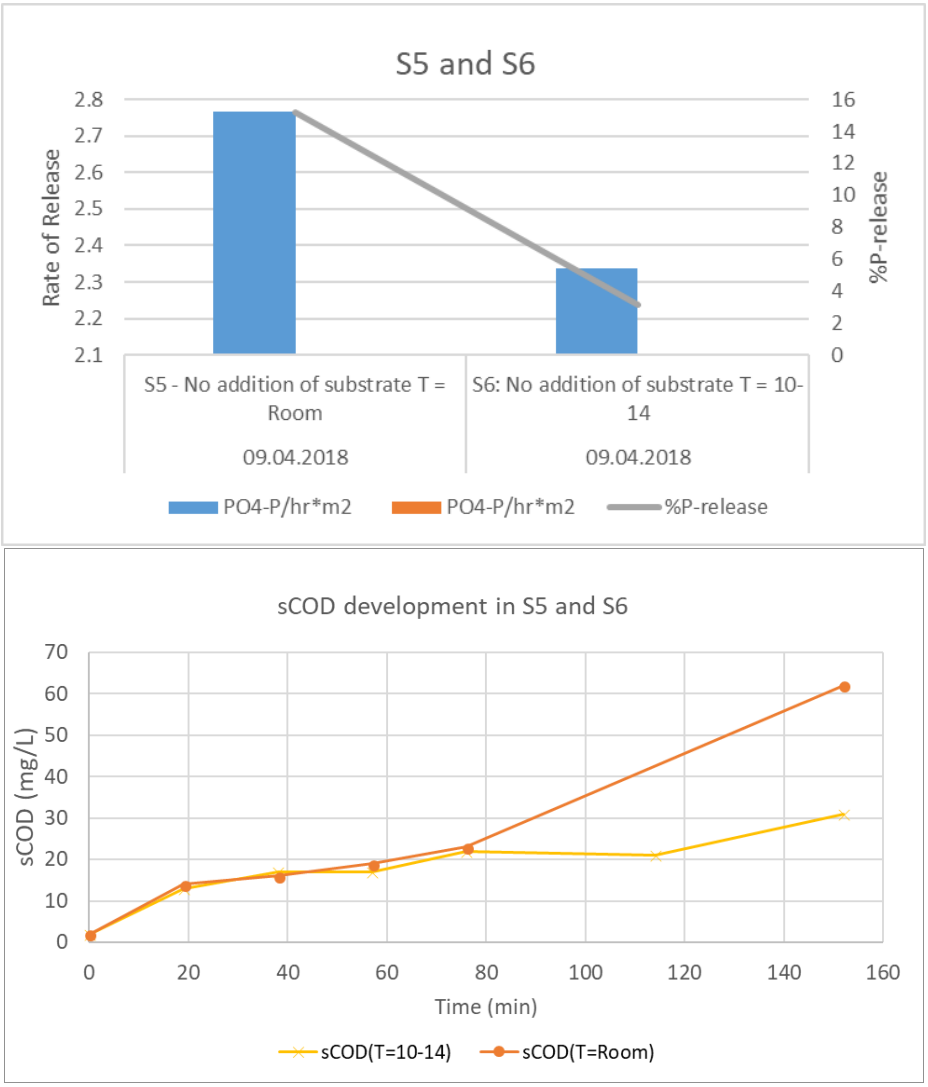


Figure 39 - Rate of release in S5 and S6 with no addition of substrate and sCOD production within the same kinetic over the anaerobic period. The biomass is fermenting and producing sCOD.

Fig. 40 shows the reduced efficiency of the process 1 month apart; before and during dilution. Despite improved biomass the effect of dilution is more prevalent for the removal efficiency. Acetate was added in excess as during both experiments mixed with wastewater. However due to the biomass not utilizing acetate to a large degree in the anaerobic zone the remaining sCOD content for the biomass to utilize was approximately 67 % less in W+S8; 49 vs 151 mg sCOD/L. It is possible to see that the rate of release for these two experiments were only minimally higher than the rate of release given in Fig. 39, despite the availability for substrate in W+S4 and W+S8. This leads to the understanding that a large quantity of the biomass does not prefer acetate as a substrate source and the reason for the increases removal in W+S4 was that dilution conditions was not yet prevalent and therefore the influent wastewater contributed enough substrate, regardless of acetate as sCOD addition.

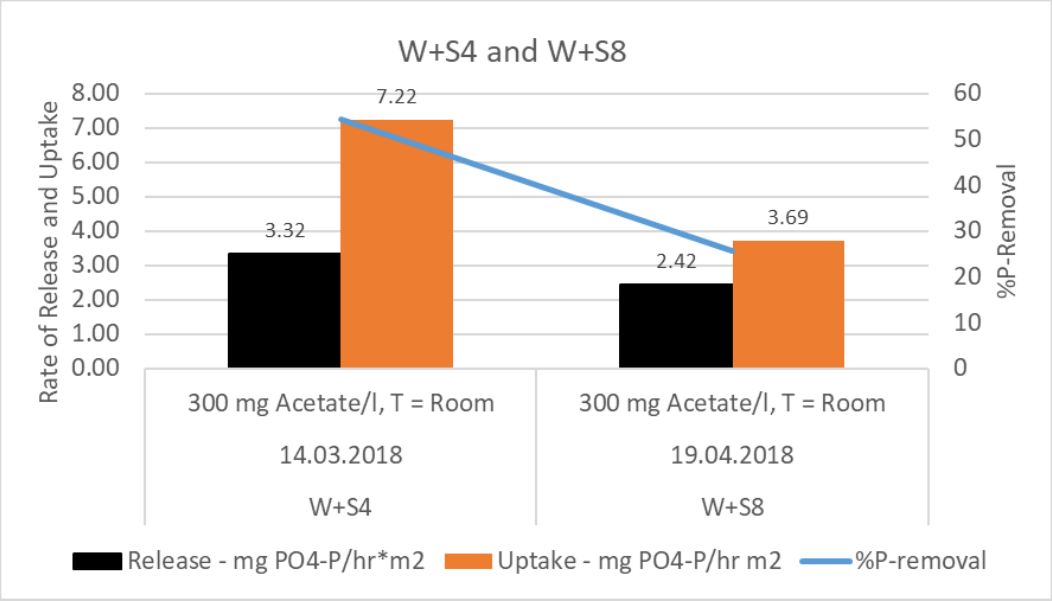


Figure 40 - Release and Uptake rate before and during dilution in wastewater with addition of 300 mg acetate as sCOD/L.

This is also shown as W+S8 was performed with two other kinetics at the same time. Substrate sources was added as 300 acetate and 300 mg glucose as sCOD to influent wastewater, while a remaining beaker had no substrate addition. All experiments contained the same wastewater. Results show that the release and uptake rate, between the wastewater with no acetate addition and the wastewater with, was almost alike. This indicates that the biomass does not depend on the acetate addition to a large degree however, the P-removal is higher in W+S8 which probably due to some PAOs present in the biomass being able to take up VFA, and due to large carry-over of VFA into the aerobic zone immediately utilized by OHOs. Barnard et al., (2017) found that some Tetrasphaera can take up VFA, but it is not the preferred substrate for storage of carbon, and when they utilize VFA they are likely to be effective they do not cycle poly-P when utilizing VFA.

As we currently have little knowledge about the microbiology on the carriers there is high reason to believe that it inhabits a low population of Accumulibacter as this strain utilizes acetate to a large degree. As the biomass shows a larger affinity to utilizing glucose, see Fig. 41, it potentially the bacteria strain Tetrasphaera which is present, which is a broad class of bacteria. Some of this species can utilize VFA, however it is not the preferred food source and instead they are more willing to ferment carbon for storage. Tab. 17 presents experiment W+S6, W+S7 and W+S8 rate of sCOD removal in the anaerobic zone.

Table 17 - Rate of sCOD removal within the anaerobic zone within 3 kinetics with different substrate addition.

	Rate of substrate removal as sCOD (mg sCOD/hr*m^2)
S+W7	-0.06
S+W8	-0.024
S+W6	-0.007

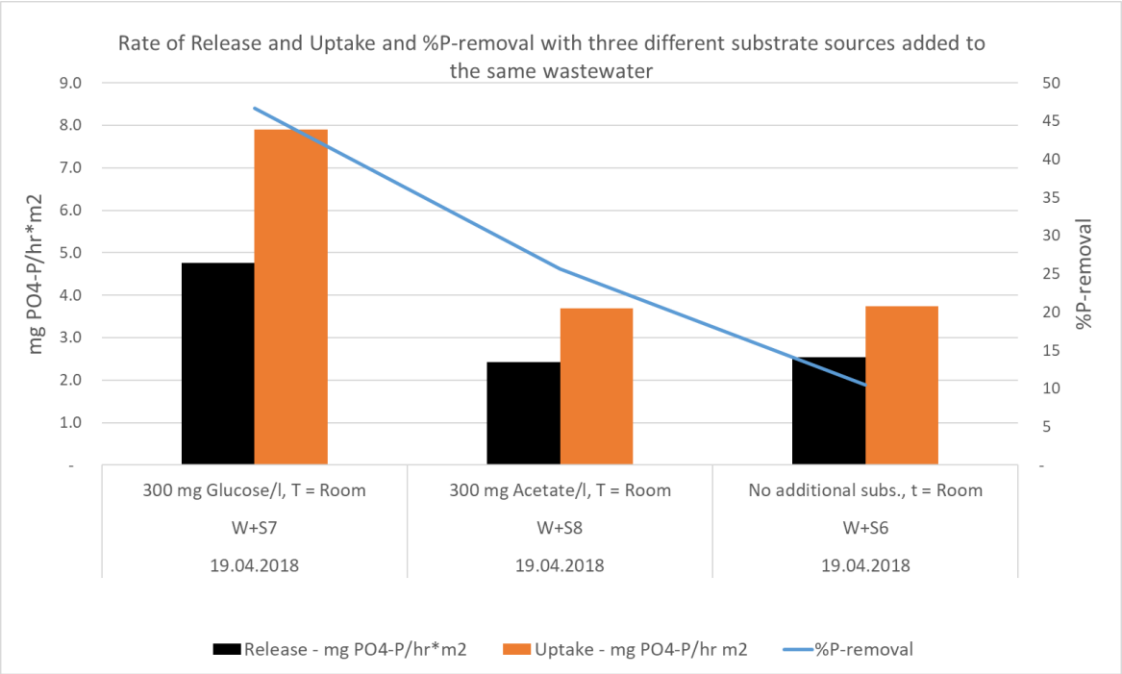


Figure 41 - The effect of adding glucose, acetate and no substrate to the same influent wastewater

From the rates presented in Fig. 39, 40 and 41 it seems that the abundance of bacteria mostly utilized the more complex organic molecules in the influent water to ferment, or its stored carbon instead of available VFA which leads to a net uptake of sCOD of zero. Only when glucose is available is the uptake of sCOD considerable in the anaerobic zone, see Tab. 17.

Lopez-Vazquez et al., (2009) and Oehmen et al., (2007 discussed that the relationship between the type of VFA and the PAO and GAO bacteria strand is affected by different influent VFA types and that these relationships are not one-dimensional but affects many areas when changed. Presence of various organic substrates seems to be a key parameter that determine the occurrence of the various PAOs, and it seems this is primarily acetate and propionate for Accumulibacter and glucose and amino acids for Tetrasphaera, although some also consume acetate (Nielsen et al., 2010; Kristansen et al., 2013). This is highly relevant for the pilot as it seems that the bacteria developed here are developed in an environment without easy access to VFA and therefore has adapted and developed other mechanism for carbon storage than what is normally seen in EBPR. Experiments performed with glycerol showed comparatively to the other experiments approximately the same rate of release, however with low uptake rates and a negative net uptake of P, see experiment W+S9 and W+S10 in Fig. 60. This could potentially indicate GAO presence utilizing the glycerol. This needs to be looked further into.

5.4 Results from daily measurements of influent and effluent concentrations

5.4.1 Phosphate, Ammonium and sCOD

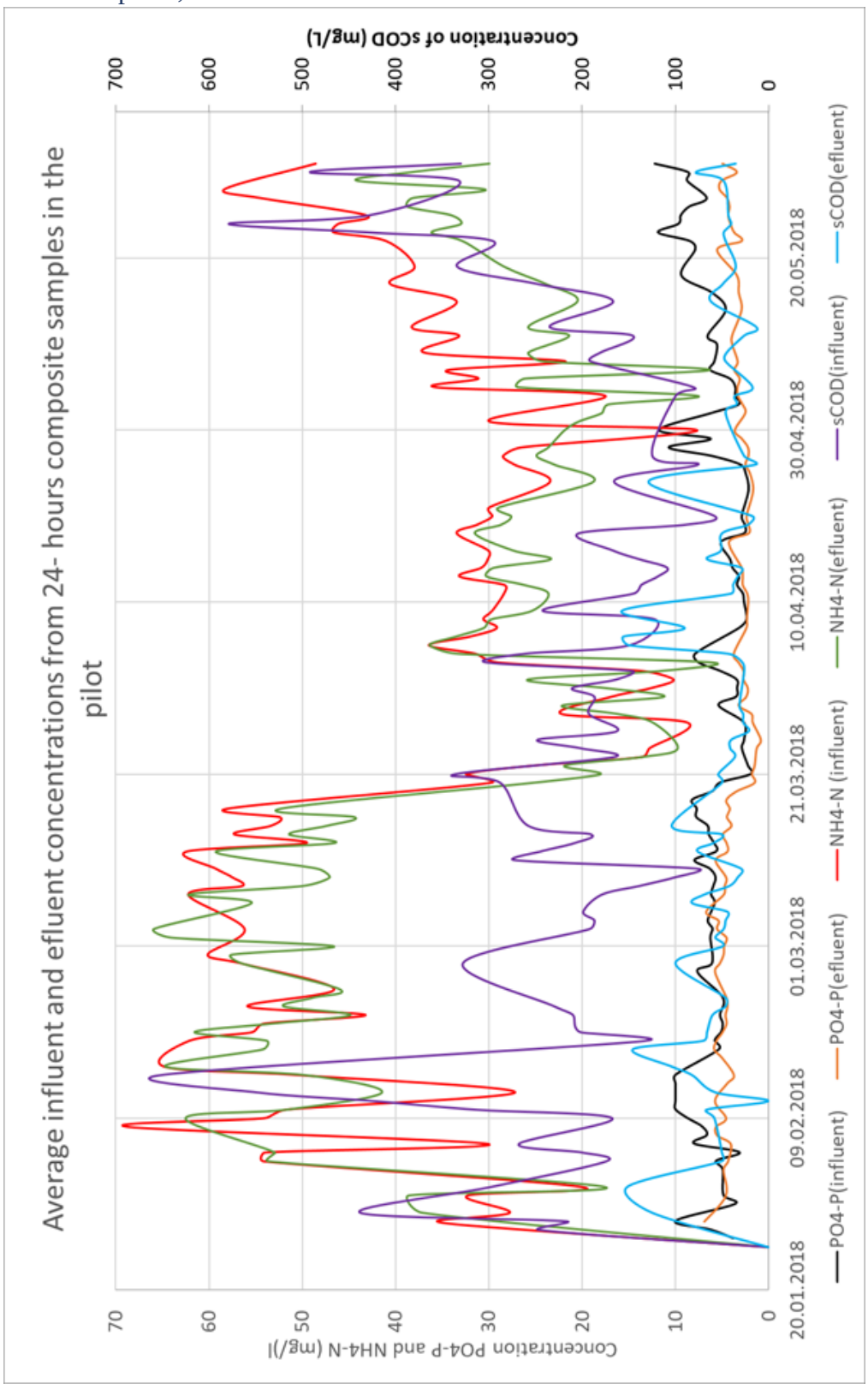


Figure 42 - Influent vs. Effluent PO4-P, NH4-N and sCOD concentrations from 25.01.2018 - 31.05.2018

5.4.1 PO4-P

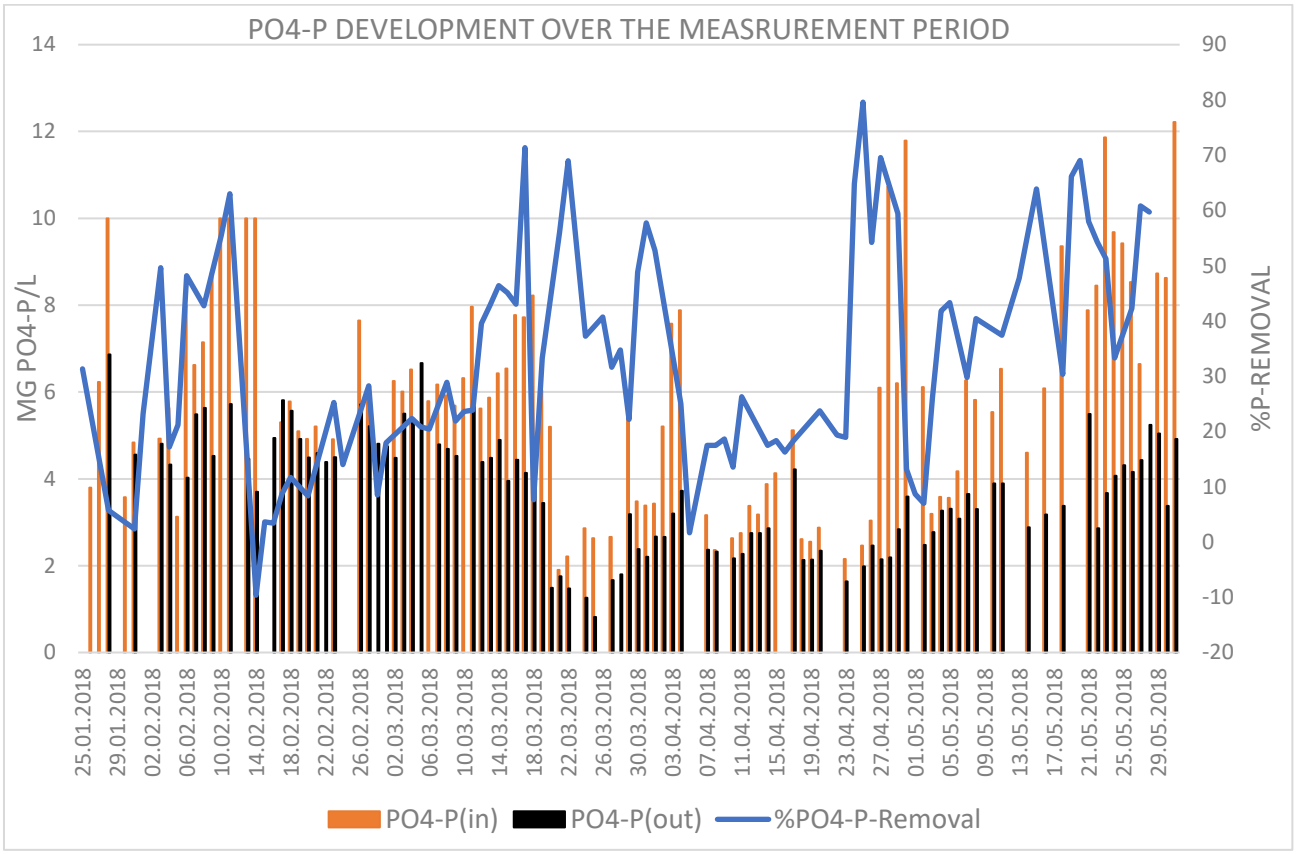


Figure 43 - PO4-P in- and out and %P-removal from 25.01.2018 - 31.05.2018

Table 18 – Average influent PO4-P concentrations and %P-removal throughout the measurement period. Measurements before dilution starting 25.01.2018 until 20.03.2018, measurement during dilution starting approximately 21.03.18 and finishing 26.04, and measurements after dilution starting 27.04.2018 until 31.05.2018

	In 25.01-21.03	Out 25.01-21.03	In 22.03-26.04	Out 22.03-26.04	In 27.04-31.05	Out 27.04-31.05
mg PO4-P/L	6.31 +/- 1.87	4.63	3.58 +/- 1.50	2.36	7.32 +/- 2.62	3.68-
%P-Removal	26.64		33.89		49.73	

During the startup there were some technical and mechanical running problems. Still the average removal from January 25th to March 22nd is 26.64 %. Right after dilution started due to snowmelt there was continued high removal, despite low influent concentrations, but after one week the removal decreased by approximately 20%, so the continued removal after dilution had been prevalent for a week was only approximately 10-20%.

From Fig. 43 one can see that removal has started to increase from May 8th. As well as increases in concentrations.

5.4.2 sCOD

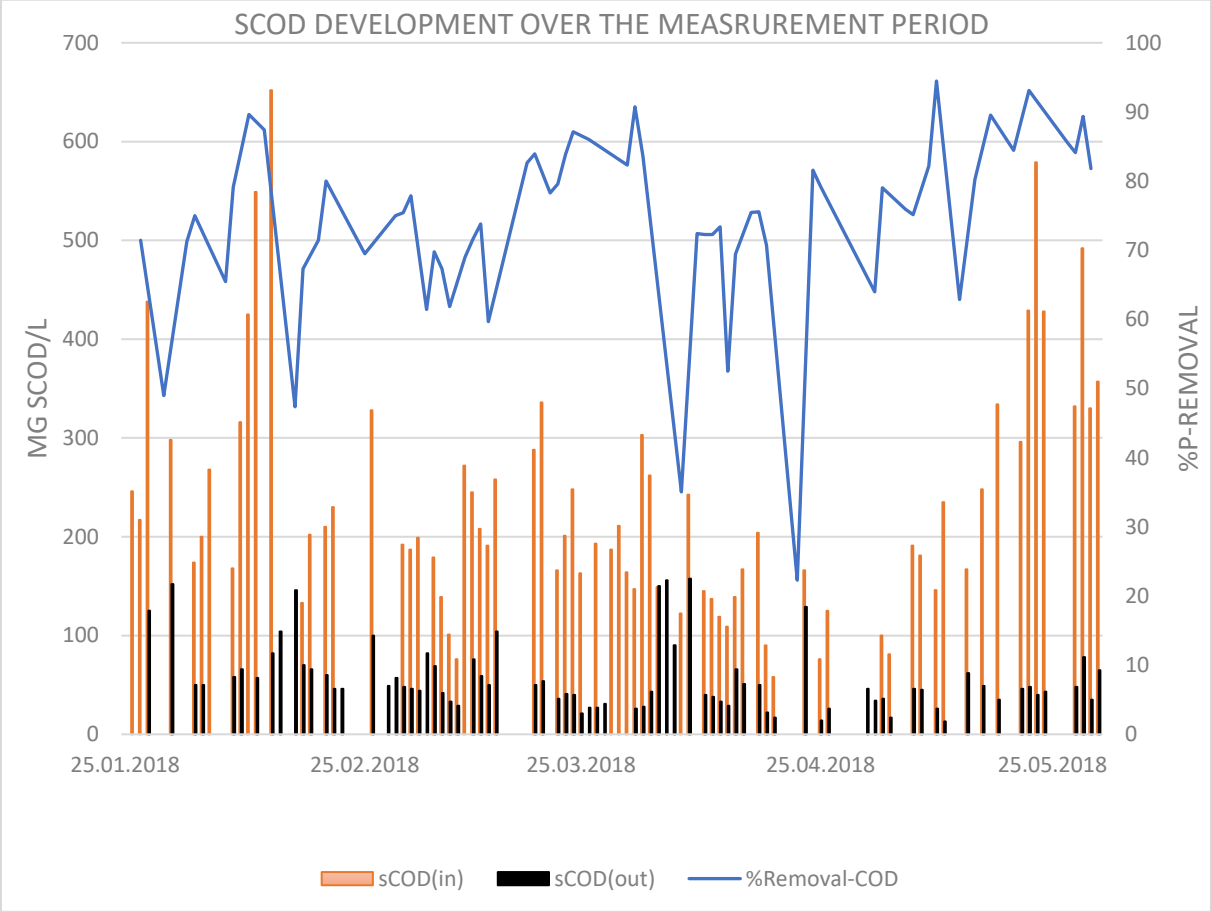


Figure 44 - sCOD in- and out from 25.01.2018 to 31.05.2018

5.4.2.1 sCOD Fluctuations

The sCOD removal has continuously been between 50 and 80% except for some drops. sCOD concentrations were affected by dilution, however not to the same degree as PO4-P and NH-N concentrations. Still, small drops in carbon loading leads to decreases of the rbCOD loading, which most probably is low beforehand. Even small decreases to the substrate loading can mean that advantageous substrate sources such as rbCOD, already low for an effective process, is even more in demand. When these sources decrease the use of substrate can be shifted so that the biomass's focus is on survival rather than utilizing its broad specter of potential for EBPR (Carvalheira et al., 2014). One can see that during the period where sCOD concentration were lowest; from 06.04.2018 to 19.04.2018 is also when the %P-removal was most affected. The P-removal decreased with 59% during the period with lowest sCOD loading, from the week prior to it. Overall it seems the dilution and decreases in substrate loading, see Tab. 20 led to decreased efficiency of the process. Currently influent sCOD concentrations are higher than before dilution and correlates well with increase P-removal.

Table 19 - The difference in substrate loading concentration to the pilot before, during and after snowmelt

Average sCOD influent before snowmelt	Average sCOD influent during snowmelt	Average sCOD influent after snowmelt
255.64 +/- 123.5	165.15 +/- 57.8	289.69 +/-140

On one hand the anaerobic COD-loading rate should be kept low enough to avoid competition from OHOs, while the COD-loading rate should be so that a sufficient PHA amount is stored for P-uptake and a net growth of biomass (Helness and Ødegaard, 2001). Studies looked at in this review are studies in suspended biomass and not biofilm. As MBBRs commonly have a higher loading rate then AS, as well as highly different diffusion limitations it might be that EBPR systems with biofilm react differently to a decrease in COD/TP inlet concentrations than literature looked at for AS. A high COD:TP ratio is needed for an efficient EBPR (US-EPA, 2010) and also shown in chapter 2.1.3, however there is always the question of how much influent COD is needed for uptake vs how the diffusion constraints affected should be considered up against each other. During the dilution period the sCOD/PO4-P ratio was 47. And during the semester sCOD/PO4-P ratio was 41. This is quite low in comparison to values from literature, see Ch. 2.1.3, which normally in the range of COD/PO4-P> 40 (Carrera et al., 2001).

What can be seen is that even though the sCOD/PO4-P ratio increased as snowmelt began in the pilot and in kinetic experiments this ratio alone is not good enough to predict the efficiency of the process here. This is probably due to the fact that the increase in sCOD/PO4-P ratio was due to decrease of both substances. Decrease of sCOD leads to less availability of rbCOD and a less efficient process.

5.4.2.2 Calculation of the bsCOD-concentration

Uncertainty Surrounding the average bsCOD concentration in the influent needs to be investigated. For the discussion here, a simplification has been made for estimation of bsCOD. bsCOD is here defined as the difference between the remaining unutilized carbon at the end of the aerobic zone and the influent sCOD tracked during kinetic experiments with no substrate addition. From this definition of bsCOD the bsCOD/PO4-P ratios can be calculated using Eq.5.1.

$$\mathrm{bsCOD} = \frac{\mathrm{sCOD(t0)} - \mathrm{sCOD(t12)}}{sCOD(t0)} \qquad \text{(Eq. 5.1)}$$

bsCOD = biodegradable soluble fraction defined by the difference between sCOD concentrations

sCOD(t0) = sCOD concentration at time zero

sCOD(t12) = sCOD concentration at time step 12

From Tab. 20 the bsCOD has been calculated using the equation 5.1

Table 20 - Using the definition of sbCOD in Eq. 5.1 the average bsCOD level in the wastewater during kinetics has been calculated

Name	bsCOD	Average bsCOD	Standard deviation
WW7	0.664	0.512	0.100
WW5	0.582		
WW4	0.496		
WW3	0.493		
WW2	0.375		
WW1	0.459		

For all kinetic experiments performed with substrate additions, substrate had been dosed in excess. In Tab. 20 the bsCOD/PO4-P ratios in beaker experiment with no substrate additions has been presented. Helness (2007) states that a minimum rbCOD/PO4-P ratio should be 10 for functioning EBPR. The ratios present in the influent to the pilot is clearly very low. Measurements done at HIAS shows the bsCOD/PO4-P ratio to be >40. Tab. 21 shows the ratios in the wastewater during kinetic experiments with no substrate additions.

Table 21 - bsCOD/PO4-P ratos in kinetic experiments with no substrate addition. WW1 and WW2 R2<0.8

Name	bsCOD/PO4-P ratio
WW7	17.20
WW5	20.43
WW4	11.16
WW3	15.60
WW2	17.11
WW1	8.14

From the ratios of bsCOD/PO4-P the two of the three kinetics with the lowest ratios; WW3,-4 and 5 were the ones to perform the best, as speed of uptake and release as well as total P-removal was the highest there. See Fig. 61. As DO and T has been controlled and pH has not differed outside of theoretically advantageous range, the dilution factor stemming from large degrees of snowmelt seems to be the contributing factor for WW7 low reoval. Despite the biomass development, it seems the net effect of improved biomass cannot compete with the reduced conditions due to dilution, see Fig. 45 and 46. As Carvalheira et al., (2014) found that PAOs showed the ability to survive during low organic loading conditions, however the specific anaerobic activity and aerobic uptake decreased. Therefore, even if PAOs did survive during low periods of loading, to ensure effective EBPR the loading must increase to sufficient levels.

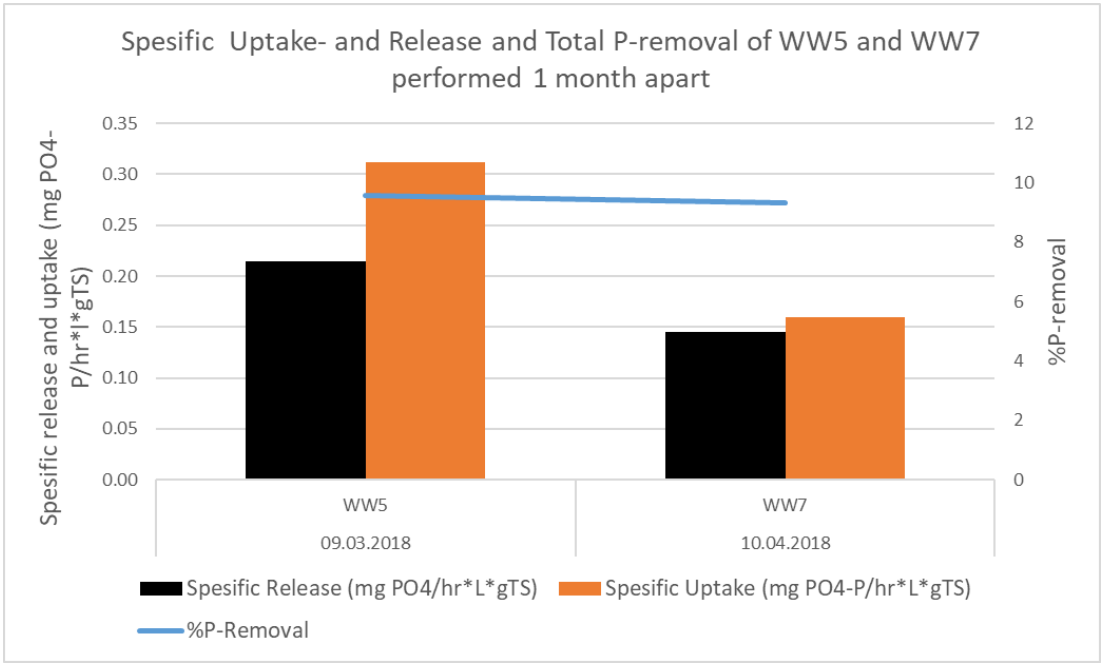


Figure 45 Shows the development of specific uptake and release in two experiments performed 1 month apart. Despite improved biomass the dilution affects the performance.

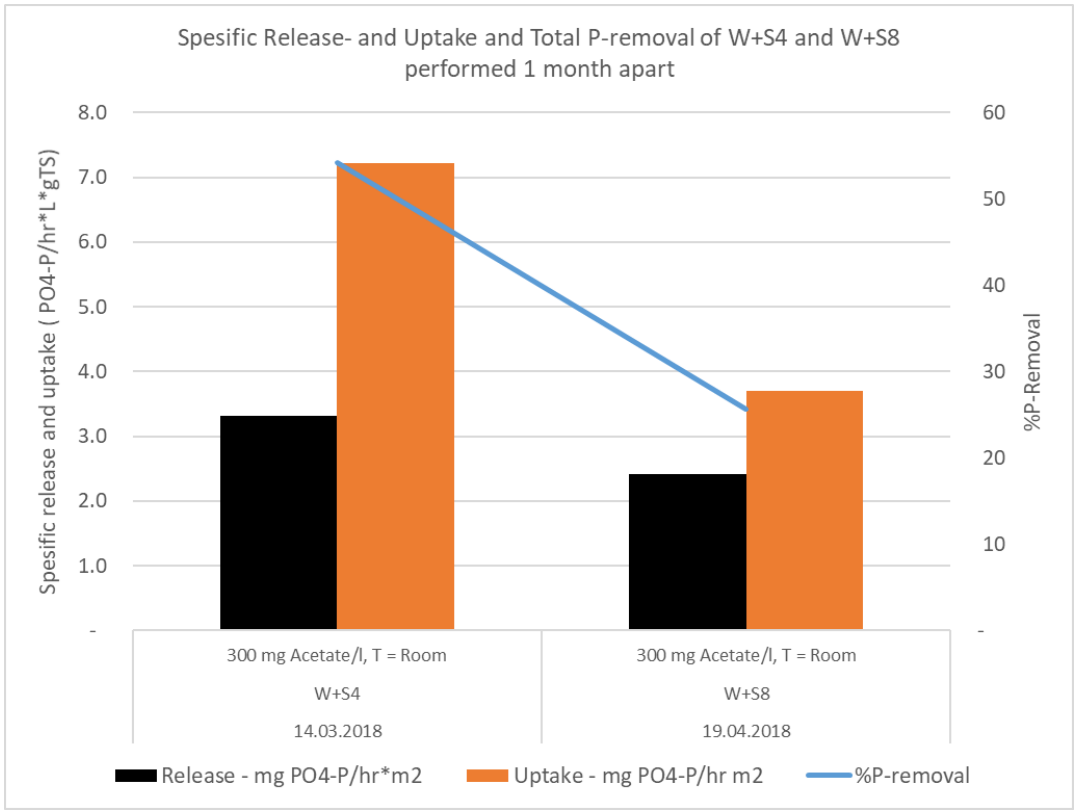


Figure 46 - Development of specific uptake and release in two experiments performed 1 month apart under the same conditions.

5.4.2.3 sCOD utilization

The removal efficiency of sCOD is quite high throughout the measurement period, however most probably it is due to OHOs in the aerobic zone. In a continuous MBBR carriers move through the differing zones with the flow of the liquid bulk, for process efficiency it is therefore essential that these move at the same speed. If there is a delay of carrier movement in comparison to liquid bulk movement this can affect the diffusion possibilities from the liquid bulk to the carriers. If there are is a high organic load with carryover of rbCOD from the anaerobic zone to the aerobic zone OHOs can proliferate. If the PAO biomass has been transported to the aerobic zone prior to the liquid bulk, they may not have had enough time to take up carbon and store this.

Generally, a high organic load not fully utilized lead to carry over of easily biodegradable carbon from the anaerobic zone. The competition from OHO will increase, as OHOs always will be present in the biomass and can experience growth inhibiting PAOs sustainment as these bacteria can hinder the PAOs uptake of both oxygen and the release of phosphate due to constraints with diffusion and thickness of biofilm (US-EPA, 2010). This operational issue is also highlighted by Helness (2007) and Helness & Ødegaard (2001), which states that the removal of rbCOD should be so that the full completion of this happens within the anaerobic zone. This is of relevance for this study when looking at the continuous MBBR as the process needs to be optimized in a way for hindering carry-over of rbCOD to the aerobic zone as this currently is most probably happening due to net usage of sCOD almost equal to 0 in the anaerobic zone.

Through batch tests in the pilot following its performance it became clear that the utilization of available sCOD is not high, or that production by the biomass it self is so high the sCOD stays flat. Batches were taken from each chamber with 38-minute intervals, method described in Ch. 4.2, 4.7 and 4.12. What these results showed is that the sCOD concentration does not decrease throughout the anaerobic zone, see Fig. 48. The removal rate of sCOD within the anaerobic zone is 0.003 and 0.0029 mg sCOD/m^2*hr for P1 and P2 respectively. Despite this fact the release rate is 1.56 mg PO4-P/m^2*hr for P1 and 0.7 mg PO4-P/m^2*hr for P2. The lower release rate during experiment P2 is probably due to low substrate loading at that time, while a higher uptake rate; 1.9 and 2.5 might be due to improved biomass, see Ch. 5.3 and improved DO and T at that time in the pilot.

To account for assimilation of P by OHOs a rate of 0.015 g PO4-P/g VSS is used (Henze et al., 2008). From Ch. 5.3 one can see that the VSS/L measured in the biomass at this time is 0.31 g VSS/L. Assimilation due to OHOs is accounted to be 0.465 mg PO4-P/L. This is contributory to about 28% of the P-uptake for both P1 and P2 in the aerobic zone and therefore it seems other microbial structures must be accountable for the P-uptake as well.

Despite no net use of sCOD the total %P-removal is 18 and 27 % for P1 and P2, see Fig. 47. As some release is present this entail that the bacteria strain present is not Accumulibacter as they do not ferment more complex organic molecules.

Tetrasphaera, a different strain of PAOs encompasses the ability to ferment complex organic molecules such as amino acids. They can store this carbon in the process and utilize this, see

Ch. 2.1 and Ch. 2.1.1. As the removal rate of sCOD is approximately 0.003 mg PO4-P/hr*m^2 this entail that there is a presence of Tetrasphaera that ferments organic molecules, while also utilizing these creating a net uptake of practically 0 mg sCOD/hr*m^2.

As there is little substrate in the influent utilized this reduced the effectiveness of the process. When the biomass has to ferment its preferred carbon source, as there is little to no availability of preferred substrate in the influent, the efficiency will be reduced due to less time for P-release and P-uptake and high energy usage for fermentation.

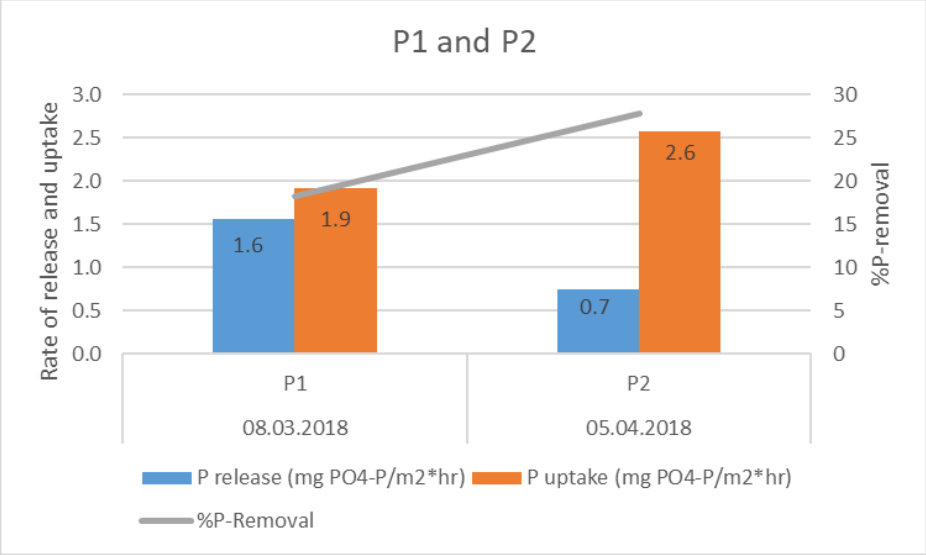


Figure 47 - Rates for release and uptake, and %P-removal in batch kinetic in the pilot with HRT 38 minutes.

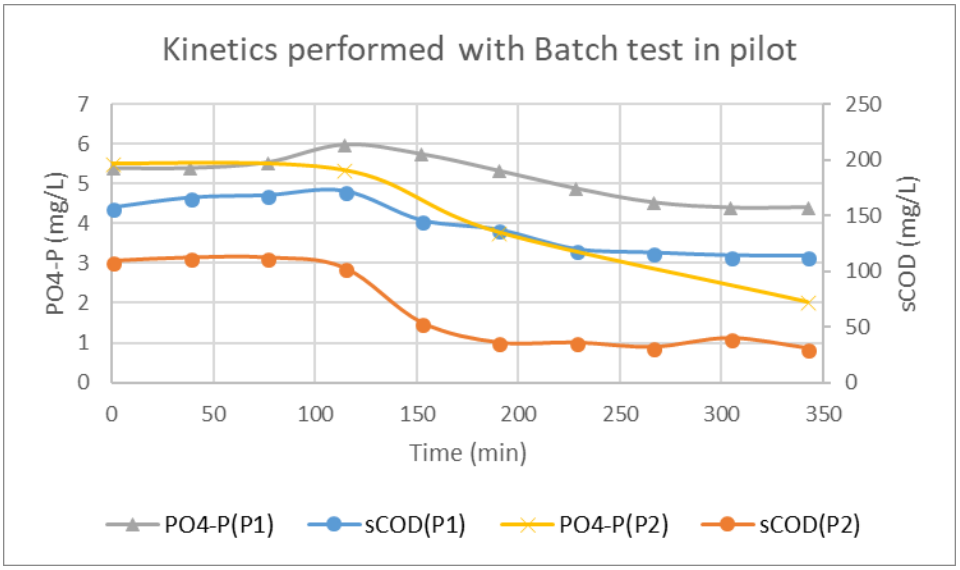


Figure 48 - Batch kinetic following reactor performance. sCOD stays completely flat throughout the anaerobic zone while P approximately 28% of the P uptake is due to assimilation.

These results are also experienced in beaker experiments following P release and P uptake where both influent wastewater, influent wastewater with acetate addition and synthetic wastewater with acetate additions experience practically no sCOD removal within the anaerobic zone, see Tab. 22. Decreases in sCOD was not measured through the samples, although activity

of P-release and P-uptake was present. After having run many kinetics and established a pattern the rate of the sCOD in the anaerobic zone was established for all experiments run with acetate and glucose. See Tab. 22.

The experiments run with glucose have on average 15.5 times higher sCOD removal in the anaerobic zone than experiments with acetate or only wastewater.

Table 22 - Net utilization of carbon sources as sCOD in the anaerobic zone

Name	Removal rate of sCOD in anaerobic zone for wastewater and synthetic water with acetate additions (mg sCOD/m^2*hr)	Name	Removal rate of sCOD in anaerobic zone with glucose additions (mg sCOD/m^2*hr)
W+S1	0.033	S7	-0.061
W+S2	0.002	W+S5	-0.065
W+S3	-0.007	W+S8	-0.060
W+S4	0.001		
S1	0.002		
S4	-0.004		
S3	0.008		
WW3	0.007		
WW4	0.004		
WW5	-0.006		
WW7	0.003		
Average	0.004	Average	-0.062

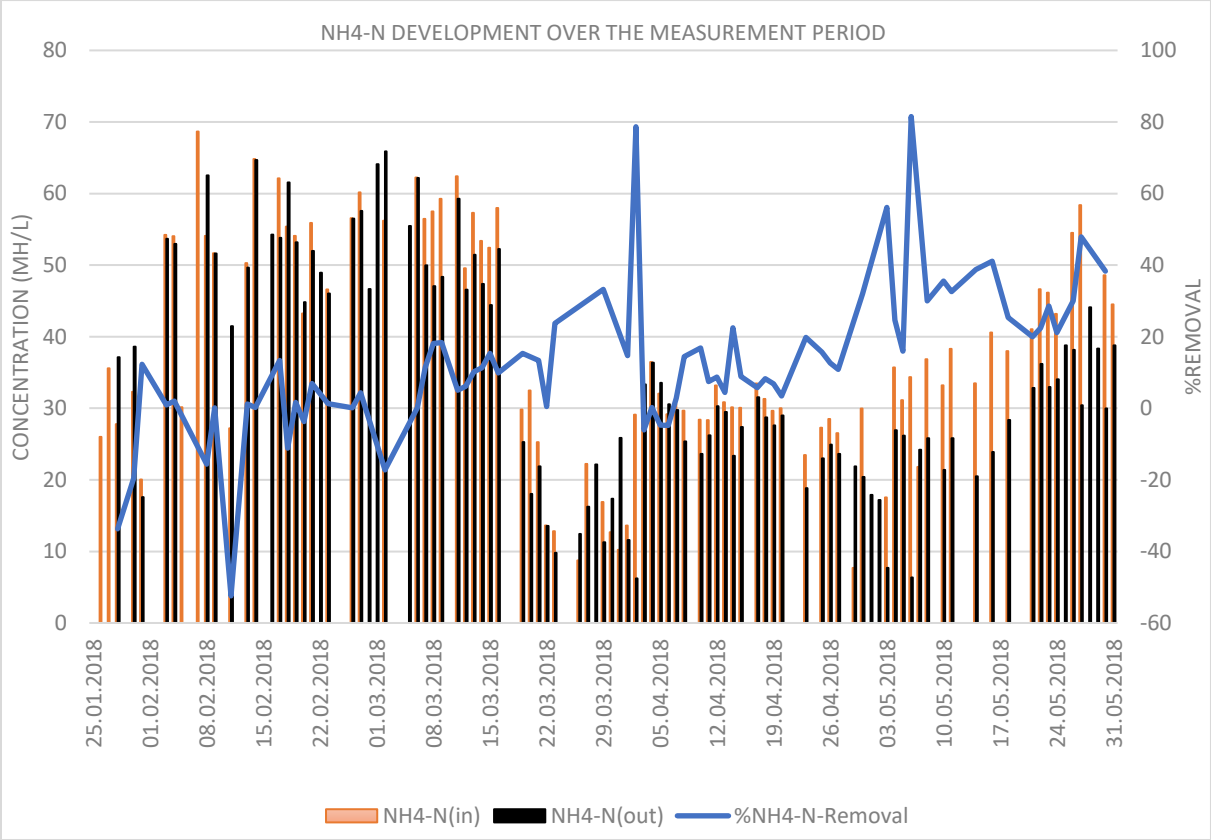
Due to the low sCOD removal within the anaerobic phase, this must entail a large degree of carry over of substrate sources from the anaerobic to the aerobic zone, which is highly undesired.

5.4.2.4 P mol/C mol

Calculation of the mol P/mol C ratio has been performed. Values from literature states that a mol P/mol C ratio of <0.25 should indicate GAO domination and >0.50 indicate PAO domination (Loosdrecht et al., 2016). This ratio is based on experimental conditions with addition of acetate, at pH 7 and 20 °C. Experiment S1 is fitting of the criteria of these conditions. The difference between the influent concentration and concentration at the end of the anaerobic zone has been used to calculate the ratio, see appendix I for calculations.

The P mol/C mol ratio is 0.0263 which according to Loosdrecht et al., (2016) entails a high dominance of GAOs as its << 0.25. This does not seem to be the case here however, as no consumption of sCOD has been detected in the anaerobic zone, which GAOs would have utilized. Other parameters in the pilot indicate that the conditions are preferential for PAOs, see Ch 5.5 on T, pH and DO effect. Either the rate of release- and uptake and net P-removal of 20%, can be caused due to other strains of PAOs that does not utilize the acetate, which seems likely, or OHOs. The P mol/C mol ratio is more relevant for Accumulibacter, which utilizes acetate. From this ratio it can be possible to conclude that the Accumulibacter dominance is low, but this does not state that the abundance of PAOs utilizing other substrate sources is low. It is highly likely that as the sCOD-removal rate in the anaerobic zone was not detectable fermenting PAOs were present, creating a net uptake of sCOD almost equalling zero.

5.4.3 Ammonium



Daily and during all kinetics experiments measurements of ammonia has been analyse. These results have shown no signs of consistent NH-N removal. Results from daily analysis of in-and out shows a removal of ammonia of 8 % +/- 20 up to May 7th, and a removal of 35 % +/- 15 from May 7th until 31st of May. The removal of ammonia has started to significantly increase. NO3-N samples have now started to be taken for influent and effluent samples in the pilot but will not be included in this thesis.

Table 23 - Effect of dilution on ammonium concentrations.

Average NH4-N concentration before	Average NH4-N concentration during	Average NH4-N concentration after dilution
49.5 +/- 13.7	25.8 +/- 8.1	36.8 +/- 11.7

Figure 49 -Kinetic experiment run with input of NO3-N instead of oxygen in aerobic zone

To assess for how the biomass reacted to NO3-N as electron acceptor, and if the biomass was able to utilize this for P-uptake NO3-N was used in experiment S2 instead of oxygen, see Fig 49.

Substrate dosage in this experiment was 300 mg acetate/L in synthetic water. As has been discussed above, the PAOs does not utilize acetate to a large degree and instead ferment other more complex organic molecules. During this synthetic experiment, as with others discussed; when acetate was added it seems a large quantity of biomass utilize their storage for degradation of carbon instead of the available acetate as the sCOD curve therefore stays flat during the anaerobic zone. Although the loading of VFA was unsatisfactory the release rate in S2 was 1.62 mg PO4-P/hr*m^2, while release rate in S1, one day prior, was 1.41 mg PO4-P/hr*m^2 under equal conditions in the anaerobic zone, see Fig. 50. Despite the release rate being lower in S1 the total P-removal was 41 % compared to 6% in experiment with NO3-N as an electron acceptor. From the results is was therefore concluded that there was no indication that the biomass could utilize NO3-N at this point. This needs to be reviewed as there currently is removal of ammonium within the reactor.

The removal of 6% were most likely due to OHOs as sCOD and NO3-N is immediately utilized in the aerobic zone. The removal due to assimilation was approximately 0.525 mg PO4-P/l and total P uptake was 0.60 mg PO4-P/L.

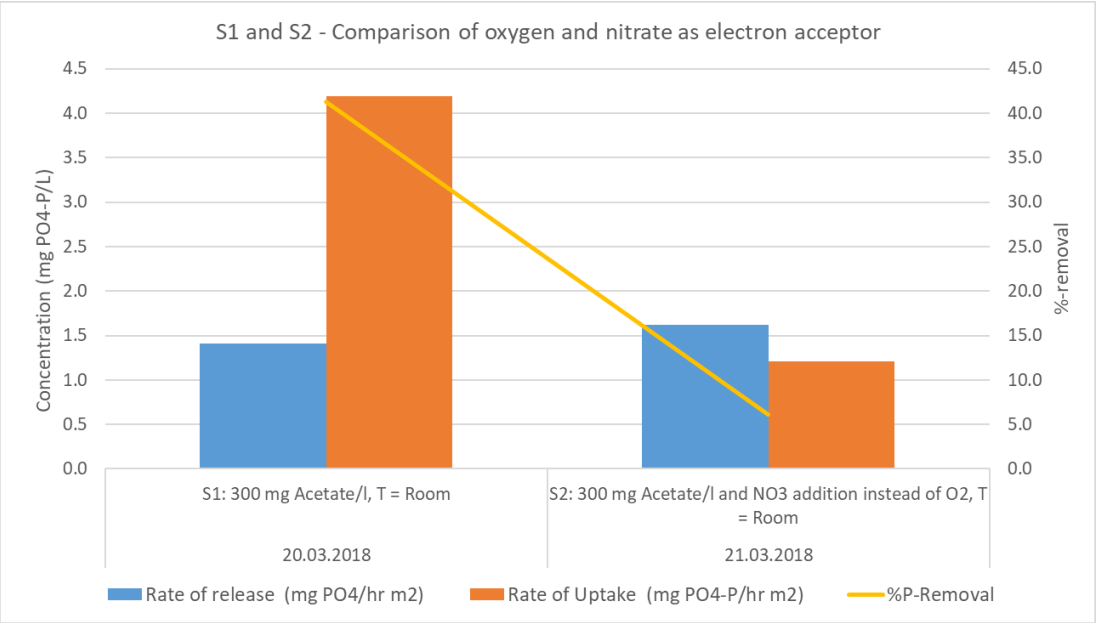


Figure 50 - Comparison of use of O2 and NO3-N as electron acceptors- %P-removal was reduced from 40 to 6%

5.5 Results from the daily monitoring of Dissolved Oxygen, pH and T

5.5.1 Dissolved Oxygen

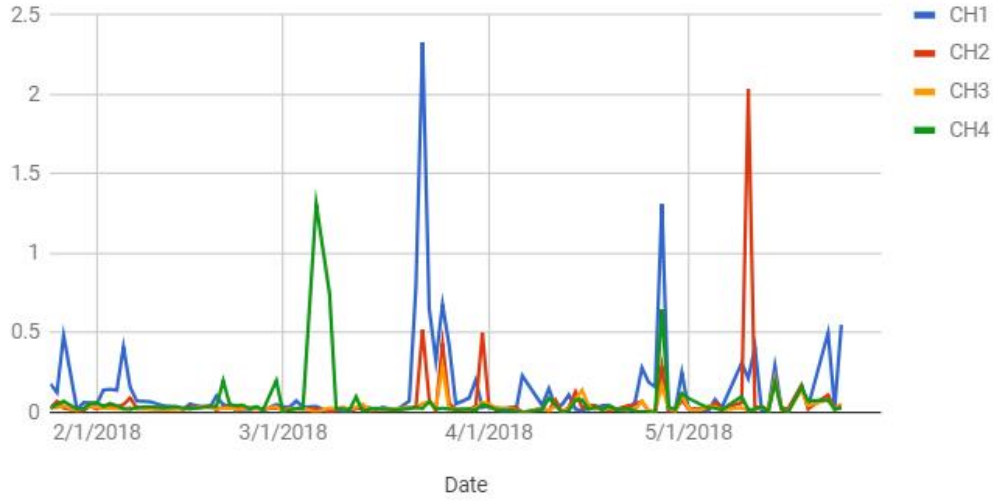


Figure 51 DO-development (mg/l) within all anaerobic chambers from 25.01.2018 - 01.06.2018

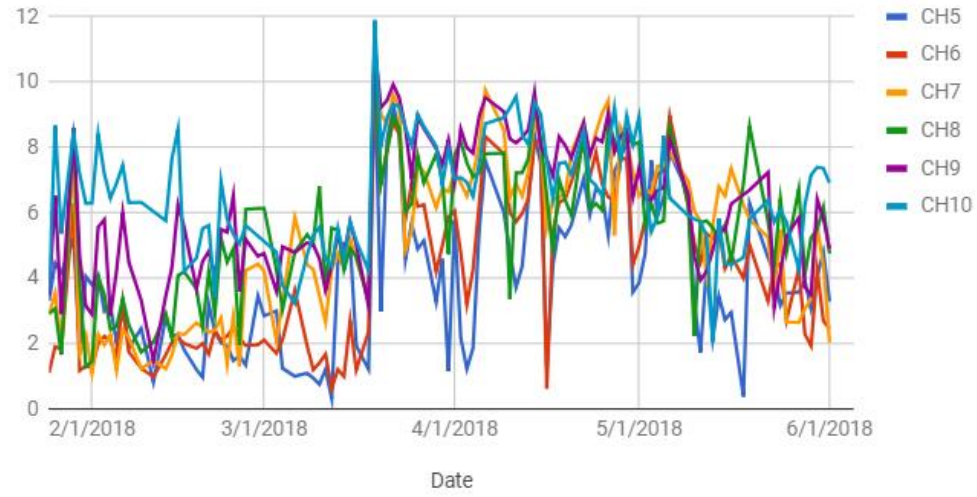


Figure 52 - DO-development (mg/l) within all aerobic chambers from 25.01.2018 - 01.06.2018

Aeration is one of the biggest operational costs in wastewater treatment plants, it is therefore of importance to minimize this to improve the cost-effectiveness of the process (Carvalheia et al., 2014). Aeration above necessary levels can also lead to higher turbulence, and an increased risk of erosion of active biomass. During experiment erosion of biofilm has been experienced.

Fig. 51 and Fig. 52 shows how the various DO concentrations present, in the anaerobic and aerobic chambers respectively, have fluctuated. These are very efficient to use to look back at and establish which periods that have had snowmelt and rainwater intrusion. Problems

stemming from operation of the reactor with clogging and accumulation of carriers have led to high DO levels in chamber 1 in periods. There have been periods where struggles with backflow from chamber 5 into chamber 4 has led to increased DO levels, see Fig. 51.

In late March the snow-melt started and a sharp increase in DO-concentrations can be clearly seen from the figure as well as how rainwater intrusion has been a recurring problem in April. Rainwater have high DO-levels in comparison to wastewater and will often lead to more aerobe levels in anaerobe zone. Increases in DO concentrations in aerobe zone most probably stems from reduced oxygen consumption, as influent concentrations were low due to dilution leading to reduced utilization of oxygen.

Before dilution the average DO concentration was 4.0 mg/L +/- 1.8 within all the aerobic chambers. During dilution there was a sudden upward shift in DO concentrations to 7.1 +/- 1. There is no indication in literature that high DO levels are negative for PAOs utilization, however that their competitive advantage may decrease with increased DO. In literature it is stated that the diffusion limitations with regards to oxygen are smaller in AS than in MBBR it might be that it is necessary with a higher oxygen feed than what literature states for AS. This can influence erosion parameters as increased aeration will lead to increased sheer stress. If the diffusion limitations are higher the lower DO-concentrations experienced in January until March may have been too low for optimized EBPR in an MBBR, as can also be seen from results from beaker experiments as increased DO-concentration was beneficial. However, the sudden upwards shift in DO-concentrations may also have been a shock to the biomass. P-removal was in the days after dilution started (March 22nd) 44% +/- 13. sCOD concentration was still not as highly affected by dilution yet which probably played the main role in the good removal, but the increase in DO-concentrations may have been beneficial for the increased removal as well. However, the following week with still high DO levels the removal efficiency declined which suggest that the sCOD load is of higher relevance.

As more removal has been experienced in May one can also see a drop in Fig. 52 around May 8th which is also when looking at Fig. 43 and 58 showing an increase in P-removal. The drop in DO-concentration is most probably explained by the increase in oxygen consumption as removal efficiency has started to increase, as no changes has been done to the air-flow. The average DO-concentration after dilution until 1st of June has been 5.5 +/- 1.19

Carvalheira et al., (2014) stated low DO levels, below 3, could be beneficial for the process as literature here shows PAO become more competitive over GAOs at lower DO levels. From May 7th the removal has been increasing in the reactor despite high DO levels. This could either mean that GAOs make up a small quantity of the biomass and is not competitive because of their small population even at higher DO concentrations, or that the PAO culture in the reactor is competitive with GAOs even at higher DO concentrations.

In a beaker experiment the effect of oxygen loading to release, uptake and %P-removal was tested. As presented in theory in Ch. 2.2.6 airflow should be above 2 for EBPR.

Levels of 3 and 7 mg DO/L were therefor set in the experiment to attest for differences. If results varied widely, a kinetic with 5 mg DO/L would be performed.

Through the kinetic experiment with average DO-values of 3.3 and 7.3 mg DO/L it did seem that a higher DO-concentration benefited the rate of release and uptake. This does also seem to correlate with the results from the daily in and-out as after DO increased in the pilot improved performance followed the week after dilution began, and it was suggested that DO could play a small role in this. The DO load is currently 5.5 +/- 1.2 which is 27% higher than levels before dilution. Increase in removal efficiency is most probably a function of several factors but a DO increase can have been beneficial, see Fig. 53. Despite increases in the rates between 25-35%, %P-removal only increased by 10%.

Due to these results the DO concentration was always kept above 5 mg/L and preferably at 7 mg/L during kinetic experiments.

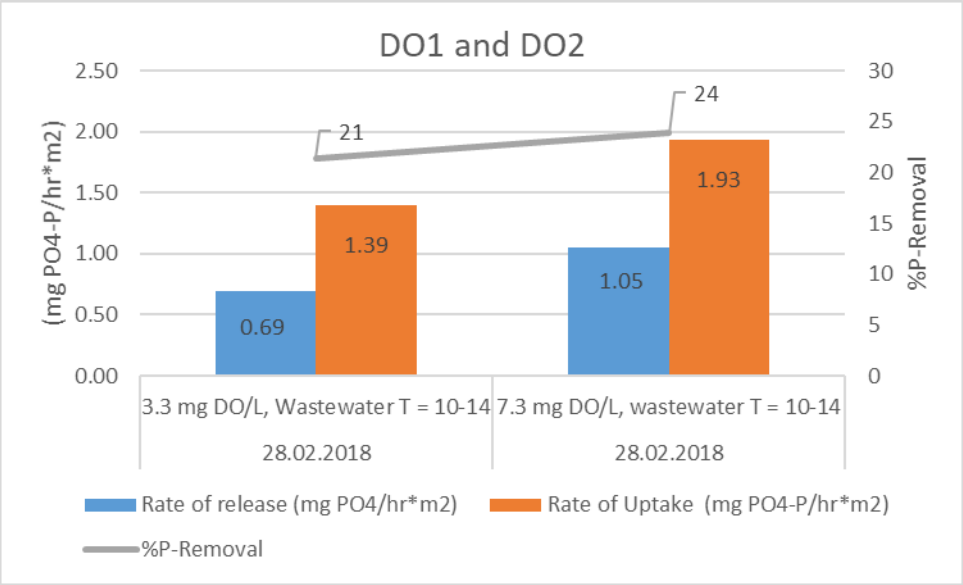


Figure 53 - Rate of release and uptake at two different DO-concentrations. Despite higher rates %P-removal is almost unaffected

Experiment P1 and P2 performed as batch kinetics in the pilot before and during dilution shows that during dilution the rate of uptake increased, this could be due to the increase in DO-levels as average DO level during experiment P2 was 7.2 mg DO/l vs. 3.7 during P1see Fig. 47. The biomass has also improved over time and could be part of the reason for increased uptake rate in P2.

5.2.2 pH

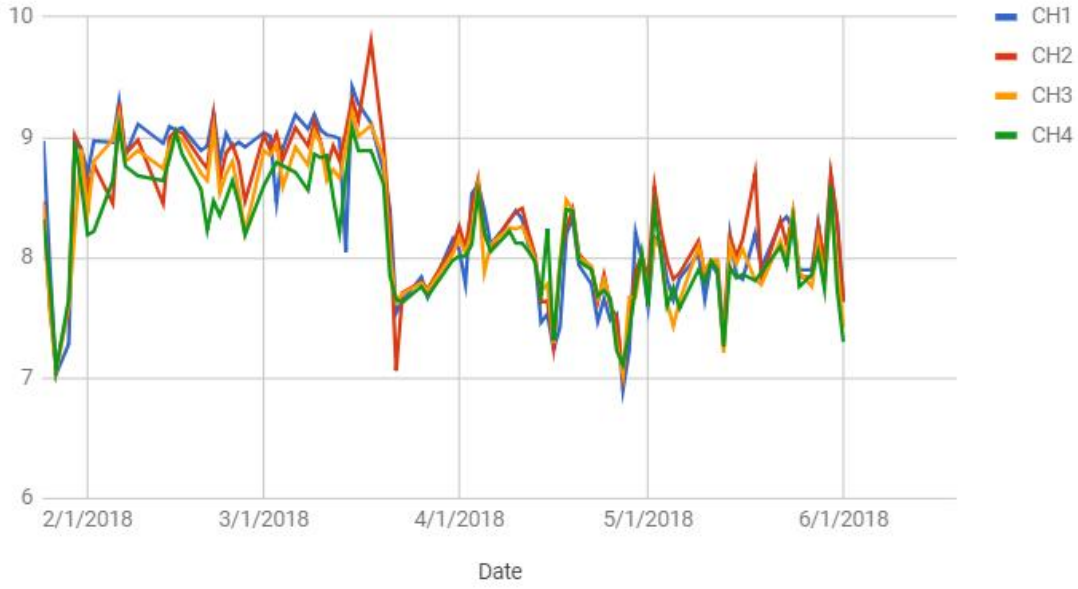


Figure 54 - pH development within all anaerobic chambers over the period 25.05.2018 - 31.05.2018

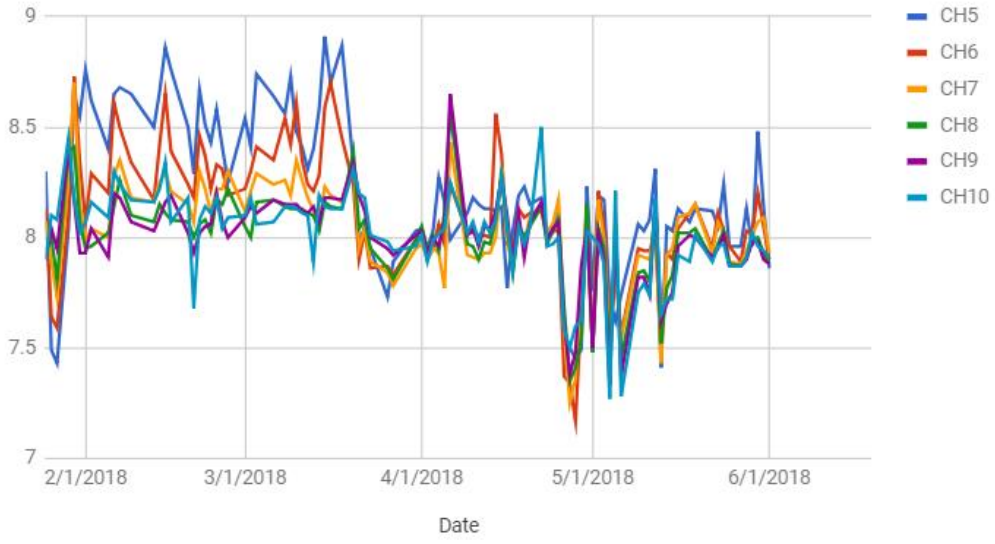


Figure 55 - pH development within all aerobic chambers over the period 25.05.2018 - 31.05.2018

After the period of snow-melt started the drop in pH was immediate. After this the pH has never recovered and returned up to its levels prior to snow melt and rainwater intrusion.

The pH in the wastewater for the pilot here at NTNU is characterized as high with an average pH in all chambers in the reactor of 8.17 +/- 0.3 from 25.01.2018-01.06.2018. This seems to be beneficial based of literature. Filipe et al., (2001) concludes that it is preferential to keep the pH high in the EBPR system to control the competition between PAOs and GAOs, as PAOs inhibit more energy than GAOs. Which also is in accordance with many of the other reviews read on this topic, see Ch. 2.2.2..As the pH normally is quite high in Norwegian wastewater this seems

beneficial for the process as the pH in the pilot is in accordance with average values from Norweian WWTP. For the prospect of implementation of EBPR in Norway this is optimistic results.

Wang et al., (2013) found that a biomass developed at initial pH of 7.8 contained a larger PAO than GAO population, than biomass developed at initial pH of 6.6. In the pilot the biomass has continuously been above seven and has been developed in an environment with a pH average above 8. This can have led to low growth of GAO in the biomass, which again can explain results from DO measurements that despite DO concentrations being beneficial for GAO competition the net-effect of a higher pH is more beneficial for the PAO competition. This can also be explained by both PAOs and GAOs utilizing oxygen and increases in DO increases both PAOs and GAOs uptake rates. While pH is not something that the microbial community utilizes, it merely just lives in it. Therefore, whichever microbial community functions best under these circumstances, PAO, has an advantage, while there is no advantage for the GAO. Which can explain that the net effect of a higher pH being competitively advantageous over DO. Since the pH has been so high it might be that the biomass has never been beneficial for GAOs growth.

Zhang et al., (2005) found that a slight shift in pH from 7.0 to 6.5 completely altered the microbial composition in the biomass and led to highly reduced phosphate removing capabilities. In the reactor the average pH dropped suddenly by approximately 0.5 during snowmelt, see Tab. 24 and Fig, 54 and 55. pH is still well within PAOs advantageous range. However, a sudden drop to pH can have led to changes in the microbial community and their composition as it was such a sudden lasting change, as Zhang et al., (2005) showed that a sudden shift did lead to changes in microbial composition. This is something that can be evaluated when the biomass will be sent for microbial determination. However comparing with results from (Tu & Schuler, 2013) it seems unlikely, as they found that between pH 7.4-8.4 the biomass was PAO dominated while GAOs dominated at pH below 7.

Table 24 - Average pH values before and during snowmelt. After snowmelt the pH have never reached levels prior to snowmelt. The drop was sudden, and did not decrease over time.

Average pH before snowmelt	Average pH during snowmelt
8.42 +/- 0.24	8.00 +/- 0.14

During beaker experiments the pH has been stable and above 7 for all kinetic experiments, except for experiment S7, with 300 mg Glucose as sCOD/L. Here the pH is decreasing throughout the anaerobic zone down to a level of 6.6 as the solutions buffer capabilities is lower in the synthetic water than the wastewater. Despite utilization of the glucose as sCOD during the kinetic experiments which can be seen in appendix D, the overall removal is low despite an improvement of the biomass over time seen in Ch. 5.3. During this experiment it is clear throughout the anaerobic zone that the rate of release and uptake is minimally higher than S3, run under the same conditions with addition of 300 mg Acetate as sCOD/L. Total %P-removal

is for both experiments 20%. This can however most probably not be attributed to the low pH during S7, although Zhang et al., (2005) investigated the effect of pH on EBPR with a slight decrease of pH from 7 to 6.5 and noted a reduced removal 99 % to 17 %, this reduced removal was experienced two weeks after the pH reduction. During the kinetic experiment the timeframe is so short that the pH is most probably not the contributing factor to the low release and uptake.

Rather during experiment S7, the biomass has been experiencing low substrate loading for some time, as it was performed in the middle of snowmelt. The glucose as sCOD is utilized in the anaerobic zone is therefore most probably utilized by the biomass for their own growth and storage, and not utilized by the biomass for effective EBPR.

5.5.3 Temperature

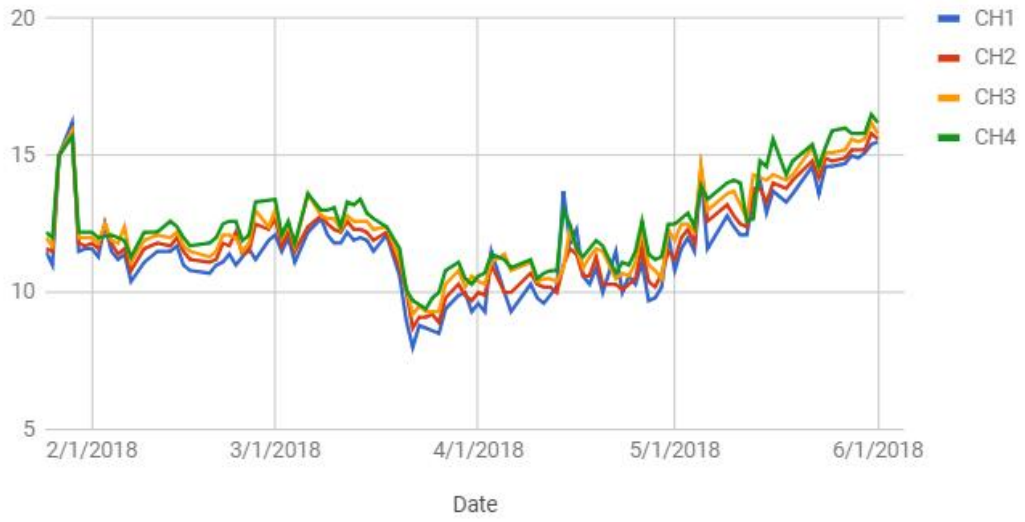


Figure 56 - Temperature development within all anaerobic chambers over the period 25.05.2018 - 31.05.2018

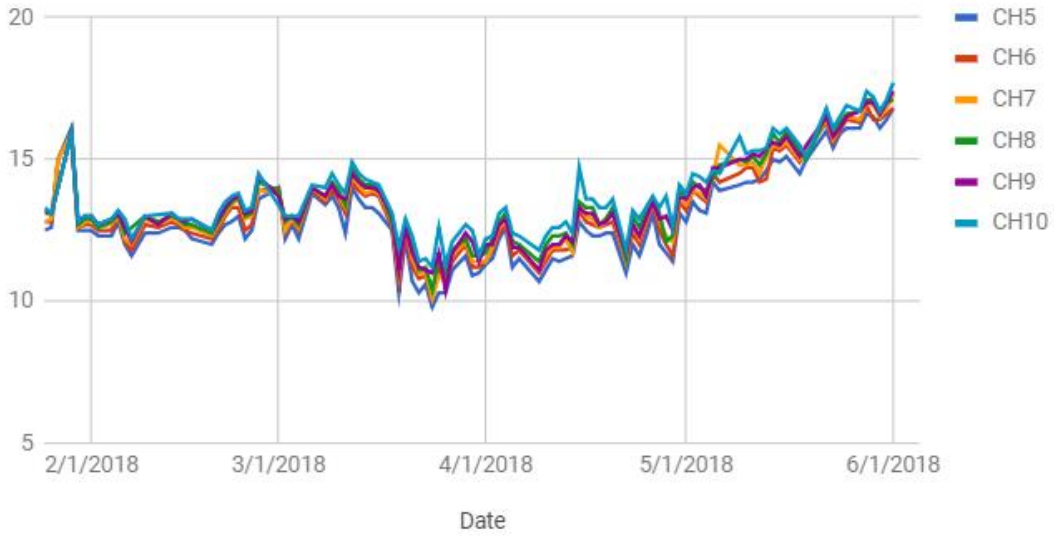


Figure 57 - Temperature development within all aerobic chambers over the period 25.05.2018 - 31.05.2018

The temperature effect on EBPR is widely known and difficult to assess as the results are contradicting. See Ch. 2.2.1 for literature on temperatures effect on EBPR.

From the Fig. 56 and 57 it is clear to see that the snow-melt and intrusion of rainwater into the system in mid-March affected the pilot in what can be seen as a sharp drop in temperatures. Any sudden change to operational conditions for the biomass can lead to adaptations and changes within the biomass. Little literature has looked at the effect of reduced temperatures on microbial population shift. Lopez-Vazquez et al., (2009) found that the effect temperature has on rates and removal were similar between different strains of bacteria. A conclusion to what a temperature change does to the population over time was not found. It will be interesting to see, when results sent for microbial determination arrive back, the biomasses evolution over time. After the sudden drop the average temperature throughout the pilot has increased steadily from mid-March. Throughout the semester the average temperature has been 12.7 +/- 1.278 °C. From Tab. 25 it is possible to see that the temperatures have varied widely.

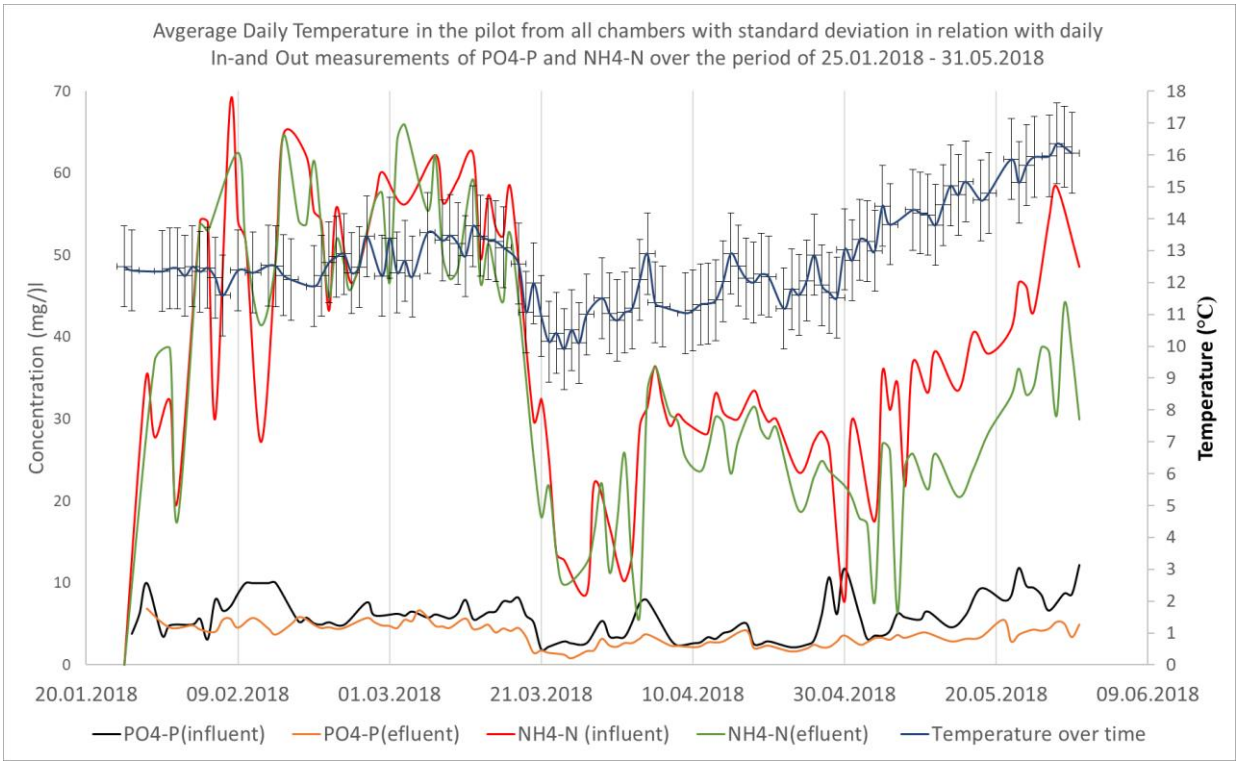


Figure 58 -Daily in and out measurements of PO4-P and NH4-N in relation with the average temperature in the pilot plotted with standard deviation. Measurement period: 25.01.2018 – 31.05.2018

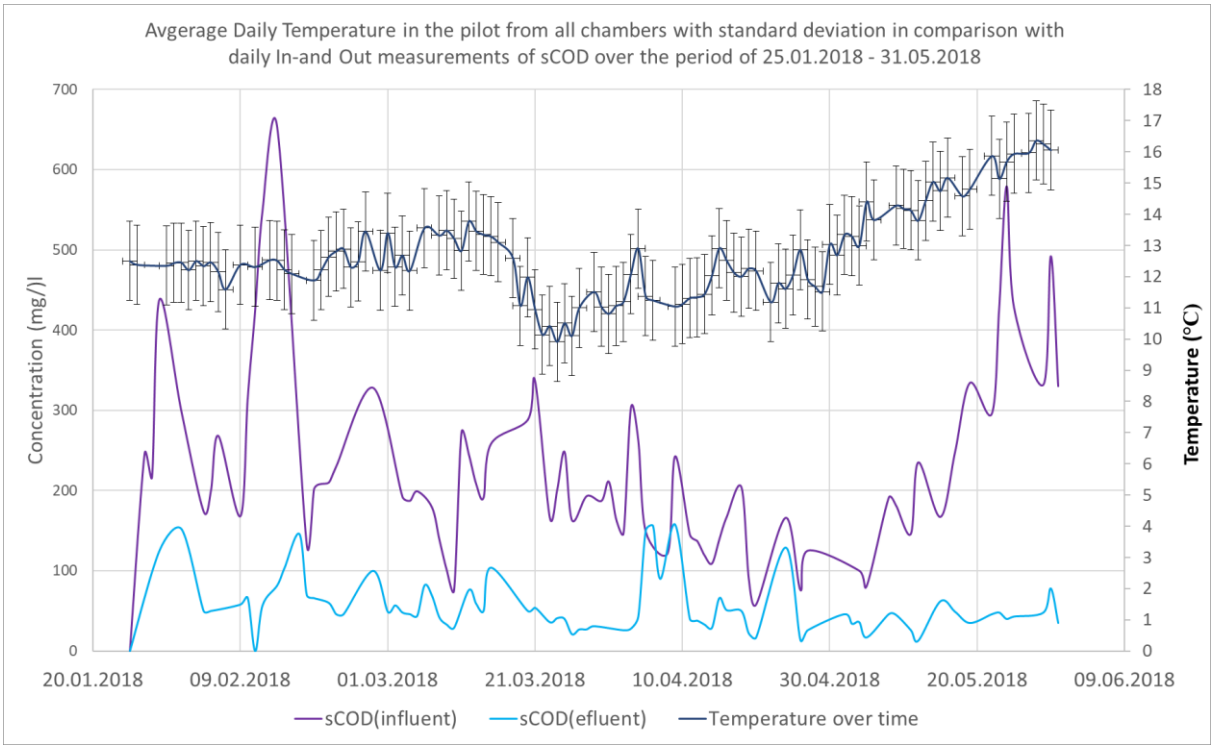


Figure 59 - Daily in and out measurements of sCOD in relation with average temperature in the pilot with standard deviation. Measurement period: 25.01.2018 – 31.05.2018.

There is reason to believe that the increase in removal efficiency From May 7th is due to temperature increases in the pilot, see Fig. 58 and increases in sCOD loading, see Fig. 59, and increased removal is a function of these two parameters especially. As pH and DO levels can explain low microbial competition and a good environment for PAOs to thrive, the increase in temperature has most probably increased biochemical reaction rates as temperatures at the same time has been low enough to also inhibit GAO competition.

Table 25 - Temperature variations throughout the semester before, during and after dilution

Average temperature for the whole measurement period	Average temperature before dilution	Average temperature during dilution	Average temperature after May 7th.
12.70 +/- 1.28	12.56 +/- 0.61	11.49 +/- 0.78	15.21 +/- 0.8

Literature states that temperatures below 15-20 degrees is beneficial for the EBPR process, see Ch. 2.2.1. This is due to the decrease in competition from GAOs as PAOs have their competitive advantage at lower temperatures, so despite lower biochemical reaction rates the net benefit is positive for EBPR. In the pilot, the sharp increase in temperatures in May does seems to have led to an increased efficiency. As PAOs is more competitive at lower temperature this can have led to them outcompeting the GAOs. The increased removal efficiency with increased temperatures might then showcase that the temperature is still at a level where GAO competition is low, or that even if GAO competition now is higher their dominance in the biomass is so incremental that they therefore are uncompetitive. Nielsen et al., (2010) found in

their study that the low GAO population might stem from generally low temperatures in the plants. This might mean that even if the temperature start to increase the growth of GAO has been inhibited for a while and their competition will not be immediately noticeable. However, some GAO will always be present in the anaerobic zone with municipal wastewater. Therefore, if the temperatures continue to rise GAOs might proliferate. Lopez-Vazquez (2009) found that GAOs is in favour of carbon source uptake at temperatures above 20°C. This is also in accordance with Erdal et al., (2003) results presented in Ch. 2.2.1 This might lead to increased competition from GAO over time, if the temperature within the reactor continue to rise. Further research is needed on this.

Temperature effect on beaker experiments run in parallel have been clearly visible. The Arrhenius equation presented in Ch. 2.2.1 shows the relationship between rate constants of temperatures at standard 20 °C and temperatures above and below this value.

Calculation of the temperature coefficient has been done with Eq. 5.2. By using the Arrhenius equation, the temperature coefficients in parallel experiments under the same conditions but with different temperatures, been calculated. Experiments run at controlled temperature between 10-14 has been set with an average of T= 12 °C. Due to the long time-lag between experiment W+S1 and W+S2 these experiments has not been used to calculate the Arrhenius temperature coefficient with, due too many factors that can have led to varying results, not just temperature. Otherwise all experiments experiments fitting the criteria has been used, see Tab. 26.

$$ß = \left(\frac{k(T)}{k(20)}\right)^{(1/T-20)} \qquad \text{(Eq. 5.2)}$$

ß = Temperature coefficient from 12 to 20 °C

k(T) = Rate of release and rate of uptake at 12 °C

k(20) = Rate of release and rate of uptake at 20 °C

T = Temperature not at standard 20 °C

Table 26 - Temperature coeffisienct calculated by the Arrhenius equationfoion

Name	Experiment conditions	Temperature coefficient (Anaerobe)	Temperature coefficient (Aerobe)
W+S3	T=10-14 (acetate)	1.065	1.000
W+S4	T=Room (acetate)		
S3	T=10-14 (acetate)	1.091	1.130
S4	T=Room (acetate)		
W+S5	T=10-14 (glucose)	1.031	1.085
W+S6	T=Room (glucose)		
W+S9	T=10-14 (glycerol)	1.069	1.099
W+S10	T=Room (glycerol)		
S5	T=10-14	1.021	
S6	T=Room		

The uptake rate for acetate seems comparable to literature, albeit a little high. It was difficult to find comparable results from literature in regard to anaerobic uptake rate and effect by temperature for glucose and glycerol in PAOs. The results does however correlate to Lopez-Vazquez et al., (2009). However, from the results it seems that the biomass present react to increase in temperature with increased uptake and release rates, however not increased removal.

The three kinetic experiments performed on synthetic water S1, S3 and S4 were all performed with synthetic wastewater and addition of 300 mg acetate as sCOD and under 3 temperature ranges; 10-14°C, Room temperature, 28-32°C. These results show that the rate does not necessitate good removal. The %P-removal was better in S1, while the highest rate was present in S4, despite having the lowest removal of the three, see Fig. 60.

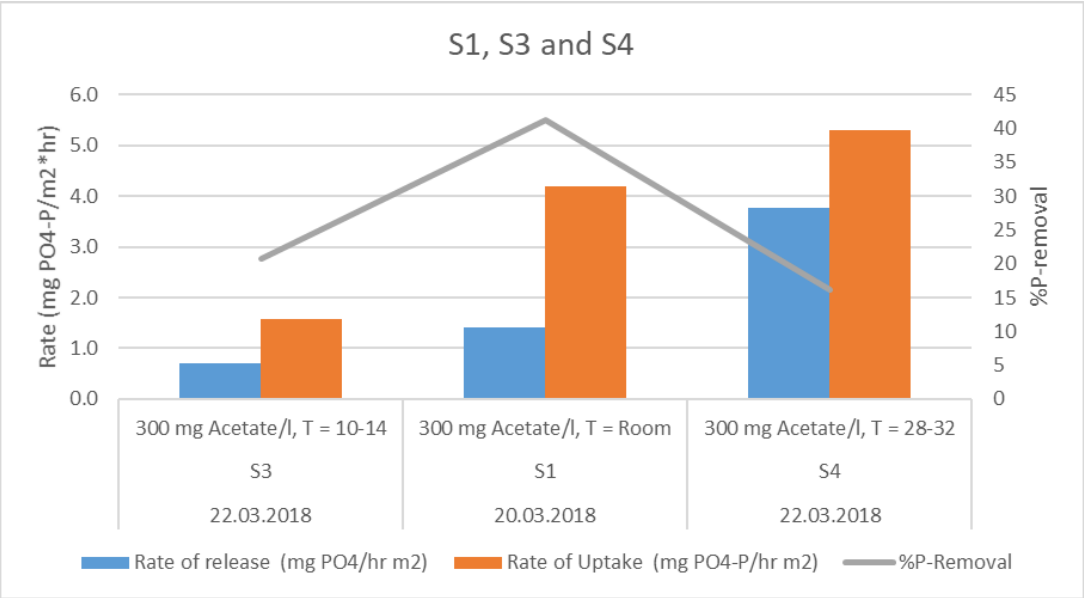


Figure 60 - Release- and Uptake rates and %P-removal for three synthethic experiments under the same conditions except for changes to temperature

5.5.4 - Dilution The combined effect on the process

Based on the evaluation of the pH, T and DO over time it seems that PAOs have had competitive conditions to thrive and proliferate to a higher degree than GAOs. Despite this the removal efficiency has not been dependable. This might show that it is not due to competition nor lack of PAO population, but start-up problems and the sudden abrupt change that affected them due to dilution. This is highly visible in four experiments; WW5, WW7 and W+S4, W+S8 run one month apart under the same experimental conditions, except for the change in the influent wastewater affected by dilution and the difference in biomass on the carriers in that month. See Fig. 45 and Fig. 46 in for results on these experiments.

Carvalheira et al., (2014) found that PAOs possess a higher substrate affinity than GAOs. The work presented also showed that the PAOs were less likely and prone to decay during periods of low organic loading. This was perhaps mainly due to the preference of PAOs for maintaining

reserves for maintenance energy purposes. Based on the discussion above it seems that the conditions within the reactor are of the sort that should enable PAOs to thrive and accumulate, but their focus has during dilution been to sustain themselves. When glucose was added during W+S8 they biomass showed great utilization of this, but maybe due to a long period of starving they utilized it solely for their own growth and not EBPR-efficiency.

When comparing W+S4 and W+S8 it is possible to see the dilution effect. When considering this there is reason to believe experiment W+S7 could have performed better if it had been performed one month prior as well, see Fig. 60. This is due to W+S7performing significantly better than W+S8. Experiments with glucose as substrate addition therefore performed best if this is taken into account. This is not valid for experiment where glucose was the only substrate source, see Fig. 61. These results also coincides with what was found by (Kristiansen et al., 2013).

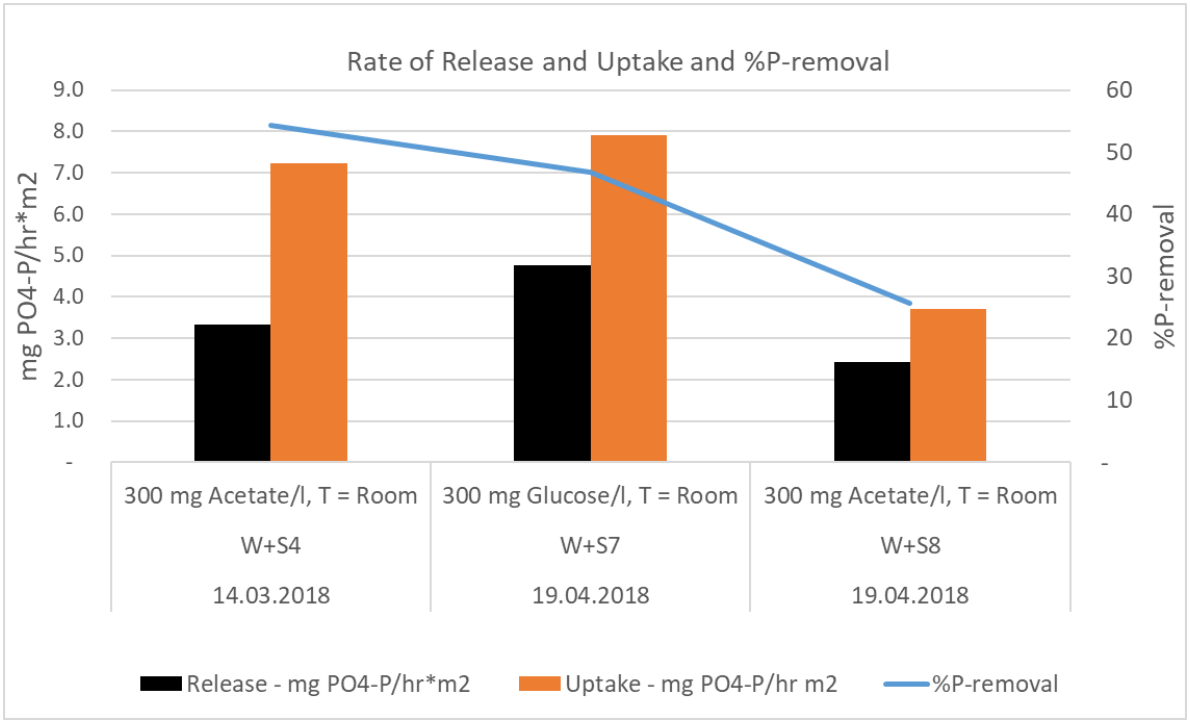


Figure 61 - Experiment performed with glucose addition during dilution compared compared to experiments with acetate addition before and during dilution

5.5.6 Comparing release and uptake rates with literature

Comparable literature is of course difficult to be found on release and uptake rates of PO4-P in biofilm systems other than the release and uptake rates presented by Saltnes et al., (2016), see Tab. 27. Fig. 62, 63 and 64 shows release and uptake rates in accordance with %P-removal for all beaker experiments.

Table 27 - Release and uptake rates in Bio-P continuous biofilm system at HIAS (Saltnes et al., 2016)

Anaerobic (mg PO4-P/m2*hr)	Aerobic (mg PO4-P/m2*hr)
77.5	63.75

The average release rates in kinetic experiments with substrate addition is 3.1 mg PO4-P/hr*m2, 1.6 with influent wastewater and 2.1 for synthetic run experiments. As can be seen the release rates are more than 25, 48 and 37 times lower respectively than rates at HIAS. The rate of uptake differs much more widely throughout the experiments; 5.3 mg PO4-P/h3*m2 for wastewater with substrate addition, 2.32 for influent wastewater and 2.1 for synthetic water. This is 12, 27.5 and 30 times lower than the rates experiences at HIAS.

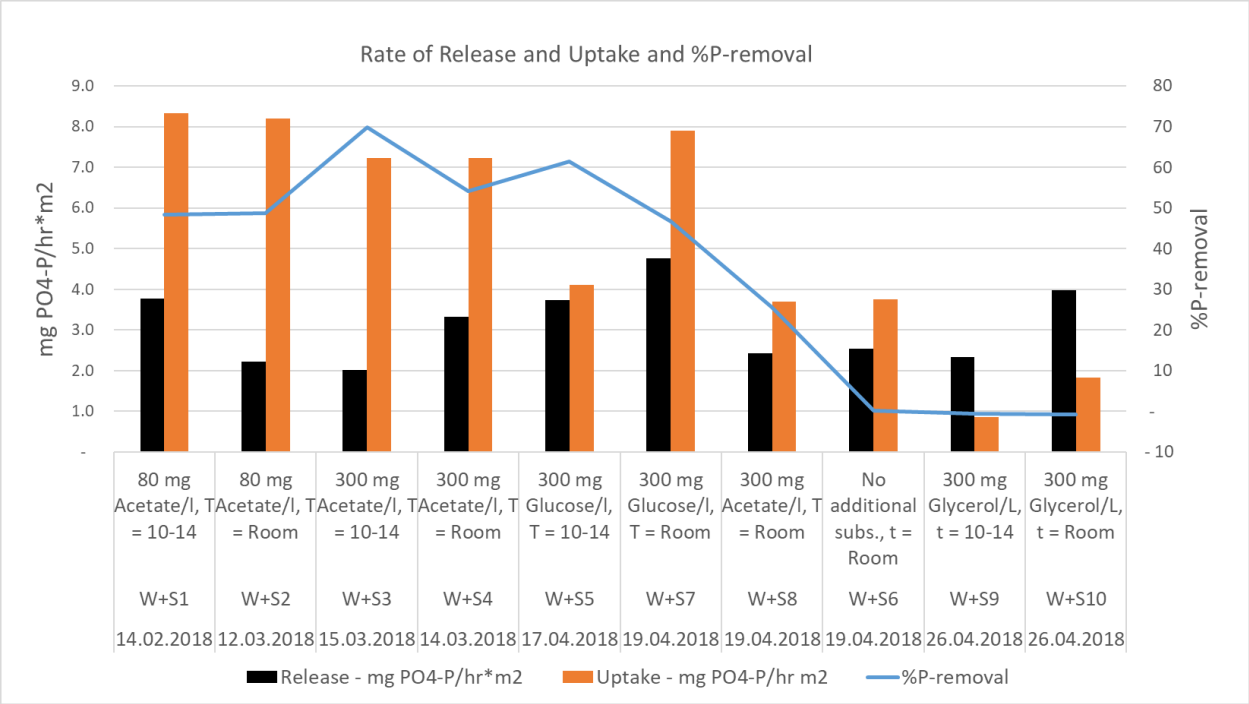


Figure 62 - All experiments with substrate additions in wastewater.

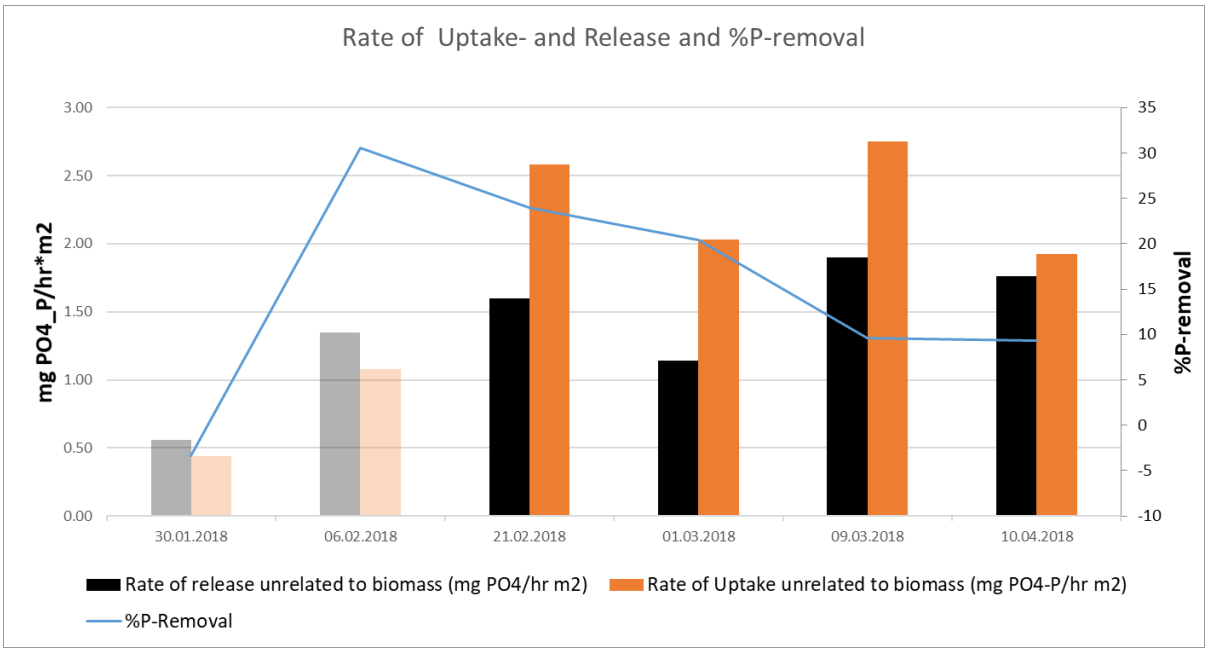


Figure 63 - All experiments without addition of substrate estimating pilot performance. WW1 and WW2 are not included due to their low R^2 value

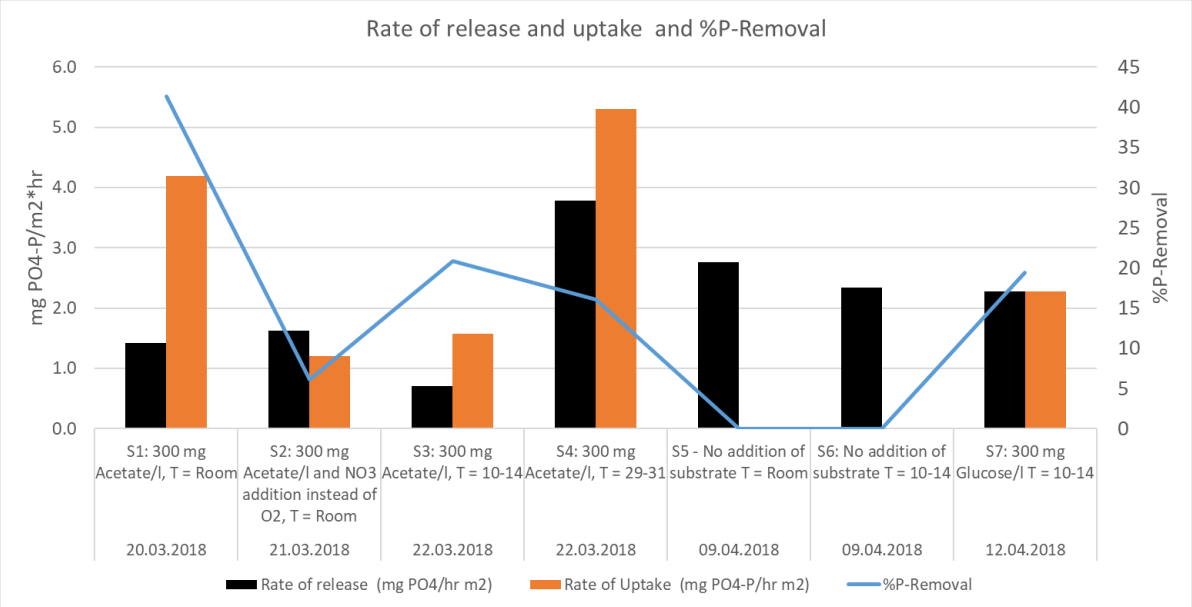


Figure 64 - All synthetic experiments

These results show that the process has a long way to go before efficient EBPR can be experienced.

Although results also show that high release and uptake rates do not necessitate the higher %P-removal, however in the long run it is necessary to establish more reliable release and uptake rates. The beaker experiments performed without additions of substrate are highly unreliable with low release and uptake rates compared to experiments performed with additions of substrate.

5.5.7 Hydrolysis of sludge

Helness (2007) pp. 112 stated that hydrolysis of COD in the process increased the amount of biodegradable soluble COD, but that the process itself was better with soluble compounds available beforehand. This can explain reduced efficiency of the pilot as the biomass, especially during dilution, has not been fed adequate substrate sources and therefore has had to use resources fermenting this within the reactor. Had this been available in the water phase maybe more of the biomasses focus could be shifted towards efficient EBPR.

The benefit of the moving bed process for removal is primarily connected to low residence times and high loading. At high organic loads and residence times < 60 min, one cannot expect hydrolysis to take place to a large degree (Ødegaard et al., 2001). It therefore seems based in the discussion above that not a large degree of hydrolysis can be expected in the anaerobic chamber of the continuous MBBR pilot here at NTNU.

Baetens et al., (1999) states that one must consider that the carbon at wastewater treatment plant has to be fermented to some degree, and that this process occurs naturally in some systems (Baetens et al., 1999). In the pilot set up here at NTNU there may be some hydrolysis in the tank where sewage sludge is pumped in before it is released to the pilot. The rate of this process is dependent upon various parameters one being the temperature in the liquid. If the temperature continues to rise throughout the summer months more hydrolysis of the sludge can be expected.

For further work it would be very interesting to look at how the bacteria performs during kinetics with fermented and hydrolyzed influent sludge as their food source. This could give an indication if any of these processes can enhance the efficiency of the EBPR.

5.6 Results from VFA-analysis

Filtrated samples sent for IC-testing at the analytical lab did not give expected results and the standard deviation was large. Because of high workloads in the lab during the spring semester filtrated VFA samples collected through kinetic experiments and from influent wastewater, normally had to be kept in the fridge for 1 to 2 days. Due to the volatility of the samples it would probably have been best to analyse them directly. Results did therefore not give a good indication of the VFA concentrations.

All the results and output from the Titra-5 Programme using the 5-point Titration Method given in Moosbrugger et al., (1993), see appendix H, are results that one their own makes sense. Most of the samples receive output results from the Titra-5 program which could be accurate estimations of VFA. However, when duplicate and triplicate samples are accounted for the standard deviations were normally in the range of +/- 1 to 2 times first sample tested. Meaning that if triplicate samples were tested the two subsequent samples were normally in the range of +/- 1 to 2 times the initial sample. All samples on their own were however normally within the range of what could have been acceptable VFA-influent concentrations, so had not duplicate or triplicate samples have been performed we would have thought we could trust these. When performing the titrations, it is of utmost importance not to leave samples for more than 15 minutes so that VFA concentrations change, and that stirring of samples is not performed at a high velocity so that CO_2-diffusion alters the results. Titrations experiments were performed meeting these requirements.

The VFA levels need to be tested again, as there is no certainty to what these values are at the current time. Throughout the process it has become clear that the values of VFA in the influent wastewater has not been of as high relevance as previously thought. Other important components to evaluate is the BOD and the rbCOD.

6 Conclusion

This master thesis provides basis for future work on the pilot placed in the wastewater laboratory at NTNU. The pilot had just been set up at the laboratory before my master thesis work started. Throughout the process fluctuations in its performance has been observed and documented. Hopefully some of this documentation can lead to better understanding and knowledge for future work on the pilot and process. Although a lot of the documentation has been on the performance of the pilot, some have also been on optimizing the process to get a better understanding into what parameters affect its performance the most. As the pilot at NTNU has so largely different wastewater characteristics entering its treatment facility, from the one at HIAS, the work documented here shows how widely different these processes operate based on location.

Norwegian wastewaters are normally characterized as undiluted and low in nutrients. This has been one of the main reasons for discourage in implementing EBPR in Norwegian WWTP.

The results presented in this master thesis with regards to the biomass preferring other substrates sources to VFA can have large implications on the way we look at, and think about EBPR, especially in Norway. Characterization previously performed of the wastewater in the lab showed that the wastewater characteristics can be established to be similar to the typical Norwegian wastewater, which is normally diluted and cold, due to rainwater and snowmelt intrusion, and typically have quite low influent concentrations of VFA. Temperatures and pH values are also typically in the same range as average Norwegian WWTP. The fact that there is documented total P-removal occurring in the pilot shows signs that the biomass has been able to establish itself in a way where it is able to thrive and grow despite these harsh conditions. It seems that the biomass has adapted to the conditions in a way that makes it able to perform EBPR despite the conditions being far from what literature states as optimal. This is very interesting results when looking at the possibility of introducing EBPR processes into Norwegian Municipalities and our way of looking at the wastewater treatment system and the possibilities to reuse and utilize the resources present there.

The daily monitoring of DO, Temp, pH, was useful tool to observe the changes in the quality of the influent wastewater and use this information to understand process performance better. The main results from evaluation of the daily influent and effluent concentrations of phosphorus, ammonium and sCOD showed signs of the process being affected by dilution in the water. It is suggested that a potential shift in the microbial population before and after dilution due to a sudden shift in parameters of DO, T and pH occurred.

Based on the results found in this master thesis there is reason to believe that it will perform different throughout the different seasons as varying weather patterns most probably will affect performance as changes in temperature- and dilution patterns have been documented to affect the pilot's performance this spring.

In this thesis the results presented shows that the PAOs should proliferate and be highly competitive against GAOs in the environment in the pilot. Results indicate a mixed microbiology which can survive and thrive under differing conditions. It seems from the results that the dependency on VFA in the process is not as prevalent as previously expected and that one strain of bacteria potentially inhibiting the carriers is Tetrasphaera.

Based on results from kinetic experiments in beaker with wastewater and acetate it was almost zero net consumption of sCOD. Removal rate within the anaerobic chambers were on average is 0.00047 mg sCOD/m2*hr, yet EBPR performance was observed. Instead it was observed that PAOs present in the cMBBR potentially instead ferment other more complex organic molecules.

PAOs survived the low organic loading, thou the anaerobic activity and net uptake decrease potentially due to focusing all their energy on survival and growth, outcompeting GAOs. This is positive results regarding initiating this process in the average Norwegian treatment plants as low organic carbon loading is a recurring difficulty to overcome, but it seems PAOs can be competitive.

The biomass has shown the ability to utilize glucose in the anaerobic zone and consequently increased P-removal, however net uptake was not improved when used at the sole carbon source.

Results from beaker experiments show P release rates is in range of 0.6 – 4.7 mg PO4-P/m2*hr and P uptake rates between 1 and 8.2 mg PO4-P/m2*hr which is significantly lower than comparable literature.

The results also show the strong correlation between temperature and increased rate of release and uptake with a temperature coefficient of 1.077 for anaerobe release of acetate and 1.031 for glucose.

The fact that there is documented total P-removal occurring in the pilot shows signs that the biomass has been able to establish itself in a way where it is able to thrive and grow despite conditions being far from what literature states as optimal.

Microorganisms has helped up treat municipal wastewater for a long time. Still we lack a lot of understanding of how these microbial communities work. Currently the knowledge of the microbial community in the pilot is based on the results and literature, so results determining microbial population will be very interesting to see when they arrive.

7 Limitations

Due to the 20-weeek limitation of the master thesis there are limitation for how much practical work could done outside of the weeks spent in the laboratory from February to May.

The main limitation regarding the auto-sampler are the anaerobic conditions that can emerge in the sampling bottles. The DO level has been measured in the bottles at various times. The anaerobic levels in the bottles can lead to release of phosphorous. This is a practical limitation which has been difficult to improve as more advanced technical solutions, such as filters before the sample or cooling systems within the sampler, needs to be looked in to. This is also correlated with how much of the biomass erode of the carriers, and how we control this erosion process. It is important that inactive biomass is disengaged from the carriers, however here it is collected in the autosampler. The higher the ratio of collection of biomass the higher the release in the autosampler can be. Currently we have no knowledge of what this ratio might be, and this should probably be looked more into.

The results regarding TP content in the biomass have only been performed with one TP sample, which is inadequate, and should have been performed with duplicates.

In May changes were performed to the mixers to achieve complete mixing within all chambers as some dead-zones was spotted. Carriers trapped have not been completely mixed, and therefore not utilized properly in the process. This is now improved and can have ripple effects on the pilot's performance, as more biomass is in movement. The biomass which may not have been in much movement before this problem was fixed may have to go through some developmental stages before it is as effective has biomass which have circulated though anaerobic and aerobic conditions for a while.

Although VFA it is not an as important parameter for the process here as initially thought analysis of the VFA can provide information about the VFA concentrations in the wastewater and give a better understanding of why and how the current microbiology has been established.

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Appendix A

Brief description of how the cuvette tests are performed (HACH, 2018; Richardsen, 2017)

- *sCOD: Addition of 2 mL filtrated sample to cuvette before digestion for 2 hours in thermostat.*
- *PO4-P: Addition of 0.5 mL filtrated sample diluted 1:2 and reagent. The sample reacts with the reagent and creates a reduction of ascorbic acid permitting the evaluation of PO4-P levels.*
- *TP: Addition of sample before the cuvette is digested for 1 hour. After digestion reagent is added.*
- *NH4-N: Addition of 0.2 mL filtrated sample which react with reagent hypochlorite ions, salicylic ions and sodium nitroprucide for 15 minutes.*
- *NO3-N: Additions of 1 mL filtrated sample. The solution contains sulfuric acid and phosphorous acid and a reagent added permits the measuring of nitrate after 15 minutes*

Appendix B

Set parameters for the pilot:

1. Through counting it has been established: 236 carriers = 200 mL → 1000 mL = 1180 carriers
2. Through measurements it has been established that the working volume of water is 794 mL pr. 1000 mL when there is a filling degree of 60% of carriers. The working volume of the pilot is then calculated using values in Tab. 5.
3. 1060 L * (794 mL/1000 mL) = 842 L.
4. Inflow should, when the pilot operates adequately, equal effluent. From Tab.B.3 one can read of what the measured flow should equal with different HRT.
5. Through calculation based of the average number of carriers in each chamber in the pilot, see Tab. B.5, the necessary movement of carriers based of HRT is calculated, see Tab. B.4.
6. The effective HRT in the pilot is based on the working volume. 842 L/10 chambers = 84.2 L.
   Effluent flow has been measured frequently in the pilot from 25.01.2018 – 31.05.2018. Through these measurements the average effluent flow has been established to be 2.12 L/min. By utilizing the working volume as well as the average flow the effective HRT is 84.2 L / 2.12 L/min = 38.27 min = 38 min. 16 sec.
7. Surface Area (SA) is calculated with Eq. B1:

$$\mathrm{SA\ (m2)} = \mathrm{V} * \mathrm{FD} * \mathrm{SSA} \qquad \text{(Eq. B.1)}$$

SA = Surface Area (m2)

V = Volume (m3)

FD = Filling degree (%)

SSA = Specific Surface Area of carriers. For this reactor K1, Kaldnes carriers (m2/m3)

Tables showing fixed parameters for the pilot:

Table B 1 - Surface Area in the pilot based on the pilots' parameters

Surface Area (SA) in the pilot (m2)	318	m2
Filling degree of carriers	0.6	m3 Biomedia/m3 MBBR-reactor
Volume of pilot	1.06	m3 volume in the reactor
Spesific SA of K1 carriers	500	m2/m3

Table B 2 - Surface Area in 1 kinetic experiment based on kinetic experiment parameter

Surface Area (SA) in 1 L kinetic experiment (m2)	0.3	m2
Filling degree of carriers	0.6	m3 Biomedia/m3 kinetic
Volume of Kinetic experiment	0.001	m3 volume in 1 kinetic
Spesific SA of K1 carriers	500	m2/m3

Table B 3 - Flow in the pilot at different retention times

Inflow (Top: l/hr Bottom: l/min)		
HRT=8h	**HRT= 6h**	**HRT=4h**
133.29	177.72	266.58
2.22	2.96	4.44

Table B 4 - Necessary movement of carriers between chambers at differing HRTs

Nessesary movement of carriers between chambers with three different HRT		
9436.76	Carriers/h	
157.28	Carriers/min	HRT=8h
2.62	Carriers/sec	
12582.34	Carriers/h	
209.71	Carriers/min	HRT=6h
3.50	Carriers/sec	
18873.51	Carriers/h	
314.56	Carriers/min	HRT=4h
5.24	Carriers/sec	

Table B 5 - Permanent characteristics for the pilot. Number of carriers are calculated based on carriers/1000 mL = 1180

Chamber	Total Vol (L)	% filling	Vol of carriers (L)	L (cm)	W (cm)	H (cm)	Empty space (cm)	# of carriers
1	109.3	60	65.58	49.4	29	45.77691	50	77384.4
2	110.6	60	66.36	48	29	47.67241	48	78304.8
3	109.9	60	65.94	49	29	46.40394	50	77809.2
4	106.3	60	63.78	48.4	29	45.4403	51	75260.4
5	107.3	60	64.38	48.2	29	46.05809	50	75968.4
6	105.8	60	63.48	48.2	29	45.41422	51	74906.4
7	106	60	63.6	48.5	29	45.21863	51	75048
8	103.9	60	62.34	48.5	29	44.32279	52	73561.2
9	104.8	60	62.88	48.3	29	44.89184	51	74198.4
10	102.4	60	61.44	49.2	29	43.0614	53	72499.2
Total/Average	1066.3	60	63.978	48.57	29	45.42605	51	75494.04

Table B 6 - Specifications for carriers used in the pilot

Carriers	Kaldnes K1	
Diameter (D)	10	mm
Width	7	mm
Spesific Area (SA)	500	m2/m3
Effective SA	300	m2/m3
Material	Polyethylene	

Appendix C

Table C 7 - Kinetic Beaker Experiments performed

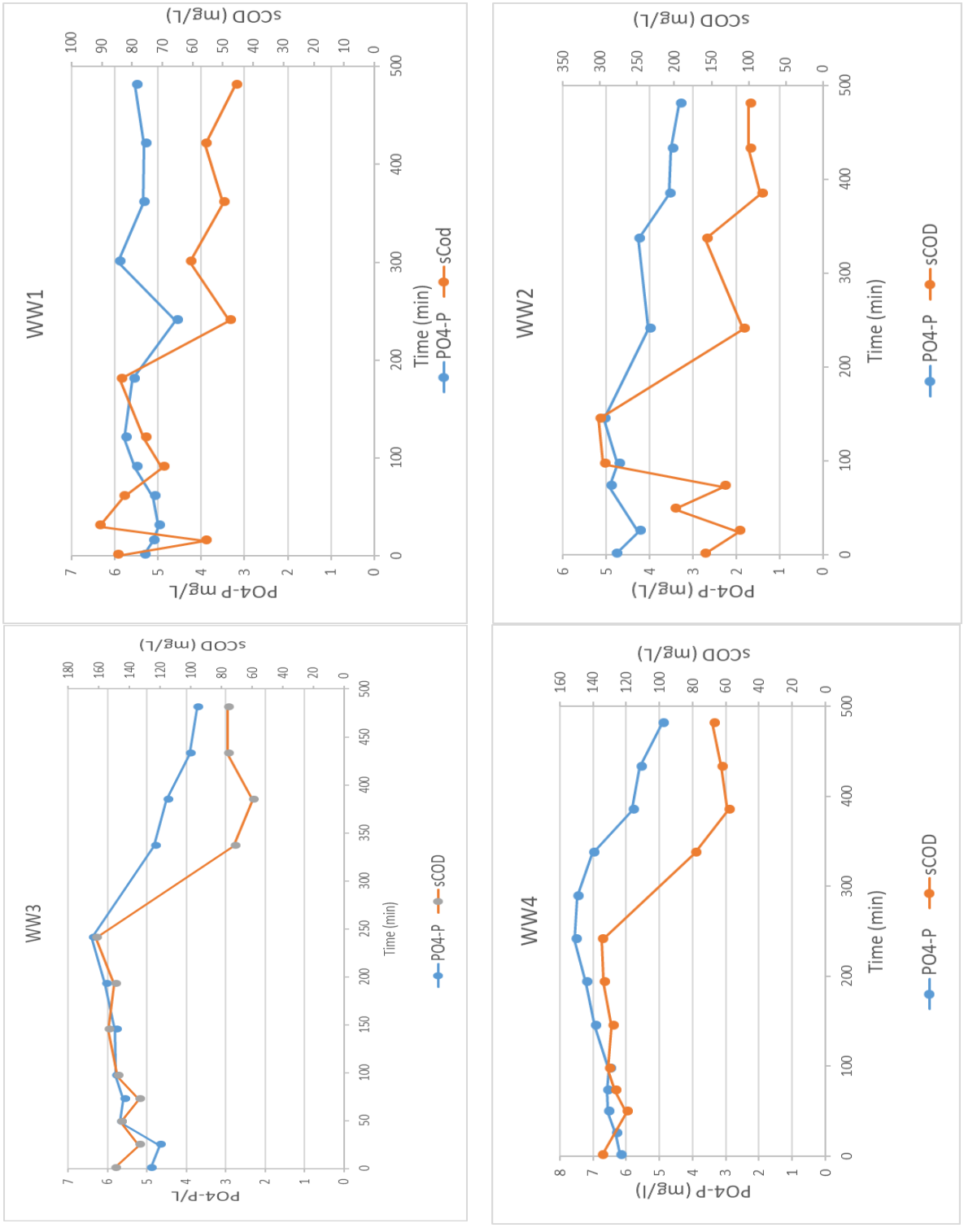
Date	S2	Kinetic experiment in beaker of 1 L with synthehtic wastewater with different carbon soures as well as temperature differations	Kinetic experiment in beaker with wastewater and additions of different carbon sources as well as temperature differiations	Kinetic experiment in beaker with only influent wastewater and no additions	Kinetic experiment following the Pilot with batches directly from each chamber after calculated HRT	Kinetic experiment in beaker with only influent wastewater and no additions with different DO values
05.04.2018	**P2**				x	
26.04.2018	W+S10		x : 300 mg Glycerol - T = 10-14			
26.04.2018	W+S9		x : 300 mg Glycerol - T = 19-22			
19.04.2018	**W+S8**		**x : 300 mg Glucose - T = 19-22**			
19.04.2018	**W+S7**		**x : 300 mg acetate - T = 19-22**			
19.04.2018	**W+S6**		**x : No substrate addition - T = 19-22**			
17.04.2018	W+S5		x : 300 mg GLUCOSE - T = 10-14			
12.04.2018	**S7**	**x : 300 mg Glycogen addition - T = 10-14**				
10.04.2018	WW8			x - T = 10-14		
09.04.2018	**S6**	**x : No substrate addition - T = 19-22**				
09.04.2018	**S5**	**x : No substrate addition - T = 10-14**		**x - T = 10-14**		
22.03.2018	S4	x : 300 mg acetate - T = 19-22				
22.03.2018	S3	x : 300 mg acetate - T = 29-32				
21.03.2018	**S2**	x : Addition of NO3 instet of O2	x : 300 mg acetate - T = 10-14			
20.03.2018	S1	**x : 300 mg acetate - T = 10-14**				
19.03.2018	**WW7**			**x - T = 10-14**		
15.03.2018	W+S4		x : 300 mg acetate - T = 10-14			
14.03.2018	**W+S3**		**x : 300 mg acetate - T = 19-22**			
12.03.2018	W+S2		x : 80 mg acetate - T = 19-22			
09.03.2018	**WW6**			**x - T = 10-14**		
08.03.2018	P1				x	
01.03.2018	**WW5**			**x - T = 10-14**		
28.02.2018	DO2					x - DO = 3
28.02.2018	DO1					x - DO = 7
21.02.2018	**WW4**			**x - T = 10-14**		
14.02.2018	W+S1		x : 80 mg acetate - T = 10-14			
13.02.2018	**WW3**			**x - T = 10-14**		
06.02.2018	WW2			x - T = 10-14		
30.01.2019	**WW1**			**x - T = 10-14**		

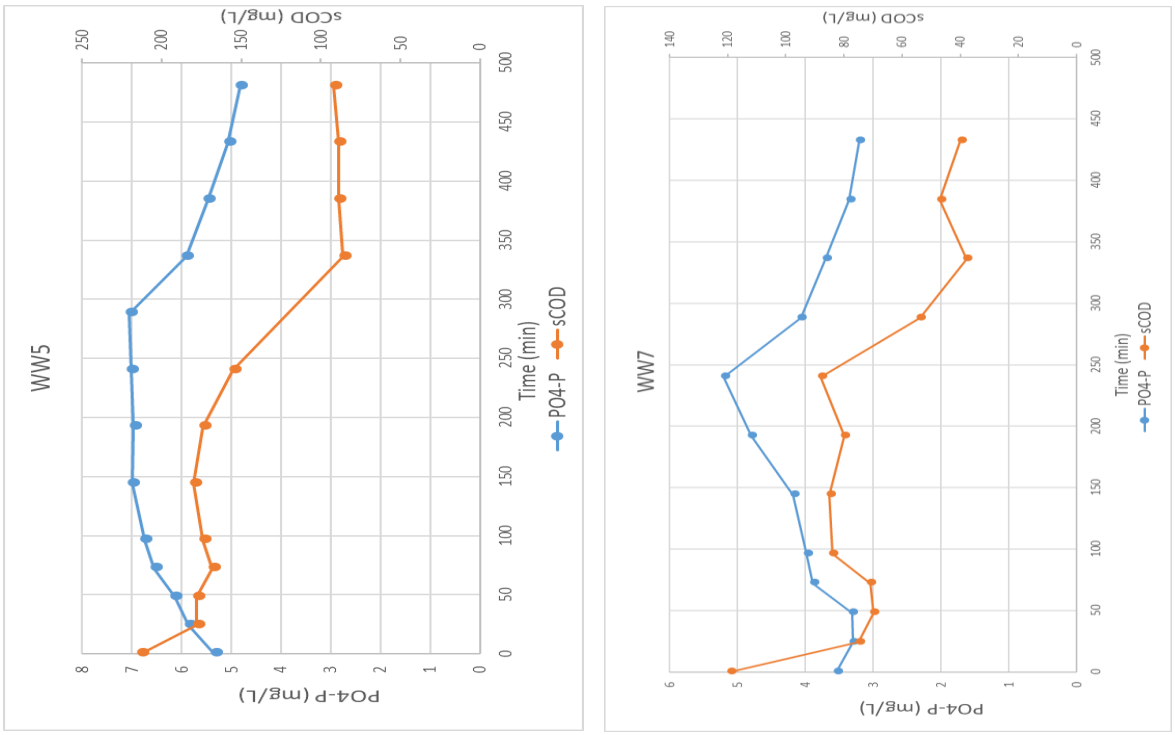
Appendix D

Graphs showing results from kinetic experiments

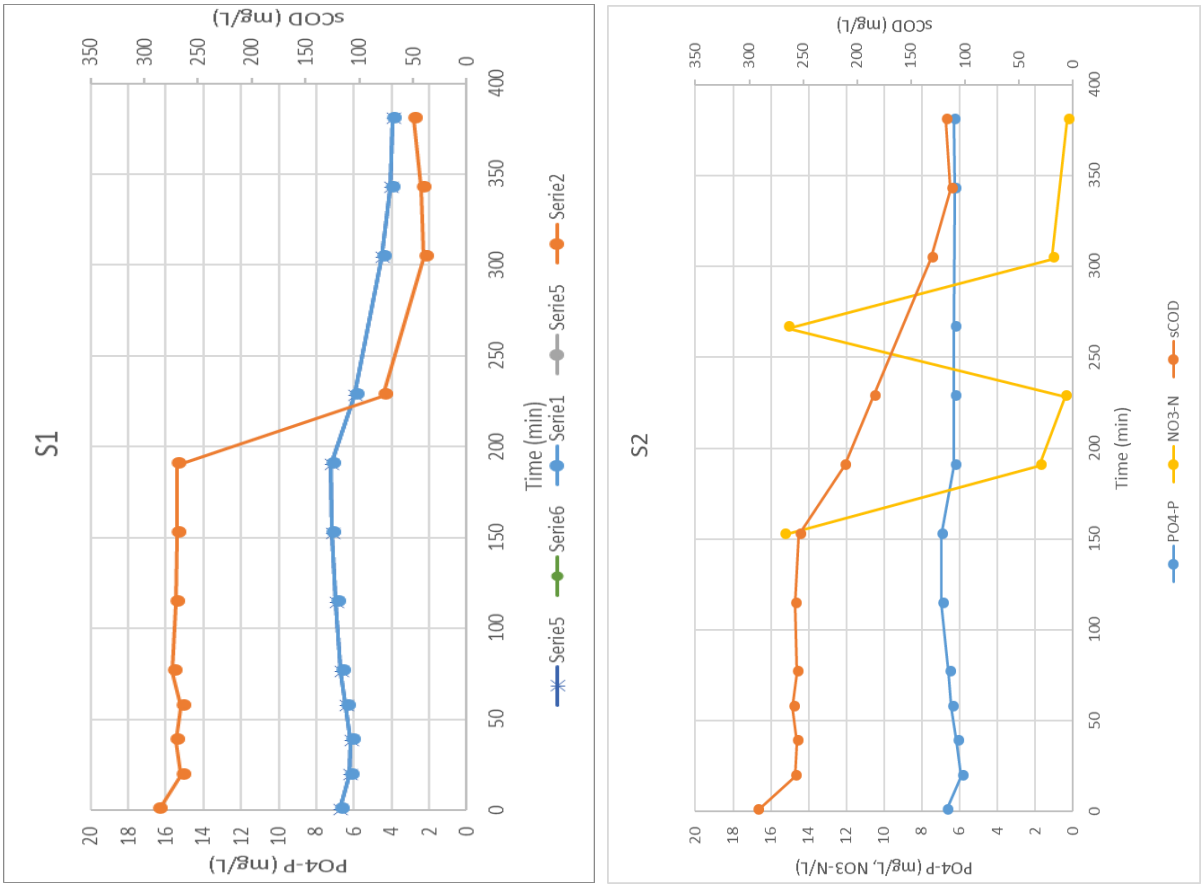
NH4-N values, Temperature, NO3-N and pH has not been included as they have stayed constant throughout and would only appear as flat lines. DO concentrations have been kept above 5 mg DO/L.

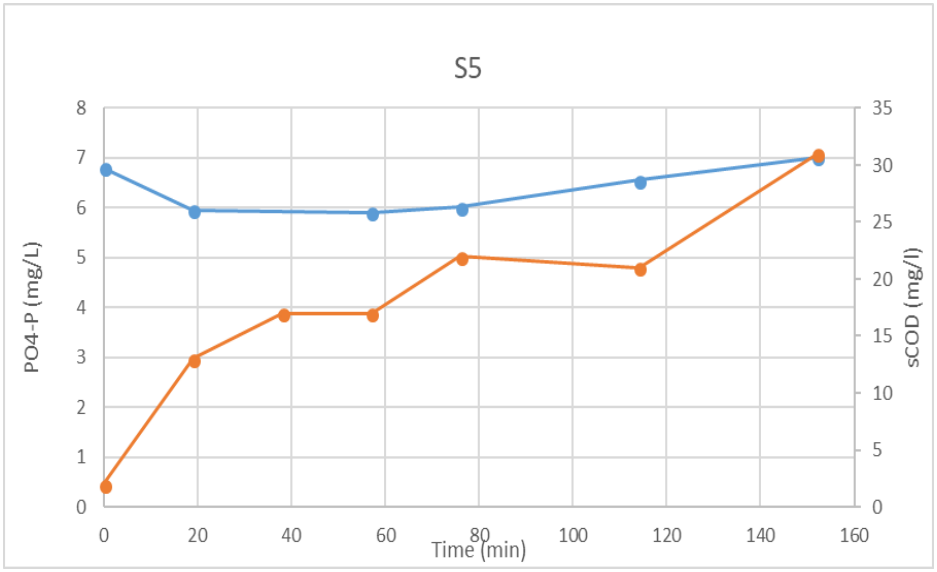
Appendix D 1 - Results from experiments mimicking the pilot-

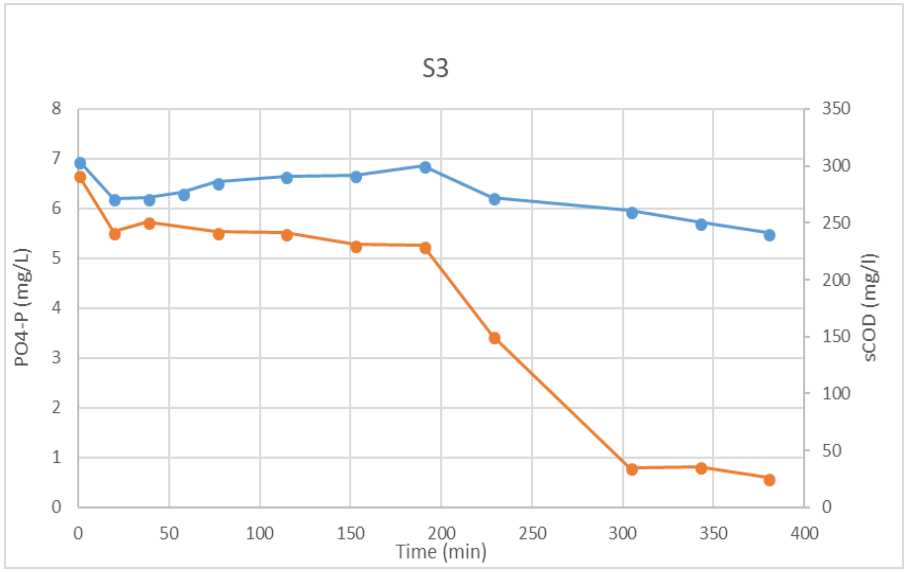




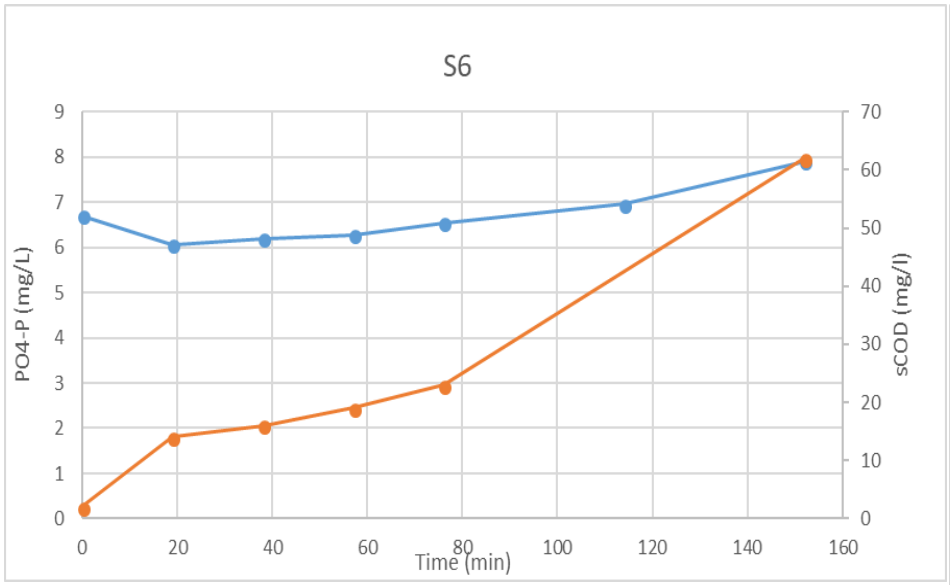
Appendix D 2 - Results from beakers experiments with synthetic wastewater

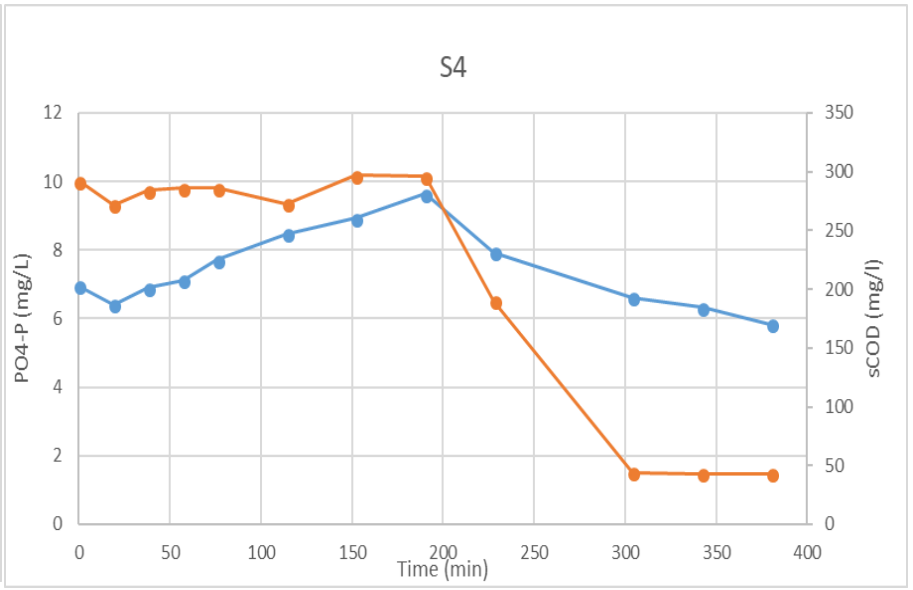


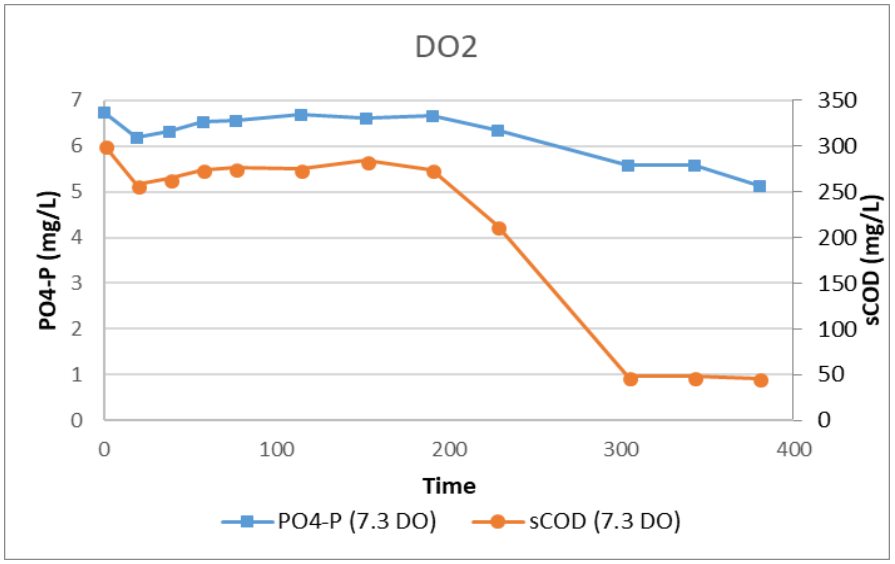

S5
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
0 1 2 3 4 5 6 7 8
0 5 10 15 20 25 30 35
0 20 40 60 80 100 120 140 160

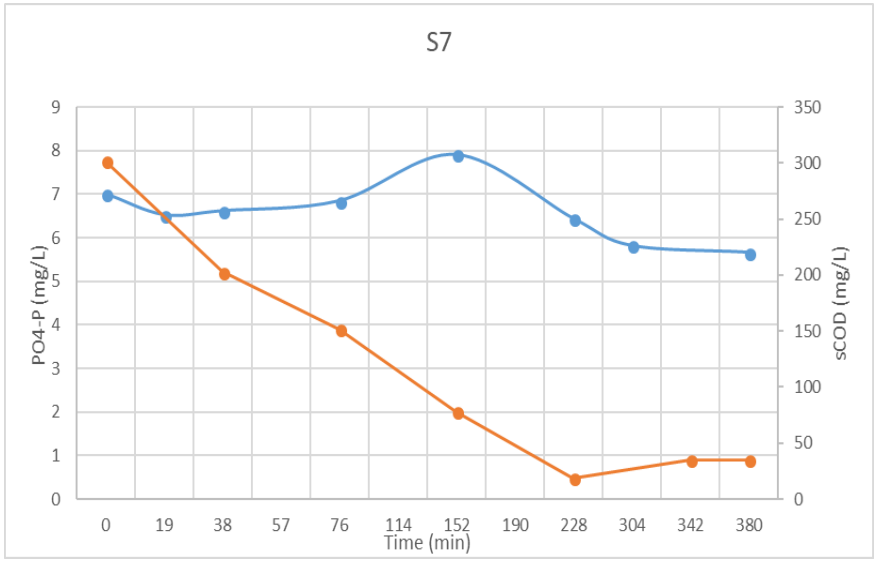

S3
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
0 1 2 3 4 5 6 7 8
0 50 100 150 200 250 300 350
0 50 100 150 200 250 300 350 400

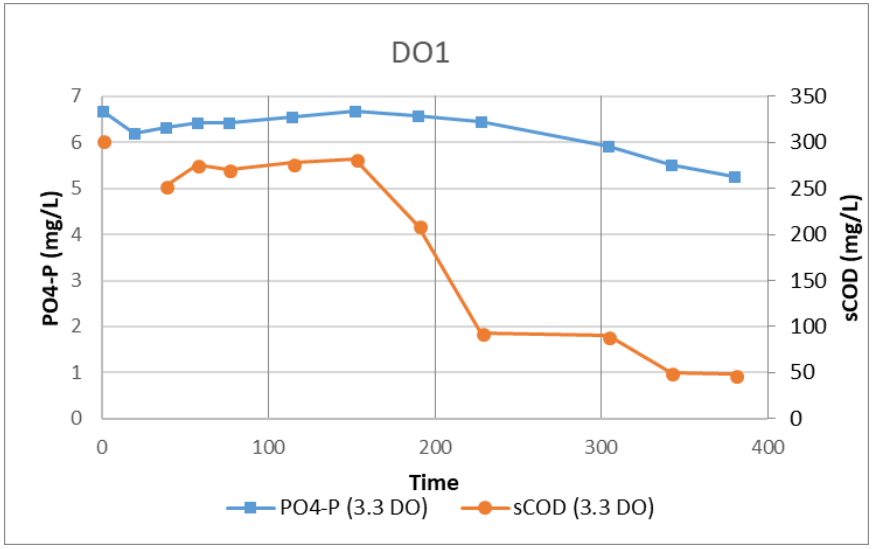
x


S6
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
0 1 2 3 4 5 6 7 8 9
0 10 20 30 40 50 60 70
0 20 40 60 80 100 120 140 160

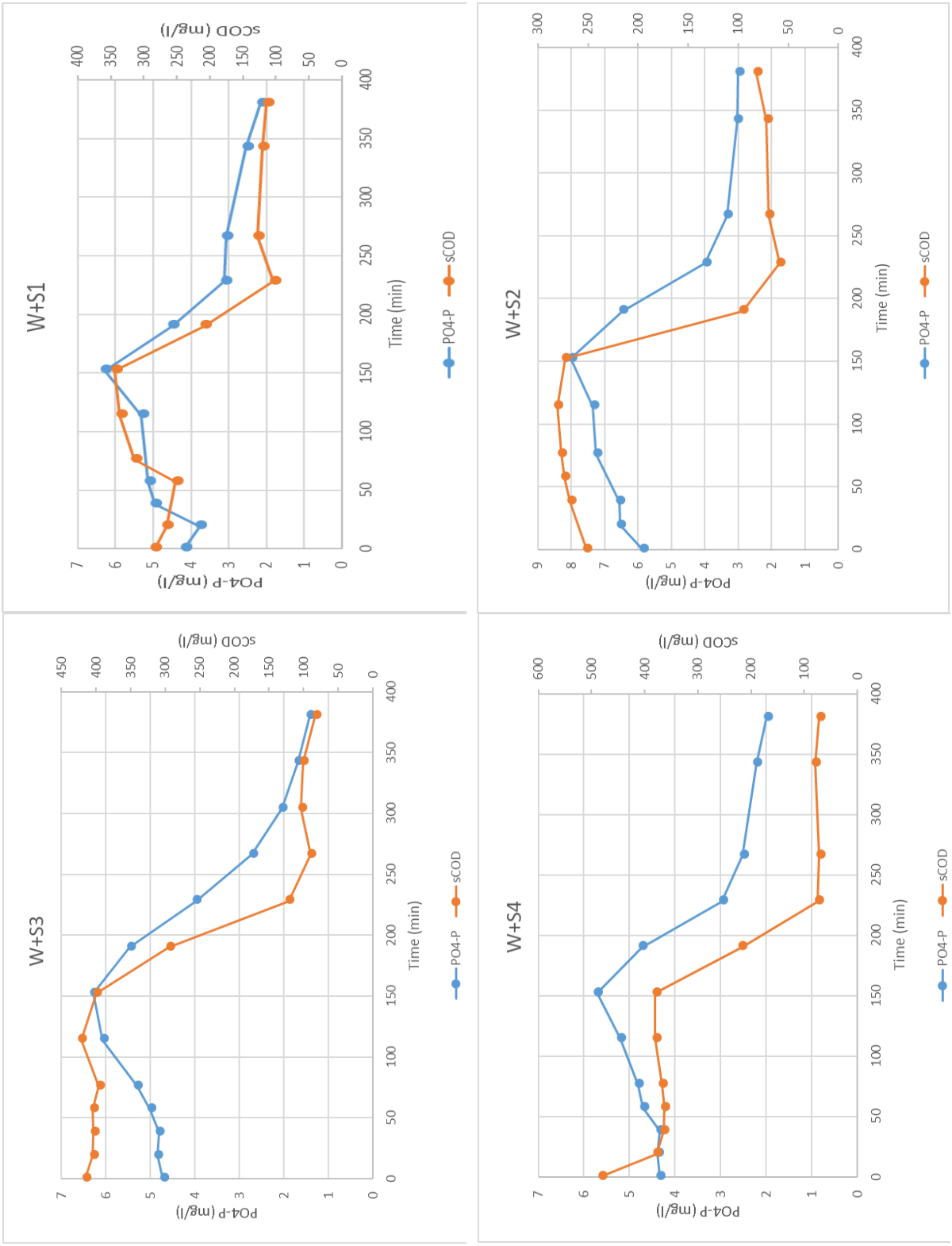

S4
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
0 2 4 6 8 10 12
0 50 100 150 200 250 300 350
0 50 100 150 200 250 300 350 400


DO2
PO4-P (mg/L)
sCOD (mg/L)
Time
PO4-P (7.3 DO)
sCOD (7.3 DO)

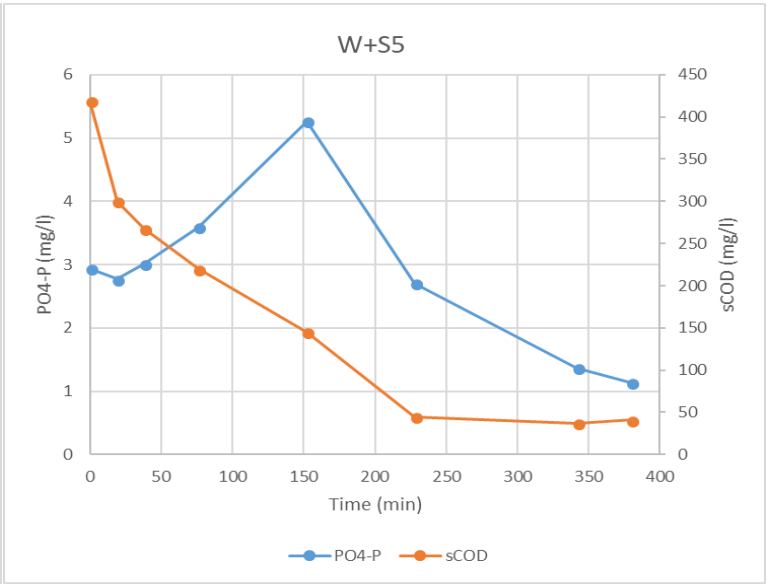

S7
PO4-P (mg/L)
sCOD (mg/L)
Time (min)

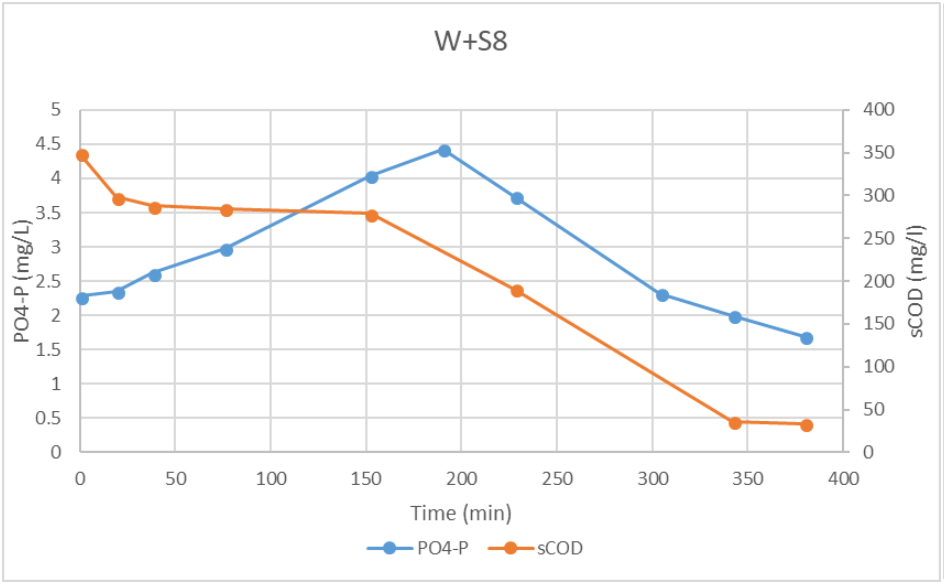

DO1
PO4-P (mg/L)
sCOD (mg/L)
Time
PO4-P (3.3 DO)
sCOD (3.3 DO)

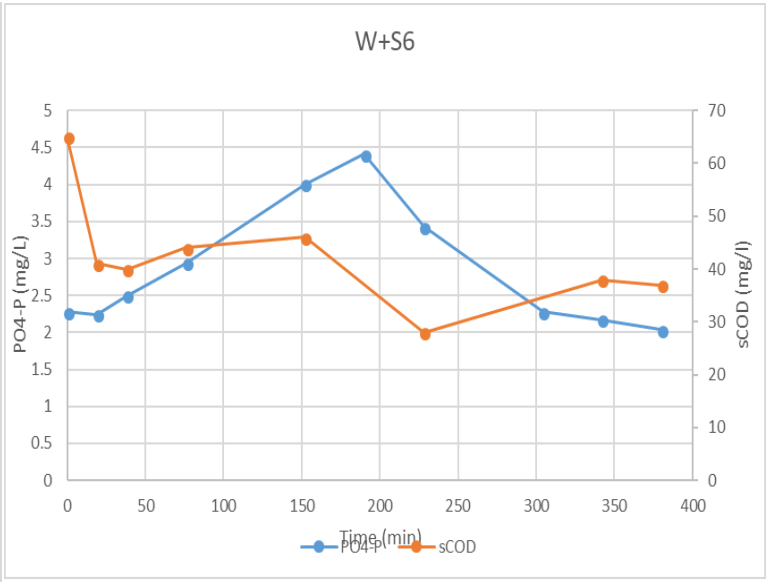
Appendix D 3 - Results from beaker experiments with influent wastewater and substrate additions




W+S7
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
PO4-P
sCOD


W+S5
PO4-P (mg/l)
sCOD (mg/l)
Time (min)
PO4-P
sCOD

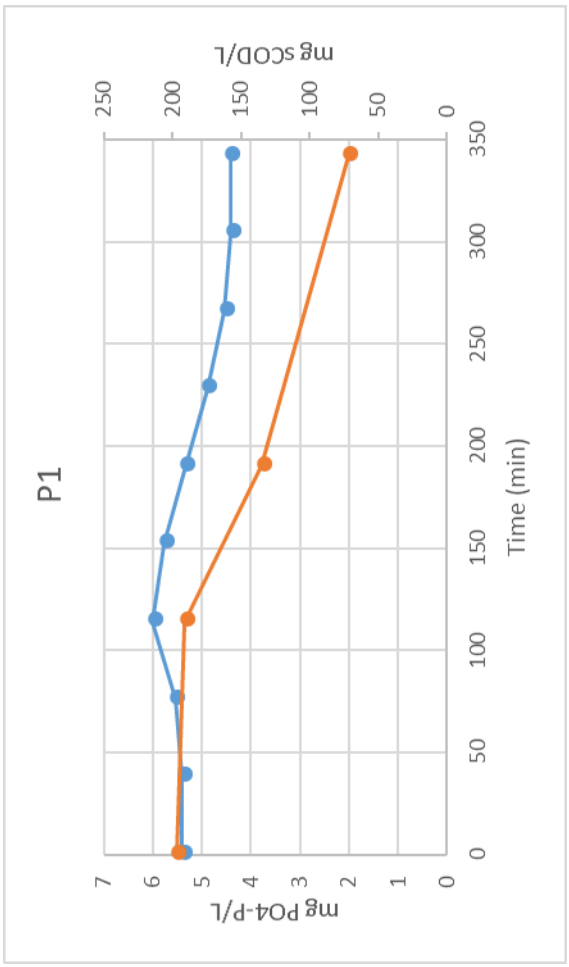

W+S8
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
PO4-P
sCOD

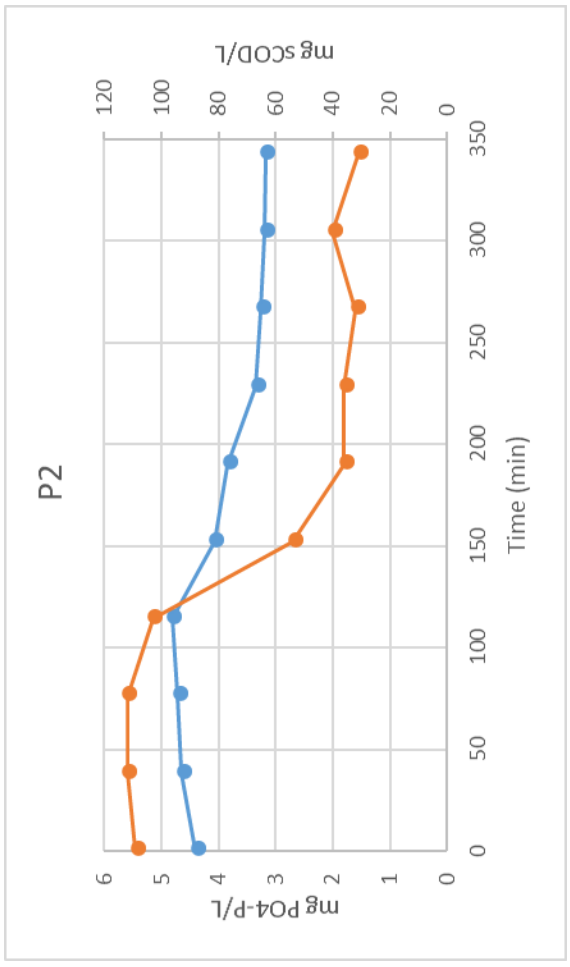

W+S6
PO4-P (mg/L)
sCOD (mg/l)
Time (min)
PO4-P
sCOD

W+S9
PO4-P (mg/l)
sCOD (mg/l)
Time (min)
PO4-P
sCOD
0
1
2
3
4
5
6
0
50
100
150
200
250
300
350
400

W+S10
PO4-P (mg/l)
sCOD (mg/l)
Time (min)
PO4-P
sCOD
0
0.5
1
1.5
2
2.5
3
3.5
4
0
50
100
150
200
250
300
350
400

Appendix D 4 - Results from batch experiments in the pilot





Appendix E

Calculations for additions of salt for synthetic experiments as well as calculations for substrate additions in the form of acetate, glucose and glycerine.

PO4-P has been added through the salt disodium phosphate; Na_2-H-PO_4. Calculations of PO4-P additions has been done according to Eq. E.1 and Eq. E.2

$$\frac{141.96\ g\ Na2-H-PO4/mol * 0.007\ g\ PO4-P/L}{30.93\ g\ PO4-P/mol} = 0.0321\ \text{g salt/l} = 32.1\ \text{mg Salt/l} \quad \text{(Eq. E.1)}$$

MW of Na_2-H-PO_4 = 141.96 g salt/mol
Target value of PO4-P in kinetic = 7 mg PO4-P/l
MW of P = 30.93 g P/mol
MW of Na2-H-PO4 = 141.96 g Na2-H-PO4/mol = 141.96 g salt/mol

$$\frac{0.0321\ g\ salt/L * 0.794\ L}{1L} = 0.0255\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.2)}$$

Target concentration of PO4-P = 0.0321 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

NH4-N has been added through the salt ammonium chloride; NH_4Cl. Calculations of NH_4-N additions has been done according to Eq. E.3 and Eq. E.4

$$\frac{53.59\ g\ salt/mol * 0.050\ g\ NH4-N/L}{18.037\ g\ NH4-n/mol} = 0.191\ \text{g salt/l} = 191\ \text{mg Salt/l} \quad \text{(Eq. E.3)}$$

MW of NH4Cl = 53.49 g salt/mol
Target value of NH4-N in kinetic = 50 mg NH4-N/l
MW of NH4 = 18.037 g P/mol

$$\frac{0.191\ g\ salt/L * 0.794\ L}{1L} = 0.151\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.4)}$$

Target concentration of NH4-N = 0.191 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

Magnesiumsulfide has been added through the salt magnesium sulphate; $MgSO_4$-$7H_2O$. Calculations of Mg^{2+} additions have been done according to Eq. E.5 in accordance with method in Smolders et al., (1994). In the method the salt stipulated is the same that has been used. Recalculation of salt concentration pr. liter is therefore not necessary.

$$\frac{0.090\ g\ salt/L * 0.794\ L}{1L} = 0.071\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.5)}$$

Target concentration of $MgSO_4$ = 0.090 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

Calcium chloride has been added through the salt calcium chloride; $CaCl_2$. Calculations of calciumchloride additions have been done according to Eq. E.6 and Eq. E.7. The total of calcium chloride to be added to the solution was in the form of calcium chloride dihydrate; $CaCl_2$-2H2O, and a concentration of 14 mg/l of this salt, in accordance with method in Smolders et al., (1994).

$$\frac{110.98\ g\ CaCl2/mol * 0.014\ g\ CaCl2-2H20/L}{147.01\ g\ CaCl2-2H20/mol} = 0.0105\ \text{g salt/l} = 10.5\ \text{mg salt/l} \quad \text{(Eq. E.6)}$$

MW of $CaCl_2$ = 110.98 g $CaCl_2$/mol
Target value of $CaCl_2$-2H_2O in kinetic = 14 mg $CaCl_2$-2H_2O/l
MW of $CaCl_2$-2H_2O = 147.01 g $CaCl_2$-2H_2O/mol

$$\frac{0.0105\ g\ salt/L * 0.794\ L}{1L} = 0.0084\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.7)}$$

Target concentration of $CaCl_2$ = 0.0105 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

Micro-Nutrients have been added according to the recipe given in E.8, which states that 0.3 mL of micronutrient need to be added pr. liter.

$$Micronutrient\ addition/beaker\ experiment = 0.3\ mL * \frac{794\ mL}{1000\ mL} = 0.2382\ mL \quad \text{(Eq. E.8)}$$

Addition of micronutrients = 0.3 mL/L
Volume of water in kinetic = 794 mL
Volume of full kinetic = 1000 mL

The synthetic wastewater was made equal for all batch experiments, while the one component changed has been the substrate addition. Different substrate sources used has been; acetate, glucose and glycerol.

Nitrate has been added through the salt Sodium-Nitrate; $NaNO_3$. Calculations of NO_3-N additions have been done according to Eq. E.9 and E.10.

$$\frac{84.99\ g\ NaNO3/mol * 0.015\ g\ NO3-N/L}{14\ g\ NO3-N/mol} = 0.091\ g\ salt/L \quad \text{(Eq. E.9)}$$

MW of $NaNO_3$ = 84.99 g $NaNO_3$/mol
Target value of NO_3-N = 15 mg NO_3-N/l
MW of NO3-N = 14 g NO3-N/mol

$$\frac{0.091\ g\ salt/L * 0.794\ L}{1L} = 0.072\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.10)}$$

Target concentration of $NaNO_3$ = 0.015 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

Acetate has been added through the salt; $C_2H_3NaO_2$-$3H_20$.
Glucose has been added through the salt; $C_6H_{12}O_6$.

All carbon sources are presented as a concentration as sCOD.

Acetic Acid + 2-02 = 2-CO2 + H2O
$2O_2$ = 64 g/mol
Acetate = 60 g/mol
1 mg of acetate = 64 g O_2/60 g Acetate = 1.0845 mg COD
It is necessary with 1.0845 g oxygen to degrade 1 g of acetate (Baumann et al., 1997)

Target concentration of 300 mg Acetate as sCOD/L.

$$\frac{300\ mg\ COD/l}{1.0845\ mg\ COD/mg\ acetate} = 276.6\ mg\ acetate/L = 0.2766\ g\ acetate/L \quad \text{(Eq. E.11)}$$

$$\frac{136.08\ g\ salt/L * 276.6\ acetate/L}{59\ g\ acetate/mol} = 0.6379\ \text{g salt/l} = 637.9\ \text{mg Salt/l} \quad \text{(Eq. E.12)}$$

MW of Salt = 136.06 g C_2H_3NaO2-3H20/mol
Target value of Acetate in kinetic = 276.6 mg Acetate/L
MW of Acetate = 59.0 g acetate/mol = 141.96 g salt/mol

$$\frac{0.6379\ g\ salt/L * 0.794\ L}{1L} = 0.506\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.13)}$$

Target concentration of acetate as sCOD = 0.506 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

Target concentration of 300 mg Glucose as sCOD/L.
1 g Glucose → 1.07 g COD

$$\frac{300\ mg\ COD/l}{1.07\ gsCOD/mg\ glucose} = 280\ mg\ glucose/L = 0.28\ g\ glucose/L \quad \text{(Eq. E.14)}$$

$$\frac{180.2\ g\ salt/L * 280\ mg\ glucose/L}{180.02\ g\ glucose/mol} = 0.28\ \text{g salt/l} = 280\ \text{mg Salt/l} \quad \text{(Eq E.15)}$$

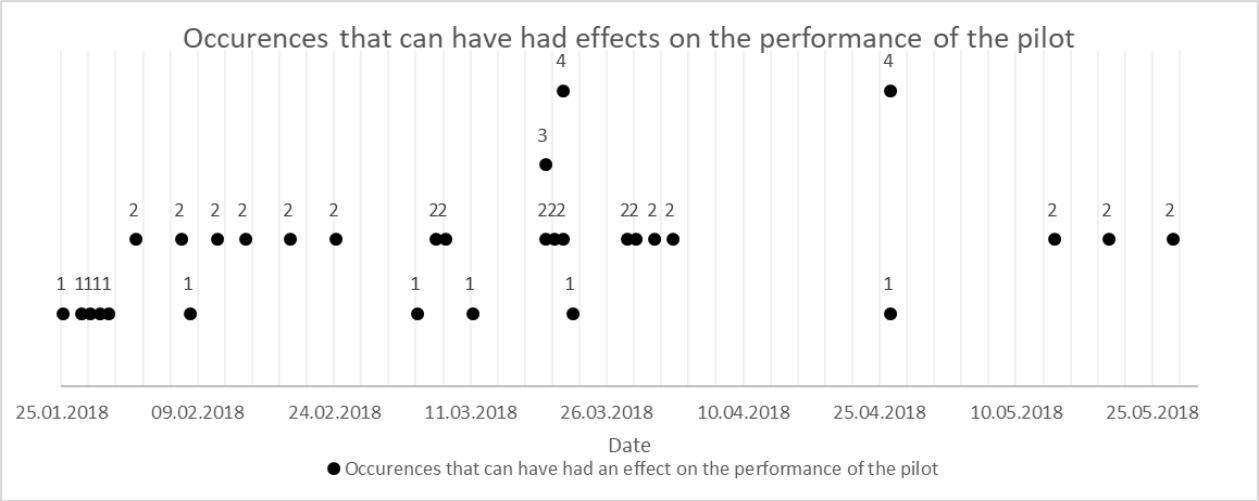
MW of Salt = 180.02 g salt/mol
Target value of glucose in kinetic = 280 mg glucose/L
MW of glucose = 180.2 g glucose/mol

$$\frac{0.28\ g\ salt/L * 0.794\ L}{1L} = 0.2223\ g\ salt\ added\ to\ solution \quad \text{(Eq. E.16)}$$

Target concentration of glucose as sCOD = 0.28 g salt/L
Water Volume in the kinetic = 0.794 L
Total volume of the kinetic including water and carriers = 1 L

Appendix F

Table F 1 – Occurrences in the pilot



1 Struggle with in pumping of water. Mainly stemmed from clogging of influent pipe to the reactor or clogging of influent pipe from the manhole to the holding tank.

2 Accumulation of carriers in 1 or more chambers - Manual redistribution. Accumulation normally stemmed from clogging of the movement holes between chambers, problems with mixers or air-diffusors. Sometimes air-diffusors changed direction and lead to direction changes for the carrier movements, and sometimes mixers were not mixing properly.

3 Rainwater intrusion

4 Snowmelt intrusions; start and end

Between each chamber there are openings where the water and the carriers flow. The mixers and the aeration system are parts of what control the movement, as well as the flow. When these openings get clogged accumulation of carriers and build-up of water within chambers starts. The size of the openings has been calibrated throughout the semester and the pilot is still in the process where these openings are optimized.

Appendix G

Notes concerning the auto-samplers use and operation:

- The autosampler should be always settled in the same position as any movement of the physical location will most probably will lead to the necessity of calibration.
- As the 24 samples are mixed in altogether four bottles, these need to be cleaned and washed daily so that there always are enough bottles to fill up the sampler. When the sampler is filled with bottles the bottles are more stable and there is less chance that there will be experienced spills.
- The autosamplers needs to be calibrated regularly, and it has been calibrated throughout the semester. The calibration of the autosampler is related to the length of the suction line and the suction head.

The inlet pipe for the influent wastewater was placed in the holding tank so that it was above the sedimented particles to avoid in-pumping of these, but at the same time placement had to be below the water line at all times to avoid pumping of air. During some periods large amount of sludge accumulated in the holding tank and samples consisted of a thick sludge which was difficult to filtrate. The holding tank was then drained and cleaned before the next in pumping.

When the auto-samplers for the influent and effluent was finished with its 24-hour program it automatically started the sampling for the next day.

Appendix H

Method for Titration experiment

Throughout the semester titration with the Titra-5 point method has been used several times to estimate the VFA-concentration of the influent wastewater by using the values given in the experiment in the Titra-5 Programme. The samples that has been analysed for VFA by the 5-point titration method described by Moosbrugger et al., (1993) is samples from the same wastewater as used in the kinetic experiments.

At low values of VFA concentrations in the water, the analysis of the VFA becomes more uncertain with this method (Moosbrugger et al., 1993). Because of the growth of bacteria and the different type of bacteria present in the reactor there is reason to believe that the presence of VFA is low in the influent to the pilot, see Ch. 5.3 and 5.4.2.

The 5-point titration procedure can be used for determining VFA and alkalinity in the water
The necessary supplies for the method is a; Titrator (drop and suction), a wastewater sample with duplicates or triplicates, HCl acid, distilled water, magnetic mixer, VFA Samples diluted accurately, pH-meter, conductivity meter, temperature meter,

The method requires a normality of the HCl-solutions of 0.05 N and a volume of 500 mL.
Eq. H.1 was used to calculate the addition of HCl. 0.77 mL HCl to 499.23 mL of distilled water.

$$C(1) * V(1) = C(2) * V(2) \qquad \text{(Eq. H.1)}$$

V(2) = 500 mL – Necessary titrant volume

C(2) = 0,05 M – Molarity = Normality for HCl since equivalents is equal to 1.

C(1) = 32,36 M - Molarity = $\frac{1180\ g\ HCl/L}{36{,}46\ g\ HCl/mol}$ *= 32,36 M, (1 L HCl = 1180 g HCl/L. Mass of HCl = 1 g H/mol + 35,46 g Cl/mol = 36,46 g HCl/mol)*

Required volume for the methods is 50 mL per sample, diluted or undiluted. The appropriate volume for each sample is then filtered in a 0.45 µm filter. Filtered samples were put on a magnetic stirrer between 60-100 rotations pr. minute. Low rotations is necessary to reduced CO_2 influence. All samples were performed at room temperature. Conductivity was measured and used as input in the program.

The initial pH was recorded. The method states that if the pH is less than 6.7, NaOH is to be added until the pH is above 6.7 +/- 0.1. However, this was never the case for any experiments performed here. Titration with HCl down to pH 5.9 +/- 0.1 was performed. When pH 5.9 was reached the volume of acid added was read of the titrator. The titration was repeated to pH 5.2 +/- 0.1 and 4.3 +/- 0.1 and the volumes of acid added was read off.

Calculation of results with the computer program Titra-5 was then performed.

An examination of the input-parameters in the 5-point-titration tool was performed to evaluate if any of the input-parameters was the reason for the high deviations in results.

Input parameters outside of pH and titration volume was Nitrogen, inorganic phosphorous, sulphide, TDS and temperature.

As seen in the tables below there had to be quite significant differences in input parameters outside of pH and titrant-volume before this affected the VFA-results of the tool Titra-5 programme. The effect variations in parameters had on alkalinity were much larger which also was expected.

Table I 1 - Test of all input parameters in the Titra-5 Program, except for pH and titration volume to account for their effect on Titra-5 programs VFA-output. This was to attest for how these parameters affect VFA result output from the program.

Nitrogen:

Concentration (mg/L)	VFA (Acetic acid)	Alcalinity (CaCO3)
0	184,2	6,6
5	184,2	6,5
20	184,3	6,3
40	184,3	5,0
80	184,4	5,3
120	184,5	4,6

Inorganic phosohorous:

Concentration (mg/L)	VFA (acetic acid)	Alcalinity (CaCO3)
0	184,2	6,6
5	184,8	2,3
10	183,5	-6,7
15	183,8	-9,8
20	184	-12,8

Sulphide:

Concentration (mg/L)	VFA	Alcalinity
0	184,2	6,6
5	184,8	2,1
10	183,5	-7,2
15	183,8	-10,5
20	184	-13,7
50	185,5	-33,4

TDS :

Concentration (mg/L)	VFA	Alcalinity
0	184,1	5,9
200	184,1	6,2
450	184,2	6,6
600	184,3	6,8
800	184,4	7
1000	184,5	7,2
1500	184,7	7,6

2000	184,9	7,8

Temperature

Concentration	VFA	Alcalinity
25	184,1	6,7
21	184,2	6,6
15	184,3	6,5
10	184,3	6,4
5	184,2	6,1
0	184,1	5,9

Appendix I

Calculation of P mol/ Cmol -ratio

- 1 g P = $\frac{1}{30.97\ g/mol} = 0.0322854\ mol$
- 1 g C = $\frac{1}{12\ g/mol} = 0.083259\ mol$

MW of P = 30.97 g/mol

MW of C = 12 g/mol

$$\frac{1\ mg\ P}{1\ mg\ C} = \left(\frac{1}{30.97\ g/mol}\right) / \left(\frac{1}{12\ g/mol}\right) = 0.3877\ \frac{mol\ P/mg\ P}{mol\ C/mg\ C} \qquad \text{(Eq. A.I.1)}$$

$$P = PO4\text{-}P(t\text{-}0) - PO4\text{-}P(t\text{-}end\ anaerobic\ zone) \qquad \text{(Eq. A.I.2)}$$

PO4-P = Concentration difference between start and finish in anaerobic zone (mg P/L)

$$C = COD(t\text{-}0) - sCOD(t\text{-}end\ anaerobic\ zone) * \frac{24\ g\ C}{59\ g\ acetate} * 1.0845 \qquad \text{(Eq. A.I.3)}$$

sCOD = Concentration difference between start and finish in anaerobic zone (mg P/L)

2 Carbons in acetate = 24 g C

MW of acetate = 59 g/mol

Additions of salt as sCOD = 1.0845 Conversion factor for salt added as sCOD to be converted back to added as acetate

$$\frac{mol\ P}{mol\ C} = \frac{P}{C} * \frac{mol\ P/mgP}{mol\ C/mgC}$$

P = Concentration change in PO4-P (mg/L)
C = Concentration change in sCOD (mg/L)
Conversion factor between mol P and mol C = 0.38 (mol P/mg P)/(mol C/ mg C)

Appendix J

Table J 1 - Timeline for when samples for microbial community estimation has been taken