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Enhanced Biological Phosphorus Removal in Typical Norwegian Wastewater

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Preface

The research done in this master thesis was carried out at the Department of Hydraulic and Environmental Engineering at the Norwegian University of Science and Technology (NTNU), spring 2017. I would like to thank my supervisor Professor Stein W. Østerhus for giving me the opportunity to write my thesis in the field of wastewater engineering, and for all the help and discussion along the way.

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Abstract

Discharge of wastewater containing excess amounts of nutrients such as phosphorus (P) cause eutrophication of the receiving waterbodies. The consequences related to eutrophication can vary in severity, from esthetical issues to medical threats. The increasing focus on P as a non-renewable resource and the necessity for recovering and recycling P from nutritious wastewater has made biological phosphorus removal (EBPR) in wastewater a more favorable method compared to chemical precipitation. EBPR is a complex process where several essential process conditions and prerequisites make Norwegian wastewater, which typically is diluted and low in nutrients, less ideal for EBPR.

In this study, the possibility of applying EBPR in a local and presumably typical Norwegian wastewater (Lerkendal wastewater) was examined. The objectives of this work were to characterize the unknown wastewater in terms of relevant substances according to EBPR, and perform batch experiments to assess the bio-P activity in the wastewater and subsequent potential for EBPR.

The main results from characterizing the Lerkendal wastewater show that there is a resemblance to typical Norwegian wastewater as there were no significant deviations when compared to literature values. The wastewater is consistent for hourly and daily variation, where the concentration of soluble chemical oxygen demand (sCOD), constituting the substrate for Polyphosphate-Accumulating Organisms (PAOs), showed no signs of diurnal pattern or vulnerability to excess amounts of precipitation. The influent ratio of sCOD:PO_4-P and COD:BOD_5 is 60.2 and 1.8 respectively, reflecting the biodegradability character of the wastewater and indicate high potential for EBPR.

The main results from batch experiments show a low P release and uptake in the wastewater both with and without external addition of acetic acid (100 mg sCOD/L). The P release rates were between 1.32 and 1.73 P/g VSS*h without acetic acid, which is classified as moderate rates. This indicates poor bio-P activity and negligible fraction of PAOs. Luxury uptake was not detected even with high P uptake rates (6.04 to 10.65 mg P/g VSS*h). The results are related to the condition of the bio-P sludge used in the experiments, which gives reason to suspect that better results could have been obtained if activated sludge was used instead of MBBR sludge.

The results presented give reason to conclude that there is potential for EBPR in the Lerkendal wastewater, however it is limited due to the low concentration of organic substrate naturally present in the wastewater. The concentration of organic substrate can be easily enhanced by installing a hydrolysis tank as a preliminary step before anaerobe bioreactor, and thus increase the potential for EBPR in the Lerkendal wastewater, and presumably Norwegian wastewaters.

Sammendrag

Utslipp av avløpsvann med høyt innehold av næringsstoffer som fosfor (P) fører til eutrofiering av vannresipienten. Konsekvensene forbundet med eutrofiering kan variere stort, fra redusert estetikk til alvorlige helseproblemer. Det økende fokuset på P som en ikke-fornybar ressurs og nødvendigheten av resirkulering og gjenbruk av P fra næringsrikt avløpsvann har ført til at biologisk fosforfjerning (EBPR) er mer gunstig sammenlignet med kjemisk utfelling. EBPR er en kompleks prosess hvor mange viktige prosessforhold og forutsetninger gjør norsk avløpsvann, som typisk er fortynnet og næringsfattig, mindre ideelt for EBPR.

I denne studien ble muligheten for anvendelse av EBPR i et lokalt og antatt typisk norsk avløpsvann undersøkt. Hovedmålene for dette arbeidet var å karakterisere det ukjente avløpsvannet (Lerkendal avløpsvann) for substanser relevant for EBPR, og utføre batch-eksperimenter for å vurdere den biologiske aktiviteten i avløpsvannet og potensialet for EBPR.

Hovedresultatene fra karakteriseringen av Lerkendal avløpsvann viser at det minner om typisk norsk avløpsvann ettersom det ikke ble vist noen signifikant forskjell når sammenlignet med litteraturverdier. Lerkendal avløpsvann er konsekvent i time og daglig variasjon, hvor konsentrasjonen av det løselige kjemiske oksygenforbruket (sCOD), som utgjør substratet for Polyfosfat Akkumulerende Organismer (PAOer), viste ingen tegn på døgnmønster eller sårbarhet for store mengder nedbør. Forholdet mellom sCOD:PO_4-P og COD:BOD_5 er 60.2 og 1.8, som reflekterer den biologiske nedbrytningskarakteren i avløpsvannet og indikerer høyt potensial for EBPR.

Hovedresultatene av batch-eksperimentene viser lavt P slipp og opptak i avløpsvannet både med og uten ekstern tilsetning av acetat (100 mg sCOD/L). Raten av P slipp er mellom 1.32 og 1.73 P/g VSS*h uten tilsetning av acetat, som er klassifisert som moderate rater. Dette indikerer dårlig biologisk aktivitet og ubetydelig fraksjon av PAOer i det biologiske slammet. Luksusopptak av P ble ikke oppdaget, selv med høye P opptaksrate (6.04 til 10.65 mg P/g VSS*h). Resultatene er relatert til tilstanden til det biologiske slammet som er brukt i eksperimentene, noe som gir grunnlag for å anta at bedre resultater kunne vært oppnådd hvis aktivslam ble anvendt i stedet for MBBR slam.

De resultatene som presenteres gir grunnlag for å konkludere at det er potensial for EBPR i Lerkendal avløpsvann, men det er begrenset på grunn av den lave konsentrasjonen av organisk substrat naturlig til stede i avløpsvannet. Konsentrasjon av organisk substrat kan forbedres ved installasjon av en hydrolysetank som et innledende steg før anaerob reaktor, og dermed øke muligheten for EBPR i Lerkendal avløpsvann, og antakeligvis i norske avløpsvann generelt.

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List of abbreviations

Bio-P sludge	Biological Phosphous-rich sludge
BOD	Biological Oxygen Demand
BSCOD	Easily Biodegradable Soluble Organic Material
COD	Chemical Oxygen Demand
DNPAOs	Dentrifying PAOs
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
GAOs	Glycogen non-Accumulating Organisms
MBBR	Moving Bed Biofilm Reactor
MLVSS	Mixed Liqour Volatile Suspended Solids
MQ water	Milli-Q water
N	Nitrogen
N_2	Nitrogen gas
NADH	Nicotinamide Adenine Dinucleotide
NH_4-N	Ammonia
NO_2-N	Nitrite
NO_3-N	Nitrate
OHOs	Ordinary Heterotrophic Organisms
Ortho-P	Orthophosphate
P	Phosphorus
PAOs	Polyphosphate Accumulating Organisms
PHA	Poly-Hydroxy-Alkanoate
PO_4-P	Phosphate
Poly-P	Polyphosphate Accumulating Organisms
sCOD	Soluble Chemical Oxygen Demand
tCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved Solids
TN	Total Nitrogen
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

1 Introduction

1.1 Phosphorus problematics

Excess discharge of nutrients such as phosphorus (P) and nitrogen (N) from wastewater can cause serious contamination of the receiving waterbodies. The result of excessive nutrient discharge is eutrophication, which is the growth of bacteria and algae who consume large amounts of oxygen due to breakdown of the discharged P and N. Eutrophication has become a global problem with consequences varying from decrease in the aesthetics of the water to serious medical threats due to toxicity (Seviour et al., 2003).

There are substantial amounts of phosphorus in domestic wastewater. In urine, there is approximately 0.3 kg phosphorus per person per year and in feces an additional 0.2 kg phosphorus per person per year originating from P in the consumed food (Milhelcic et al. 2011, Balmér. 2004). Industry and commercial sources, such as synthetic detergents and other cleaning products also contribute to the total phosphorus concentration in wastewater. The concentration is affected by the degree of disturbances such as the degree of dilution in the wastewater, contribution of industrial waste and the size of the area producing the wastewater. The discharge limit for P will vary depending on the sensitivity of the receiving water. The general limit in Norway is below 1 mg P/L (Forurensningsforskriften 2005).

Phosphorus is an essential nutrient for all life forms, and it essential for food production. The increased focus on P as a non-renewable recourse and the necessity for recovering and recycling of P from nutritious wastewater has improved the technology and knowledge related to sustainable use of P. Wastewater could possibly be a major source of P recycling for global P sustainability (Naidu, R. et al., 2012).

1.1.1 Phosphorus removal methods

P removal from wastewater will depend on the ability to convert dissolved phosphates into suspended P, which then is separated from the water. This is typically achieved by chemical P removal, biological P removal or a combination (Morse et al. 1997).

1.1.1.1 Phosphorus removal by chemical precipitation

Chemical P removal, also referred to as chemical precipitation, was traditionally the most commonly used method. The method is divided into four steps: precipitation, coagulation, flocculation and separation. Precipitation is achieved by dosing the system with metal salts of typically aluminum or iron with the ability to form precipitation of dissolved P. The precipitated metal bound phosphate forms particles during coagulation, where the subsequent flocculation develops flocs that are large and heavy enough to be easily separated from the liquid phase (Ødegaard et al., 2014). Commonly used separation methods are sedimentation or flotation. Chemical precipitation is an efficient and easily implemented method where the level of P removed is determined by the amount of chemicals dosed to the system. However, the method is associated with large production of chemical sludge with high metal content, as well as high operating costs as coagulants are expensive. The re-use of the chemical sludge is limited as the high metal content can harm the environment (Morse et al. 1998, Driver et al. 1999).

1.1.1.2 Phosphorus removal by biological processes

Biological phosphorus removal is based on exposing the wastewater to microorganisms with the ability to remove P from the wastewater, due to consumption for internal biochemical processes. The method began in the USA in the 60's (Ødegaard et al., 2014), and is generally referred to as Enhanced Biological Phosphorus Removal (EBPR). There are two main types of EBPR; P removal by maintaining suspended growth in the system (activated sludge) and by maintaining attached growth in the system (biofilm). EBPR is a relatively new technology which is becoming favorable to chemical precipitation due to the elimination of chemicals and thus also eliminate the production of chemical sludge. The method produces biological sludge with high P content (bio-P sludge). Excess bio-P sludge is removed from the process after separation from the liquid phase, and is optimal for P recovery as the sludge has a higher bioavailability compared to chemical sludge, and does not pose an environmental threat (Janssen et al. 2002).

1.2 EBPR overview

EBPR is based on microorganisms with the ability to assimilate the P that is in the wastewater for cellular growth, hence removing the P from the liquid phase. These organisms are referred to as Polyphosphate Accumulating Organisms (PAOs) (Mino et al. 1998).

In EBPR, PAOs are exposed to alternating anaerobe and aerobe or anoxic conditions. The biochemical processes occurring inside the PAOs rely on the presence of several compounds, such as glycogen, organic material, carbon source, polyphosphate (poly-P), that during the anaerobic-aerobic or anoxic cycle are accumulated and stored internally in the bacteria cell and subsequently degraded (Helness, 2007). EBPR is a two-step system where P is firstly converted to soluble P, and secondly assimilated in the PAOs cells (Janssen et al. 2002, Mino et al. 1998).

1.2.1 Biochemical anaerobic processes

The first step in the EBPR process is to convert P to soluble P and release it to the liquid phase. Figure 1.1 is a simplified illustration of the biochemical processes occurring under anaerobic conditions.

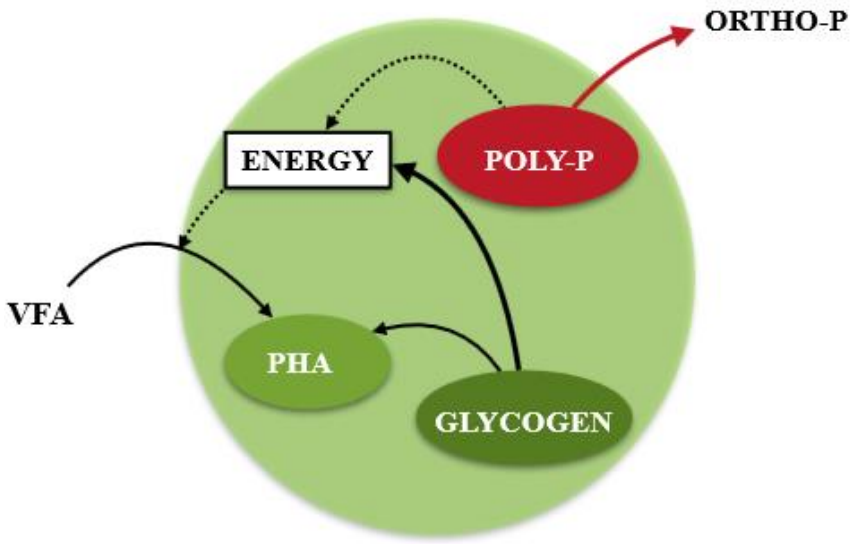


Figure 1.1. Simplified biochemical model for PAOs under anaerobe conditions (modified from Wentzel et al., 2008).

PAOs require easily biodegradable soluble Chemical Oxygen Demand (BSCOD), such as volatile fatty acids (VFA) as carbon source for the biochemical processes to occur under anaerobe conditions. The amount of BSCOD available in the wastewater will depend on the

concentration of soluble chemical oxygen demand (sCOD). The amount of sCOD is an indirect measure of the BSCOD as a portion of the sCOD is inert and not biologically available (Saltnes et al., 2016). By splitting the internally stored poly-P and glycogen reserves, PAOs are provided with the energy needed for VFA uptake from the liquid phase and through the PAOs cell membrane. Glycogen is used for glycolysis and the conversion of VFA into long and complex poly-hydroxy-alkanoate (PHA) carbon molecules, which becomes carbon storage compounds (Oehmen et al., 2007). PHA is a more reduced polymer than VFAs, therefore the conversion requires reducing power in the form of nicotinamide adenine dinucleotide (NADH) (Janssen et al., 2002). The generally accepted theory for NADH production in PAOs is the Mino model, which proposes degradation of already stored glycogen as the main source of reducing power for anaerobic conversion of VFA to PHA in PAOs (Mino et al., 1995).

Due to the splitting of the internally stored poly-P reserves, orthophosphate (ortho-P) is released to the liquid phase. This increases the concentration of ortho-P in the liquid phase which is a prerequisite for luxury uptake of P in aerobic phase (Wentzel et al., 2008).

The concentration of BSCOD and subsequent VFA naturally present in the system will depend on the condition of the wastewater. Research has found that Norwegian wastewaters are typically diluted and has low values of BSCOD (Raspati, 2016, Ødegaard, 1999). This is known to be one of the main disadvantages of Norwegian wastewater when assessing the potential for EBPR. VFA is fermented from the fermentable COD by heterotrophic organisms during anaerobe conditions. Fermentation is the degradation of organic matter under oxygen and nitrate fatigue environment (Janssen et al., 2002).

The ratio of BSCOD consumed to phosphate removed has been found to vary in literature. Theoretically, an EBPR process will require 7 mg VFA to remove 1 mg PO_4-P (Janssen et al., 2002). This theoretical ratio will vary depending on the type of VFA present in the system. The most common VFAs are acetate, butyrate, propionate and valeriate (Janssen et al., 2002, Helness, 2007). There are contradicting results as to which organic substrate is the most effective, but acetate has been used as a carbon source in many studies and is generally a well-known carbon source for EBPR (Smolders et al., 1994). Balamane-Zizi and Ait-Amar (2012), in accordance with several other studies, report propionate as the most favorable organic substrate for EBPR as it is found to give higher P release compared to acetate.

1.2.2 Biochemical aerobic processes

The second step in EBPR involves aerobic assimilation of the ortho-P in the liquid phase as PAOs utilize the intercellular stored PHA for energy to create a luxury uptake of P (Wentzel et al., 2008). Figure 1.2 is a simplified illustration of the biochemical processes occurring under aerobic conditions.

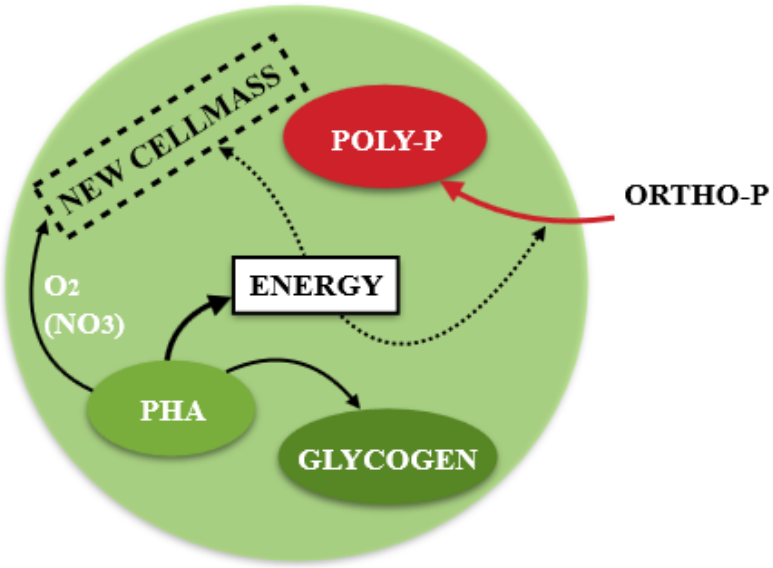


Figure 1.2. Simplified biochemical model for PAOs under aerobe conditions (modified from Wentzel et al., 2008).

Under aerobic conditions, the PAOs receive the ability to utilize the intercellular stored PHA as a source of energy for growth of new cells, giving them the ability to take up more phosphate than what was released during anaerobe phase (Wentzel et al., 2008). This creates a luxury uptake of phosphate where the PAOs are able to take up all the released ortho-P as well as the phosphates originally present in the influent wastewater. PAOs take up ortho-P from the liquid phase and regenerate the poly-P storage needed as energy source when the condition is altered to anaerobe again. Glycogen reserves are replenished due to degradation of PHA. In activated sludge, excess bio-P sludge is removed after aerobe phase as the fraction of PAOs is increasing due to stimulation of cellular growth. The rest of the bio-P sludge is returned to anaerobe phase where it is mixed with fresh influent wastewater (Janssen et al., 2002, Wentzel et al., 2008).

Figure 1.3 illustrates the two-step system of EBPR and the biochemical processes occurring by showing how the concentration of ortho-P, VFA, PHA, glycogen and poly-P vary as a function of time and process conditions in both liquid phase and biomass. The curve illustrating the

variation of poly-P indicates how the biomass will contain a high concentration of poly-P when it reaches the clarifier and subsequently returned to anaerobic phase (Wentzel et al., 2008).

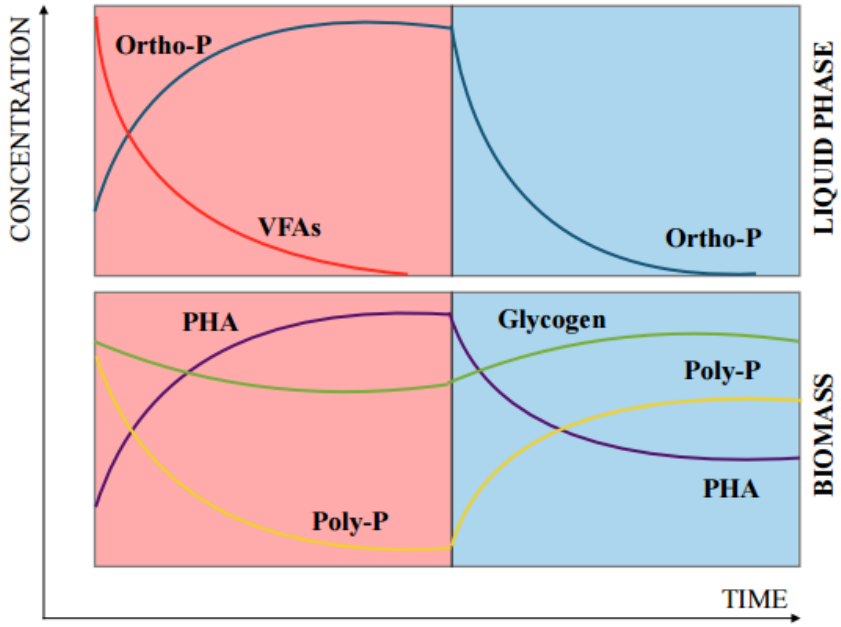


Figure 1.3. Schematic overview showing variation in liquid phase and biomass as a function of time in concentration of VFA, ortho-P, PHA, glycogen and poly-P in liquid phase and biomass under anaerobic and aerobic conditions (modified from Wentzel et al., 2008)

1.2.3 Microbial diversity

Contradicting results from studies dedicated to clarifying the microbiology of PAOs indicate that PAOs are relatively complex microorganisms. Current available literature generally agrees that there are two types of bacteria with the ability to take up large amounts of phosphate; poly-P organisms and PAOs (Janssen et al., 2002). Poly-P organisms only take up and store phosphate for maintenance purposes, thus not removing P from the wastewater in excess (Mino et al., 1998). Originally, the common understanding was that *Acinetobacter* and *Microthrix parvicella* were the main PAOs (Helness, 2007, Blackall et al., 2002). Later research has contradicted this assumption as Acinetobacter and Microthrix Parvicella were identified as poly-P organisms and cannot function as the main PAOs responsible for bio-P removal (Helness, 2007). PAOs are therefore referred to as the "real" phosphate bacteria and is the only bacteria able to create the luxury uptake and thus remove large amounts of P from the wastewater. *Candidatus Accumulibacter phosphatis* is one of the most common PAOs known today (Janssen et al., 2002).

As PAOs are the bacteria responsible for P removal in EBPR, the process is dependent on maintaining a maximum and continuous concentration in relation to the total bacteria culture in the system. Other bacteria in the system will compete with PAOs for the available organic matter and thus have the ability to reduce the performance of the process. Numerous studies have investigated the total bacteria culture occurring in EBPR and tried to clarify their involvement with PAOs. The results prove that the microbial community in EBPR is naturally diverse and complex as internal processes, such as denitrification and nitrification, alter the presence and function of the total bacteria culture (Mino et al., 1998).

1.2.3.1 GAOs

Glycogen non-polyphosphate Accumulating Organisms (GAOs) are defined as "organisms that store glycogen aerobically and consume it anaerobically as their primary source of energy for taking up carbon sources and storing them as PHAs" (Mino et al., 1995). GAOs are similar to PAOs, but have a distinct different metabolism and function. GAOs consume the organic substrate anaerobically and convert it into PHA similar to PAOs, but without releasing or removing P from the wastewater. GAOs degrade the stored glycogen via glycolysis to gain the energy needed for VFA uptake and conversion of PHA. GAOs oxidize the stored PHA aerobically to provide energy for replenishment of glycogen and cell growth (Kjørlaug, 2013). The microbiological characteristics of GAOs are not yet fully understood, but it is documented that GAOs cause deterioration of the EBPR process when they are able to grow due to their competition with PAOs for the available substrate (Erdal et al., 2003). As will be discussed later, there are several operational conditions and parameters that give GAOs a competitive advantage over PAOs.

1.2.3.2 OHOs

Ordinary Heterotrophic Organisms (OHOs) are not able to accumulate poly-P and therefore do not remove P in excess from the wastewater. Under anaerobe conditions OHOs are not able to utilize the available VFA as they require external oxygen and/or nitrate as electron acceptor. If the EBPR process is configurated optimal and the return of biomass does not return oxygen and/or nitrate, OHOs do not compete with PAOs for the available VFA. However, if oxygen and/or nitrate is recycled to the anaerobe reactor, OHOs can use the available fermentable COD for energy and growth. The OHOs use 1 mg O_2 to consumes 3 mg fermentable COD (Wentzel

et al., 2008). This reduces the fermentable COD significantly thus leaving a low concentration of COD available for VFA conversion. This results in a low PAOs storage of VFA and subsequent low P release, P uptake and net P removal.

1.2.3.3 DNPAOs

P uptake under anoxic conditions indicate that the PAOs have denitrifying capability, and are then referred to as Denitrifying PAOs (DNPAOs). DNPAOs have the capacity to use either oxygen or nitrate as an oxygen agent to consume VFA and take up P from the liquid phase (Zeng et al., 2016).

Both PAOs and DNPAOs require VFA to carry out the internal biochemical processes. In wastewater treatment plants (WWTPs) where simultaneous P and N removal is desired, DNPAOs are of advantage because they remove P while denitrifying nitrate (NO_3-N) using the same substrate under anoxic conditions. As VFA generally is limited in wastewater, the competition for substrate is relieved (Shoji et al., 2003).

DNPAOs may not play a primary role in EBPR, but they can be crucial in scavenging P left from aerobic phase and be responsible for producing an effluent of very low P concentration. Compared to PAOs, DNPAOs are hypothesized to be 40 % less efficient in energy generation, which would lead to a 20 to 30 % lower cell yield and an overall lower sludge production (Zeng et al., 2003).

1.3 EBPR in typical Norwegian wastewaters

At the time of writing this thesis, EBPR is not widely implemented in Norwegian wastewater treatment. There are many contradictions to why, but the main argument is that Norwegian wastewater characteristics are not optimal for EBPR as it typically is diluted and low in nutrients. It is therefore assumed that the wastewater will not enhance the functionality of PAOs in relation to the diverse microbiology of EBPR, and therefor give low efficiency of P removal.

1.3.1 Typical Norwegian wastewater

Norwegian wastewater is typically cold and diluted due to the high amount of precipitation and runoff during transportation. The configuration of the transport system affects the degree of dilution as combined transport system allows the wastewater to be diluted by stormwater. The

concentrations found in wastewater are a combination of pollutant load and the amount of water with which the pollutant is mixed (Henze et al., 2008).

It is not possible to give an accurate characterization of wastewater as the composition will vary significantly from one location to another, as well as vary with time. However, Table 1.1 shows the comparison of statistical average values for Norwegian wastewater (Raspati, 2016), typical values for diluted and concentrated wastewater (Henze et al., 2008) and potentially concentrated wastewater from Hias (Saltnes et al., 2016).

Table 1.1. Comparison of typical average wastewater values in Norway, typical values for diluted and concentrated wastewater in general, and concentrated wastewater from Hias (Henze et al., 2008, Raspati, 2016, Saltnes et al., 2016)

Parameter	Average Norwegian wastewater*	Concentrated wastewater**	Diluted wastewater***	Hias wastewater****
TSS (g/L)	0.22	0.6	0.25	-
tCOD (mg/L)	494	1200	500	553
sCOD (mg/L)	-	480	200	298
TN (mg/L)	37	100	30	-
NH_4-N (mg/L)	24.7	75	20	53
TP (mg/L)	5	25	6	-
PO_4-P (mg/L)	3.3	15	4	5.1
BOD_5 (mg/L)	195	560	230	-

* Statistical average Norwegian wastewater

** Typical composition of concentrated wastewater with low water consumption and/or infiltration

*** Typical composition of diluted wastewater with high water consumption and/or infiltration

**** Wastewater from Hias, Hamar, where EBPR is operated successfully. Wastewater is affected by industrial discharge and is therefore referred to as concentrated

Statistical values for average Norwegian wastewater is based on the presentation held by Gema Sakti Raspati, Trondheim 2016, where the information about WWTPs names, size and location is unavailable. It is here assumed that the values are based on WWTPs with capacity > 2000 PE.

Table 1.1 indicate that the average Norwegian wastewater is characterized as diluted when compared to typical values for diluted and concentrated wastewater (Henze et al., 2008). Hias wastewater is higher in concentration for all the analyzed parameters, which is to be expected as this wastewater is receiving industrial discharge which makes it more concentrated and higher in nutrients (such as ammonium and phosphorus). Hias wastewater composition is more similar to typical diluted wastewater when compared to the values from Henze et al. (2008). Hias currently has successful operation of EBPR, therefore it is interesting to compare a wastewater that is to be assessed for EBPR to these values.

The average values for Norwegian wastewater does not include the concentration of sCOD, hence an estimated value for BSCOD is not available. According to Henze et al. (2008), the typical value for diluted wastewater is 200 mg sCOD/L. As substrate is the most vital substance for PAOs and therefore the most important parameter in EBPR, it is essential that the sCOD concentration is high so that there is potential for a high fraction of BSCOD and VFA in the wastewater. sCOD is therefore an important parameter to determine when assessing the potential for EBPR in a certain wastewater. As Norwegian wastewater typically is low in nutrients and organic material when there is a limited amount of discharge from industry, sCOD is generally known to be the limiting factor for implementation of EBPR in Norway. However, there are several processes that can be configurated into the process to increase the VFA concentration. An example and commonly used process configuration is a hydrolysis tank as a preliminary step before the wastewater reaches the anaerobe process conditions.

1.3.2 Important aspects of EBPR

EBPR is a complex process where several environmental and operational conditions affect the process differently depending on where in the process they occur. There are numerous parameters and conditions to assess when investigating the potential for EBPR in any wastewater. The complexity of the EBPR process makes it difficult to fully understand how certain conditions and parameters affect the overall process.

One of the main highlights when assessing the potential for EBPR for any wastewater, is the composition and characteristics of the influent. This will reveal whether the wastewater possesses the right conditions and characteristics that are optimal for EBPR, such as the amount of BSCOD and substrate available for consumption by the PAOs. Other important aspects of

the influent are whether the operating conditions, such as temperature and pH, provides PAOs with a competitive advantage in relation to the diverse microbiology in the system.

1.3.2.1 Composition of influent wastewater

Organic material, carbon source and substrate

VFA is as mentioned one of the major concerns for optimal EBPR. sCOD is the only compound that will be fermented into VFA under anaerobe conditions as the retention time is not long enough to ferment suspended COD. As PAOs require VFA to be able to successfully synthesis PHA and glycogen, and thus creating the luxury uptake that is EBPR, sCOD is one of the most important substances in EBPR.

The amount of organic material in the influent can be determined by measuring COD, biochemical oxygen demand (BOD) or volatile suspended solids (VSS). Both BOD and COD analysis determined the amount of organic material, but COD analysis includes also organic material that is not biodegradable and the value will therefore be higher. COD analysis is less time-consuming than BOD, and is therefore more suitable as an operational parameter.

In theory, 7 mg VFA is required per 1 mg PO_4-P for EBPR (Janssen et al., 2002). Norwegian wastewater typically suffers from a generally low sCOD concentration, with variation depending on the amount of industrial waste or the condition of the wastewater. As the Norwegian wastewater is transported in systems affected by the varying topography, the dissolved oxygen (DO) levels in the wastewater is often high and therefore potentially inhibits the fermentation of VFA.

As PAOs are not the only microorganisms with the ability to consume VFA under anaerobe conditions, the initial low concentration of VFA and the lack of fermentation is a potential problem for EBPR in Norway. Low concentration of sCOD in conjunction with the competition for available substrate provides the PAOs with insufficient source of energy to accumulate an excess amount of P, thereby reducing the EBPR functionality.

However, a study performed by Saltnes et al. (2016) indicate that the influent VFA concentration is not the decisive factor for the amount of PO_4-P removed through EBPR, as none of the WWTPs in the study had the theoretically required VFA:PO_4-P ratio of 7:1. According to the conclusion, this indicates that PAOs have the potential for utilizing carbon

sources that are not sCOD, or that other microorganisms present in the wastewater are able to disintegrate the organic matter into some form of sCOD that is available for PAOs to consume. However, more research is needed on the topic.

C:P

The ratio between organic material and the concentration of P in the influent assesses the amount of organic material that is required to remove P by PAOs. Experience have proven that a BOD:P ratio above 15 – 20 guarantees the possibility for efficient biological P removal (Janssen et al., 2002, Saltnes et al., 2016). When the influent ratio between organic material and P is high, it means that it will be easy to remove the P biologically. According to Carrera et al. (2001) the optimal sCOD:PO_4-P in the influent is 42.

The amount of organic material and P in the wastewater is affected by several factors such as the area producing the wastewater, climate and the amount of industrial waste. These factors contribute to diluting or concentrating the wastewater (Henze et al., 2008). Norway is typically influenced by high precipitation of snow or rain depending on the temperature and subsequent stormwater infiltration which results in wastewater with diluted characteristics. This will however not affect the C:P ratio, therefore it is possible to assess the influent quality using this factor (Henze et al., 2008).

Metal ions

The presence of metal ions in influent such as potassium (K), calcium (Ca) and magnesium (Mg) are required for successful EBPR. These ions affect the operation of PAOs as they act as anti-ions for the negatively loaded phosphate ions (Janssen et al., 2002). The bio-P kinetics are strongly correlated with the concentration of K and Mg as low concentration of these ions negatively affects the uptake of poly-P and will results in low P removal (Rickard and McClintock, 1992). The ratio between K:P and Mg:P is 0.33 mg K/mg P and 0.26 mg Mg/mg P, respectively (Janssen et al., 2002). Norwegian drinking water is referred to as “hard water” because of the high concentration of Ca and Mg (Ødegaard, 2014). Because wastewater constitutes of drinking water, it is assumed that the typical Norwegian wastewater will not suffer from Mg and Ca deficiency.

Variation in composition

The characteristics of a wastewater varies according to season, day and hour (Ødegaard, 2014). Large variation in the wastewater composition can influence the EBPR process and give the EBPR process operational problems.

The assessment of the potential for EBPR in a wastewater cannot be limited to only one day, week or season. The characteristics of the wastewater changes, thus will also the potential. If parameters and ratios such as C:P vary depending on time of day and season, it must be assessed if the influent has an overall potential for EBPR. Nutrients in influent is affected by the consumers as a city will naturally have higher nutrient levels in the wastewater compared to a small apartment complex. Discharge of industrial waste increase the influent nutrient concentration significantly and give higher concentration of organic matter. It is therefore important to investigate the continuity of industrial discharge as it will alter the operational aspects of the process.

Analyzing the hourly and daily concentration of the wastewater and subsequent compounds can provide important information of the characteristics and wastewater conditions. The general flow in wastewater on an hourly basis is illustrated in Figure 1.4.

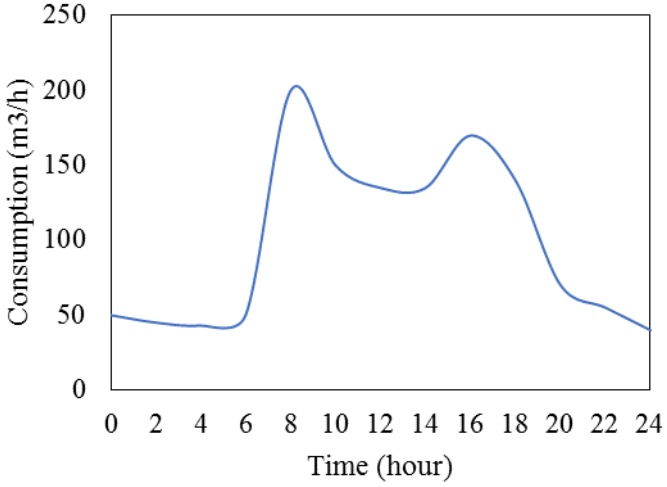


Figure 1.4. General flow in wastewater on an hourly basis (modified from Ødegaard et al., 2014).

Depending on the compounds analyzed, it is expected that the concentrations in the influent are affected by the consumption and follows the same curvature as the flow. Usually, the concentration is consistently low between mid-night and 6 am due to low consumption of water

(Ødegaard et al., 2014). A prominent peak will occur between 6 am and 9 am and around 6 pm which is related to the daily habits of the inhabitants. Mid-day concentrations are typically low as there is little consumption. Comparing hourly variation during a day will uncover if the wastewater variation is consistent and can provide a general introduction to the wastewater composition of important compounds related to successful EBPR.

Characterization of the influent on a daily basis will prove information concerning the vulnerability to weather changes such as high precipitation periods. Daily characterization can be compared to characterization conducted during a different season to assess if seasonal change is affecting the composition of the influent.

1.3.2.2 Temperature

Temperature is a complex operating condition that affects EBPR differently due to the diverse microbial mixture and subsequent optimal growth temperature. Studies have shown contradicting results as to the affect temperature has on the overall EBPR process as it will affect various processes and wastewater characteristics in the system simultaneously (Janssen et al., 2002).

Low temperature is typical for Norwegian wastewaters. During winter the average wastewater temperature is 5 °C whilst during spring the average wastewater temperature is 0 °C (as a function of leakage water) (Ødegaard et al., 2009). As low temperatures generally reduce biological reaction rates, this indicates that the cold Norwegian influent can slow down and/or inhibit biological processes.

EBPR has been proven to work efficiently at higher temperatures (20 °C – 37 °C). However, numerous studies have proven that biological P removal will function better under influence of low temperatures (5 °C – 15 °C) (Erdal et al., 2003). This is due to the other bacteria's present in the liquid bulk who are more vulnerable to low temperatures than PAOs, which will give PAOs a competitive advantage (Janssen et al., 2002, Helmer and Kunst, 1998). This is further supported by the observation of an obvious competitive advantage for substrate uptake by GAOs temperatures higher than 20 °C (Zheng et al., 2014), hence decreasing the P removal efficiency in the system. GAOs tend to consume substrate more efficient than PAOs at high temperatures, while PAOs growth and substrate uptake is favored at low temperatures (Oehmen et al., 2007).

This indicates that Norwegian wastewater temperature is optimal for EBPR. However, temperature cannot be treated as an isolated condition, and it is therefore not possible to conclude that the wastewater temperature makes Norwegian wastewater optimal for EBPR. Even though the temperature gives PAOs the competitive advantage over other microorganisms, the cold temperature makes fermentation of COD less efficient, and therefore provide the wastewater with insufficient substrate.

1.3.2.3 pH

Optimal pH in the influent is an important parameter for controlling the competition between PAOs and GAOs in the overall process. pH at 6.5 was found to take up P approximately 40 % less efficient compared to P uptake found at pH 7.0, also resulting in a reduced degradation of PHA and growth of biomass (Filipe et al., 2001a, Filipe et al., 2001b). This will subsequently give lower P removal capacity. GAOs are found to thrive in a such an environment, as the VFA originally meant for PAOs will be consumed by the GAOs. It is therefore recommended to keep a high pH in aerobe phase of EBPR as it is more beneficial for PAOs (Janssen et al., 2002, Filipe et al., 2001a, Filipe et al., 2001b).

PAOs ability to take up substrate under anaerobe conditions is independent of pH in the range between 6.5 and 8.0, while GAOs are strongly affected within this pH range (Filipe et al., 2001b). The fraction of PAOs can be enhanced in the anaerobe phase by increasing the pH, which will reduce the growth and substrate uptake by GAOs significantly. GAOs consume VFA faster than PAOs at pH lower than 7.25, while PAOs consume VFA faster than GAOs at pH higher than 7.5 (Filipe et al., 2001a).

Smolders et al. (1994) introduced a linear relationship between P release and VFA uptake, and pH. Results from the study indicate that pH ranging between 5.5 and 8.5 gives a P release variation of 0.25 – 0.75 mol P/mol C. According to Janssen et al. (2002), energy required for VFA transport through the cell membrane is reduced at low pH, which results in a lower phosphate release. This results in a waste of energy as the energy in the poly-P storage will not be utilized for converting VFA into PHA, but rather for the transportation of VFA through the cell membrane. It is therefore recommended to maintain a relatively high pH (> 7.5) in the overall EBPR process, since this will shift the microbial competition from GAOs to PAOs.

Study performed by Zhang et al. (2005) showed how a lowering of pH from 7.0 to 6.5 gave a P removal efficiency reduction from 99.9 % of P to 17 % of P in 14 days.

Norwegian wastewater is typically low buffered with a pH around 7 – 8 (Ødegaard et al., 2014, Saltnes et al., 2016), where the topography gives the wastewater oxidizing conditions. When combining this with literature, it is reasonable to assume that the typical Norwegian wastewater pH will not pose a problem for EBPR as it is within the optimal range and will favor the accumulation of PAOs. However, there are other compounds and conditions in the EBPR process that can alter the pH and therefore it is not necessarily so that the pH in the wastewater will be the most decisive factor for the overall pH in the process. If the pH is not optimal and causes deterioration in the process, chemicals can be added as a method for manipulating pH. However, as there are several advantages associated with a pure biological process, adding chemical therefore counteracts these advantages.

1.3.2.4 Dissolved oxygen

DO can either disturb or enhance the EBPR process depending on where DO is present. If DO is limited during aerobe phase of the process, the PAOs will not take up ortho-P. According to study by Shehab et al. (1996), the DO concentration should be 3.0 – 4.0 mg/L in the aerobic reactor, any higher will be a waste of energy and will not benefit the process. Over-aeration causes PHA depletion and subsequent lower P uptake rate.

Norwegian wastewater has typically high DO concentration due to the topography, heavy rainfall and groundwater infiltration that supplies the wastewater with oxygen, hence the wastewater is generally aerobe at the inlet of the WWTPs. This oxygen must be removed before the wastewater enters the anaerobe phase otherwise the process will deteriorate. Oxygen is removed by heterotroph turnover by bacteria as they consume oxygen by degradation of organic material. For each mg of oxygen removed, 2 mg COD is consumed. The concentration of the sCOD and subsequent BSCOD becomes limited for PAO consumption, resulting in less phosphate removed (Janssen et al., 2002).

1.3.3 Batch experiments for P activity and kinetics

To obtain more information on the potential for EBPR in the influent wastewater, laboratory P release under anaerobic conditions and P uptake under aerobic and anoxic experiments can be performed. These tests will provide information about the capacity of P release and P uptake

and subsequent potential of EBPR. The tests can also indicate whether P in an activated sludge system is removed, the activity of PAOs or give information on the presence of DNPAOs. Figure 1.5 illustrates the typical course of PO_4-P during anaerobic P release test, aerobic P uptake test and anoxic P uptake test. The linear area of the curves is used to determine the rate of P release and P uptake under anaerobe, aerobe and anoxic conditions.

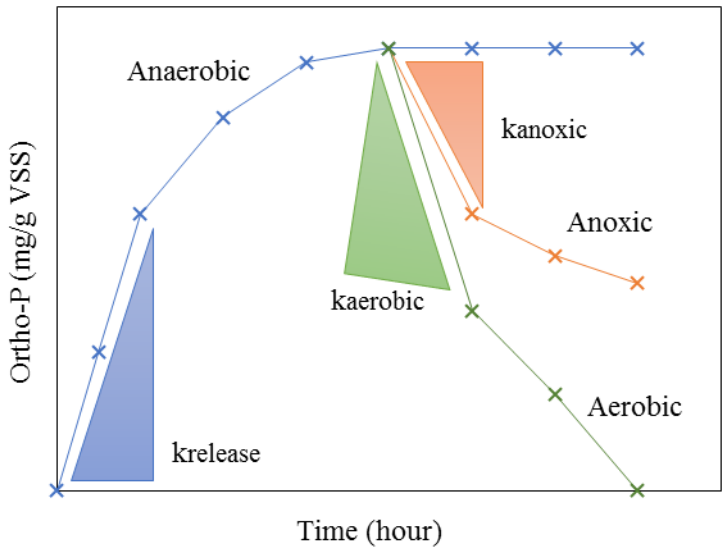


Figure 1.5. Kinetics of PO_4-P in batch experiment during anaerobic P release test, aerobic P uptake test and anoxic P uptake test, as well as linear area for P uptake and release rates (modified from Janssen et al., 2002).

The results obtained from performing these experiments, including the release and uptake reaction rates, show little direct relation to the bio-P process efficiency, but yield information related to the actual bio-P activities in the associated WWTP (Janssen et al., 2002). If the test is performed regularly at one plant the results can be used for optimization of the operation. The test results can be used for modelling or as a simple tool for identifying periods with problems (Janssen et al., 2002).

1.3.3.1 Bio-P sludge importance in relation to batch experiments

The sludge used in such experiments is of importance when assessing the results from these experiments and obtaining representative results. When using activated sludge, it is guaranteed that the sludge and PAOs are fresh and active as the basis for activated sludge is returning the active accumulated fraction of the biomass back into the cycle. Bio-P sludge obtained from Moving Bed Biofilm Reactor (MBBR) carriers is in relation to activated sludge not characterized as fresh, as this biomass is the fraction that has detached from the carriers after

adhering over a longer period. The biomass and subsequent PAOs obtained from MBBR carriers are therefore not necessarily as fresh or vital as what is optimal for optimal results.

1.4 Alternative EBPR process configurations

1.4.1 EBPR with activated sludge

EBPR combined with activated sludge is the most used method for EBPR, where P removal is obtained by maintaining suspended growth in the system. Bio-P sludge flows with the wastewater through alternating conditions, stimulating the growth of PAOs. Figure 1.6 illustrates the most basis configuration of EBPR combined with activated sludge where the bio-P sludge is returned to anaerobic phase after a separation step.

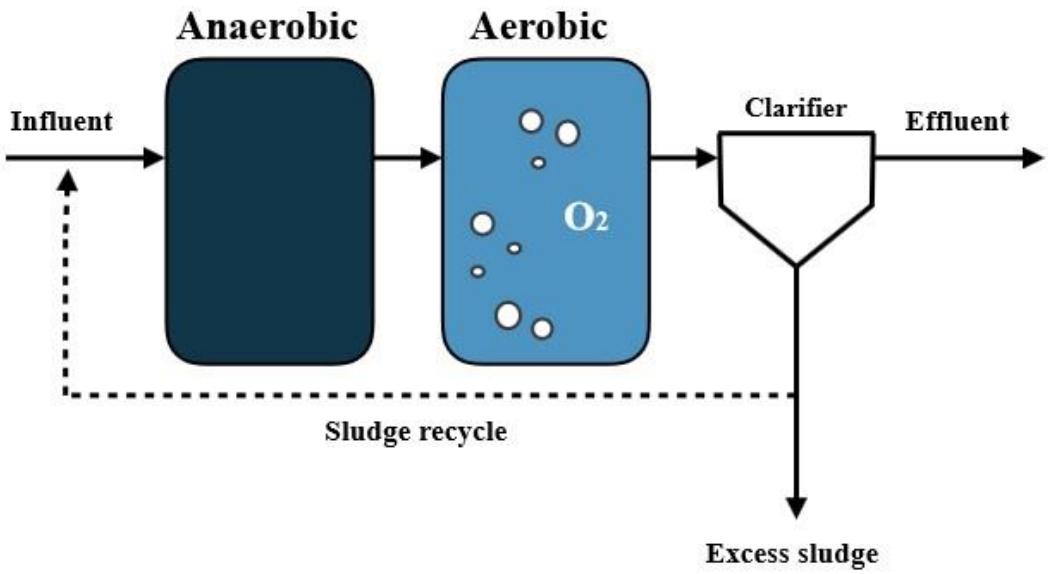


Figure 1.6. Basic EBPR process configuration (Modified from Janssen et al., 2002).

In a strictly aerobe active sludge system the P incorporated in the sludge can reach a theoretical value of 0.02 mg P/mg VSS, which has been found to remove of 15 – 25 % of P in municipal wastewater (Wentzel et al., 2008). By combining activated sludge and EBPR as in Figure 1.6, the incorporation of P in the biomass can increase to 0.06 – 0.15 mg P/mg VSS and give a higher P removal from the wastewater (Wentzel et al., 2008).

Successful operation of EBPR is achieved when the concentration of PAOs in the system is continuous and high. In activated sludge, this is ensured by returning some part of the bio-P

sludge from aerobe phase back to anaerobe phase, where it is mixed with fresh influent wastewater from the inlet and repeats the cycle (Janssen et al., 2002, Wentzel et al., 2008). When the EBPR is designed appropriately, the PAOs can make up about 40 % of the active organisms present, which will remove about 10 – 12 mg P/L per 500 mg influent COD/L (Henze et al., 2008).

The bio-P sludge must be separated effectively from the treated wastewater before it is returned to anaerobe phase. The degree and type of separation influences the quality of the bio-P sludge as low separation causes release of biomass to the effluent, and a subsequent low return of PAOs. The most common used method is sedimentation by clarifies.

The net removal of P from the wastewater is achieved by removing excess bio-P sludge after sedimentation. Routinely removal of excess sludge is necessary to maintain the optimal sludge age that allows growth of PAOs while avoiding nitrification in the sludge. It is important that the excess sludge removed is less than what is produced, otherwise the amount of bio-P sludge returned to anaerobe phase becomes low and results in process deterioration.

EBPR with activated sludge has numerous different configuration options. The most widely used process options is the A/O process which includes anaerobic, anoxic and aerobic reactors in series with aerobic mixed liquor recycled to the anoxic reactor and return of sludge recycle to the anaerobic reactor, see Figure 1.7.

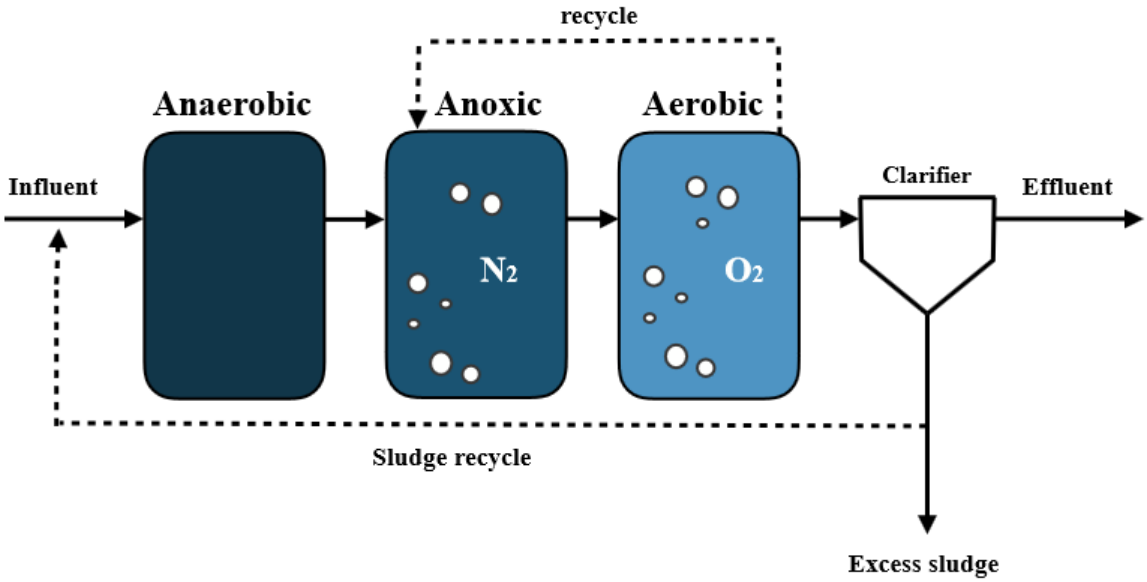


Figure 1.7. Basic A/O configuration (Modified from Wentzel et al., 2008).

A different, but also much used process configuration is the UTC process which includes anaerobic, aerobic and anoxic reactors in series with aerobic mixed liquor recycle to the second stage anoxic reactor, first stage anoxic mixed liquor recycle to the anaerobic reactor and return of sludge recycle to the first stage anoxic reactor (Wentzel et al., 2008). See Figure 1.8.

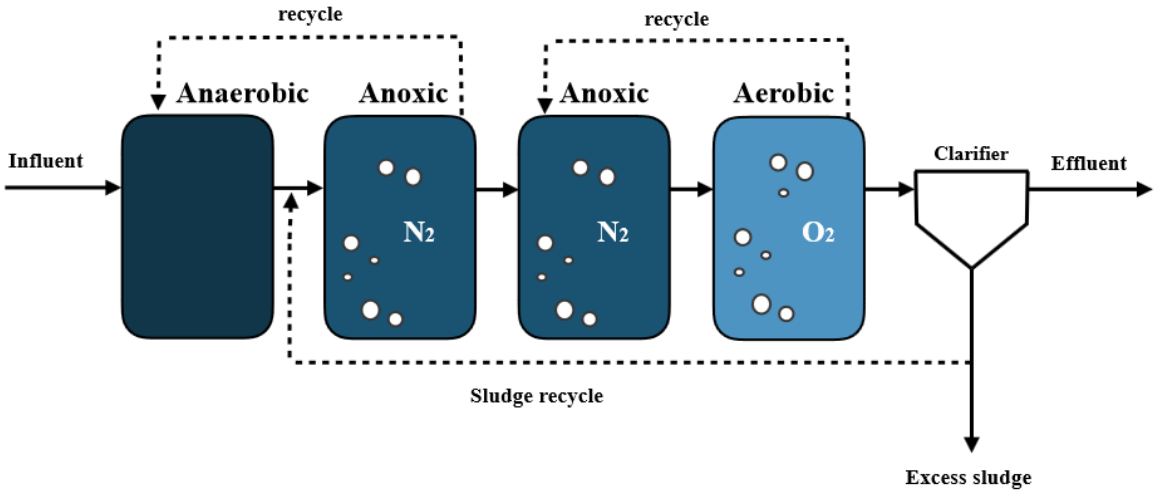


Figure 1.8. Basic UTC configuration (Modified from Wentzel et al., 2008)

1.4.2 EBPR with suspended biofilm

1.4.2.1 EBPR with MBBR

When EBPR is combined with MBBR, continuous biofilm on MBBR carriers flows with the wastewater through the process conditions. The carriers must be exposed to alternating conditions of anaerobe and aerobe and/or anoxic conditions. This is achieved by alternating the conditions in the same reactor (Figure 1.9) or by moving the carriers between several reactors (Figure 1.10).

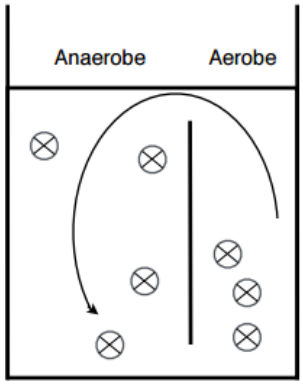


Figure 1.9. Configuration with carriers are circulating in one reactor.

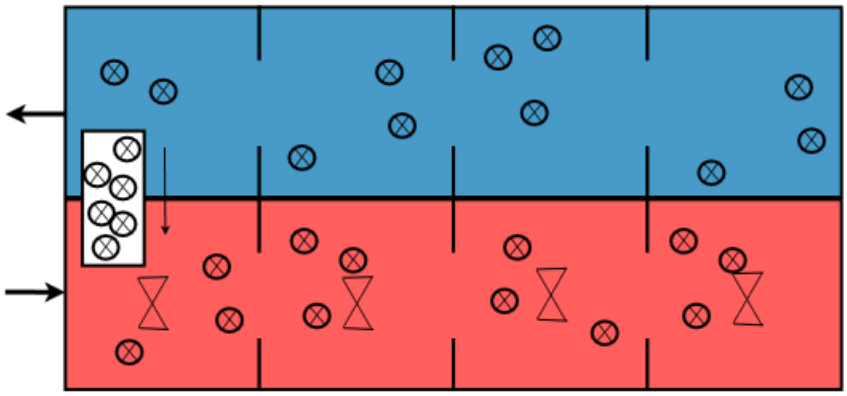


Figure 1.10. Configuration with carriers moved between several reactors. MBBR carriers are circulated with the wastewater through aerobe (red) and anaerobe(blue) conditions. A mechanical conveyer is installed to move the carriers from anaerobe back to aerobe reactors (modified from Torgeir Saltnes (Hias) presentation at RECOVER meeting, Århus 10 – 11. August 2016).

One of the advantages of EBPR with MBBR is the possibility for simultaneous N removal due to nitrification and denitrification. Due to the configuration of the carriers, nitrifying bacteria can function in the outer layer of the biofilm where excess of oxygen is available, while denitrifying bacteria can function in the inner layer where oxygen is limited.

Nitrification

Wastewater typically contains a high amount of ammonia (NH_4-N) and organic bound N, with minor contribution of NO_3-N and nitrite (NO_2-N). Biological N removal occurs in two steps; nitrification and denitrification.

Nitrification is the biological process by which NH_4-N is converted to NO_3-N under aerobic conditions. Nitrifying bacteria, also referred to as autotrophic bacteria, are slow growing. The key requirement for nitrification to occur is that the net rate of accumulation of biomass (hence also the net rate of withdrawal of biomass) is less than the growth rate of nitrifying bacteria, which means a high sludge age (Ødegaard et al., 2014). Nitrification requires the presence of NH_4-N and oxygen, and a limited amount of COD as carbon inhibits nitrification from occurring. Optimum pH for nitrification is between 8 and 9, where low pH decreased the nitrification rate. Nitrification is described by equation 1,

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (1)$$

Denitrification

Denitrification is the biological reduction of NO_3-N to nitrogen gas (N_2) under anoxic conditions. Heterotrophic bacteria need carbon source to be able to use nitrate as electron acceptor when organic matter is oxidized. The requirements for denitrification to occur are presence of COD and NO_3-N, and limited presence of oxygen. Optimal pH for denitrifying bacteria is around 7 to 9. Denitrification is described by equation 2,

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \quad (2)$$

Combing EBPR with MBBR ensures optimal process conditions for EBPR without adding the disadvantages of activated sludge. The process is more compact as return of sludge is not needed. The configuration makes it possible for the entire reactor volume to be utilized, thus reducing the space requirements. Separation of sludge and wastewater is made easier, and the separation step does not affect the biological process, as there is no sludge recycle (Arvidsson, 2015). Hias WWTP in Hamar, Norway has for several years performed experiments on EBPR, which has led to an ongoing EBPR process using MBBR carriers.

As PAOs and DNPAOs utilize the same carbon for P uptake and denitrification, the overall carbon necessary for performing the biochemical processes is reduced. DNPAOs take up carbon anaerobic and use the stored carbon in anoxic layers in the biofilm in aerobic phase. Since the organic material in aerobic phase is reduced, the nitrification is increased. Combining EBPR with MBBR allows the volume of the treatment process to be reduced as both nitrification and denitrification can occur in aerobic zone.

1.5 Aim

The aim of this master thesis was to investigate the potential for implementation of EBPR in a local, typical Norwegian wastewater where the characteristics are unknown. The main tasks to be performed were:

- Characterization of the wastewater by determining the hourly and daily variation of parameters relevant to EBPR implementation.
- Investigate the kinetics of phosphorus release and uptake in batch experiments by using bio-P sludge.

The results from performing the tasks would give basis to answer the following questions:

- What are the characteristics and the condition of the local wastewater?
- What anaerobe phosphate release rate can be achieved using the local wastewater?
- What aerobe phosphate uptake rate can be achieved using the wastewater?
- It the wastewater optimal for EBPR?

1.6 Outline of the work

The master thesis work started with a literature study and practice in experimental methods for characterization of the wastewater to obtain the relevant knowledge and experimental skills. In late February, one week of daily characterization was performed, followed by the hourly characterization three weeks later. The hourly characterization of the wastewater was time-consuming as the BOD_5 analysis took three weeks to complete as the instrument only had space for six samples (including blank seed and dilutions) and each analysis took seven days to perform. The batch experiments were carried out in April/May and took a total of 15 days.

2 Methods and material

This chapter describes the materials and methods used for the experiments and tests performed during this master thesis. The methods and experiments performed are divided into three parts. Firstly, the methods used for characterization of the influent wastewater are described. Secondly are the methods used for characterization of the bio-P sludge upon arrival from Hias WWTP. Thirdly are the methods used for investigating the bio-P kinetics in the wastewater.

2.1 Characterization of influent wastewater

2.1.1 Influent wastewater origin

The wastewater to be characterized was raw municipal wastewater originating from a local apartment complex area of Lerkendal (Gnr/Bnr 64/17), Trondheim (Norway). Figure 2.1 is a satellite photo showing the three apartment complexes (inside red square) which contributed to the production of wastewater. Each complex house 48 apartments. The location of the wastewater laboratory is located inside the blue square.

Figure 2.1. Satellite photo of the location of wastewater production showing the apartment complexes (framed in red) producing the influent wastewater. Blue framed building is the wastewater laboratory where wastewater is pumped (source: Google Maps).

The wastewater was transported from the apartment complexes to the wastewater laboratory by pumping the wastewater from the local pump pit into a pre-installed holding tank inside the

laboratory, see Figure 2.2. In-pumping frequency could be changed depending on the need for fresh wastewater. The holding tank has a capacity of 3.5 m^3 (3500 L) of wastewater. The holding tank was not equipped with mechanical stirring, which causes biomass to settle as a function of time. Inlet and outlet is leveled at the bottom of the tank. The valve located at the outlet was used for retrieving sample manually. The in-pumping of wastewater was continuous for 3 minutes, where the excess wastewater goes to overflow.

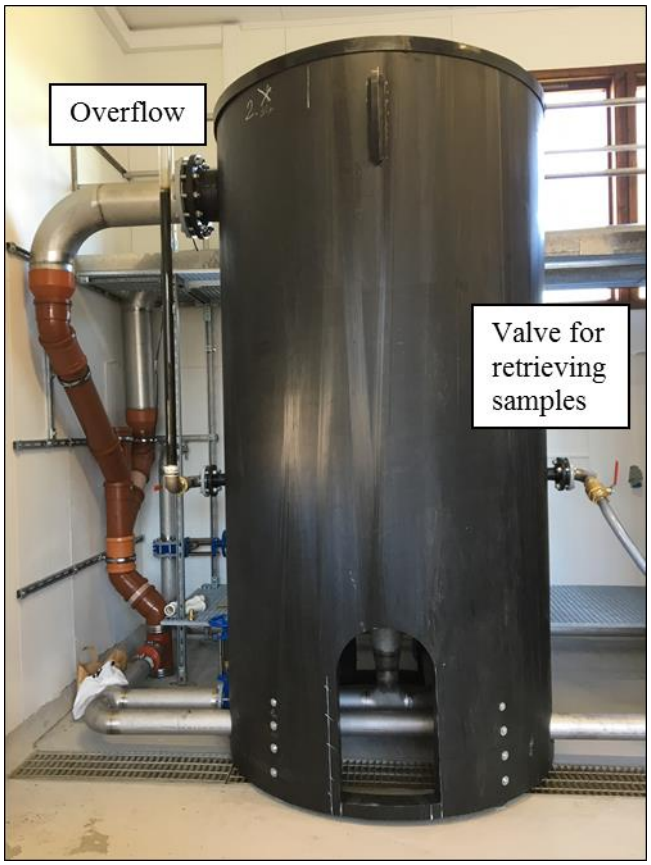


Figure 2.2. Holding tank located in the wastewater laboratory used for retrieving wastewater samples for analyses.

The holdup time in the pump pit causes a delay in wastewater delivery to the holding tank. This resulted in a delay in wastewater characterization as the wastewater arriving in the holding tank at 8 am would be the real-time wastewater at approximately 7 am etc.

Table 2.1 presents all the parameters that the influent wastewater was analyzed for. Duplicates were performed for all the samples and parameters, and the final results was determined by calculating the average.

Table 2.1. Overview of the parameters analyzed for characterization of wastewater

Parameters analyzed for influent wastewater characterization	
NH_4-N	sCOD
NO_3-N	TS
NO_2-N	TDS
TN	TSS
PO_4-P	VSS
TP	BOD_5
tCOD	

2.1.2 Sampling techniques

2.1.2.1 Daily wastewater variation

Daily wastewater variation was determined by collecting wastewater grab samples at approximately 9 am for five continuous days (Mon 20th Feb. – Fri. 24th Feb.). During this sampling period, the pumping frequency was every three hours. Grab samples can give highly variable results as they represent the concentration at the specific time of collection. As the holding tank was not emptied before each in-pumping (due to there was no continuous consumption), the samples are characterized as composite samples of the entire sample day. The samples are therefore reliable for characterization of the daily variation of substances in the wastewater.

When collecting the samples, it was essential to let the wastewater drain for a couple of minutes before taking the sample. This is to avoid a higher fraction of biomass due to the lack of mechanical mixers and subsequent settling. Each sample was analyzed for all the parameters on the day of collection when possible. When direct analysis was not possible, the samples were frozen after preparation according to the parameters in question.

2.1.2.2 Hourly wastewater variation

For determining of the hourly wastewater variation, an automatic sampler (Isco Avalanche Multi-bottle Refrigerated Portable Sampler) was used to collect samples over 24 hours (Figure 2.3). During the characterization, the pumping frequency was increased to every hour. One sample per 2nd hour was collected over 24 hours. The samples were kept refrigerated for 24

hours from sampling time at 3 ± 1 °C in separate containers of 800 mL. The characterization of the hourly wastewater variation was carried out the next day for all parameters, except for BOD_5 which was performed over a period of three weeks due to instrumental space limitations and analysis duration. Continuous wastewater consumption was installed to avoid the generation of composite wastewater samples and allowed concentration peaks to be detection. The exact wastewater consumption rate was unknown as a manual value was used to ensure consumption, but complete draining of the holding tank was achieved before each in-pumping.

The automatic sampler was positioned at the top of the holding tank, with the suction tube emerging under the water table, see Figure 2.3.

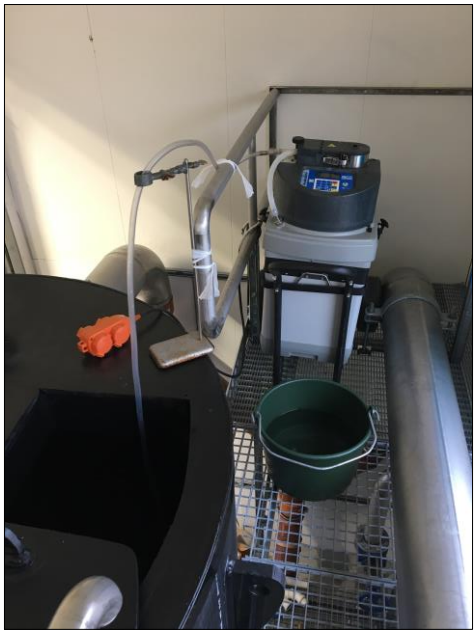


Figure 2.3. Set up for automatic sampler used for collecting fresh wastewater samples every two hours. Suction tube emerged in wastewater at all times.

After each sampling, the sampler purged the suction tube to prevent residual wastewater from mixing with new samples and give less representative results. The headloss will affect the automatic sampler's ability to completely purge the suction tube. Table 2.2 presents the schedule for sampling and in-pumping of fresh wastewater.

Table 2.2. Schedule for automatic sampler. Shows time of start and end of in-pumping of fresh wastewater, and time sample was collected.

Pumping start	Pumping stop	Time of sampling
8:31 am	8:34 am	8:37 am
10:37 am	10:40 am	10:44 am
12:43 am	12:46 am	12:50 pm
14:49 pm	14:52 pm	14:57 pm
16:55 pm	16:58 pm	17:04 pm
19:01 pm	19:04 pm	19:11 pm
21:07 pm	21:10 pm	21:18 pm
23:13 pm	23:16 pm	23:25 pm
1:19 am	1:22 am	1:32 am
3:25 am	3:39 am	3:39 am
5:31 am	5:46 am	5:46 am
7:37 am	7:53 am	7:53 am

The schedule show how the samples were taken after an in-pumping of fresh wastewater was done. This gives very reliable results as there was a natural circulation of the wastewater and therefore no biomass settling.

2.1.3 Cuvette tests for influent wastewater

The parameters NH_4-N, NO_3-N, NO_2-N, TN, TP, PO_4-P, sCOD and tCOD were determined by using Hach Dr. Lange cuvette tests according to the user manual presented by the manufacture. Table A.1 in appendix A shows the used Hach Dr. Lange methods and measuring ranges.

2.1.3.1 Procedure

The samples were used directly or diluted depending on the method and measuring range. Samples for NH_4-N, NO_3-N, NO_2-N, PO_4-P and sCOD were filtrated using 0.45 µm cellulose and nitrate filter (Sartorius), while samples for tCOD, TN, and TP was used directly. The 0.45 µm cellulose and nitrate filters used were prepared by rinsing the filters with 10 mL of Milli-Q water (MQ water) three times according to standard procedure.

DR 3900^{TM} (Hach) spectrophotometer was used to measure the results from the prepared cuvette tests. The instrument has an integrated system for barcode reading of the prepared tests, with ten measurements per rotation for elimination of wrong reading caused by the preparation

of the cuvettes. Digestion of TP, tCOD, sCOD and TN samples was performed in a heating block instrument (DRB200 Thermostat). The Hach Dr. Lange cuvette tests are calibrated for ISO 15705:2002.

The following is a short description of the methods used to determine the different parameters in the wastewater by using Hach Dr. Lange cuvette tests:

tCOD and sCOD

Determination of tCOD and sCOD is performed by adding 2 mL of prepared sample to the cuvette, which is digested for 2 hours at 148 °C. After cooling, the cuvette is inserted into the spectrophotometer and measured.

NH_4-N

Determination of NH_4-N is performed by adding 0.2 mL of filtrated sample to the cuvette to react with the hypochlorite ions and salicylic ions in presence of sodium nitroprucide. After 15 minutes the cuvette is inserted into the spectrophotometer and measured.

NO_3-N

Determination of NO_3-N is performed by adding 1.0 mL of filtrated wastewater sample to react with 2,6-dimethylphenol in presence of sulfuric acid and phosphorus acid. After 15 minutes the cuvette is inserted into the spectrophotometer and measured.

NO_2-N

Determination of NO_2-N is performed by adding 2.0 mL of wastewater sample to react with the primary amines in an acidic solution. After 10 minutes the cuvette is inserted into the spectrophotometer and measured.

TN

Determination of TN is performed by adding 1.3 mL of wastewater sample to react with the nitrate ions and 2,6-dimethylphenol in a solution of sulphuric and phosphoric acid, which forms nitrophenol. The sample is digested for 1 hour at 100 °C. After cooling, the cuvette is inserted into the spectrophotometer and measured.

TP and PO_4-P

Determination of phosphates is performed by adding 0.5 mL of prepared wastewater sample to react with molybdate ions and subsequent reduction by ascorbic acids. Samples for TP are digested for 1 hour at 100 °C. After cooling, the cuvette is inserted into the spectrophotometer and measured. PO_4-P is not digested.

2.1.4 Determination of Biological Oxygen Demand in Influent Wastewater

The procedure for determination of BOD_5 in the wastewater was adapted from Standard Method 5219 D Respirometric Method (American Public Health et al., 1960) and Hach Standard Method procedure for BOD using the user manual (Hach Company, 2013). The test determines the relative oxygen requirements for biochemical degradation of organic material in the wastewater by measuring the molecular DO utilized during a selected incubation period (5, 7 or 10 days). The instrument used was BODTrak™ Respirometric BOD Apparatus (Hach), which measured the DO concentration in the incubation bottles over the duration of 7 incubation days.

External atmospheric pressure change is prevented by sealing the incubation bottles. The bacteria in the sample use oxygen when they consume organic matter that is in the wastewater. This oxygen consumption caused the pressure in the bottle head space to drop, which is monitored by the instrument. The pressure drop correlates directly to BOD. During a test period, stir bars mix the sample and cause oxygen to move from the air in the bottle to the sample. This helps simulate natural conditions (User manual BODTrak II^{TM}).

At the end of the incubation period, the concentration of DO was displayed as a function of time. The standard method for BOD determination is BOD_5, but 7 days of incubation was used to display the extended DO variation curve even though the BOD analysis would be calculated for 5 incubation days.

2.1.4.1 Procedure

All wastewater samples to be analyzed for BOD_5 were frozen from the time of sampling to preserve the microorganisms and prevent low BOD_5 values due to microbial degradation. The samples were defrosted and brought to room temperature before preparing the BOD_5 samples for the experiment.

The BOD samples were prepared according to Table 2.3, modified from BODTrak IITH User Manual (Hach Company, 2013). The range used for sample preparation was 0 to 700 mg O_2/L as influent wastewater samples with unknown BOD concentration typically fall within this range. Each BOD sample was prepared by adding 45 mL of wastewater sample at room temperature and 35 mL of seed (fresh wastewater) measured into a graduate cylinder, giving a total volume of 80 mL.

Table 2.3. Sample and seed volume required according to BOD range (Modified from Hach Standard Method)

BOD range (mg/L)	Sample volume (mL)	Seed volume (mL)	Final volume (mL)	Dilution factor
0 to 35	370	35	405	1.09
0 to 70	305	35	340	1.11
0 to 350	110	35	145	1.32
0 to 700	45	35	80	1.77

To ensure a representative BOD_5 test the wastewater sample was shaken thoroughly to achieve homogeneity before extraction. The seed was fresh raw wastewater collected from the holding tank the day of analysis, and was added to revive and ensure a sufficient amount of viable microorganisms after being frozen, and give representative BOD_5 results. The BOD sample was transferred from the graduate cylinder to a beaker for further preparation.

Oxidization of reduced forms of N was prevented by adding 1 mL of nitrification inhibitor N-Allylthiourea (ATU) per liter of prepared BOD sample. See appendix E, section E.5., for method used for preparation of ATU solution.

Temperature and pH of BOD sample was detected using sensION^{TM} PH3, with input accuracy ≤ 0.01. The temperature of the BOD sample was adjusted to 20 °C by warming the sample. pH was adjusted to pH 7 by adding 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH). Temperature of sample deviating from 20 ± 1°C may inhibit the microorganisms and give low BOD results. pH outside the range of 6 to 8 may influence the BOD results as well (User manual BODTrak IITH).

The prepared BOD_5 sample with temperature of 20 °C and pH 7 was moved from the beaker to a 300 mL BOD incubator bottle. Settling of biomass throughout the experiment was prevented by continuous mixing with a magnetic stirring bar. The incubator bottle was sealed by placing a plastic seal cup into the bottle neck. The seal cup contained two potassium hydroxide pellets (KOH) to absorb carbon dioxide (CO_2), which is a result of the oxidation process and can interfere with a measurement. The incubator bottle was placed on the BODTrak™ chassis. The bottle neck was greased, connected to the applicable tubes and sealed airtight by attaching the caps. The instrumental set up is illustrated in Figure 2.4.

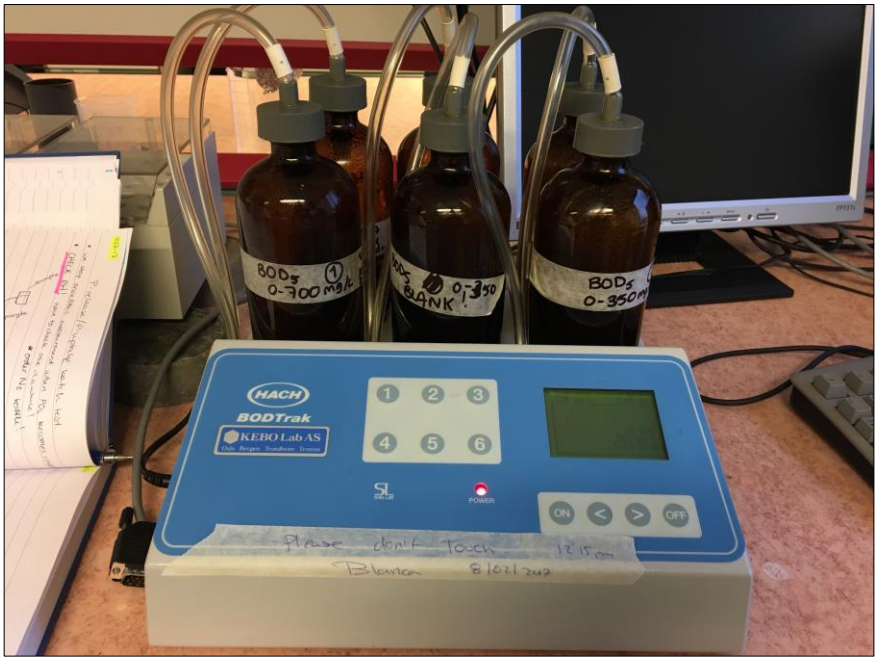


Figure 2.4. Experimental setup for BOD analysis of the wastewater using BODTrak™. The picture shows six incubator bottles with prepared BOD samples ready for analysis.

A seed blank BOD_5 sample was prepared in addition to the BOD_5 samples to correct the seeded BOD sample results. The seed blank was prepared by mixing 35 mL of seed, 45 mL of distilled water and adding a same nitrification inhibitor. The seed blank contained the same seed used for seeding the BOD samples. Temperature and pH was also corrected.

The BODTrak™ was programmed for the correct BOD range and test duration according to BODTrak II™ User Manual. The BOD range was set to 0 to 700 mg/L, and the duration was set to 7 days to analyze the complete curve illustrating the DO concentration. Ideally the BODTrak™ instrument with attached incubator bottles is kept in an incubator to ensure 20 ± 1°C for the entire duration of the experiment. An incubator was not available during the tests;

hence the experiment was conducted without incubation and in room temperature of approximately 20 °C.

2.1.4.2 Interpretation of results

After the experiment was completed, the results were presented as a curve for each of the BOD samples and seeded blank BOD sample illustrating the DO concentration as a function of incubation time. The results were extracted from the display by photographing the BODTrak™ display and digitalizing the curve using software Plot Digitizer. A polynomial trendline of third degree was fitted to the digitalized curve. By selecting 5 days of incubation as the x-value in the polynomial equation, the value for observed BOD_5 was calculated, see equation 1.

$$\boldsymbol{BOD_{5,obs} = a_1 x^3 + a_2 x^2 + a_3 x + a_4} \quad (1)$$

Where:

$BOD_{5,\,obs}$ = Observed BOD_5 concentration, mg/L

a_1, a_2, a_3, a_4 = non-zero value

x = duration of desired BOD analysis

The real BOD_5 value was calculated using equation 2. The observed BOD_5 must be corrected for the dilution factor which is based on the BOD range and was found in Table 2.3. In this experiment, the dilution factor was 1.77 as 45 mL of wastewater sample is diluted with 35 mL of seed. The observed BOD_5 value was therefore multiplied by 1.77. The real BOD_5 must be corrected for the added seed.

$$\boldsymbol{Real\ BOD_5 = BOD_{5,obs} * D - (BOD_{5,seed} * \frac{V_{seed}}{V_{wastewater}})} \quad (2)$$

Where:

Real BOD_5 = Real BOD_5 concentration of the wastewater, mg/L

D = Dilution factor

$BOD_{5,obs}$ = Observed BOD_5 after corrected with dilution factor, mg/L

$BOD_{5,\,seed}$ = BOD_5 concentration for seed, mg/L

V_{seed} = Volume of seed added to sample, mL

$V_{wastewater}$ = Volume of wastewater added to sample, mL

2.1.5 Determination of solid in influent wastewater

Characterization of biomass in the wastewater was performed by analyzing the amount of solid matter present in a wastewater sample. Solid matter refers to matter suspended or dissolved in water and consists of total solids (TS), total suspended solids (TSS), total dissolved solids (TDS) and volatile suspended solids (VSS). Biomass, or solids analysis is important for implementation of EBPR and all biological wastewater treatment processes as it is the key parameter in designing, modelling and operating (Henze et al., 2008).

The total solid is a measure of TDS and TSS in wastewater. Too high or low values of TS can reduce the efficiency of wastewater treatment plants. TS can vary widely, depending on weather, rainfall or snowmelt events, and is therefore an indicator on whether the wastewater is diluted or not.

Determination of TDS in wastewater indicates the collective content of all inorganic and organic substances. TDS is the solids that pass through when wastewater is filtered through a 2 µm filter. TSS analysis determines the quantity of material suspended in the wastewater. Decaying plants and animals are components that increase the TSS value. TDS is differentiated from TSS as TSS is the matter that cannot pass through a water filter with pores smaller than 2 µm.

Analysis of VSS determines the amount of organic solids in the wastewater. Determination of VSS make it possible to assess the amount of biologically organic matter, which is important when discussing the amount of organic matter available for PAOs. The greater the concentration of VSS, the stronger the wastewater is.

2.1.5.1 Procedure

Determination of all solids in wastewater were performed according to Standard Methods 209 A, C, D and E (American Public Health et al., 1960).

TS

80 mL of homogeneous wastewater sample was measured in a graduate cylinder, and added to a clean, pre-weighed evaporating porcelain dish. The sample was evaporated to dryness in a drying oven at 105 °C for 24 hours. The dish with dry wastewater sample was cooled in

desiccator to balance temperature and was then weighed. The value of TS was calculated using equation 3,

$$\boldsymbol{TS} = \frac{(\boldsymbol{W_{dried\ residue} - W_{dish}})}{\boldsymbol{V_{sample}}} \quad (3)$$

Where:

TS = Concentration of total solids, g/L

$W_{dried\ residue}$ = Weight of dried residue + dish, g

W_{dish} = Weight of dish, g

V_{sample} = Volume of sample, L

Total Suspended Solids and Total Dissolved Solids

80 mL of homogenous wastewater sample was measured in graduate cylinder. The measured sample was filtrated through a pre-weighed 1.2 µm pore size GF/C filter in the filtration setup, as illustrated in Figure 2.5. The filter was washed with MQ water with continuous suction to ensure filtration of all solids.

Figure 2.5. Experimental filtration setup for TDS, TSS and VSS analysis. Experiment was performed using suction filtration.

The portion of solids retained by the filter represents TSS. After complete drainage, the filter was carefully removed and transferred to an aluminum pre-weighed dish and placed in an oven

at 105 °C to dry. The dry filter in the aluminum dish was then cooled in a desiccator to balance temperature before weighing the filter with the retained solids. TSS value was calculate using equation 4,

$$\boldsymbol{TSS} = \frac{(\boldsymbol{W_{dried\ residue} - W_{blank\ filter,\ 105°C}})}{\boldsymbol{V_{sample}}} \quad (4)$$

Where:

TSS = Concentration of TSS, g/L

$W_{dried\ residue}$ = Weight of dried residue + dish, g

$W_{blank\ filter,\ 105\ °C}$ = Weight of blank filter after 105 °C, g

V_{sample} = Volume of sample, L

The portion of the wastewater sample that passed though the filter represents TDS. The total filtrate (with volume from washing) was transferred to a clean and pre-weighed evaporating porcelain dish and placed in an oven. The sample was evaporated to dryness at 105 °C for 24 hours. The dry evaporated sample was cooled in a desiccator and then weighed. The TDS value was calculated using equation 5,

$$\boldsymbol{TDS} = \frac{(\boldsymbol{W_{dried\ residue} - W_{dish}})}{\boldsymbol{V_{sample}}} \quad (5)$$

Where:

TDS = Concentration of TDS, g/L

$W_{dried\ residue}$ = Weight of dries residue and filter, g

W_{dish} = Weight of empty dish, g

V_{sample} = Volume of sample, L

Volatile Suspended Solids

Determination of VSS was performed by igniting the filter containing retained solids from wastewater sample (TSS) at 550 °C in muffle furnace for 30 minutes. The aluminum dish with the filter was cooled in desiccator to balance temperature before weighing. VSS value was calculating using equation 6,

$$\boldsymbol{VSS} = \frac{(\boldsymbol{W_{dried\ residue,105} - W_{dried\ residue,550}})}{\boldsymbol{V_{sample}}} \quad (6)$$

Where:

VSS = Concentration of VSS, g/L

$W_{dried\ residue,\ 105}$ = Weight of dries residue and filter after 105 °C, g

$W_{dried\ residue,\ 550}$ = Weight of dried residue and filter after 550 °C, g

V_{sample} = Volume of sample, L

2.1.5.2 Considerations

All solid determinations were performed in duplicates. The final solids concentration was the averaged concentration of the duplicates. To ensure representative values of TS, TDS, TSS and VSS, all evaporating porcelain dishes, aluminum dishes and filters were pre-weighed. Calculations of TSS and VSS was performed by determining the weight of a blank filter soaked in MQ water after 2 hours in oven at 105 °C and after 30 minutes in muffle furnace at 550 °C. The filters were cooled in desiccator and weighed.

2.2 Characterization of bio-P sludge

2.2.1 Bio-P sludge origin

Bio-P sludge used in this thesis was received from the Hias WWTP, and the characterization of the sludge was conducted immediately upon arrival to determine the initial sludge composition. See table B.1 in appendix B for all parameters and methods used in characterization of bio-P sludge.

The bio-P sludge from Hias is produced in an EBPR process using MBBR carriers, which gives the sludge certain characteristics that are different from activated sludge. As the bio-P sludge received is sludge that has detached from the carriers due to shear forces, the bio-P sludge is not necessarily as fresh or vital as activated sludge. In EBPR using activated sludge, the active part of the biomass is recycled back to the anaerobe phase to repeat the cycles, which is not the case for MBBR carries.

To thicken the bio-P sludge, a cationic organic polymer was added at Hias WWTP. The presence of the polymer may probably alter the sludge characteristics slightly. Therefore, in order to avoid possible interference of the polymer in the VSS determination, the bio-P sludge was shaken to a homogenous state before extracting sample to ensure a representative and equal amount of solids throughout all the experiments.

2.2.2 Determination of solids in bio-P sludge

Bio-P sludge is typically very concentrated and solid, which makes it difficult to determine solids according to Standard Methods 209 (American Public Health et al., 1960) due to clogging of filter. Determination of solids in bio-P sludge was therefore only conducted for TS and VSS using EPA Method 1684.

2.2.2.1 Procedure

TS

25 - 50 g homogenous bio-sludge sample was measured using an analytical balance, and added to a clean, pre-weighed evaporating porcelain dish. The samples were evaporated to dryness in a drying oven at 105 °C for 24 hours. The dish with dry bio-sludge sample was cooled in desiccator to balance temperature and was then weighed. Dried samples were weighed quickly to prevent absorption of moisture into the samples when exposed to air. The percentage and the

mg solids/kg sludge of TS in the bio-sludge was calculated using equation 7 and equation 8 accordingly,

$$\boldsymbol{Percentage\ TS = \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} * 100} \quad (7)$$

$$\boldsymbol{W_{TS} = \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} * 1,000,000} \quad (8)$$

Where:

Percentage TS = The percentage of TS in bio-P sludge, %

W_{TS} = Weight of TS in bio-P sludge, mg TS/kg bio-P sludge

W_{total} = Weight of dried residue and dish after 105 °C, g

W_{dish} = Weight of dish, g

W_{sample} = Weight of wet sample and dish, g

VSS

The evaporating porcelain dishes containing the dried residues from TS determination was transferred to a muffled furnace and ignited at 550 °C for 30 minutes. The residue was cooled in a desiccator to balance the temperature and was weighed. The percentage and the mg solids/kg sludge of VSS in the bio-sludge was calculated using equation 9 and equation 10 accordingly,

$$\boldsymbol{Percentage\ VSS = \frac{W_{total} - W_{dried\ residue}}{W_{total} - W_{dish}} * 100} \quad (9)$$

$$\boldsymbol{W_{VSS} = \frac{W_{total} - W_{volatile}}{W_{total} - W_{dish}} * 1,000,000} \quad (10)$$

Where:

Percentage VSS = The percentage of VSS in bio-P sludge, %

W_{VSS} = Weight of VSS in bio-P sludge, mg VSS/kg bio-P sludge

W_{total} = Weight of dried residue and dish after 105 °C (TS), g

$W_{dried\ residue}$ = Weight of dried residue and dish after 550 °C, g

W_{dish} = Weight of dish, g

2.3 Batch experiments

Batch experiments were performed to obtain information about the bio-P activity in the wastewater and to assess the potential for EBPR in the Lerkendal wastewater. The experiments were performed under anaerobe, aerobe and anoxic conditions using real bio-P sludge from Hias WWTP which was characterized previous to the experiments. Loosdrecht et al. (2016) recommends that all batch experiments with activated sludge should be performed with a mixed liquor volatile suspended solids (MLVSS) concentration of 2000 mg/L. The bio-P sludge from EBPR MBBR process (Hias) was therefore diluted to a concentration of 2000 mg MLVSS/L to mimic the concentration of MLVSS in an activated sludge configuration.

2.3.1 Experimental setup

All batch experiments were executed in bioreactors consisting of 1 L beakers with airtight lids, magnetic bar and magnetic mixer at 150 revolutions per minute (rpm) to ensure homogenous and representative samples. The bioreactor was equipped with a portable pH meter sensION^{TM} PH3 and oxygen meter WTW Oxi 3315 for monitoring pH and dissolved oxygen concentration during the experiments.

Table 2.4 presents the batch experiments performed and the conditions the experiments were exposed to. Going forward the experiments will be referred to as listed in the table (Experiments 1, 2A, 2B, 3A, 3B, 4 and 5).

Table 2.4. Overview of the batch experiments performed. Table shows the different experiments performed and the experimental name that will be used onward in the thesis, sample preparation, the amount of acetate added to the experiment in t = 0, and the experimental conditions the experiment was exposed to.

Experiment	Sample preparation	Acetate dosage (mg sCOD/L)	Experiment conditions
1	353 mL bio-P + 647 mL MQ water	300	Anaerobe
2A	353 mL bio-P + 647 mL MQ water	100	Anaerobe – Aerobe
2B	353 mL bio-P + 647 mL MQ water	100	Anaerobe – Anoxic
3A	353 mL bio-P + 647 mL wastewater	-	Anaerobe – Aerobe
3B	353 mL bio-P + 647 mL wastewater	-	Anaerobe – Anoxic
4	353 mL bio-P + 647 mL wastewater	300	Anaerobe – Aerobe
5	353 mL bio-P + 647 MQ water	300	Anaerobe – Aerobe

Batch experiments referred to with letter A and B (2A and 2B, 3A and 3B) were performed in parallel. Experimental setup for batch experiments in parallel is illustrated in Figure 2.6. The setup is representative for all batch experiments performed, but without the parallel when anaerobe – anoxic conditions were not tested.

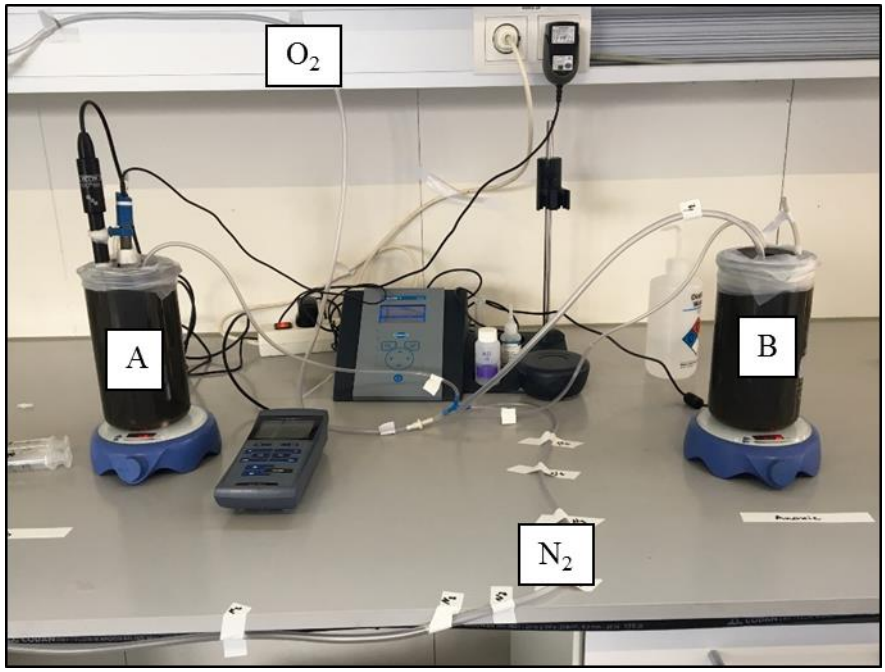


Figure 2.6. Experimental setup for batch experiments performed in parallel. Experiment A (left) was exposed to anaerobe – aerobe conditions and experiment A (right) was exposed to anaerobe – anoxic conditions. pH electrode and DO electrode was switched between the ongoing experiments. Beakers are seal airtight with homemade lids and parafilm.

2.3.2 Procedures

Batch experiments were performed based on the protocols of Loosdrecht et al. (2016) and Janssen et al. (2002). Prior to execution of the experiments, the samples were prepared by collecting 353 mL of bio-P sludge from the sludge container and transferring it to the bioreactor where it was diluted with MQ water or wastewater according to the experiment performed (Table 2.4). This ensured a MLVSS concentration of approximately 2000 mg/L. The prepared sample in the bioreactor was measured for pH and DO. All experiments were conducted at the constant pH of 7.5 and room temperature. pH was corrected by dosing 1 M HCl and 1 M NaOH to the sludge mixture. DO levels in the aerobic zone was also recorded to ensure an DO concentration above 5 mg/L for aerobe conditions and below 0.018 mg/L for anaerobe conditions. Temperature and pH in bioreactor was observed and recorded continuously to

ensure the correct values during all batch experiments. The samples were aerated for 30 minutes to reach an initial DO concentration higher than 5 mg/L before initiating the batch experiments.

Batch experiments 1, 2A and 2B were performed to obtain information about the bio-P kinetics in the sludge when the bio-P sludge was mixed with 647 mL of MQ water and external acetate was dosed to the system. Batch experiments 3A and 3B investigated the bio-P kinetics in the wastewater when 353 mL of bio-P sludge was mixed with 647 mL of wastewater without any additional acetate. Experiment 4 investigated the bio-P kinetics of the wastewater when 353 mL of bio-P sludge was mixed with 647 mL of wastewater and dosed with additional acetate. Experiment 5 determined the bio-P kinetics of 15-day old bio-P sludge mixed with 647 mL MQ water and additional acetate dosage identical to experiment 2A as there was suspicion of inactive PAOs at this time.

The following is a description of the procedures used for all the batch experiments performed. The procedures are explained individually, but some of the procedures are similar if not identical. The activity in the experiments was tracked by using Hach Dr. Lange cuvette tests and determination of TS and VSS, and the parameters analyzed are mentioned in the procedure. TS and VSS were determined before starting the experiments and after ending the experiments. TS and VSS were performed in duplicates.

2.3.2.1 Experiment 1

Prior to start of experiment 1 the bioreactor was filled with 1 L prepared sludge mixture with MQ water. Samples were collected at t = 0 (after aeration but before acetate dosing) to determine the initial concentration of TN, NO_3-N, NO_2-N, NH_4-N, TP, PO_4-P, tCOD, sCOD.

The experiment was initiated by dosing an excess of acetate (approximately 300 mg sCOD) to the sludge mixture. Acetate was dosed in excess to determine the PAOs maximum P release capacity. Samples were collected at t = 10, 20, 30, 60, 120 minutes, and analyzed for PO_4-P and sCOD until maximum PO_4-P release was reached. Sample t = 120 minutes was determined for all parameters (TN, NO_3-N, NO_2-N, NH_4-N, TP, PO_4-P, tCOD, sCOD).

The pH of the sludge mixture was constantly measured and corrected during the experiment. If necessary the sludge mixture was purged with nitrogen gas to avoid oxygen from disturbing the PAOs metabolisms under anaerobic conditions. All samples for determination of NO_3-N, NO_2-

N, NH_4-N, PO_4-P and sCOD were directly filtrated through 0.45 µm cellulose and nitrate filter (Sartorius). Hach Dr. Lange LCK cuvette tests and DR 3900^{TM} portable spectrophotometer were used for the analysis of the cuvette tests.

2.3.2.2 Experiment 2A

Prior to experiment 2A the bioreactor was filled with 1 L prepared sludge mixture with MQ water. The experiment started by repeating experiment 1, but adding a required amount of acetate instead of acetate in excess. The required amount of acetate was based on a P release/P uptake rate of 0.4 mg/P mg acetate COD (Janssen et al., 2002).

After t = 120 minutes the anaerobic phase ended by initiating aerobic phase by supplying the bioreactor with compressed air. DO concentration was kept above 5 mg/L for the duration of the experiment using oxygen meter.

To determine the aerobic kinetic parameters, samples for determination of sCOD and PO_4-P was collected at t = 120, 130, 140, 150, 180 and 240 minutes. Samples at t = 120, 130 and 140 minutes were analyzed for NO_3-N, NO_2-N and NH_4-N to determine the presence of DNPAOs. Sample t = 240 minutes was determined for all parameters (TN, NO_3-N, NO_2-N, NH_4-N, TP, PO_4-P, tCOD, sCOD). The samples were prepared and parameters were determined as experiment 1.

2.3.2.3 Experiment 2B

Prior to start of experiment 2B the bioreactor was filled with 1 L prepared sludge mixture with MQ water. The experiment started by repeating experiment 1, but adding a required amount of acetate instead of acetate in excess. The required amount of acetate was based on a P release/P uptake rate of 0.4 mg/P mg acetate COD.

After t = 120 minutes the anaerobic phase ended, and the anoxic phase was initiated by dosing the sludge mixture with approximately 10 mg NO_3-N. To ensure that no oxygen disturbed the bio-P metabolism the bioreactor was purged with nitrogen gas.

To determine the anoxic kinetic parameters, samples for determination of $_S$COD and PO_4-P was collected at t = 120, 130, 140, 150, 180 and 240 minutes. Samples at t = 120, 130 and 140

minutes were analyzed for NO_3-N, NO_2-N and NH_4-N to determine the presence of DNPAOs. Sample t = 240 minutes was determined for all parameters (TN, NO_3-N, NO_2-N, NH_4-N, TP, PO_4-P, tCOD, sCOD). The samples were prepared and parameters were determined as experiment 1.

2.3.2.4 Experiment 3A

Prior to start of experiment 3A, the bioreactor was filled with 1 L prepared sludge mixture with fresh wastewater. The experimental procedure was identical to experiment 2A, except for acetate dosage. Acetate is not dosed in this experiment as the natural occurring bio-P kinetics of the wastewater was of interest. See experiment 2A for procedure.

2.3.2.5 Experiment 3B

Prior to start of experiment 3B, the bioreactor was filled with 1 L prepared sludge mixture with fresh wastewater. The experimental procedure was identical to experiment 2B, except for acetate dosage. Acetate is not dosed in this experiment as the natural occurring bio-P kinetics of the wastewater was of interest. See experiment 2B for procedure.

2.3.2.6 Experiment 4

Prior to start of experiment 4, the bioreactor was filled with 1 L prepared sludge mixture with fresh wastewater and acetate. The experimental procedure was identical to experiment 2A, including amount of acetate dosed to the system. See experiment 2A for procedure.

2.3.2.7 Experiment 5

Experimental procedure for experiment 5 was identical to experiment 2A. This experiment was performed to compare the initial activity in bio-P sludge to 15-day old bio-P sludge. The experiment revealed any changes in the bio-P sludge kinetics.

2.3.3 Considerations

Depending on the amount of sample extracted from the sample mixture during the batch experiment, the total volume will decrease. It is recommended to optimize for a remaining volume of approximately 90 % of the initial volume as all parameters are measured as mg per liter of sample. A significant reduction in sample volume will therefore give unreliable and incomparable results.

2.3.4 Sludge maintenance

The batch experiments were performed over a period of 15 days. PAOs in bio-P sludge are dependent on alternating anaerobic and aerobic conditions as well as VFA to survive. To keep the bio-P sludge and PAOs active during the 15 days, an automatic timer was installed to aerate the bio-P sludge to ensure aerobic conditions and stop aeration to create anaerobe conditions in three cycles. Mechanical mixing was installed to avoid biomass from settling. The setup is illustrated in Figure 2.7.

Figure 2.7. Experimental setup for maintenance of bio-P sludge during the experimental period. Mechanical mixer is emerged into container holding 25 L of bio-P sludge to prevent settling. Automatic timer is installed to ensure alternating anaerobe and aerobe conditions. Bio-P sludge is fed with acetate three times a day.

For providing the PAOs with the VFA they need for survival and bacteria growth, 30 mL of acetate solution equivalent to 15 mg acetate/L was injected to the bio-P sludge at the beginning of each anaerobic phase according to a planned schedule, as presented in Figure 2.8.

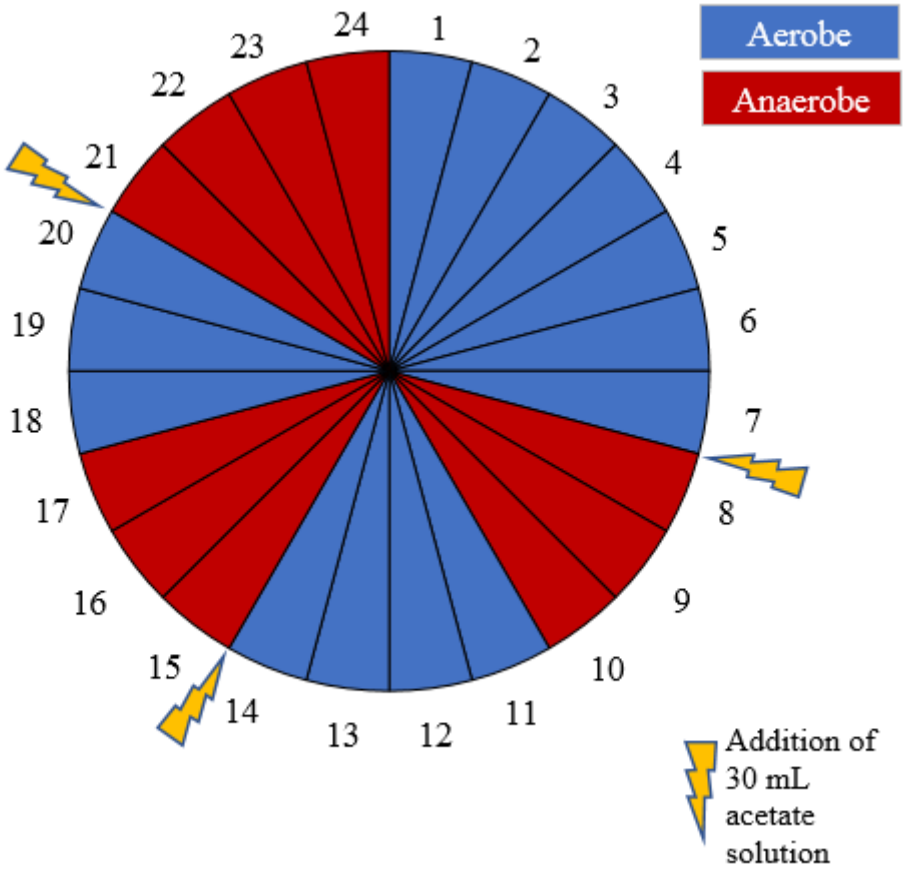


Figure 2.8. Schedule for bio-P sludge maintenance during the 15 days of experiments. Diagram is divided into 24 hours. Red part of the diagram indicates anaerobe conditions, while blue part indicate aerobe conditions. Yellow lightning indicates when 30 mL of acetate solution was fed to the sludge container.

2.4 Source of error in the experimental methods

2.4.1 Instrument resolution

All instruments have finite precision that limits the ability to resolve small measurement differences. Each Hach Dr. Lange cuvette has a measuring range that is the maximum and minimum detectable values the instrument can give accurate results for. If LCK 348 is used for measuring PO_4-P concentration that is expected to be 0 mg PO_4-P/L, then the device would not be able to give this result. This means that the analysis would indicate that there still is a concentration of PO_4-P in the solution, even though the correct result is that there is no concentration of PO_4-P.

2.4.2 Physical variation

Ideally, experiments should always be performed multiple times to reveal trends and deviations that might otherwise go undetected. For characterization of the wastewater, multiple tests were performed for almost all the analysis, where they were combined to find an average value. The batch experiments were not performed multiple times, which makes it difficult to conclude if results show trends and/or deviations. Results that deviate from the normal trend should be closer examined, but since batch experiments are ongoing experiments it is not possible to retrieve the sample once more.

Sampling of wastewater is challenging due to the variation in flow and component concentrations. The sample taken will represent the concentration at that time, and is not a representation of the wastewater as a whole as the results can vary considerably on a daily and hourly basis. The best representation is obtained when characterizing of the wastewater is performed over a longer period where several parallel tests are conducted.

2.4.3 Sampling deviation

Collecting samples from the holding tank does not guarantee a homogenous sample. Several conditions such as settling of biomass, the retention time of the wastewater or variation in flow can give varying results even though the samples are taken at the same time. It is not possible to ensure the same amount of solids or bacteria in all samples when measuring out a volume. This can give deviating results in the parameters as large particles will give the impression of a higher concentration of certain parameters that are not filtrated such as solids, TN, TP or tCOD. For this reason, we consider average values.

2.4.4 Incomplete definition

Measurements are not always clearly defined. Two people may measure the same volume, but have different results because each person may have a different definition of measurements. Other possible errors are contamination in pipet, and leaving droplets in the walls that cause a smaller amount of solution to be delivered. This error was minimized by ensuring that all pipettes used were new and not contaminated, as well as using the same person for measuring out and preparing sample when possible.

3 Results and discussion

3.1 Lerkendal wastewater; characteristics and variations

One of the aims of the master thesis work was to characterize and investigate the composition of a local and presumably typical Norwegian wastewater, for assessing the potential for biological phosphorus removal using EBPR. The concentration of substances in wastewater varies with time. The characterization of hourly and daily concentration of substances give a good basis for an evaluation of the overall composition of wastewater. Using the methods and materials that were previously presented, hourly and daily variation of the Lerkendal wastewater was characterized to assess the composition, trends, deviations and condition in relation to typical Norwegian wastewaters and the theoretically necessary compounds for implementing EBPR.

3.1.1 Overview of hourly variation

The samples analyzed for a full day wastewater characterization consists of the real-time wastewater as the holding tank was equipped with a constant consumption that emptied the holding tank completely before in-pumping of new wastewater. Composite samples were therefore avoided and the results made it possible to detect concentration peaks as well as a representative presentation of the wastewater composition for 24 hours. Figure 3.1 presents all parameters analyzed (with the exception of TS, TDS and TSS) assembled for hourly variation.

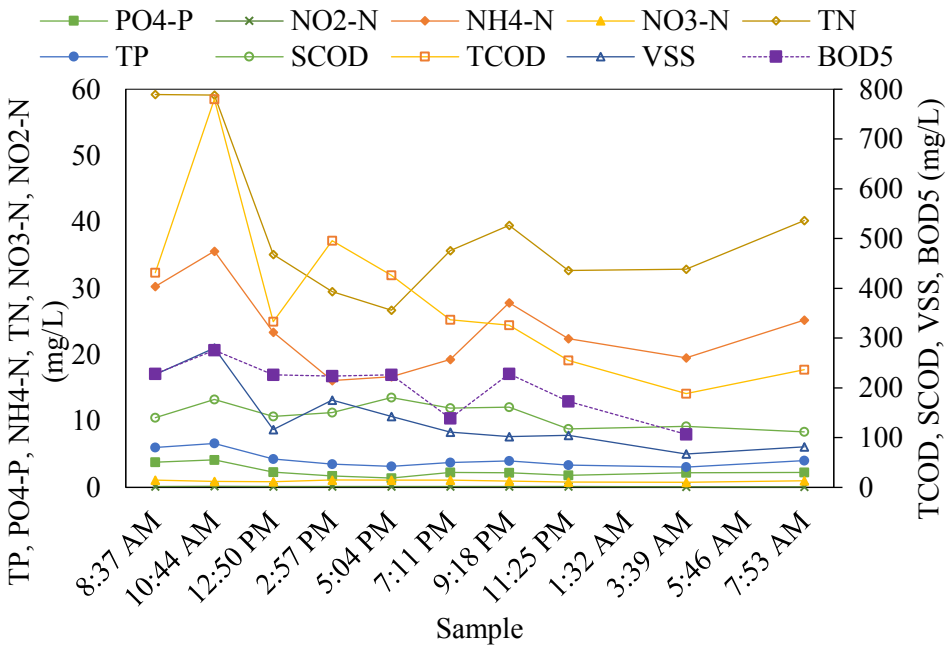


Figure 3.1. Overview of all parameters analyzed for hourly variation of wastewater composition. Only VSS is represented for solids.

The analysis shows an hourly variation in substance concentration, where a prominent peak is detected at 10:44 for tCOD, NH_4-N, TN and VSS. According to literature, this was to be expected due to inhabitant's general daily habits (Ødegaard et al., 2014). In theory, the peak concentration usually occurs around 6 - 8 am, which indicates that there is a delay in the presented results. This is presumably caused by the transport system configuration and subsequent in-pumping schedule.

Figure 3.1 indicate a diurnal pattern for the hourly variation of NH_4-N. This trend is associated with the knowledge that urine is the main source of NH_4-N. The increase in TN is in relation to the trend of NH_4-N as TN is the sum of all nitrogen (NH_4-N, NO_3-N, NO_2-N, organic and reduced nitrogen).

tCOD include both particulate and soluble COD. As it is an analysis for measuring the oxidation potential for the organic material in the wastewater, while VSS is a measure for the biological organic material in the wastewater, also known as the volatile components to successfully be digested under anaerobe conditions - it is not surprising that these parameters follow the same diurnal trend. The interesting observation is the trend of sCOD which was not variating on an hourly basis in the wastewater. It is interesting how it is consistent and does not follow the diurnal trend.

It is not expected to detect a prominent peak in the evening in the general concentration of the wastewater. This is probably related to a natural discrepancy related to inhabitant's nightly routines. It is therefore assumed that the consumption in the evening is spread over a larger timeframe compared to in the morning when most people will leave for their daily activities at a more concurrent time. The community producing the incoming wastewater is relatively small compared to what is referred to in literature, therefore it cannot be expected that the peaks and variation will be immense. However, the 9:18 pm analysis indicate that the NH_4-N and TN concentration is following the diurnal trend as discussed.

3.1.2 Overview of daily variation

Analysis of the daily variation in wastewater composition was based on samples taken at 9 am the four days the analyzes were performed. As there was no continuous consumption of the wastewater in the holding tank, the samples constitute composite samples due to mixing of influent wastewater and pre-existing wastewater during the entire day. The results are comparable as the samples were taken at the same time each day. Figure 3.2 shows all parameters analyzed (with the exception of TS, TDS and TSS) assembled for daily variation.

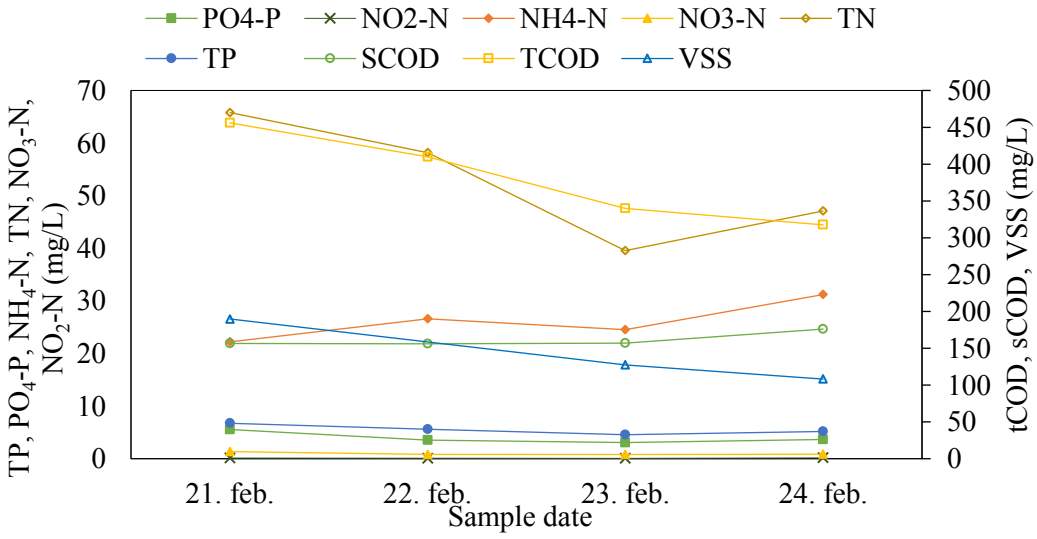


Figure 3.2. Overview of all parameters analyzed for daily variation of wastewater composition. Only VSS is represented for solids.

The analysis indicates that a decreasing trend is occurring on the 23rd of February for parameters TN, tCOD and VSS. The concentration of substances in the wastewater is a combination of pollutant load and the amount of water which the pollutant is mixed with (Henze et al., 2008).

The composition of the composite wastewater samples must therefore be seen in context with the precipitation that occurred that day to be able to evaluate if the compounds are vulnerable to dilution. During the characterization period the weather was typical for Norwegian winter and spring, which means high precipitation and varying temperature. Figure 3.3 presents the precipitation and temperature recorded during the week the wastewater was analyzed.

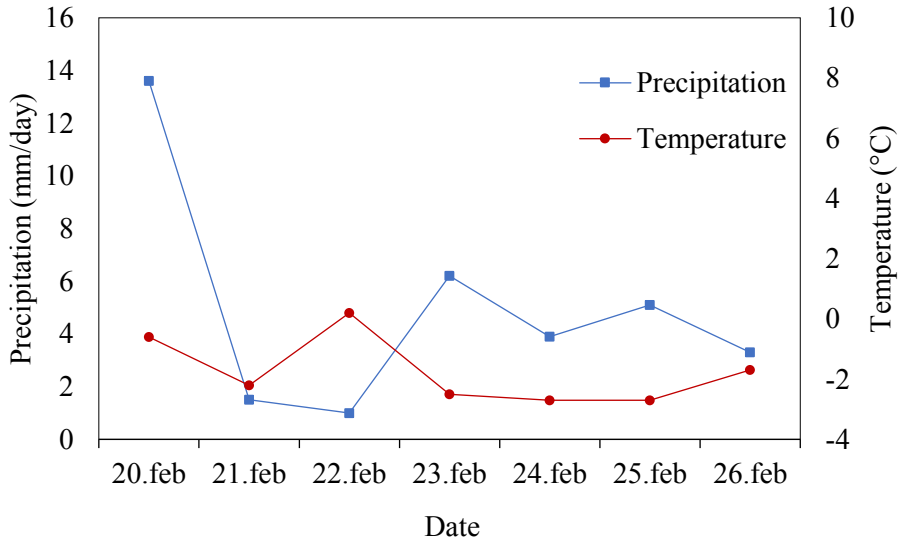


Figure 3.3. Precipitation and temperature during week of daily characterization. Precipitation in mm/day and temperature in °C.

The average temperature of the week analyzed was -1.7 °C, which indicates that the precipitation would most likely accumulate. On the 22nd of February, there occurred an increase in temperature to 0.2 °C, which results in ablation and subsequent high runoff effected by the large amount of accumulated precipitation. The trend in TN, tCOD and VSS is therefore not surprising as the wastewater sample taken in the morning of the 23rd of February is most likely subjected to a large amount of runoff and therefore can be showing signs of vulnerability to external water infiltration. The quantities of runoff and infiltration of stormwater will depend on the configuration of sewage system (combined or separate).

3.1.3 In-depth analyzation of Lerkendal wastewater composition

The composition of the Lerkendal wastewater and subsequent assessment of the wastewater condition is based on comparing the results from the hourly and daily variation in Table 3.1. BOD_5 was not determined for daily variation of the wastewater.

Table 3.1. Comparing average results from hourly and daily wastewater characterization. Includes standard deviation.

Parameter	Hourly average	Daily average
TS (g/L)	0.47 ± 0.11	0.44 ± 0.04
TSS (g/L)	0.20 ± 0.09	0.13 ± 0.04
TDS (g/L)	0.30 ± 0.03	0.30 ± 0.04
VSS (g/L)	0.17 ± 0.08	0.14 ± 0.03
tCOD (mg/L)	380.90 ± 161.55	380.88 ± 55.18
sCOD (mg/L)	146.25 ± 20.65	161.38 ± 8.45
TN (mg/L)	39.06 ± 11.34	52.65 ± 10.07
NH_4-N (mg/L)	26.63 ± 6.21	26.13 ± 3.32
NO_3-N (mg/L)	0.97 ± 0.13	0.97 ± 0.22
NO_2-N (mg/L)	0.13 ± 0.02	0.12 ± 0.03
TP (mg/L)	4.18 ± 1.20	5.53 ± 0.79
PO_4-P (mg/L)	2.83 ± 0.96	3.97 ± 0.94
BOD_5 (mg/L)	202.92 ± 49.90	-
pH	7.79 ± 0.42	7.94 ± 0.26

The comparison analysis indicate that the analyzed parameters are consistent for the Lerkendal wastewater as the values are reasonably similar. The comparison shows that almost all the parameters analyzed for hourly variation (except NO_3-N and NO_2-N) have a higher standard deviation than the daily characterized parameters. This indicates that the compounds in the wastewater is not affected immensely by the runoff, which can mean that the wastewater does not receive an excessive external amount of stormwater. A lower standard deviation was expected for the daily variation as the samples were composite of the entire day and the results will represent "a gliding average" of the daily wastewater composition, and not give large detectable deviations.

The TSS for daily characterization was found to be lower than VSS. This analytical error is presumably caused by inaccurate measurements as the samples were not ensured constant weight by differing results of less than 4 %. VSS cannot be higher than TSS as VSS constitutes the amount of TSS that is volatile.

See table C.1 in appendix C for all results from hourly characterization, and table D.1 in appendix D for all results from daily characterization of the Lerkendal wastewater.

The average composition of the Lerkendal wastewater is further discussed in the following subchapters by dividing the substances into organic material and biomass, and nutrients.

3.1.3.1 Organic matter and biomass

BOD represents the organic contaminants in the wastewater, either as soluble or particulate, by measuring the oxygen used for oxidation of the organic material. The standard method for BOD analysis is BOD_5, and the results from the BOD_5 analysis is presented in Figure 3.4 as hourly variation.

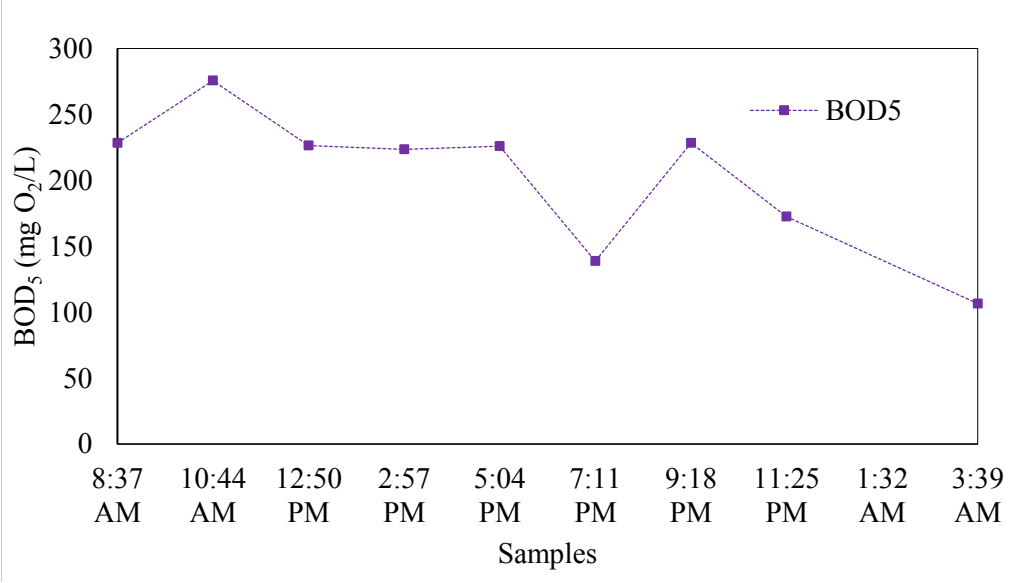


Figure 3.4. Variation of BOD_5 for all samples analyzed for hourly variation, represented in mg O_2/L.

The results from the analysis indicate that the highest oxygen demand occurred in the 10:44 am sample, where the value is 275.58 mg O_2/L. This indicates that the amount of biodegradable organic matter available in the wastewater is highest in the morning. This is to be expected as human waste is the main contributor to organic material. This corresponds to the observation of the lowest BOD_5 value of 106.55 mg O_2/L, which is detected in sample 3:39 am where the human activity is low.

BOD_5 was calculated from the BOD analysis presented in Figure 3.5. See appendix E, section E.3 for an example of how to calculate BOD_5 from the BOD analysis used, as well as table E.2

for a complete table of all calculated BOD_5 including fitted polynomial equations, observed BOD_5 values and corrected BOD_5 values.

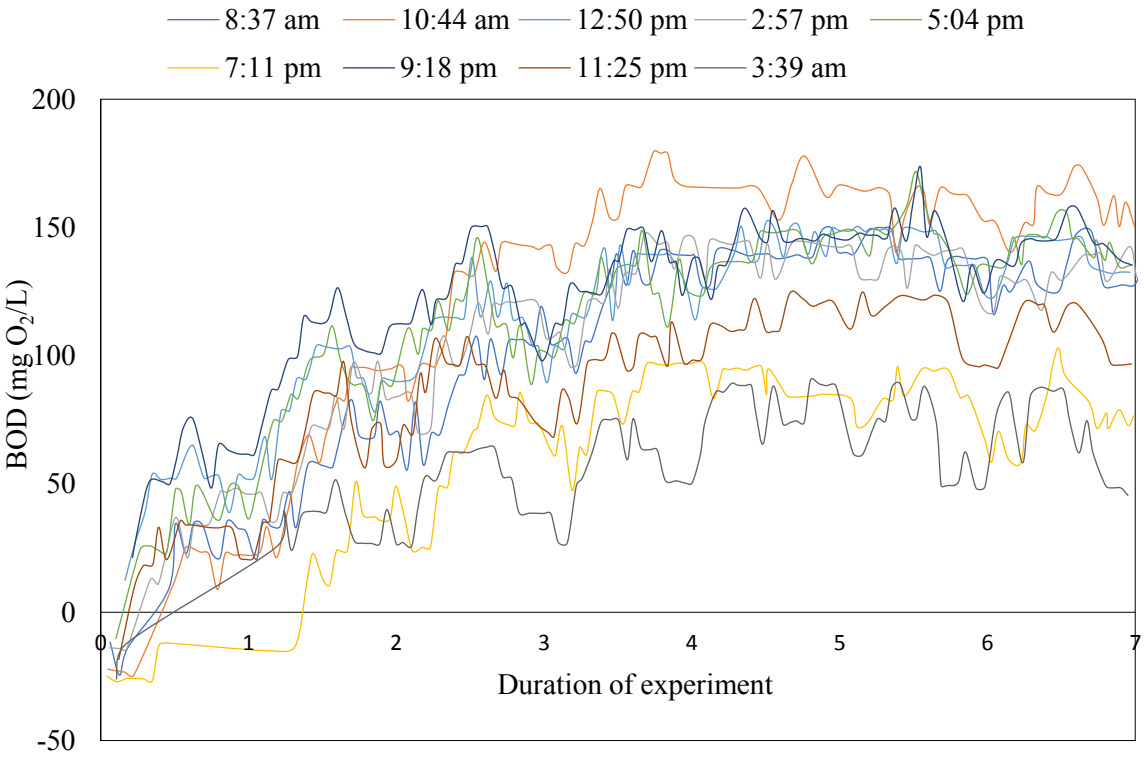


Figure 3.5. Development of BOD for all samples analyzed for hourly variation of BOD in the wastewater.

In theory, BOD gives a lot of information about the potential for EBPR. However, some BOD is particulate and will be removed before entering a biological treatment step. This means that a part of the measured BOD is not biologically available within the normal retention time of a few hours, therefore the results are not directly representable.

A disadvantage of BOD testing is the time-consuming aspect of the method. As BOD analysis measures the oxygen used for oxidation of a portion of the organic matter, further analysis can be performed to determine biodegradable organic matter in wastewater. COD is a measure for the chemical oxygen demand, and is referred to as tCOD when discussing the total influent COD concentration. sCOD is measured to further determine the concentration of soluble organic matter in the wastewater. Figure 3.6 shows total, particulate and soluble COD for a) daily variation and b) hourly variation.

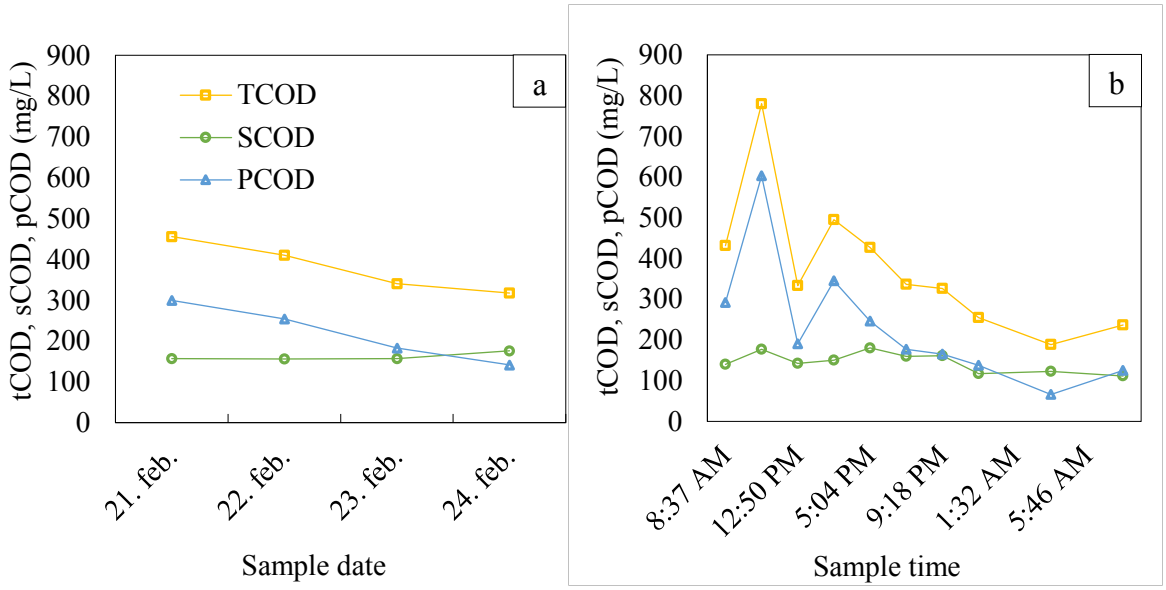


Figure 3.6. Variation of tCOD, sCOD and pCOD for a) daily and b) hourly wastewater characterization in mg/L.

The average value for hourly and daily sCOD concentration is 146.25 ± 20.65 mg/L and 161.38 ± 8.45 mg/L, respectively. It is interesting to observe that the sCOD concentration is approximately the same and consistent for both characterizations, even when there is a definite decrease (figure 3.6a) and diurnal variation (figure 3.6b) in other related parameters. This emphasizes the consistency and stability of the wastewater in terms of sCOD even if there are large amounts of rainfall, difference in sampling techniques or different time of sampling. This is a promising observation as the BSCOD presumably also is consistent.

Some organics measured in COD tests are resistant to biological oxidation. This results in a higher COD concentration compared to BOD. In typical diluted wastewaters, the ratio of BOD:COD is between 0.3 and 0.8. Based on the results from characterization the BOD:COD ratio in the Lerkendal wastewater is 0.53. This indicates that the organic material in the wastewater is considered easily treatable by biological means (Henze et al., 2008).

Total solids are all the solids present in wastewater, both suspended and dissolved. In EBPR it is relevant to know the amount of biological organic matter (VSS) as this is the amount of biological organic material present in the wastewater or biomass (Henze et al., 2008). When discussing activity related to EBPR, all parameters are presented per gram VSS, which in theory means per gram organic material. Figure 3.7 shows the variation in VSS present in the wastewater for both analysis, as well as TS, TDS and TSS.

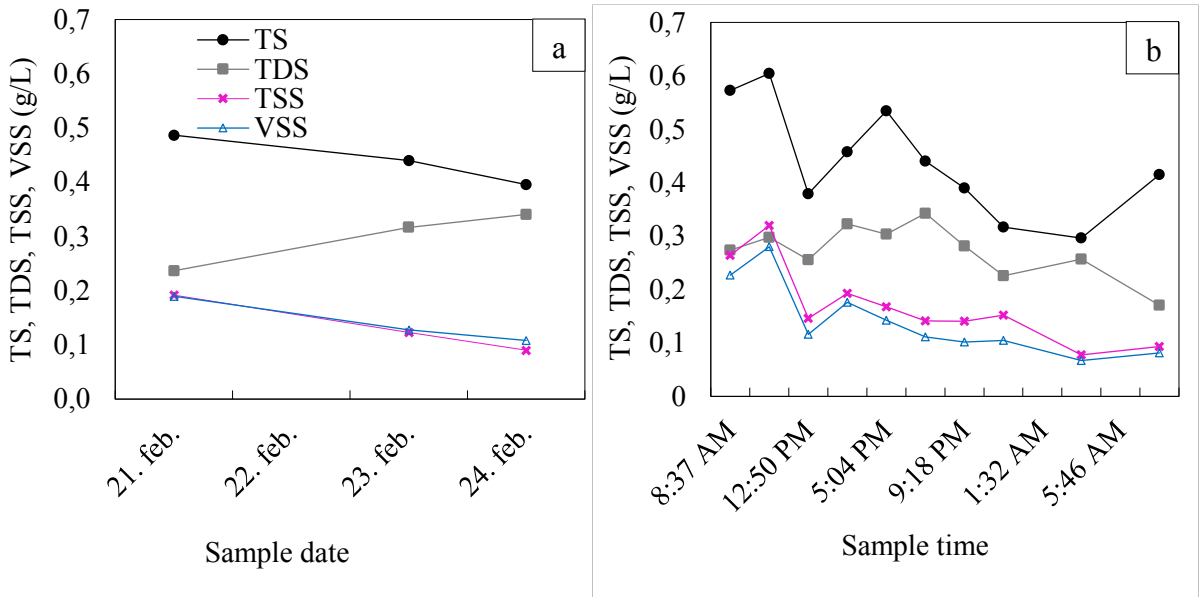


Figure 3.7. Variation of TS, TDS, TSS and VSS for a) daily and b) hourly wastewater characterization in g/L.

For both analysis TSS and VSS are approximately identical (figure 3.7a and figure 3.7b). The percentage of TSS that is VSS is a general measure of the fraction of the TSS that is associated with organic material. According to Henze et al (2008), a high percentage of VSS means that there is a high amount of solids that can be successfully digested under anaerobic conditions. The typical ratio of TSS:VSS in diluted wastewater is typically 0.4 – 0.6. The concentration of TSS and VSS is on average 0.20 and 0.17 and 0.13 and 0.14 g/L, respectively. As discussed previous, the TSS values cannot be lower than the VSS, therefore the hourly variation is used for determining the TSS:VSS ratio. The ratio is found to be 0.85, which actually indicates that the Lerkendal wastewater is concentrated and that the organic material has a high potential for successful digestion under anaerobe conditions.

3.1.3.2 Nutrients

The fraction of nutrients in the wastewater has influence on the treatment options for the wastewater. Since most nutrients are normally soluble, they cannot be removed by mechanical removal processes such as settling or filtration (Henze et al., 2008). The distribution between soluble and suspended nutrients is therefore of importance in relation to the characterization of wastewater.

Phosphorus

As the Lerkendal wastewater is to be assessed for the possibility of EBPR, it is important to evaluate the presence of P. Measuring TP estimates the total amount of phosphate in the

wastewater, both particulate and soluble. The soluble phosphate present in the wastewater is a measure for PO_4-P available for uptake by the PAOs. A low PO_4-P concentration will equal a low P uptake rate as there is little PO_4-P to be consumed by the PAOs under aerobe conditions. Figure 3.8a show that TP and PO_4-P follow the same curvature, which indicates that the relationship between TP and PO_4-P is consistent.

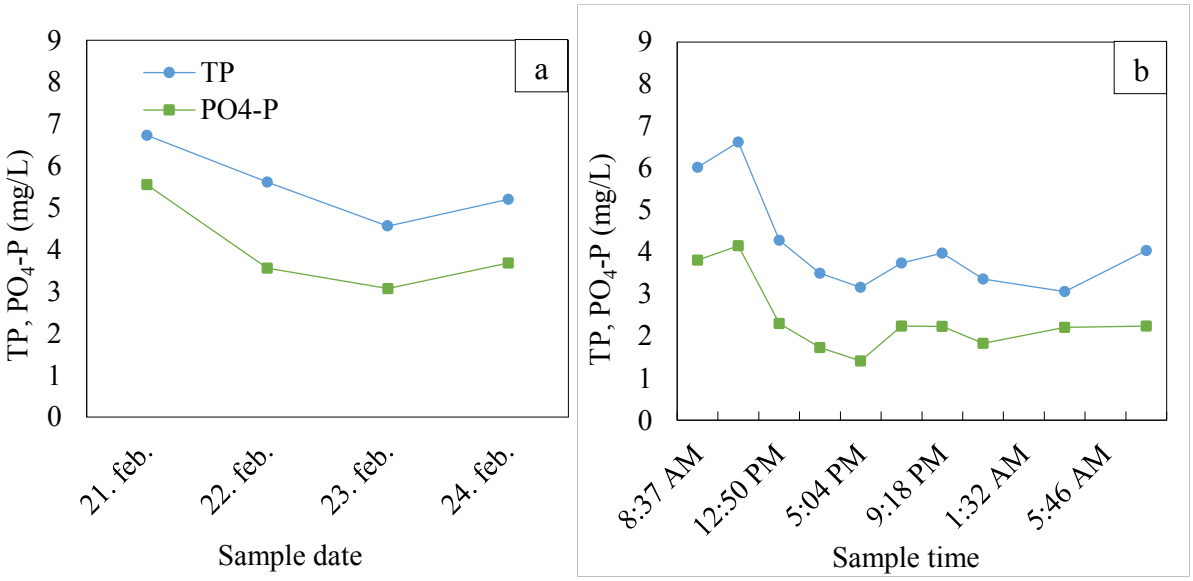


Figure 3.8. Variation of TP and PO_4-P for a) daily and b) hourly wastewater characterization in mg/L

The hourly variation (Figure 3.8b) shows a prominent peak at 10:44 am for both TP and PO_4-P. This is to be expected as the main source of phosphorus is human waste, which at this time will be concentrated due to human daily habits. Last analysis (7:53 am) show that concentrations of both PO_4-P and TP is increasing to form a second peak due to people's morning routines. The average hourly and daily concentration of PO_4-P is 2.83 mg/L and 3.97, respectively. The average ratio between TP and PO_4-P is between 0.58 – 0.72, which shows that the amount of soluble phosphate in the Lerkendal wastewater can potentially be more than 50 % of the total phosphates. This indicates that if particulate P is removed from the wastewater due to pre-treatment, there is a high amount of PO_4-P left in the liquid phase that is available for P removal by EBPR.

Nitrogen

The presence of nitrogen in the wastewater can indicate the need for nitrogen removal as a high total nitrogen concentration can have an unfortunate effect on the environment such as eutrophication. Total nitrogen in wastewater constitutes the fraction of NH_4-N, NO_3-N, NO_2-N

and organic N. High concentration of NO_3-N in the wastewater is not desired when applying EBPR as NO_3-N inhibits the uptake of P.

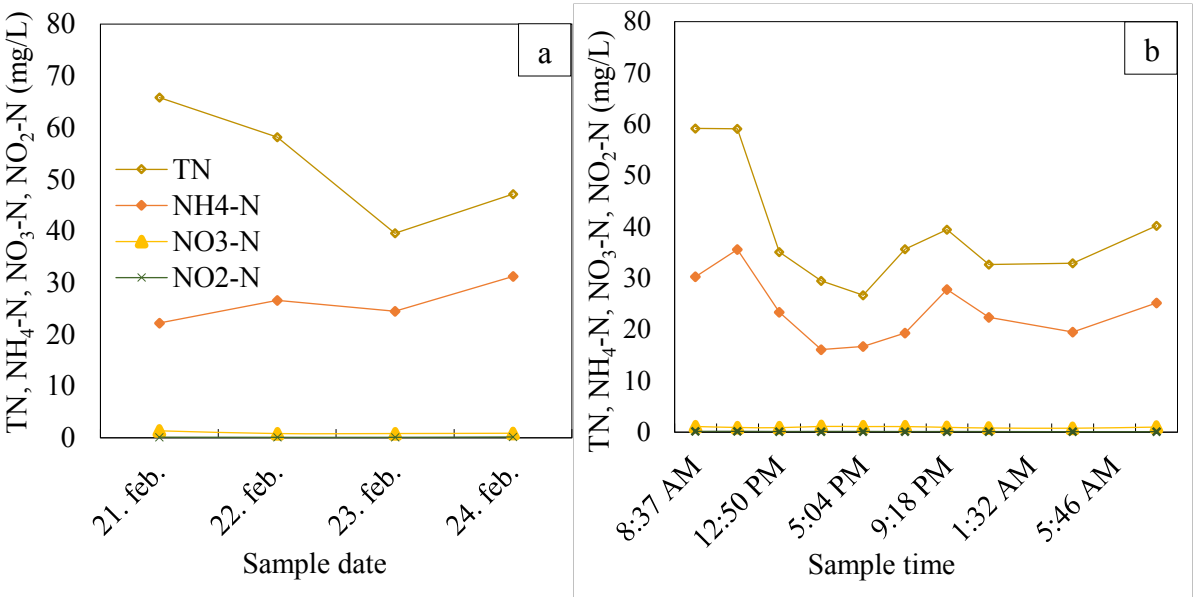


Figure 3.9. Variation of TN, NH_4-N, NO_3-N and NO_2-N for a) daily and b) hourly wastewater characterization in mg/L.

The hourly and daily NO_3-N variation is 0.97 ± 0.13 and 0.97 ± 0.22 mg/L, respectively. The hourly and daily NO_2-N variation is 0.13 ± 0.02 and 0.12 ± 0.03, respectively. This, as well as Figure 3.9a and b, show that a variation in NO_3-N and NO_2-N is negligible. This indicates that there is a low grade of nitrification occurring. As the Lerkendal wastewater is not transported over a long distance before reaching the laboratory, compared to regular wastewater treatment, nitrification is not expected. The wastewater will have a short retention time in the transportation system, which in combination with lack of bacteria, will give not optimal conditions for nitrification to occur.

The concentration of NH_4-N is consistently high for both analysis around an average of 26 mg/L. Figure 3.9b illustrates a NH_4-N concentration peak occurring in the morning. The main source of NH_4-N is urine, which indicate that the peak is related to human disposal. NH_4-N is the most soluble N-compound which explains why NH_4-N is much higher than NO_3-N and NO_2-N. TN and NH_4-N follow the same curves for hourly variation, while for daily variation TN decreases with a prominent dip on 23rd of February and a continuous increase in NH_4-N. This is related to wastewater dilution as the amount of precipitation leading up to 23rd of February was high.

3.1.4 Assessment of the Lerkendal wastewater

3.1.4.1 Comparing Lerkendal wastewater to different wastewaters

Studies have shown that Norwegian wastewater is typically much more diluted compared to other wastewaters (Ødegaard, 1999). The results from the daily characterization show that various components decrease coherent with precipitation, which indicates that the Lerkendal wastewater is exposed to dilution. But the results also indicated the there is a concentrated amount of TS and VSS compared to TSS which gives high potential for successful digestion of the organic material under anaerobe conditions. To assess if the Lerkendal wastewater is similar in composition to typical Norwegian wastewater which is diluted, the results are compared with statistical average values for Norwegian WWTPs (Raspati, 2016, Ødegaard, 1999) and a concentrated Norwegian wastewater affected by industrial discharge (Saltnes et al., 2016) in Table 3.2. The values used for Lerkendal wastewater was the hourly characterization as it gives a more representative overview of the wastewater composition.

Table 3.2. Comparing Lerkendal wastewater to known values. Table compares the hourly characterized Lerkendal wastewater to Hias wastewater and average values for typical Norwegian wastewater (Raspati 2016, Saltnes et al., 2016).

Parameter	Lerkendal wastewater*	Hias wastewater**	Average Norwegian wastewater***
TS (g/L)	0.47 ± 0.11	-	0.22
TSS (g/L)	0.20 ± 0.09	-	-
TDS (g/L)	0.30 ± 0.03	-	-
VSS (g/L)	0.17 ± 0.08	-	-
tCOD (mg/L)	380.90 ± 161.55	553	494
sCOD (mg/L)	146.25 ± 20.65	298	< 100
TN (mg/L)	39.06 ± 11.34	-	37
NH_4-N (mg/L)	26.63 ± 6.21	53	24.7
NO_3-N (mg/L)	0.97 ± 0.13	1.1	0.1
NO_2-N (mg/L)	0.13 ± 0.02	-	0.3
TP (mg/L)	4.18 ± 1.20	-	5
PO_4-P (mg/L)	2.83 ± 0.96	5.1	3.3
BOD_5 (mg/L)	202.92 ± 49.90	-	195
pH	7.79 ± 0.42	7.8	-

* Average hourly concentration of Lerkendal wastewater
** Average concentration of Hias wastewater (Saltnes et al., 2016)
*** Average concentration of Norwegian wastewaters, (Raspati, 2016, Ødegaard, 1999)

Hias WWTP operates the local wastewater in continuous EBPR based on MBBR run on a pilot scale (Saltnes et al., 2016). Compared to the values retrieved from Hias, the Lerkendal wastewater is low in concentration for all parameters. However, Hias is naturally high on P, COD and N because it receives industrial waste (Saltnes et al., 2016), and is therefore naturally nutritious. It is reasonable to assume that the Lerkendal wastewater does not receive any industrial waste as the wastewater is produced from a housing area with only apartment complexes. It is therefore not surprising that it is has a lower nutrient concentration than Hias. This gives the definite conclusion that the Lerkendal wastewater is diluted compared to Hias WW.

TS in wastewater can vary widely, depending on weather, rainfall or snowmelt events, and is therefore an indicator on whether the wastewater is diluted or not. The TS value in the Lerkendal wastewater is 113 % higher than the average Norwegian wastewater. The average BOD:COD ratio was found to be 0.53 in the Lerkendal wastewater, while 0.39 in typical Norwegian wastewaters (according to table 3.2). This indicates that the Lerkendal wastewater is high on biologically organic material compared to the average Norwegian wastewater. The average Norwegian tCOD concentration in wastewater is 494 mg/L, according to Raspati (2016). The tCOD in the Lerkendal wastewater is not deviating to much from the average Norwegian value if the standard deviation is considered. According to Ødegaard (1999) the average sCOD concentration is below 100 mg/L for typical Norwegian wastewaters. This indicates that the Lerkendal wastewater sCOD concentration is 46.45 % higher than what is typical in Norway. By comparing the wastewater from Lerkendal with values from Raspati (2016) and Ødegaard (1999) it is reasonable to conclude that the Lerkendal wastewater is not any more diluted or any less nutritious than the average Norwegian wastewater.

3.1.4.2 C:P ratio

There are several factors influencing the potential and effectiveness of treating a wastewater with EBPR. Even if the Lerkendal wastewater is affected by external water diluting the wastewater, this will not affect the ratios between the various parameters.

The BOD:TP ratio of influent provide information about the possibilities for the application of EBPR. Figure 3.10 shows the average sCOD:PO_4-P and BOD:TP ratios for hourly characterization of the Lerkendal wastewater compared to Hias wastewater. Daily characterization is not assessed as BOD_5 was not determined.

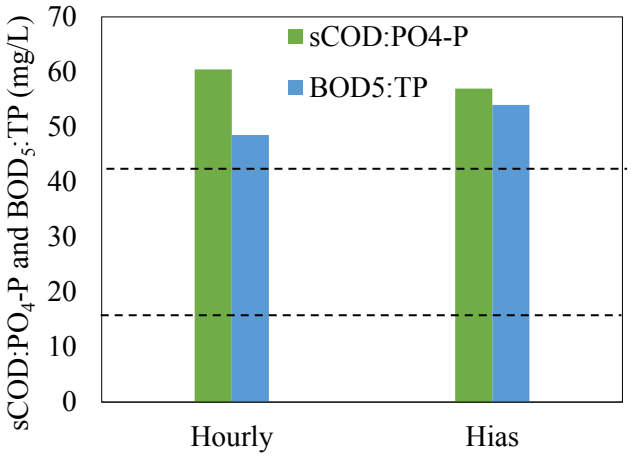


Figure 3.10. sCOD:PO_4-P ratio and BOD_5:TP ratio for hourly wastewater characterization compared to Hias wastewater (modified from Saltnes et al. (2016).

The higher the BOD:TP ratio is, the easier it is to consume the substances biologically (Janssen et al., 2002). Experience have proven that a successful EBPR process is achievable if BOD:TP ratio is higher than 15 - 20 (Janssen et al., 2002, Saltnes et al. 2016). Lerkendal wastewater has on a BOD:TP ratio of 44.5 based on the hourly wastewater composition. This is above the recommended value, which indicates that Lerkendal wastewater in theory has a high theoretical potential for biological P removal as a high degree of the substances are biological available. Study by Carrera et al. (2001) show that the optimal sCOD:PO_4-P in the influent is 42. sCOD:PO_4-P ratio in the Lerkendal wastewater is 60.4, which is higher than the optimal value and therefore the potential for EBPR is good. The charts show how the Lerkendal wastewater has an hourly higher sCOD:PO4-P ratio and lower BOD:TP ratio compared to the wastewater of Hias WWTP.

The calculated C:P ratios are not fully reliable as some part of the BOD will be removed in a pre-treatment step, and is therefore not biological available for the PAOs during EBPR. How much is available for PAOs consumption will depend on the total bacteria culture in the system and if the conditions in the system is favorable to the PAOs compared to the other bacteria. The BOD values are based on BOD_5 analysis, which means that some part of the organic material will not be biologically available during a retention time of a few hours. It should be noticed that the values for Hias in figure 3.10 is based on an annual average while the Lerkendal wastewater values are based on an hourly average. The results in figure 3.10 is therefore only

a theoretical potential, and not a direct result for the effectiveness of implementing EBPR to the Lerkendal wastewater.

3.1.4.3 C:N ratio

The BOD:TN ratio of influent provide information about the possibility for combining nitrogen removal with EBPR. Figure 3.11 show the average sCOD:NH_4-N and BOD:TN ratios for hourly characterization of the Lerkendal wastewater compared to Hias wastewater. Daily characterization is not assessed as BOD_5 was not determined.

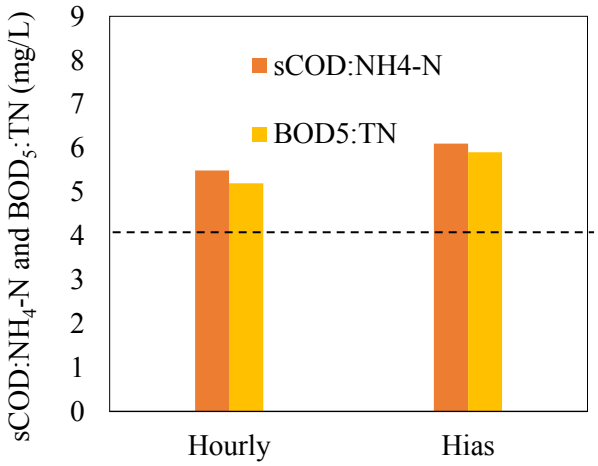


Figure 3.11. sCOD:NH_4-N ratio and BOD_5:TN ratio for hourly wastewater characterization compared to Hias wastewater (modified from Saltnes et al. (2016).

According to literature, it is recommended that the BOD:TN ratio should be greater than 4 – 5 for optimal biological N removal. Lerkendal wastewater has an average BOD:TN ratio of 5.2 based on hourly wastewater composition. This is within the range of what is recommended, and not far from the BOD:TN ratio for Hias which is 5.9. The results indicate that there is a theoretical potential for nitrogen removal in the Lerkendal wastewater.

3.1.4.4 Temperature

The average Norwegian wastewater has a temperature between 0 °C and 5 °C (Ødegaard et al., 2009). The general acceptance is that biochemical processes are inhibited at low temperatures. The operational temperature in EBPR is therefore important as this is the temperature that the microorganisms in EBPR will be exposed to. When the temperature of the influent wastewater is low, it will alter the operating temperatures. However, the operating temperature cannot be treated as an isolated condition for EBPR. Various studies indicate how EBPR is successful at

very low temperatures as there was found a higher P removal efficiency and a corresponding decrease in glycogen formation from biomass at 5°C compared to 20°C (Oehmen et al., 2007, Erdal et al., 2003). This theory is contradicting to the general acceptance that the cold temperature of the Norwegian wastewater inhibits EBPR from operating successfully.

Temperatures below 4 °C have proven to give lower hydrolysis rate (0.04/day), which explains why the sCOD concentration is low in the Lerkendal wastewater as well as in Norwegian wastewaters in general. It is possible to manipulate the amount of available VFA by fermentation, but as Yuan et al. (2011) emphasizes, the cold temperature will decrease the fermentation efficiency. This can cause operational difficulties if EBPR is used on the Lerkendal wastewater without manipulating the temperature, adding a hydrolysis tank before the treatment process or adding doses of acetate as VFA. Even though the wastewater temperature indicates that the PAOs will have a competitive advantage over GAO and thus provide the Lerkendal wastewater with the optimal operating temperatures, the available amounts of VFA brought into the EBPR process will not be optimal due to the typical Norwegian temperatures.

3.1.4.5 pH

Based on the knowledge surrounding how pH will affect the microbial mechanisms under anaerobic and aerobic conditions, it is reasonable to aim for a pH >7.5, since high pH will shift the microbial competition from GAOs to PAOs. The average pH found in the Lerkendal wastewater was 7.87, which is comparable to the average pH in Norwegian wastewater (Ødegaard et al., 2014, Saltnes et al, 2016). This indicates that the natural occurring pH in the Lerkendal wastewater is an advantage for EBPR, and indicates that there should not be necessary to correct the pH in the wastewater to reach the preferred pH for EBPR.

3.1.5 General conclusion of the Lerkendal wastewater

The characterization of the influent on an hourly and daily basis has proven that the Lerkendal wastewater is of typical Norwegian standard, where there is not a large variation in substances across the characterizations. This has proven a promising trend in consistency of the influent. The most interesting observation was that the sCOD does not follow a diurnal trend or is affected by precipitation. The sCOD in the Lerkendal wastewater is therefore consistent, and according to Ødegaard (1999) more concentrated than the average Norwegian wastewater.

Compared to the sCOD concentration in Hias WWTP, where successful EBPR is performed, the sCOD concentration is low which is due to the absence of industrial discharge. The sCOD:PO_4-P ratio is 60.4, which is higher than the optimal value. Based on the season the characterization of the Lerkendal wastewater was performed, it is possible to assume that the results are based on "worst-case-scenario" as there is a potential that the wastewater is generally diluted during winter/spring. During summer months, it is not unrealistic that the characterization if the wastewater is different and thus more concentrated. With this in mind, the observations indicate a promising trend for EBPR implementation, and the results show that the amount of sCOD, and presumably BSCOD, in the wastewater is not abnormally low when compared to average Norwegian values or values associated general diluted wastewater (sCOD of 200 mg/L in Henze et al., 2008).

It should also be mentioned that the Lerkendal wastewater is produced from a small community with approximately 144 apartments. If one housing has an average of 2 inhabitants, this equals wastewater discharge from 288 population equivalent (PE). This will naturally affect the concentration of the wastewater and simulate more diluted wastewater characteristics as there is a limited amount of people contributing to the production of wastewater. This will give lower values compared to wastewater generated by larger areas and cities as Hias, Henze et al. (2008) and Raspati (2016) values are based on. In addition to this, the Lerkendal wastewater is fresher than most wastewaters because of the short travel time before it is analyzed compared to influent wastewaters at WWTP, which is of importance for the natural occurring VFA as the anaerobe fermentation is lowered.

Performing batch experiments with bio-P sludge and the Lerkendal wastewater will give further basis to assess the potential for EBPR in practice. The results from batch experiments will indicate whether the Lerkendal wastewater has potential for EBPR in its natural state. It is then possible to increasing the VFA amount by external addition of carbon source and thus assess whether EBPR is the right treatment method for the Lerkendal wastewater.

3.2 Batch activity experiments

Batch experiments as described in materials and methods and table 2.4 were performed to investigate the bio-P activity in the characterized Lerkendal wastewater.

3.2.1 Nature of bio-P sludge (Experiment 1)

25 liters of biological sludge (bio-P sludge) from Hias was received 25th of April at 5:30 pm. The nature of the bio-P sludge was characterized upon arrival as described in materials and methods. Table 3.3 present the initial nature of the bio-P sludge received from Hias.

Table 3.3. Characterization of bio-P sludge upon arrival

Parameter	Value
TS (g/L)	6.62
VSS (g/L)	5.66
tCOD (mg/L)	11400
sCOD (mg/L)	1420
TN (mg/L)	850
NH_4-N (mg/L)	30.8
NO_3-N (mg/L)	0.59
NO_2-N (mg/L)	1.1
TP (mg/L)	160
PO_4-P (mg/L)	24.7
pH	9.10

The bio-P sludge used was produced by EBPR using MBBR and was highly anaerobic upon arrival due to overnight transportation in an airtight container. This is presumably the explanation for the high initial concentration values of PO_4-P with 24.6 mg/L (due to P-release) and sCOD with 1420 mg/L (due to hydrolysis).

The bio-P activity in the bio-sludge was investigated the following morning by performing an anaerobic batch experiment. All graphs illustrating the activity in batch experiments are presented as mg per liter as the MLVSS will vary for each individual batch experiment. When discussing the results, the values are presented as mg per L for better comparison with the graphs, while mg per g VSS is presented in parentage as this is recommended when the purpose

is to evaluate the capacity of the biomass (Tykesson and Jansen, 2005). See table F.1, F.2, F.3, F.4, F.5, F.6 and F.7 in appendix F for complete results from all batch experiments.

Figure 3.12 illustrates the P release and sCOD consumption in the bio-P sludge when diluted with MQ water and dosing of 300 mg COD/L. The experiment was conducted with an initial MLVSS = 2259.2 mg VSS/L.

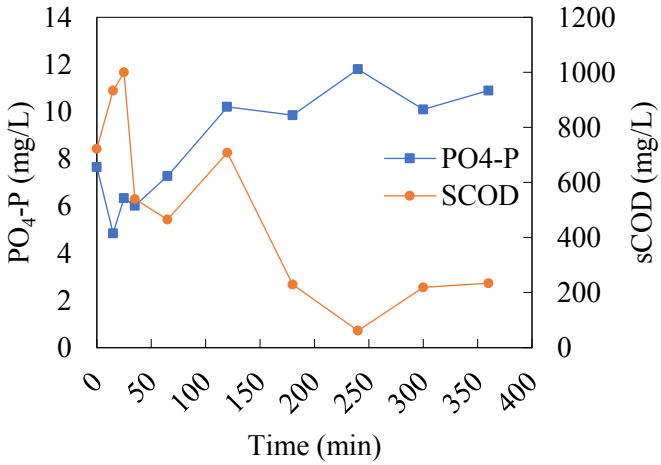


Figure 3.12. PO_4-P release and sCOD consumption for experiment 1. Blue line represents PO_4-P release and orange line represents sCOD consumption when bio-P sludge was mixed with MQ water and 300 mg COD/L. MLVSS = 2259.2 mg VSS/L

The initial concentration of sCOD in the batch experiment was 722 mg sCOD/L (319.6 mg sCOD/g VSS). Immediately after time zero the system is dosed with 300 mg COD, increasing the total concentration of sCOD to 934 mg sCOD (413.4 mg sCOD/g VSS). The PAOs release their internally stored poly-P into the mixed liquor, making an increased PO_4-P concentration from initial 8 mg PO_4-P/L (3.5 mg PO_4-P/g VSS) to approximately 10 mg PO_4-P/L. (4.4 mg PO_4-P/g VSS). After six hours 200 mg sCOD was still remaining in the batch, which indicate that the remaining sCOD is not BSCOD and is therefore not fully consumed by the PAOs.

The initial P uptake capacity of the bio-P sludge was determined by equation 11,

$$\boldsymbol{Bio - P_{capacity} = \frac{TP_{after\ aeration} - PO_4 - P_{after\ aeration}}{MLVSS}} \quad (11)$$

Where:

Bio-$P_{capacity}$ = Capacity of the bio-P to take up P under aerobe conditions, mg P/mg VSS

$TP_{after\ aeration}$ = Concentration of TP after aeration in t = 0, mg/L

PO_4-$P_{after\ aeration}$ = Concentration of PO4-P after aeration in t = 0, mg/L

MLVSS = initial concentration of LMVSS, mg/L

The bio-P capacity was calculated to be 0.024 mg P/mg VSS. Theoretically, the bio-P sludge has a P uptake capacity of 0.06 – 0.15 mg P/mg VSS (Wentzel et al., 2008). The capacity for P uptake is therefore lower than the theoretical value for bio-P sludge.

3.2.2 Kinetics of bio-P sludge with MQ water and acetate (Experiment 2A and 2B)

The initial characterization of the bio-P sludge unfolded that the bio-P sludge is naturally high in sCOD. When the bio-P sludge undergoes aerobic and anoxic conditions in batch experiments, it is essential that there is a limited amount of acetate present. An excess amount of sCOD can disturb the metabolism of the PAOs and prevent P uptake (Janssen et al., 2002). To avoid unrealistic results, 100 mg COD was added to the batch experiments to ensure limited amount of acetate in the aerobe and anoxic phase of the experiments. The required amount of acetate was based on a P release/acetate uptake-rate of 0.4 mg P/ mg acetate (Janssen et al., 2002). The required amount of acetate was derived to be 75 mg acetate, but as the sCOD concentration in time zero was found to be lower compared to the previous batch experiment, the acetate dosage was set to 100 mg sCOD/L to avoid limited conditions and a limited P release rate in anaerobic phase.

P kinetics and sCOD consumption

Figure 3.13 illustrates the activity of P release, P uptake and sCOD consumption under a) anaerobic – aerobic batch experiment and b) anaerobic – anoxic batch experiment, when 353 mL of bio-P sludge was mixed with 647 MQ water and 100 mg COD/L. The experiments were conducted with an initial MLVSS = 2028.4 mg VSS/L.

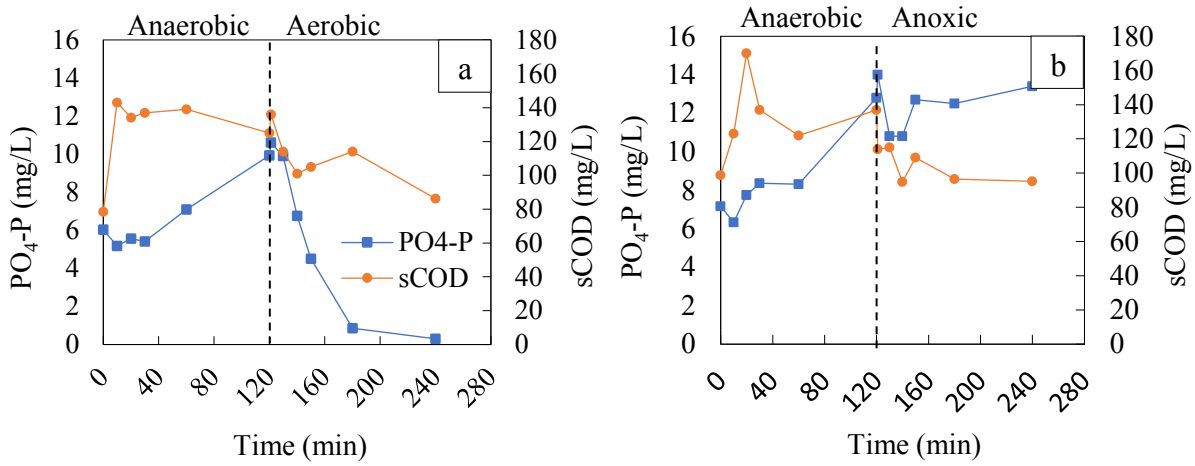


Figure 3.13. Activity of PO_4-P and sCOD consumption in experiment 2A and 2B. Activity of PO_4-P and sCOD consumption for a) anaerobe – aerobe batch experiment and b) anaerobe – anoxic batch experiment when sludge is mixed with MQ water and 100 mg COD/L. MLVSS = 2028.4 mg VSS/L.

The results from Figure 3.13a show that all PO_4-P was taken up from the mixed liquor as the PAOs consumed all the added acetate during the experiment. The initial concentration of 80 mg COD/L (39.4 mg sCOD/g VSS) increased to 150 mg COD/L (73.9 mg sCOD/g VSS) after dosing the batch experiment with 100 mg COD/L. At the end of the experiment (240 minutes) the concentration of sCOD was 82 mg/L (40.4 mg/g VSS). This indicates that the PAOs in the bio-P sludge were able to fully consume the added acetate during the experiment.

Figure 3.13b show that there is no P uptake under anoxic conditions even though the amount of sCOD added to the batch experiment is fully consumed after 240 minutes. The lack of PO_4-P uptake indicates that there are no DNPAOs present in the bio-P sludge. Since the acetate added is fully consumed during the experiment, it is reasonable to assume that there are other microorganisms and bacteria who consume the added acetate. The observation of no DNPAOs in the bio-P sludge is to be expected as Hias WWTP operates by alternating the bio-P sludge through anaerobic and aerobic conditions, and not anoxic.

Nitrification

The possibility of nitrification in the batch experiments performed was assessed. Nitrification is conversion of NH_4-N to NO_3-N and NO_2-N with absence of oxygen, where NH_4-N is fully consumed.

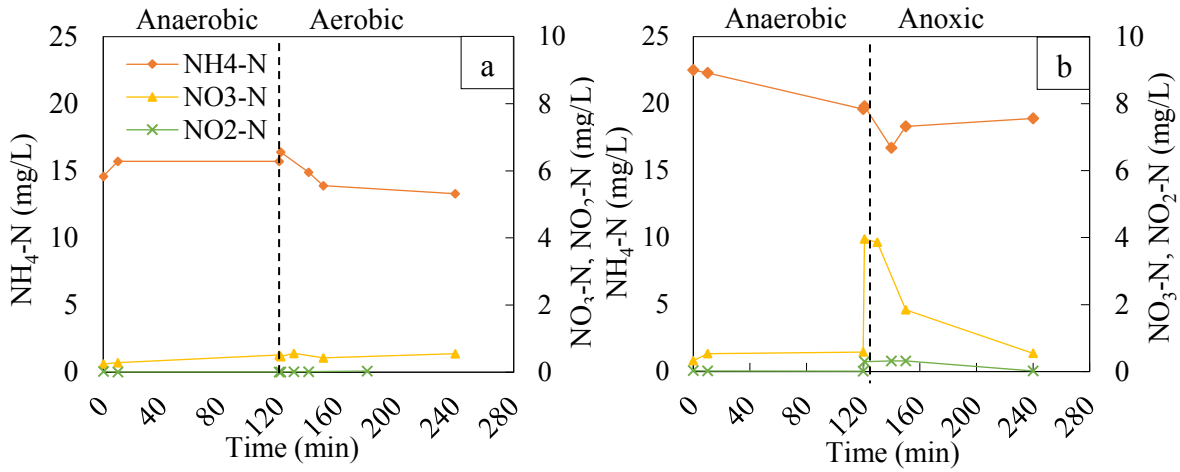


Figure 3.14. Variation of NH_4-N, NO_3-N and NO_2-N in experiment 2A and 2B. Variation of NH_4-N, NO_3-N and NO_2-N as a function of time for a) anaerobe – aerobe batch experiment and b) anaerobe – anoxic batch experiment with sludge mixed with MQ water and 100 mg COD/L

Figure 3.14a show a low consumption of NH_4-N under anaerobe conditions. This indicates that the system has a low nitrification rate, which is probably due to lack of nitrifying bacteria. sCOD is an inhibiting factor for nitrification as high concentration of sCOD prevents nitrification from occurring. Mousavi and Ibrahim (2014) report effective nitrification when C:N < 0.25. C:N ratio in this batch experiment is 0.6, thereby giving less than optimal conditions for nitrification as the sCOD is too high for nitrification to occur. There was not detected any significant change in NO_3-N or NO_2-N concentration. When the concentration of organic carbon is high, heterotrophic bacteria dominate the denitrifying bacteria, thereby reducing the nitrifying rate.

It is interesting to observe denitrifying activity in Figure 3.14b. Anoxic phase was initiated by adding 4 mg/L NO_3-N to the system, which was fully consumed after 120 minutes. As sCOD is consumed simultaneously as no P uptake is occurring, it is assumed that there are no DNPAOs present in the bio-P sludge. This indicates that there must be ordinary denitrifying bacteria present in the system that is consuming the NO_3-N and acetate added to the system.

Comparing PO_4-P activity and sCOD consumption in both experiments

As both experiments discussed is executed using bio-P sludge collected from the sludge container at the same time, it is interesting to evaluate the results against each other. Figure 3.15a show a comparison of the P release and P uptake results, while Figure 3.15b shows the comparison of sCOD consumption, obtained from the anaerobe – aerobic and anaerobe – anoxic batch experiments.

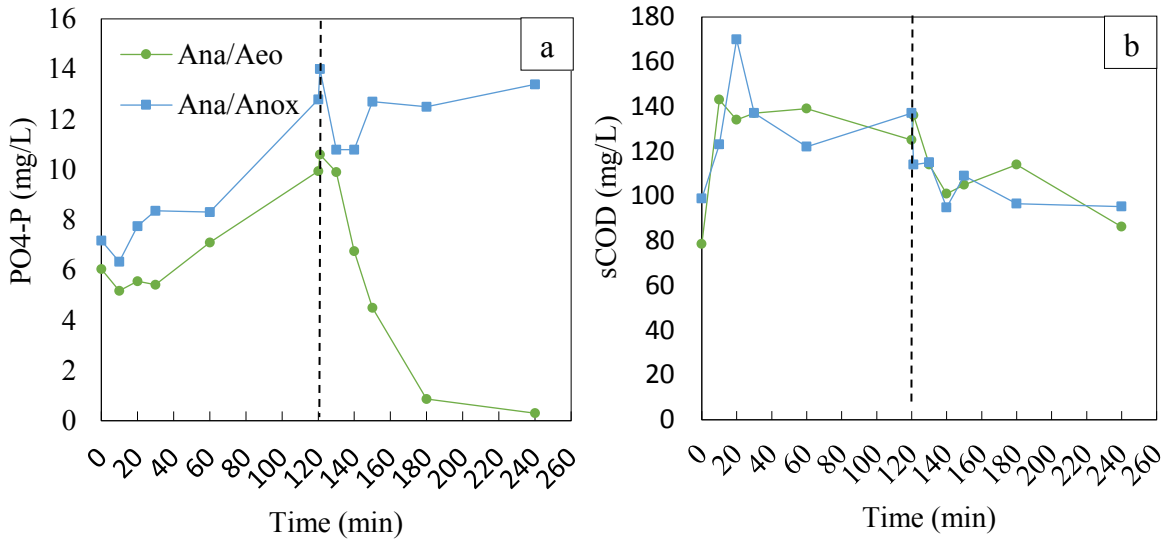


Figure 3.15. Comparison of results from experiment 2A and 2B. a) PO_4-P variation and b) sCOD consumption for batch experiments with anaerobe – aerobe conditions (green) and anaerobe – anoxic conditions (blue) for bio-P sludge mixed with MQ water and 100 mg COD/L. MLVSS = 2028.4 mg VSS/L.

According to Figure 3.15a, PO_4-P is released in a similar matter for both experiments during the anaerobic phase. The experiment conducted with anaerobe/anoxic conditions (blue points) has a higher initial value than the experiment conducted with anaerobe/aerobe conditions (green points). Theoretically, the initial PO_4-P concentration should be equal, but a deviation is expected as the bio-P samples are measured out and ensured homogenous by shaking the samples and pouring them into a measuring cylinder. This makes it difficult to ensure identical sample composition as there naturally will be a deviation in solids in the two samples. This is also the case for sCOD consumption. According to Figure 3.15b, the experiments follow the same curvature, with the exception that the experiment conducted with anaerobic/aerobic conditions (green line) has a constant lower concentration of sCOD than anaerobic/anoxic experiment (blue line).

When P uptake is initiated by aeration (aerobic conditions) and nitrogen gas (anoxic conditions) there is a clear difference in P uptake for the two experiments. There is no P uptake under anoxic conditions, which as discussed indicates that there are no DNPAOs taking up the available PO_4-P.

3.2.3 Kinetics of the bio-P sludge with wastewater

Results conducted from characterization of the Lerkendal wastewater indicate that there is a stable and consistent trend in the sCOD concentration. On an hourly- and daily basis the sCOD concertation was found to be 146.25 ± 20.65 and 161.39 ± 8.45, respectively. Continuity in sCOD concentration is an important observation for implementation of EBPR in the Lerkendal wastewater as the concentration of sCOD is an indirect representation of the VFA concentration. The amount of VFAs naturally occurring in the wastewater, and the ratio of acetate to other VFAs such as propionate or butyrate is unknown as it was not tested in this thesis work. Therefore, the amount and type of VFA naturally in the wastewater for the PAOs to successfully remove P in excess was not assessed. Batch activity tests where bio-P sludge was mixed with wastewater without external acetate dosing was performed to investigate the natural potential for EBPR in the wastewater.

3.2.3.1 Kinetics with no added acetate (Experiment 3A and 3B)

P kinetics and sCOD consumption

The activity of P release, P uptake and sCOD consumption when 353 mL of bio-P sludge is mixed with 647 mL of wastewater under a) anaerobic – aerobic batch experiment and b) anaerobic – anoxic batch experiment is presented in Figure 3.16. The experiments were conducted with an initial MLVSS of 1958.3 mg VSS/L.

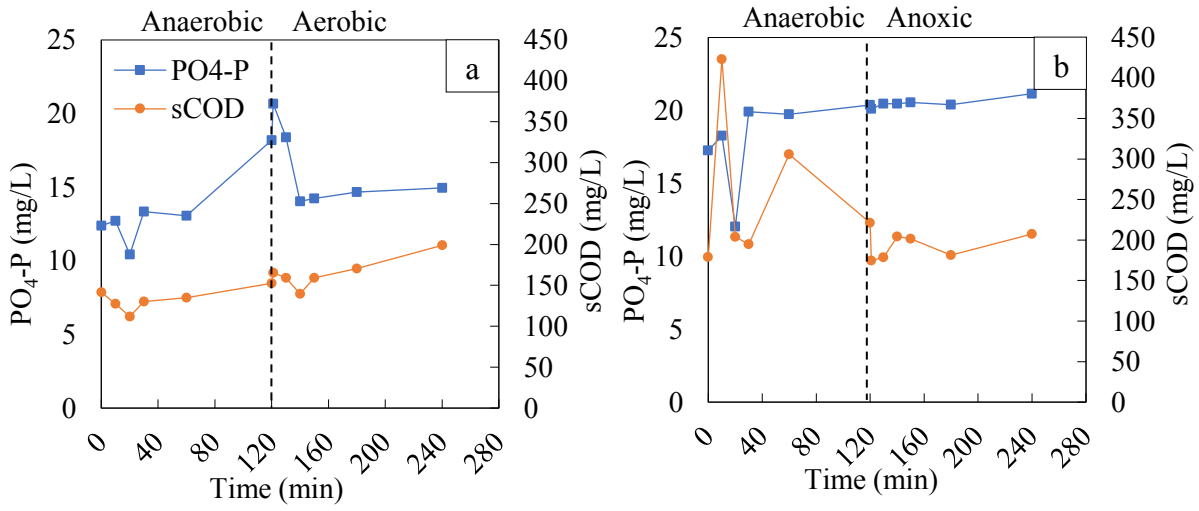


Figure 3.16. Activity of PO_4-P and sCOD consumption in experiment 3A and 3B. Activity of PO_4-P and sCOD consumption for a) anaerobe – aerobe batch experiment and b) anaerobe – anoxic batch experiment when sludge is mixed with wastewater without external carbon source. MLVSS = 1958.4 mg VSS/L.

The results from Figure 3.16a show a strange tendency in sCOD compared to previous experiment. At the end of the experiment, the sCOD concentration was detected to have increased from 150 mg sCOD/L (76.6 mg sCOD/g VSS) to 200 mg sCOD/L (102.1 mg sCOD/g VSS), which indicates a release or production of sCOD instead of consumed during the experiment. Simultaneously, PO_4-P is released in anaerobic phase and taken up under aeration by the PAOs, but there is no detectable luxury uptake. It seems as if the PAOs are able to take up the amount of PO_4-P that they released during anaerobe conditions by consuming the BSCOD naturally present in the wastewater, but without being able to create a luxury uptake. This may indicate that there is not enough VFA naturally in the wastewater.

Figure 3.16b show scatter in the sCOD results. The value of 10 minutes can be considered an outlayer, possibly due to sampling or filtration error. This is assumed as there was no acetate added during this experiment, and therefore there is no reasonable explanation for the immense increase in sCOD. When discarding this value, it becomes apparent that the sCOD is kept relatively stable around 200 mg sCOD/L (102.1 mg sCOD/g VSS) during the duration of the experiment, which indicate that there was no consumption of BSCOD under anaerobic/anoxic conditions either.

Nitrification

For analysis of the possibility for nitrification only the most important parameters associated with the batch tests were analyzed. Figure 3.17 show the variation of a) NH_4-N under anaerobe – aerobe conditions and b) NO_3-N under anaerobe – anoxic conditions for the batch experiment. As there was not detected any nitrification in experiment 2A, experiment 3A was not analyzed for NO_2-N and NO_3-N. Experiment 3B was not analyzed for NH_4-N and NO_2-N.

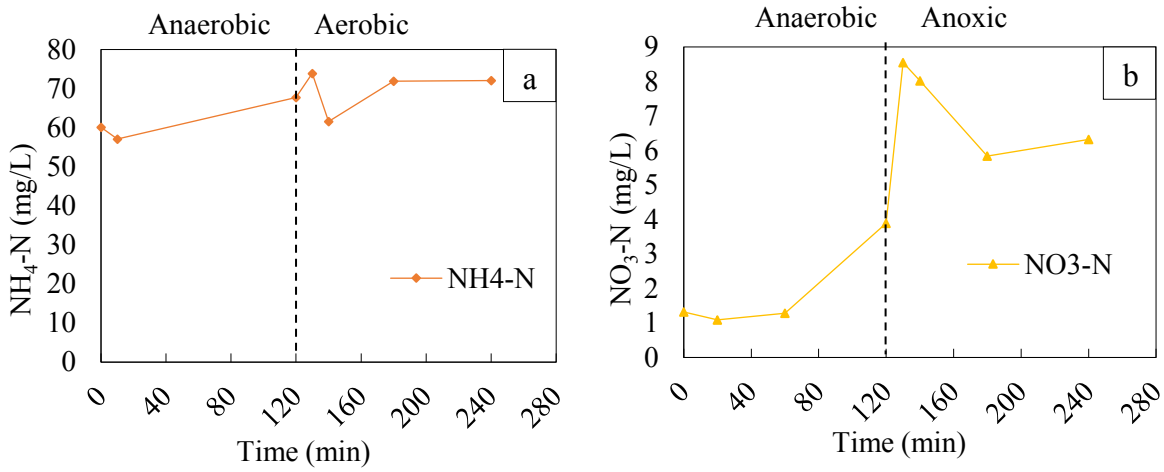


Figure 3.17. Variation of NH_4-N and NO_3-N in experiment 3A and 3B. Variation of a) NH4-N under anaerobe – aerobe batch experiment and b) NO3-N under anaerobe – anoxic batch experiment where sludge is mixed with wastewater without external carbon source

The trend in NH_4-N concentration is expected to be flat during anaerobe phase due to the lack of nitrification. The increase in NH_4-N which is observed in Figure 3.17a is assumed to be caused by measuring error as previous results have indicated that there is no nitrification occurring in the system. The variation in NH_4-N concentration is expected to be minor due to the presence of excess sCOD in the system, which inhibits nitrification from occurring.

Figure 3.17b show the same trend as experiment 2B. sCOD is consumed by ordinary denitrifying bacteria as there is no P uptake in anoxic phase and therefore no DNPAOs in the system. At time 120 minutes 4 mg NO_3-N is added to the system, which is not fully consumed at the end of the system. The NO_3-N concentration had increased from initial 1.32 mg NO_3-N/L (0.67 mg NO_3-N/g VSS) to 6.32 mg NO_3-N/L (3.22 mg NO_3-N/g VSS) by the end of the experiment.

Comparing PO_4-P activity and sCOD consumption in both experiments

Figure 3.18a shows a comparison of the PO_4-P activity, while Figure 3.18b shows the comparison of sCOD consumption, obtained from the experiment 3A and 3B.

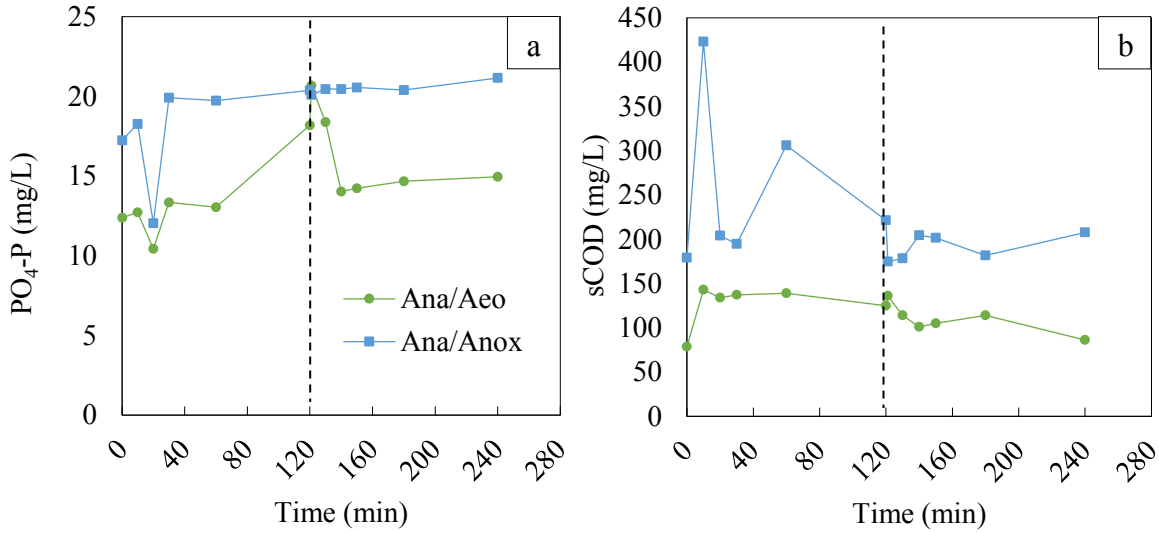


Figure 3.18. Comparison of results from experiment 3A and 3B. a) PO_4-P variation and b) sCOD consumption for batch experiments with anaerobe – aerobe conditions (green) and anaerobe – anoxic conditions (blue) for bio-P sludge mixed with wastewater and no external acetate addition

Since the bio-P sludge used in both experiments is retrieved from the same batch of bio-P sludge, and the anaerobe part of the experiments is performed both identically and in parallel, it is reasonable to expect similar anaerobic activity. However, the PO_4-P concentration is increased by 7.7 mg PO_4-P/L (4.2 mg PO_4-P/g VSS) in the anaerobe – aerobe experiment, while it only is increased by 2.7 mg PO_4-P/L (1.4 mg PO_4-P/g VSS) in the anaerobe – anoxic experiment as Figure 3.18a illustrates. The initial PO4-P concentration is higher in the anaerobe – anoxic experiments by 2.5 mg PO_4-P/g VSS, as well is sCOD concentration by 23.3 mg sCOD/g VSS. This is presumably due to inaccurate homogeneity when measuring out the bio-P sludge and wastewater volume manually.

From Figure 3.18b an increase in sCOD concentration is detected after 10 minutes of performing both experiments even though no acetate was dosed. This can be explained by fermentation of VFA in the wastewater under anaerobe conditions, and a subsequent higher sCOD concentration. The amount of substrate increased is consumed during the duration of the experiment, but the overall sCOD consumption is low. This indicates that the BSCOD naturally occurring in the Lerkendal wastewater is low.

These experiments were performed 11 days after receiving the bio-P sludge from Hias WWTP. The start concentration of sCOD for experiments 3A and 3B is higher compared to the previous experiments because the bio-P sludge had been fed with acetate three times per day since

arrival. As the results from the initial characterization of the bio-P sludge showed that there was an excess of sCOD in the sludge after aeration, it was decided to lower the acetate feeding from 29.1 mg/L to 14.55 mg/L. If there is an excess of substrate present at the end of anaerobic phase, and the sludge passes to aerobic or anoxic conditions, PAOs will not oxidize any PHA, but will rather take up acetate and store it as PHA (Janssen et al., 2002). This biochemical mechanism result in P release instead of P uptake. The obtained results from these experiments may indicate that feeding the PAOs with 15.44 mg/L caused them to starve and become exhausted as there is no sCOD consumption occurring in both experiments.

3.2.3.2 Kinetics with added acetate (Experiment 4)

As the results from the previous experiment indicate that the PAOs were exhausted and starving, the feeding regime was increased to 58.2 mg COD/L the day before performing the following experiment where 353 mL of bio-P sludge, 647 mL of wastewater and 300 mg COD/L was mixed together. The activity of P release, P uptake and sCOD consumption under anaerobic – aerobic batch experiment is presented in Figure 3.19.

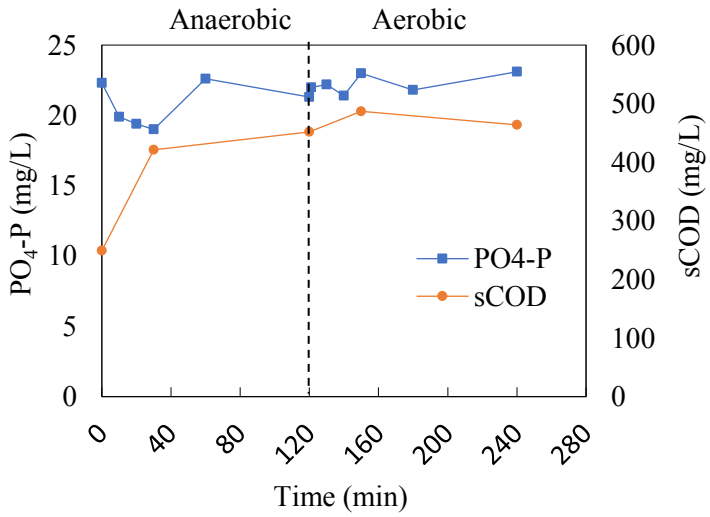


Figure 3.19. Activity of PO_4-P and sCOD consumption in experiment 4. Activity of PO_4-P and sCOD consumption for anaerobe/aerobe batch experiment where sludge is mixed with wastewater and 300 mg COD/L. MLVSS = 1346.82 mg VSS/L.

The MLVSS was 1346.82 mg VSS/L, which is lower than what the other batch experiments have been conducted with. This is caused by inaccuracy when the samples of wastewater and bio-P sludge is collected as it is difficult to ensure the identical amount of biomass in each batch experiment. There was also observed a settled biomass layer on the inside of the sludge

container, which can have resulted in a lower VSS concentration in the bio-P sludge, thus giving lower VSS concentration in the experiment. This layer of settled biomass was "pushed" back down into the bio-P sludge before the next experiment (experiment 5) was conducted, but not for experiment 4. Anaerobic – anoxic conditions were not tested as there are no DNPAOs present in the bio-P sludge. At the time of performing this experiment, it was expected that the PAOs in the bio-P sludge was inactive (based on previous results), therefore NH_4-N, NO_3-N and NO_2-N was not analyzed.

The results from this experiment indicate that there is no PO_4-P release or uptake occurring. The sCOD concentration was kept stable after adding the external acetate dose, which indicate the that PAOs are not able to consume the sCOD even though there is an excess of acetate available for consumption. Interpreting these results gives evidence to assume that the bio-P sludge is not functionating optimal, as the bio-P activity is negligible. The experiment was performed 15 days after the bio-P sludge was received from Hias WWTP. During the 15 experimental days, the bio-P sludge had been maintained in a 25 L container equipped with a constant mechanical mixer, alternating anaerobic – aerobic conditions, as well as feeding of acetate three times per day (see 2.3.4). The results from this experiment indicate that the decay in bio-P sludge freshness compromises the functionality of the PAOs and EBPR.

The lack of P uptake under aerobe conditions can be caused by the excess of sCOD present at the end of the anaerobe phase, as 452 mg sCOD/L is high compared to the results from previous experiments. It is possible that the PAOs did not oxidize any PHA, but rather took up acetate and stored it as PHA, resulting in P release instead of P uptake under the subsequent aerobe phase. This can be casing the lack of P uptake which was observed in Figure 3.19.

3.2.4 Deterioration in bio-P sludge over time (Experiment 5)

As the bio-P has been kept alive for 15 days, it is possible that the nature of the sludge has changed and therefore has affected the batch experiments results. A final batch experiment was performed using bio-P sludge mixed with MQ water and 100 mg COD/L to simulate the first batch experiment performed under anaerobe – aerobe conditions (experiment 2A). Figure 3.20 illustrates the comparison of the nature of the sludge upon arrival and after 15 days for a) activity of PO_4-P and b) sCOD consumption under anaerobe – aerobe conditions.

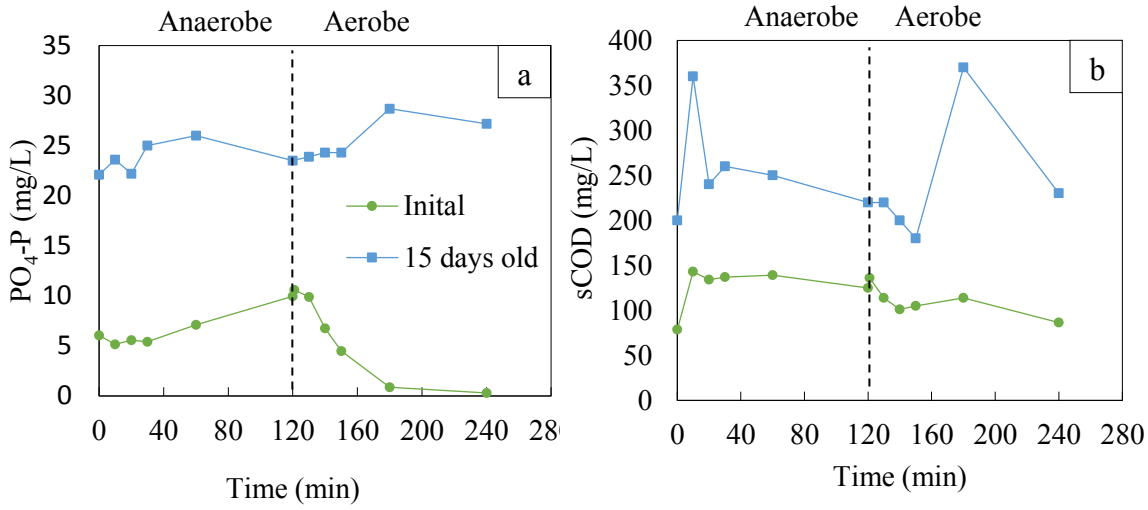


Figure 3.20. Examining the activity in bio-P sludge after 15 days. Comparison of the activity in the bio-P sludge upon arrival and after 15 days for a) activity of PO4-P and b) sCOD consumption under anaerobe/aerobe conditions.

Figure 3.20a illustrates a defining difference in PO_4-P activity. After 15 days, the PAOs have become lacy due to exhaustion and starvation, and are no longer able to release P and take up P in excess even though acetate is available for consumption. As Figure 3.20b illustrates, the nature of the sludge after 15 days gives complete consumption of the added acetate after 20 minutes, which indicate that the bacteria that are present in the bio-P sludge are starving. Compared to the initial nature of the bio-P sludge where the PAOs used 240 minutes to fully consume the added acetate, and were therefore not starving. Whether PAOs is a part of the bacteria still active in the bio-P sludge is not certain. The value of 180 minutes is considered an outlayer, possibly an error due to sampling and filtration of sCOD.

This experiment shows how there is a release of PO_4-P under aerobe conditions for the experiment performed after 15 days, which emphasized the theory of excess acetate leading to P release instead of P uptake. It is also interesting to observe a much higher initial P concentration after at the start of experiment 5 compared to experiment 2A. After 15 days, the P concentration at the start of the experiment is found to be 22.1 mg PO_4-P/L (10.53 mg PO_4-P/g VSS) compared to 5.17 mg PO_4-P/L (2.59 mg PO_4-P/g VSS) in experiment 2A. This indicates that anaerobe conditions might have occurred in the bottom of the sludge container that as given P release and thereby has increased the amount of PO_4-P in the sludge container.

3.2.5 P uptake and P release rates

It is difficult to standardize a method for calculating the P release and uptake rates in batch experiments (Tykesson and Jansen, 2005). It is important to choose a method that gives comparable values for all batch experiments performed. Bio-P activity is affected by the fraction of PAOs and the amount of available BSCOD in the system. The activity of bio-P is expressed as the rate P release and P uptake in the batch experiment.

All P release and P uptake rates were calculated using linear regression. To achieve comparable and consistent values the P release rates are calculated by including all points between 10 and 120 minutes of anaerobe phase, while P uptake rates are calculated by including all points between 120 and 140 minutes of aerobe phase as this is the most linear area for all the batch experiments.

Figure 3.21 shows how the linear area for P release and P uptake equations and R^2 values were determined using Excel linear regression tool for experiment 2A. The method is representative for all rate calculations. See appendix G for linear area for all batch experiments, linear equations and R^2 values.

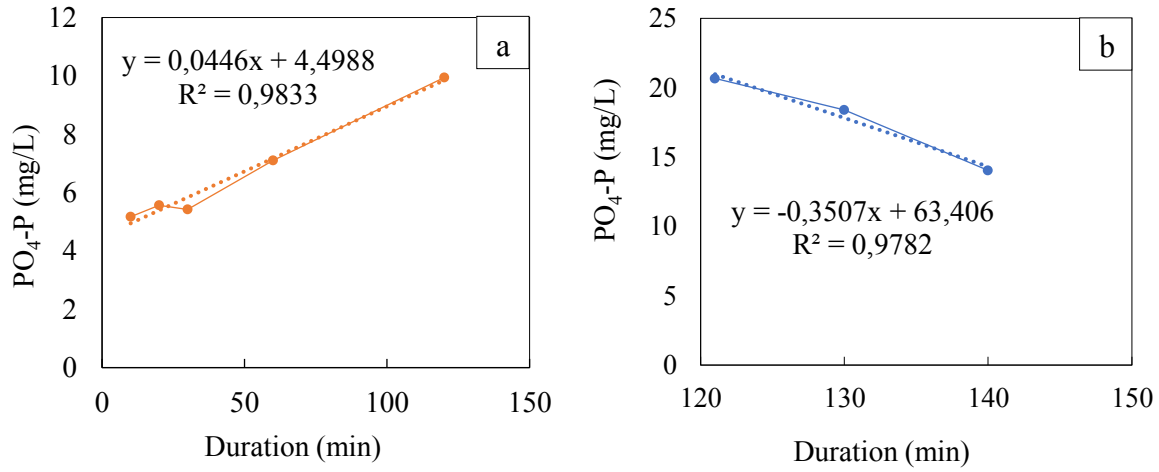


Figure 3.21. Presentation of P release and P uptake rate in experiment 2A. Illustrating method for determining the linear equation for a) P release and b) P uptake.

Calculating the P release and P uptake rates where done by multiplying the slope in the linear equation with 60 min/h and dividing by the MLVSS concentration, g VSS/L. The MLVSS concentration in experiment 2A was 1.9757 g/L. This gives a P release of 1.32 mg P/g VSS*h and P uptake rate of 6.02 mg P/g VSS*h in experiment 2A.

Table 3.4 presents the calculated P release and P uptake rates for the performed batch experiments. P uptake is not calculated under anoxic conditions due to the lack of DNPAOs. R^2 is the correlation between linear and non-linear regression, where a R^2 value close to 0 indicates a low correlation between the measured points. Calculated rates that gave R^2 lower than 0.8 is not considered reliable, and are listed as approximately zero in the table.

Table 3.4. Calculated P release and P uptake rates for batch experiments.

Experiment	MLVSS (g VSS)	P release rate (mg P/g VSS*h)	R^2 release	P uptake rate (mg P/g VSS*h)	R^2 uptake
1	2.2259	1.26	0.96	-	-
2A	2.0284	1.32	0.98	6.04	0.900
2B	2.0284	1.55	0.92	-	-
3A	1.9757	1.73	0.80	10.65	0.978
3B	1.9757	≈ 0	-	≈ 0	-
4	1.3470	≈ 0	-	≈ 0	-
5	2.4754	≈ 0	-	≈ 0	-

The classification of the bio-P sludge is related to the magnitude of the release and uptake rates, and is presented in Table 3.5 (Janssen et al., 2002).

Table 3.5. Classification of bio-P sludge based on the P release and P uptake rate (Janssen et al., 2002).

Release or uptake rate (mg P/gVSS*h)	Classification
< 3	Moderate
3 – 7	Good
> 7	Very good

As all the calculated P release rates are below 3 mg P/g VSS*h, the bio-P sludge is categorized as *moderate.* This is defined as a negligible bio-P activity as a result of a negligible fraction of PAOs in the sludge. The release rate is affected by the type and amount of organic substrate present for the PAOs. A low P release rate indicates that there is a low amount of VFA available. The P release rates obtained in Table 3.4 indicate that there is a low amount of available BSCOD or that available BSCOD does not contain enough VFA or the right kind of VFA. The organic material must be degraded before the PAOs can consummate it, resulting in low P release rates. According Kang et al. (1991), the favorable P release rate is above 2.4 mg P/g VSS*h. The

obtained P release value for wastewater without acetic acid was found to be 1.73 mg P/g VSS*h (experiment 3A), which is lower than what Kang et al. (1991) recommends.

The P uptake rates are classified as *good/very good*, which indicates a high rate of P uptake. However, this must be seen in context with the linear area which for P uptake is small. The results from the batch experiments showed that P is not removed in excess when performed with wastewater. A good to very good bio-P activity does not necessarily mean that the bio-P efficiency in the batch experiment is high (Janssen et al., 2002). The increase in P uptake rate is related to the increasing concentration of PO_4-P in the sludge container during the experimental period of 15 days.

These results can be related to the P incorporation capacity of the PAOs present in the bio-P sludge. In an EBPR activated sludge system, the amount of P incorporated in the sludge mass is increased from the normal value of 0.02 mg P/mg VSS to values around 0.06 – 0.15 mg P/mg VSS (Wentzel et al., 2008). The capacity of P incorporation in the bio-P sludge upon arrival was calculated to 0.024 mg P/mg VSS. After 15 days of bio-P sludge maintenance the P capacity incorporation was reduced to 0.014 mg P/mg VSS. This observation indicates that the capacity of the bio-P sludge resembles ordinary activated sludge, and not bio-P sludge, which can be assumed to affect the batch experiment results and the subsequent low P release and P uptake rates.

The low P incorporation capacity in the sludge can also be explained by the condition of the bio-P sludge. As the bio-P sludge is the fraction of the biomass that has detached from the MBBR carriers as a function of time, the bio-P sludge is not necessarily characterized as fresh bio-P sludge with vital PAOs. When the initial condition of the bio-P sludge is not fresh, it is not surprisingly that the PAOs are not capable to incorporate the amount that is theoretically possible. Since the bio-P sludge is not fresh initially when starting the bio-P sludge maintenance, it is assumed that the bio-P sludge will keep deterioration over time which may be causing the low potential for P removal in the Lerkendal wastewater.

Bio-P removal can be periodically less efficient due to over-aeration (Janssen et al. 2002). As the bio-P sludge is exposed to a 7-hour aeration period each night (see figure 2.8 of sludge maintenance schedule), it can be threatened by over-aeration. This can result in glycogen

consumption by PAOs, which leads to a decreased capacity to take up substrate under anaerobic conditions and lower the subsequent P uptake, and thereby give lower rates than optimal.

3.2.6 Maintenance of bio-P sludge

The results obtained from all the batch experiments indicate that the container and set-up does not provide optimal conditions for keeping the PAOs in the sludge active and fresh.

Based on the results from examining the nature of the sludge after 15 days, there should not be performed any more batch activity experiments before fresh bio-P sludge is received. Keeping bio-P sludge "alive" for more than 2 weeks under less optimal conditions results in deterioration of the sludge and does not assure active PAOs which can give less representative results.

The container does not have a flat bottom, which gives the possibility for biomass to settle and develop anaerobic conditions (despite of the mixer). If this occurs, it is possible that PO_4-P is released even though the sludge container is under aerobic conditions. PO_4-P activity in the sludge container was tracked due to suspicion of less than optimal conditions in the container. Figure 3.22 illustrates the results obtained by tracking the PO_4-P concentration in the bio-P sludge container during anaerobe and aerobe phase.

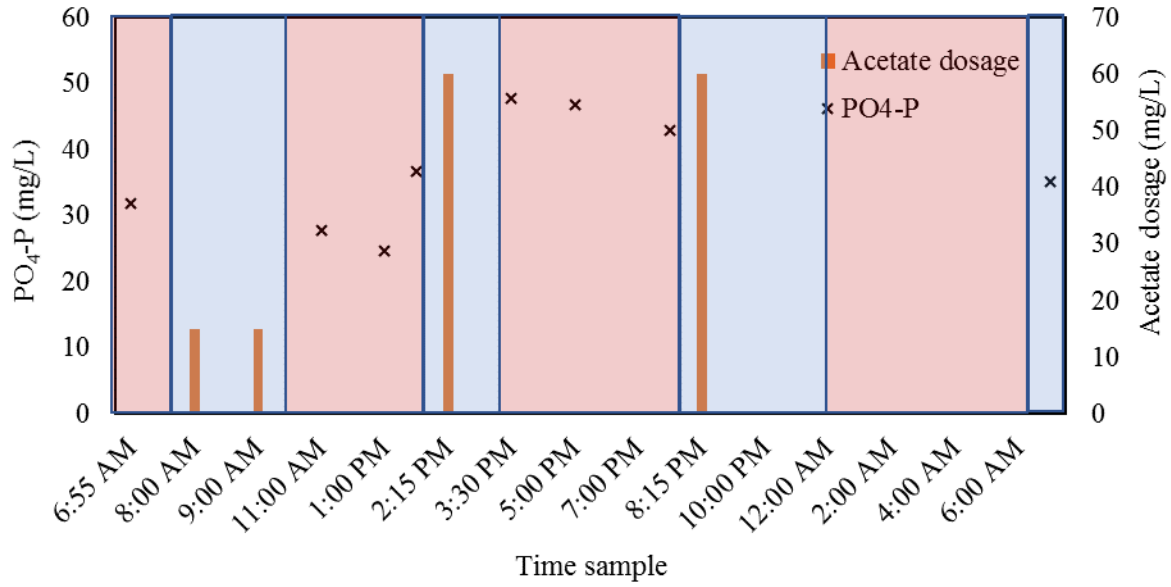


Figure 3.22. Schematic tracking of PO_4-P concentration under anaerobe (blue) and aerobe (red) conditions to investigate the state of P release and P uptake in the bio-P sludge. Amount and time of dosing acetate is also illustrated (orange bars).

The results indicate that the conditions in the sludge container are not optimal as there is no definite PO_4-P uptake during aerobic phases or PO_4-P release during anaerobe phases. This is assumed to be caused by biomass settling in the bottom of the container, providing anaerobe conditions and subsequent P release incoherent with overall container conditions.

The results from the batch experiments raised suspicions about the conditions present when acetate is dosed to the bio-P sludge container. As acetate was fed to the bio-P container immediately after aeration was stopped, it is a possibility of high levels of oxygen still present in the sludge when acetate is dosed since the system is not flushed with nitrogen gas to ensure immediate anaerobe conditions. Measuring of DO after initiating anaerobe phase gave results of high DO concentration in the sludge. Available acetate and oxygen under anaerobe conditions provide the heterotrophic bacteria with the right conditions to function optimal and consume the available acetate before PAOs. It is required 2 mg COD to remove 1 mg oxygen, and when combining this relationship with the OHOs consumption of acetate, there is a minor amount of substrate left for the PAOs. Based on these results, the PAOs in the bio-P sludge have probably been starving during the whole experimental period, and have become exhausted and inactive as a result.

4 Conclusion

The aim of this master thesis was to investigate the possibility for applying EBPR in the Lerkendal wastewater by characterizing the unknown composition of the wastewater according to relevant substances, and perform batch experiments to assess the bio-P activity in the wastewater.

The main results from characterizing the Lerkendal wastewater show that there is a resemblance to typical diluted Norwegian wastewater as there were no significant deviation when compared to literature values, both in composition and the environmental conditions temperature and pH. The Lerkendal wastewater is consistent for hourly and daily variation. The surprising observation was that the sCOD concentration showed no signs of diurnal pattern or vulnerability to excess amounts of precipitation and subsequent dilution of the wastewater.

The influent ratio of sCOD:PO_4-P and COD:BOD_5 is 60.2 and 1.8 respectively, which is higher than was is referred to as optimal for EBPR. This reflects the biodegradability character of the wastewater and indicate high potential for EBPR. As the characterization is performed during period with high precipitation, the results are assumed to indicate a "worst-case-scenario" as there is potential that the wastewater is generally diluted during this period.

The potential for EBPR in the Lerkendal wastewater was investigated using batch experiments with bio-P sludge from EBPR with MBBR (Hias WWTP). The main results obtained from the batch experiments show a low P release in the wastewater both with and without external addition of acetic acid (100 mg sCOD/L). The P release rates were between 1.32 and 1.73 P/g VSS*h without acetic acid, which is low compared to favorable release rates in EBPR. Luxury uptake was not detected even with high P uptake rates (6.04 to 10.65 mg P/g VSS*h). This indicates poor bio-P activity and negligible fraction of PAOs in the bio-P sludge used, which presumably is explained by the insufficient maintenance of the bio-P sludge over the experimental period of 15 days. The experiments were therefore most likely performed with PAOs that were not functionating optimal. The results are related to the condition of the bio-P sludge used in the experiments, which gives reason to suspect that better results would be obtained if the batch experiments with wastewater was conducted immediately when the bio-P sludge arrived, or if activated sludge was used.

The low P release rate and lack of excess P uptake does not indicate that EBPR will not function optimal in the Lerkendal wastewater and subsequent Norwegian wastewater, it rather indicates that the process is sensitive to the bio-P sludge used, and should therefore be as fresh and active as possible to prevent the process from deteriorating in the long term. The results indicate that performing batch experiments with bio-P sludge from the detached biomass from MBBR is not optimal.

The results presented give reason to conclude that there is potential for EBPR in the Lerkendal wastewater, however it is limited due to the low concentration of organic substrate naturally present in the wastewater. The concentration of organic substrate can easily be enhanced by installing a hydrolysis tank as a preliminary step before anaerobe bioreactor, and thus increase the potential for EBPR in the Lerkendal wastewater, and presumably Norwegian wastewaters. Dosing of external acetate as VFA is also a possibility, but this solution raises an economical dilemma as acetate is an expensive source of carbon to add in large quantities.

5 Limitations

This master thesis had a time limit of 20 weeks, which put limitations to how long the practical part could be carried out. The wastewater was characterized during February and March, which is typical Norwegian spring with high precipitation. Due to time limits it was not possible to carry out a characterization during summer where the wastewater presumably would have different characteristics.

The lack of equipment, such as a bioreactor, for maintenance of the bio-P sludge during the experimental batch period was another limitation. The set up used was homemade to the best ability to meet the requirements for sludge maintenance, but results would show that the bio-P sludge would deteriorate over time due to the set up. These problems could not be foreseen and were not due to lack of planning.

Ideally, the laboratory batch experiments should have been carried out within a short period to ensure the freshness of the bio-P sludge, but due to blockage in wastewater delivery system, the batch experiments using wastewater was delayed. Due to the time limitations, there was not enough time to receive new bio-P sludge, and the batch experiments with wastewater was not repeated with fresher bio-P sludge.

6 Further research

This master thesis provides basis for the future work on a pilot in laboratory scale using the Lerkendal wastewater for EBPR with MBBR (see figure 1.10). As the wastewater composition was unknown, this master thesis has provided a good indication on the compounds present in the wastewater and concentrations to be expected in the month of February and March. As the pilot will be operated year-round, it is recommended to characterize the Lerkendal wastewater during summer/autumn as the results of wastewater characterization can vary considerably according to climate and season. The batch experiments should therefore also be repeated to assess if the results using summer/autumn characterized wastewater differ from what was concluded in this
thesis.

As VFA analysis was not performed in this master thesis, it would be beneficial to analyze the percentage of acetate, propionate or other VFAs that may be naturally present in the Lerkendal wastewater as well as in the bio-P sludge. Theoretically, 7 mg VFA is required to remove 1 mg PO_4-P in EBPR. The analysis of the VFA can therefore provide information about the available VFA natural in the wastewater, and give a better understanding of the bio-P kinetics in the Lerkendal wastewater.

7 References

American Public Health, A., American Water Works, A. & Water Pollution Control, F. (1960). *Standard methods for the examination of water and wastewater: including bottom sediments and sludges.* 11th edn. New York: American Public Health Association.Standard Methods for the Examination of Water and Wastewater

Arvidsson, R. (2015). *Biological Phosphorus removal from Wastewater with a Moving Bed Biofilm Reactor Process.* Master Thesis, Lund University. Available at: https://lup.lub.lu.se/student-papers/search/publication/7454789 (accessed: 3 June 2017)

Zeng, W., Li, B., Wang, X., Bai, X., Peng, Y. (2016). Influence of nitrite accumulation on «Candidatus Accumulibacter» population structure and enhanced biological phosphorus removal from municipal wastewater. *Chemosphere.* Vol. 144, pp. 1018-1025.

Balamane-Zizi, O. & Ait-Amar, H. (2012). Biological phosphorus removal from dairy wastewater by alternating anaerobic and aerobic conditions. *African Journal of Biotechnology.* Vol. 11, No. 46, pp. 10575-10581.

Blackall, L., Crocetti, G., Saunders, A. & Bond, P. (2002). A Review and Update of the Microbiology of Enhanced Biological Phosphorus Removal in Wastewater Treatment Plants. *International Journal of General and Molecular Microbiology*, Vol. 81, No. 1, pp. 681-691.

Borkar, R., P., Gulhane, M. L. & Kotangale, A. J. (2013). Moving Bed Biofilm Reactor – A New Perspective in Wastewater Treatment. *Toxicology and Food.* Vol. 6, No. 6, pp. 15-21.

Carrera, J., Sarrà, M., Lafuente, F. & Vicent, T. (2001). Effect of Different Operational Parameters in the Enhanced Biological Phosphorus Removal Process. Experimental Design and Results. *Environmental Technology,* Vol. 22, No. 12, pp. 1439-1446.

Driver, J., Lijmbach, D. & Steen, I. (1999). Why Recover Phosphorus for Recycling, and How? *Environmental Technology,* Vol. 20, No. 7, pp. 651-662.

Erdal U.G., Erdal Z. K. & Randall C.W. (2003). The competition between PAOs (phosphorus accumulating organisms) and GAOs (glycogen accumulating organisms) in EBPR (enhanced biological phosphorus removal) systems at different temperatures and the effects on the system performance. *Water Science and Technology*, Vol. 47, No. 11, pp. 1-8.

Filipe, C. D. M., Daigger, G. T. & Grady, C. P. L. (2001a). A metabolic model for acetate uptake under anaerobic conditions by glycogen accumulating organisms:

Stoichiometry, kinetics, and the effect of pH. *Biotechnology and Bioengineering*, Vol. 76, No. 1, pp. 17-31.

Filipe, C. D. M., Daigger, G. T. & Grady, C. P. L. (2001b*).* Effects of pH on the Rates of Aerobic Metabolism of Phosphate-Accumulating and Glycogen-Accumulating Organisms. *Water Environment Research*, Vol. 73, No 2, pp. 213-222

Forurensningsforskriften (2005) *Forskrift om begrensning av forurensing.* Available at: https://lovdata.no/forskrift/2004-06-01-931 (Accessed: 3 June 2017)

Hach Company (2013) *BODTrakTH II User Manual.* 1st edn. Available at: https://www.hach.com/bodtrak-ii-respirometric-bod-apparatus/product-downloads?id=7640450995 (accessed: 3 June 2017).

Helmer, C. & Kunst, S. (1998). Low temperature effects on phosphorus release and uptake by microorganisms in EBPR plants. *Water Science and Technology*, Vol. 37, No. 4-5, pp. 531-539.

Helness H. (2007). *Biological phosphorus removal in a moving bed biofilm reactor.* Dr.art. dissertation, Norwegian University of Science and Technology. Available at: https://brage.bibsys.no/xmlui/handle/11250/242070 (assessed: 3 June 2017)

Henze, M. (2008). Wastewater Characterization*,* in Henze, M., Loosdrecht, M. C. M. V., Ekama, G. A., Brdjanovic, D. (ed). *Biological Wastewater Treatment: Principles, Modelling and Design.* London: IWA Publishing, pp. 34-52.

Janssen, P. M. J., Meinema, K. & Van Der Roest, H. F. (2002). *Biological Phosphorus Removal: Manual for Design and Operation,* London: IWA Publishing

Kang, S. J., Asfalk, T. J., Englert, C. J. & Deline, R. R. (1991). A new procedure for screening feasibility of biological phosphorus removal for a wastewater. *Water Science and Technology*, Vol. 23, pp. 595-602.

Kjørlaug, O. M. (2013). *Assessment of Biomass Functionalities in a Biofilm Membrane Bioreactor (BF-MBR) Targeting Biological Nutrient Removal.* Master thesis, Norwegian University of Science and Technology. Available at: https://brage.bibsys.no/xmlui/handle/11250/245966 (accessed: 3 June 2017)

Loosdrecht, M. C. M. V., Nielsen, P.H., Lopez-Vazquez, C. M. & Brdjanovic, D. (2016). *Experimental Methods in Wastewater Treatment.* London: IWA Publishing.

Milhelcic, J. R., Fry, L. M. & Shaw, R. (2011). Global potential for phosphorus recovery from human urine and feces. *Chemosphere*. Vol. 84, No. 6, pp. 832-839.

Mino, T., Liu, W. T., Kurisu, F. & Matsuo, T. (1995). Modeling Glycogen-Storage and Denitrification Capability of Microorganisms in Enhanced

Biological Phosphate Removal Processes. *Water Science and Technology*, Vol. 3, No. 2, pp. 25-34.

Mino, T., Loosdrecht, M. C. M. V. & Heijnen, J.J. (1998). Microbiology and Biochemistry of the Enhanced Biological Phosphorus Removal Process. *Water Research*, Vol. 32, No. 11, pp. 3193-3208.

Morse, G. K., Brett, S. W., Guy, J. A. & Lester, J. N. (1997). Review: Phosphorus removal and recovery technologies. *The Science if the Total Environment*. Vol. 212, No. 1, pp. 69-81.

Mousavi, S. A. & Ibrahim, S. (2014). Effect of COD/N Ratio on Growth and Adaption of Nitrifying Bacteria. *American Journal of Oil and Chemical Technologies,* Vol. 2, No. 6, pp. 189-196.

Naidu, R., Lamb, D. T., Bolan, N. S., & Gawandar, J. (2012) *Recovery and reuse of phosphorus from wastewater*. Available at: http://www.massey.ac.nz/~flrc/workshops/12/Manuscripts/Naidu_2012.pdf (accessed: 3 June 2017).

Oehmen, A., Lemos, P. C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L. L. & Reis, M. A. M. (2007). Advances in enhanced biological phosphorus removal: From micro to macro scale. *Water Research,* Vol. 41, No.11, pp. 2271-2300.

Raspati, G. (2016). *Data Evaluation and Trend Analysis of Wastewater Composition in Norway.*

Rickard, L. & McClintock, S. A (1992). Potassium and magnesium requirements for enhanced biological phosphorus removal from wastewater. *Water Science and Technology,* Vol. 26, No. 9, pp. 2203-2206.

Saltnes T., Sørensen G. & Eikås S. (2016) Potensialet for biologisk fosforfjerning ved norske renseanlegg. *VANN*, No. 2, pp. 148-156.

Seviour, R. J., Mino, T. & Onuki, M. (2003). The microbiology of biological phosphorus removal in activated sludge systems. *FEMS Microbiology Reviews,* Vol. 27, pp. 99-127.

Shehab, O., Deininger, R., Porta, F. & Wojewski, T. (1996). Optimizing phosphorus removal at the Ann Arbor Wastewater Treatment Plant. *Water Science and Technology*, Vol. 34, No. 1-2, pp. 493-499.

Shoji, T., Satoh, H. & Mino, T. (2003). Quantitative Estimation of the Role of Denitrifying Phosphate Accumulating Organisms in Nutrient Removal. *Water Science and Technology*, Vol. 47, No. 11, pp. 23-29.

Smolders, G. J. F., Van Der Meij, J., Loosdrecht, M. C. M. V. & Heijnen, J. J. (1994). Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. *Biotechnology and Bioengineering,* Vol. 43, No. 6, pp. 461-470.

Tykesson, E. & Jansen, J. L. C. (2005). Evaluation of Laboratory Batch Tests for Enhanced Biological Phosphorus Removal. *Vatten,* Vol. 61, No. 1, pp. 43-50.

Zeng, R. J., Saunders, A. M, Yuan, Z., Blackall, L. L. & Keller, J. (2003). Identification and Comparison of Aerobic and Denitrifying Polyphosphate-Accumulating Organism. *Biotechnology and Bioengineering,* Vol. 83, No. 2, pp. 140-148.

Zeng, W., Li, B., Wang, X., Bai, X., Peng, Y. (2016). Influence of nitrite accumulation on «Candidatus Accumulibacter» population structure and enhanced biological phosphorus removal from municipal wastewater. *Chemosphere.* Vol. 144, pp. 1018-1025.

Wentzel, M. C., (2008). Enhanced Biological Phosphorus Removal, in Henze, M., Loosdrecht, M. C. M. V., Ekama, G. A., Brdjanovic, D. (ed). *Biological Wastewater Treatment: Principles, Modelling and Design.* London: IWA Publishing, pp. 155-220.

Zhang, T., Liu, Y. & Fant, H. H. P (2005). Effect of pH change on the performance and microbial community of enhanced biological phosphate removal process. *Biotechnology and Bioengineering,* Vol. 92, pp. 173-182.

Zheng, X., Sun, P., Han, J., Song, Y., Hu, Z., Fan, H. & Lv, S. (2014). Inhibitory factors affecting the process of enhanced biological phosphorus removal (EBPR) – A mini-review. *Process Biochemistry*, Vol. 49, No. 12, pp. 2207-2213.

Ødegaard, H. (1999). The influence of wastewater characteristics on choice of wastewater treatment method, *Proc. Nordic Conference: Nitrogen removal and biological phosphate removal.* Oslo 2. – 4. February 1999

Ødegaard, H., Rusten, B, Storhaug, R. & Paulsrud, B. (2009). *Veiledning for dimensjonering av avløpsanlegg* (Norsk Vann Rapport 168-2009). Hamar: Norsk Vann. Available at: https://norskvann.no/kompetanse/va-bokhandelen/rapporter/product/172-r168-veiledning-for-dimensjonering-av-avlopsrenseanlegg (accessed: 3 June 2017).

Ødegaard, H., Norheim, B. & Norsk Vann, B. A. (2014) *Vann- og avløpsteknikk.* 2nd edn. Hamar: Norsk Vann

APPENDIX A

Table A.1. List of used Hach Lange methods and the measuring range for these methods. If needed, the samples were diluted with MQ water in order to fall into the measuring range. Foot note explains method used for filtration of samples. No footnote indicates that samples were not filtrated.

Analysis	Hach Lange method	Measuring range
NH_4-N [a]	LCK 303	2.0 – 47.0 mg/l
NO_3-N [a]	LCK 340	0.23 – 13.5 mg/l
NO_2-N [a]	LCK 341	0.015 – 0.6 mg/l
TN	LCK 138	1.0 – 16.0 mg/l
PO_4-P [a]	LCK 348	0.5 – 5.0 mg/l
TP	LCK 348	0.5 – 5.0 mg/l
tCOD [a]	LCK 114	15 – 150 mg/l
sCOD	LCK 114	15 – 150 mg/l

[a] Filtered with 0.45 µm cellulose and nitrate filter (Sartorius). Filters were thoroughly rinsed with MQ three times before filtrating the samples.

APPENDIX B

Table B.1. List of used methods used for characterization of bio-P sludge upon arrival, including Hach Lange methods, measuring range and methods used for determination of solids. Foot note explains method used for filtration of samples. No footnote indicates that samples were not filtrated.

Analysis	Hach Lange method	Measuring range
NH_4-N [a]	LCK 303	2.0 – 47.0 mg/l
NO_3-N [a]	LCK 340	0.23 – 13.5 mg/l
NO_2-N [a]	LCK 341	0.015 – 0.6 mg/l
TN	LCK 138	1.0 – 16.0 mg/l
PO_4-P [a]	LCK 348	0.5 – 5.0 mg/l
TP	LCK 348	0.5 – 5.0 mg/l
tCOD [a]	LCK 114	15 – 150 mg/l
sCOD	LCK 114	15 – 150 mg/l
TS	EPA 1684	-
VSS	EPA 1684	-

[a] Filtered with 0.45 µm cellulose and nitrate filter (Sartorius). Filters were thoroughly rinsed with MQ three times before filtrating the samples.

APPENDIX C

Table C.1 Hourly characterization of influent wastewater including average values, minimum values, maximum values and standard deviation.

Sample time	tCOD (mg/L)	sCOD (mg/L)	TN (mg/L)	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	pH
8:37 AM	431.5	140	59.2	30.3	1.11	0.151	8.4
10:44 AM	780	177	59.1	35.6	0.914	0.162	8.53
12:50 PM	333	142.5	35.1	23.4	0.878	0.129	7.88
2:57 PM	495.5	150.5	29.5	16.1	1.12	0.144	7.7
5:04 PM	426.5	180.5	26.7	16.7	1.09	0.14	7.4
7:11 PM	336.5	159.5	35.7	19.3	1.09	0.119	7.75
9:18 PM	326	161	39.5	27.8	0.942	0.127	7.42
11:25 PM	255	117.5	32.7	22.4	0.818	0.121	8.41
3:39 AM	188.5	122.5	32.9	19.5	0.772	0.093	7.71
7:53 AM	236.5	111.5	40.2	25.2	0.992	0.1	7.12
Average value	380.90	146.25	39,06	23.63	0.97	0.13	7.74
Minimum value	188.50	111.50	26,70	16.10	0.77	0.09	7.12
Maximum value	780.00	180.50	59,20	35.60	1.12	0.16	8.53
Standard deviation	161.55	20.65	11,34	6.21	0.13	0.02	0.42

Sample time	TP (mg/L)	PO_4-P (mg/L)	TS (g/L)	TDS (g/L)	TSS (g/L)	VSS (g/L)	BOD_5 (mg/L)
8:37 AM	6.02	3.81	0.5725	0.2739	0.2641	0.2268	228.40
10:44 AM	6.62	4.15	0.6044	0.2976	0.3200	0.2800	275.58
12:50 PM	4.28	2.30	0.3789	0.2558	0.1461	0.1160	226.50
2:57 PM	3.5	1.73	0.4575	0.3228	0.1931	0.1757	223.46
5:04 PM	3.16	1.41	0.53445	0.3037	0.1679	0.1426	225.97
7:11 PM	3.74	2.24	0.4401	0.3426	0.1413	0.1113	138.78
9:18 PM	3.98	2.23	0.3896	0.2813	0.1406	0.1018	228.45
11:25 PM	3.36	1.83	0.3167	0.2260	0.1518	0.1044	172.60
3:39 AM	3.06	2.21	0.2963	0.2569	0.0777	0.0671	106.55
7:53 AM	4.04	2.24	0.4150	0.1705	0.0931	0.0815	-
Average value	4.18	2.42	0.44	0.28	0.18	0.15	202.92
Minimum value	3.06	1.41	0.30	0.23	0.08	0.07	106.55
Maximum value	6.62	4.15	0.60	0.34	0.32	0.28	275.58
Standard deviation	1.20	0.88	0.10	0.03	0.07	0.06	49.90

APPENDIX D

Table D.1 Daily characterization of influent wastewater including average values, minimum values, maximum values and standard deviation.

Sample date	tCOD (mg/L)	sCOD (mg/L)	TN (mg/L)	NH_4-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)
21st February	45.00	156.50	65.80	22.20	0.12	1.35
22nd February	410.00	156.00	58.15	26.60	0.09	0.82
23rd February	340.00	157.00	39.55	24.50	0.09	0.83
24th February	317.50	176.00	47.10	31.20	0.16	0.88
Average value	380.88	161.38	52.65	26.13	0.12	0.97
Minimum value	317.50	156.00	39.55	22.20	0.09	0.82
Maximum value	456.00	176.00	65.80	31.20	0.16	1.35
Standard deviation	55.18	8.45	10.07	3.32	0.03	0.22

Sample date	TP (mg/L)	PO_4-P (mg/L)	TS (g/L)	TDS (g/L)	TSS (g/L)	VSS (g/L)
21st February	6.73	5.55	0.49	0.24	0.19	0.1897
22nd February	5.61	3.56	-	-	-	-
23rd February	4.56	3.07	0.44	0.32	0.12	0.1273
24th February	5.20	3.68	0.40	0.34	0.09	0.1081
Average value	5.53	3.97	0.44	0.30	0.13	0.14
Minimum value	4.56	3.07	0.40	0.24	0.09	0.11
Maximum value	6.73	5.55	0.49	0.34	0.19	0.19
Standard deviation	0.79	0.94	0.04	0.04	0.04	0.03

APPENDIX E

E1. PROCEDURE FOR DETERMINING BOD_5 USING HACH BODTRAK™ INSTRUMENT

This procedure for determination of BOD_5 in wastewater samples is adapted from Standard Method 5219 D Respirometric Method (American Public Health et al., 1960) and Hach Standard Method procedure for BOD using the user manual (Hach Company, 2013). The method used Hach BODTrak™ Respirometric BOD Apparatus to measure the DO concentration in incubation bottles containing prepared BOD wastewater samples over an incubation period of 7 days.

BOD sample preparation

The BOD sample are prepared according to table E.1, modified from BODTrak™ User Manual (Hach Company, 2013). BOD range used for sample preparation is 0 to 700 mg O_2/L as influent wastewater samples with unknown BOD concentration typically fall within this range. The BOD sample are prepared by adding 45 mL of wastewater at room temperature and 35 mL of seed measured into a graduate cylinder, giving a total volume of 80 mL. The seed used is fresh wastewater, and ensures sufficient viable microorganisms in the experiment. Make sure that all measured volumes are homogeneous.

Table E.1. Sample and seed volume required according to BOD range (Modified from Hach Standard Method)

BOD range (mg/L)	Sample volume (mL)	Seed volume (mL)	Final volume (mL)	Dilution factor
0 to 35	370	35	405	1.09
0 to 70	305	35	340	1.11
0 to 350	110	35	145	1.32
0 to 700	45	35	80	1.77

1 mL of ATU solution (see section E.5) is added per liter prepared BOD sample. Temperature and pH is corrected to 20 °C and 7, respectively. The prepared BOD sample containing wastewater, seed, ATU and correct pH and temperature is added to a 300 mL BOD incubator bottle. The incubator bottle is sealed by placing a plastic seal cup into the bottle neck. Two KAO pellets are placed in the seal cup for oxygen uptake. The incubator bottle is placed on the

BODTrak™ chassis. The bottle neck is greased, connected to the applicable tubes and sealed airtight by attaching the caps.

The BODTrak™ instrument is programmed to the correct BOD range used in the experiment (0 – 700 mg/L) and test duration (7 days). The duration is set to 7 days to analyze the complete curve illustrating the DO concentration.

Blank seed sample preparation

A blank seed sample is prepared for correction of the seed added to the BOD samples. The seed blank is prepared identical to the BOD sample and table E.1, but with adding 45 mL of distilled water instead of wastewater. ATU solution is added, as well as correction for temperature and pH.

E2. INTERPRETATION OF RESULTS AND CALCULATION OF BOD_5

After 7 days of incubation the O_2 concentration curve is displayed in the instrument display. The results are extracted from the display by photographing the BODTrak™ display and digitalizing the curve using software Plot Digitizer. A polynomial trendline of third degree is used to fit the digitalized BOD curve. The third degree polynomial trendline equation is determined where x is the duration of the experiment of the desired BOD analysis (x = 5 if BOD_5 is of interest), as in equation E1,

$$\boldsymbol{BOD_{5,obs} = a_1x^3 + a_2x^2 + a_3x + a_4} \quad \text{(E1)}$$

Where:

$BOD_{5,\,obs}$ = Observed BOD_5 concentration, mg/L

a_1, a_2, a_3, a_4 = non-zero value

x = duration of desired BOD analysis

The real BOD_5 value is calculated by correcting the $BOD_{5,\,obs}$ value for the dilution factor based on table E.1., and the added seed using equation E2.

$$\boldsymbol{Real\ BOD_5 = BOD_{5,obs} * D - (BOD_{5,seed} * \frac{V_{seed}}{V_{wastewater}})} \quad \text{(E2)}$$

Where:

Real BOD_5 = Real BOD_5 concentration of the wastewater, mg/L

D = Dilution factor

$BOD_{5,obs}$ = Observed BOD_5 after corrected with dilution factor, mg/L

$BOD_{5,\ seed}$ = BOD_5 concentration for seed, mg/L

V_{seed} = Volume of seed added to sample, mL

$V_{wastewater}$ = Volume of wastewater added to sample, mL

E3. EXAMPLE USING SAMPLE 9:18 PM FOR CALCULATING BOD_5

Digitized O_2 curve with fitted polynomial trendline presented in figure E.1 and polynomial third degree trendline equation presented in equation E3,

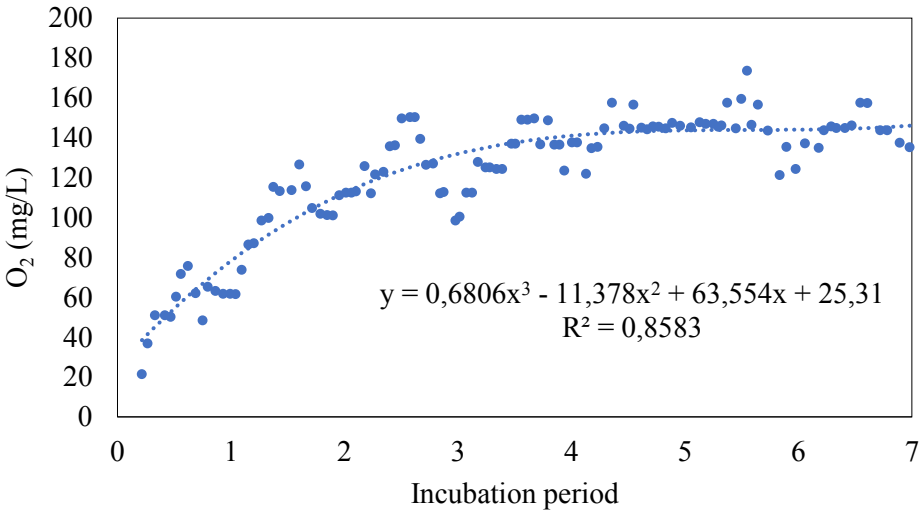


Figure E.1. Digitized O_2 curve with fitted polynomial trendline, trendline equation and R2 value is presented.

Polynomial third degree trendline equation:

$$\boldsymbol{BOD_{5,obs} = 0.6806 - 11.378x^2 + 63.554x + 23.31} \quad \text{(E3)}$$

Observed BOD_5 when x is 5 incubation days:

$$\boldsymbol{BOD_{5,obs} = 143.705\ \text{mg}\ O_2/\text{L}}$$

The real BOD_5 value is calculated using dilution factor 1.77 and correcting for seed added with concentration of 33.33 mg/L according to equation E4,

$$\boldsymbol{Real\ BOD_5 = 143.705 \frac{mg}{L} * 1.77 - \frac{35\ mL * 33.33 \frac{mg}{L}}{45\ mL}} \quad \text{(E4)}$$

$$\boldsymbol{Real\ BOD_5 = 228.45\ \text{mg}\ O_2/\text{L}}$$

E4. TABLE SHOWING ALL CALCULATED BOD_5 VALUES

Table E.2 Samples measured and calculated for BOD_5. Tables shows individual polynomial equations, the calculated observed BOD_5 value when multiplied with dilution factor and the real calculated BOD_5 value

Sample	Polynomial trendline equation	R^2	Observed BOD5 value* (mg/L)	Seed concentration (mg/L)	Corrected BOD_5 value** (mg/L)
8:37 am	$-0.2331\ x^3 - 3.0045\ x^2 + 52.277\ x - 15.162$	0.96	251.27	29.45	228.40
10:44 am	$0.4816\ x^3 - 13.289\ x^2 + 97.167\ x - 44.956$	0.96	298.49	29.45	275.58
12:50 pm	$-0.0596\ x^3 - 3.7584\ x^2 + 43.515\ x + 26.432$	0.90	252.40	33.33	226.50
2:57 pm	$0.3611\ x^3 - 9.5742\ x^2 + 69.592\ x - 14.549$	0.95	246.37	29.45	223.46
5:04 pm	$0.5402\ x^3 - 10.336\ x^2 + 66.02\ x + 3.0856$	0.91	251.89	33.33	225.97
7:11 pm	$-0.0829\ x^3 - 4.5225\ x^2 + 51.179\ x - 41.122$	0.89	161.69	29.45	138.78
9:18 pm	$0.6806\ x^3 - 11.378\ x^2 + 63.554\ x + 25.31$	0.86	254.36	33.33	228.45
11:25 pm	$0.2973\ x^3 - 7.2207\ x^2 + 51.08\ x + 0.1084$	0.85	198.51	33.33	172.60
3:39 am	$-0.2205\ x^3 - 1.0017\ x^2 + 27.453\ x - 9.8161$	0.63	132.47	33.33	106.55

* Value multiplied with dilution factor = 1.77 and x = 5 days

E5. PREPARATION OF ALLYLTHIOUREA (ATU) SOLUTION

ATU solution was prepared according to Hach Standard Procedure and NS-EN 1899-2:1998. 0.1 g allylthiourea ($C_4H_8N_2S$) was dissolved in 25 mL MQ water, before diluting the solution to 50 ml.

APPENDIX F

F1 RESULTS FROM ALL BATCH ACTIVITY EXPERIMENTS PERFORMED

Experiment 1: Initial activity in bio-P sludge under anaerobic conditions with external acetate

Table F.1. 353 mL bio-P sludge mixed with 647 mL MQ water with 300 mg sCOD/L

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	NO_3-N (mg/L)	NH_4-N (mg/L)	NO_2-N (mg/L)	TP (mg/L)	TN (mg/L)	tCOD (mg/L)	VSS (g/L)
0	7.66	722	0.551	15.2	0.027	61	210	3930	2.3
15	4.85	934	-	-	-	-	-	-	-
25	6.34	1000	-	-	-	-	-	-	-
35	6.01	540	-	-	-	-	-	-	-
65	7.27	466	-	-	-	-	-	-	-
120	10.20	709	-	-	-	-	-	-	-
180	9.86	230	-	-	-	-	-	-	-
240	11.80	61,7	-	-	-	-	-	-	-
300	10.10	219	-	-	-	-	-	-	-
360	10.90	234	0.245	13.5	0.015	11.6	216	3120	2.3

Experiment 2A: Activity in bio-P sludge under anaerobe – aerobe conditions with external acetate

Table F.2. 353 mL bio-P sludge mixed with 647 mL MQ water with 100 mg sCOD/L

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	NH_4-N (mg/L)	TP (mg/L)	tCOD (mg/L)	TN (mg/L)	VSS (g/L)
0	6.04	78.5	0.244	0.025	14.6	55.8	2000	271	2.0
10	5.17	143	0.288	0.011	15.7	-	-	-	-
20	5.56	134	-	-	-	-	-	-	-
30	5.42	137	-	-	-	-	-	-	-
60	7.1	139	-	-	-	-	-	-	-
120	9.94	125	0.512	0.011	15.7	59.3	776	337	-
121	10.6	136	0.462	0.01	16.4	-	-	-	-
130	9.9	114	0.563	0.013	-	-	-	-	-
140	6.76	101	-	0.016	14.9	-	-	-	-
150	4.5	105	0.425	-	13.9	-	-	-	-
180	0.86	114	-	0.033	-	-	-	-	-
240	0.3	86.3	0.555	-	13.3	55.9	3560	282	2.2

Experiment 2B: Activity in bio-P sludge under anaerobe – anoxic conditions with external acetate

Table F.3. 353 mL bio-P sludge mixed with 647 mL MQ water with 100 mg sCOD/L

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	NH_4-N (mg/L)	TP (mg/L)	tCOD (mg/L)	TN (mg/L)	VSS (g/L)
0	7.17	98.8	0.324	0.028	22.5	60.5	2710	271	2.0
10	6.33	123	0.532	0.015	22.3	-	-	-	-
20	7.75	170	-	-	-	-	-	-	-
30	8.36	137	-	-	-	-	-	-	-
60	8.31	122	-	-	-	-	-	-	-
120	12.8	137	0.58	0.012	19.6	57.1	3080	242	-
121	14	114	3.96	0.291	19.8	-	-	-	-
130	10.8	115	3.86	-	-	-	-	-	-
140	10.8	94.9	-	0.319	16.7	-	-	-	-
150	12.7	109	1.84	0.319	18.3	-	-	-	-
180	12.5	96.5	-	-	-	-	-	-	-
240	13.4	95.2	0.544	0.016	18.9	68	4990	310	2.1

Experiment 3A: Activity in bio-P sludge and wastewater under anaerobe – aerobe conditions

Table F.4. 353 mL bio-P sludge mixed with 647 mL wastewater

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	NH_4-N (mg/L)	TP (mg/L)	TCOD (mg/L)	TN (mg/L)	g VSS/L (g/L)
0	12.387	141.6	60.1	67.4	1770	291	2.0
10	12.717	127.5	57.1	-	-	-	-
20	10.431	111.9	-	-	-	-	-
30	13.353	130.2	-	-	-	-	-
60	13.05	135	-	-	-	-	-
120	18.18	152.7	67.7	57.8	1780	235	-
121	20.67	165.3	-	-	-	-	-
130	18.39	159	73.9	-	-	-	-
140	14.037	139.8	61.6	-	-	-	-
150	14.229	159	-	-	-	-	-
180	14.667	170.7	71.9	-	-	-	-
240	14.958	198.9	72.1	60.6	1720	229	2.3

Experiment 3B: Activity in bio-P sludge and wastewater under anaerobe – anoxic conditions

Table F.5. 353 mL bio-P sludge mixed with 647 mL wastewater

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	NO_3-N (mg/L)	TP (mg/L)	tCOD (mg/L)	TN (mg/L)	g VSS/L (g/L)
0	1725	179.1	1.32	61.4	1770	227	2.0
10	18.27	-	-	-	-	-	-
20	12.04	204.1	1.09	-	-	-	-
30	19.92	194.7	-	-	-	-	-
60	19.74	-	1.28	-	-	-	-
120	20.37	221.4	3.89	63.4	1760	216	-
121	20.1	174.6	-	-	-	-	-
130	20.46	178.5	8.55	-	-	-	-
140	20.46	204.3	8.02	-	-	-	-
150	20.55	201.6	-	-	-	-	-
180	20.4	181.5	5.84	-	-	-	-
240	21.15	207.6	6.32	57.9	1740	217	1.5

Experiment 4: Activity in bio-P sludge and wastewater under anaerobe and aerobe conditions with external acetate

Table F.6. 353 mL bio-P sludge mixed with 647 mL wastewater and 300 mg sCOD/L

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	VSS (g/L)
0	22.3	249	1.35
10	19.9	-	-
20	19.4	-	-
30	19.0	421	-
60	22.6	-	-
120	21.3	452	-
121	22.0	-	-
130	22.2	-	-
140	21.4	-	-
150	23.0	487	-
180	21.8		-
240	23.1	464	1.38

Experiment 5: Activity in 15-day old bio-P sludge under anaerobe and aerobe conditions with external acetate performed

Table F.7. 353 mL bio-P sludge mixed with 647 mL MQ water and 300 mg sCOD/L

Time (min)	PO_4-P (mg/L)	sCOD (mg/L)	NH_4-N (mg/L)	TP (mg/L)	tCOD (mg/L)	TN (mg/L)	VSS (g/L)
0	22.1	200	55.7	705	2500	198	2.1
10	23.6	360	60.3	-	-	-	-
20	22.2	240	-	-	-	-	-
30	25	260	-	-	-	-	-
60	26	250	-	-	-	-	-
120	23.5	220	56.3	676	2200	186	-
130	23.9	220	56.3	-	-	-	-
140	24.3	200	-	-	-	-	-
150	24.3	180	57.2	-	-	-	-
180	28.7	370	-	-	-	-	-
240	27.2	230	60.2	586	3000	237	2.0

APPENDIX G

G.1 P RELEASE AND P UPTAKE RATES AND ASSOCIATED R^2

All P release rates include values between t = 10 minutes and 120 minutes.

All P uptake rates include values between t = 120 minutes to 140 minutes.

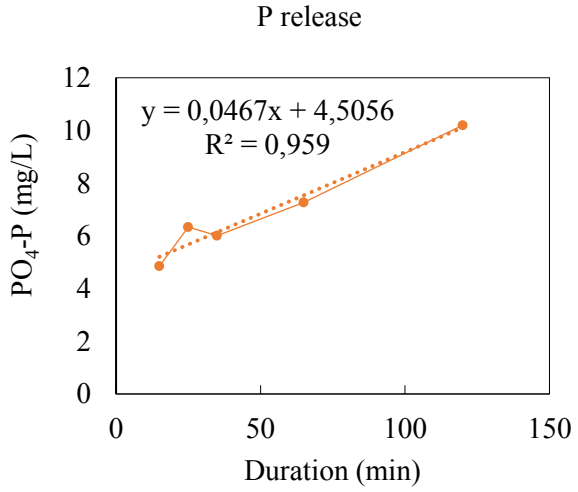
Table G.1 Calculated P release and P uptake rates for all batch experiments

Experiment	MLVSS (g VSS)	P release rate* (mg P/g VSS*h)	R^2 release	P uptake rate* (mg P/g VSS*h)	R^2 uptake
1	2.2259	1.26	0.96	-	-
2A	2.0284	1.32	0.98	6.04	0.900
2B	2.0284	1.55	0.92	-	-
3A	1.9757	1.73	0.80	10.65	0.978
3B	1.9757	≈ 0	-	≈ 0	-
4	1.3470	≈ 0	-	≈ 0	-
5	2.4754	≈ 0	-	≈ 0	-

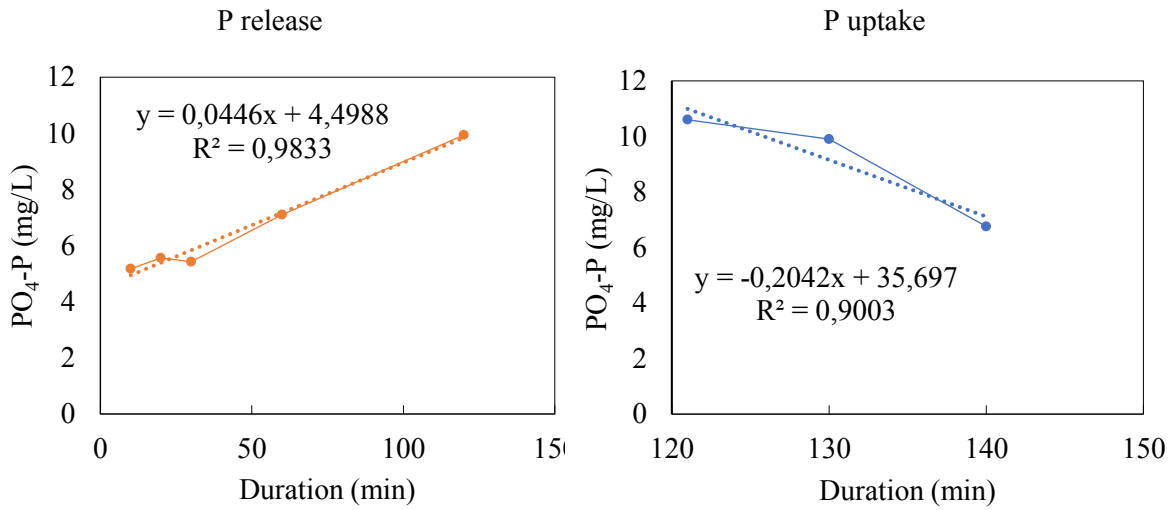
* Slope of linear line multiplied with 60 min/h and divided by the MLVSS concentration in g VSS/L.

G.2 P RELEASE AND P UPTAKE LINEAR AREA FOR BATCH EXPERIMENTS

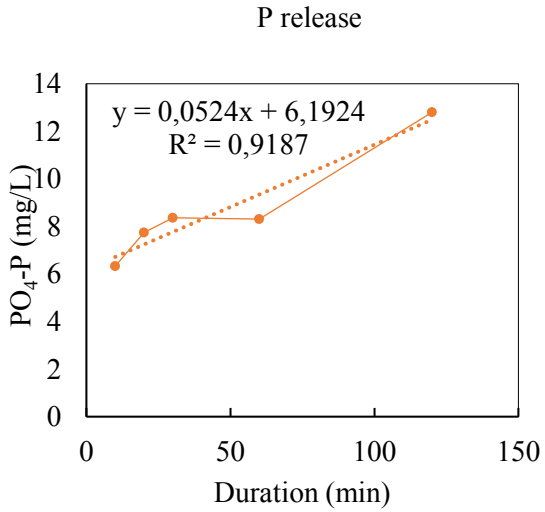
Experiment 1



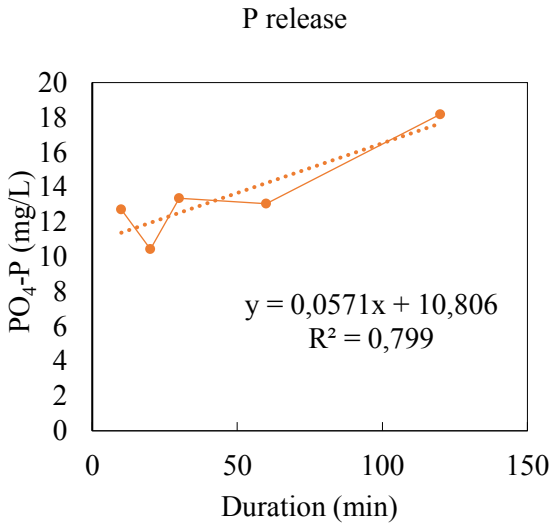
Experiment 2A

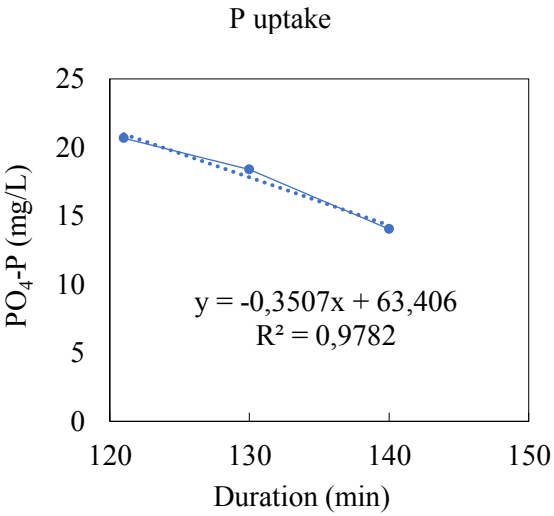


Experiment 2B

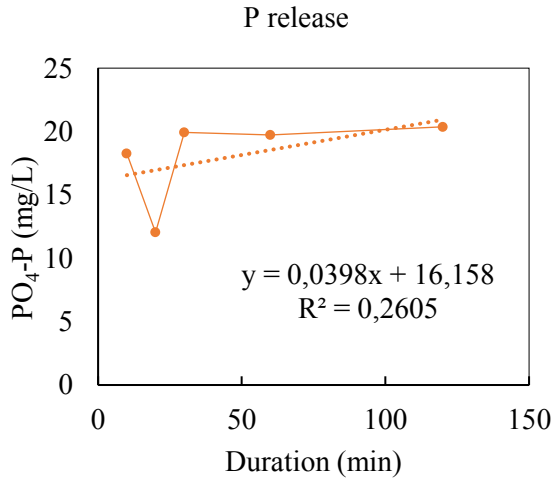


Experiment 3A

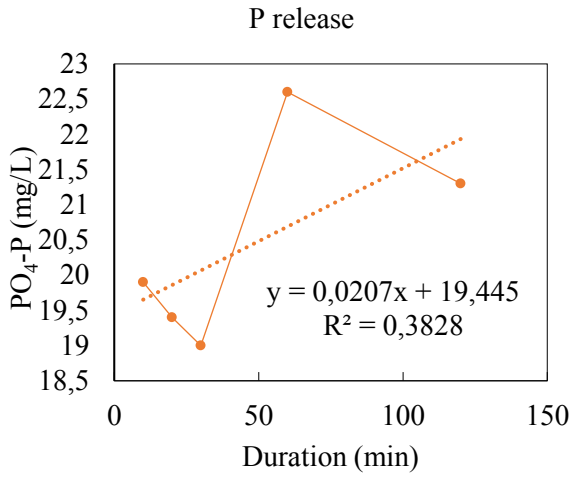


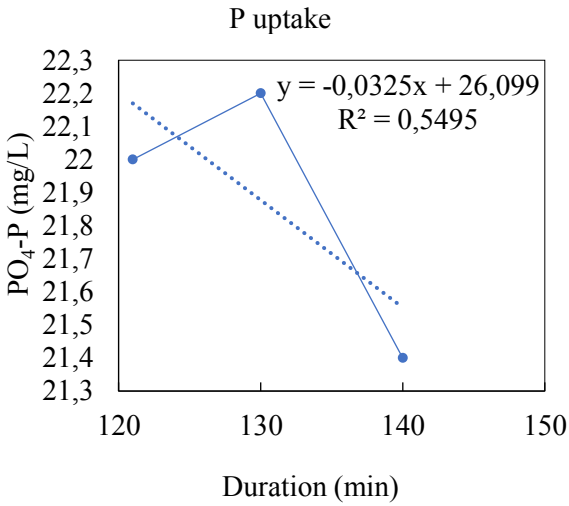


Experiment 3B



Experiment 4





Experiment 5