Potential for Enhanced Biological Phosphorus Removal in an activated sludge system for greywater

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by

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Picture on front page: Reco-Lab facility Photo by Ashley Hall

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Preface

First, I would like to thank my supervisors in this master's thesis. Thank you, Michael Cimbritz, for always giving me positive feedback and helping me to be confident during this project. A special thanks to Hamse Kjerstadius for always helping me during this project with his helpful tips and being available even during his parental leave. I would also like to thank my examiner Åsa Davidsson, who helped me a lot by providing useful resources during this project and arranged the car for us to take the samples from the Reco-Lab in Helsingborg.

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Summary

As the world's population grows, concerns about water scarcity and rising pollution such as phosphorus and nitrogen have increased significantly. Greywater has been considered by researchers as an ideal alternative to fresh water for decades, and several projects have been implemented to treat and recycle it for various reuse purposes. Greywater, which makes up about 80% of household water consumption, is treated and reused by different treatment methods. One of the parameters that should be largely eliminated is phosphorus, the large amount of which causes many problems in human health and the environment, including eutrophication. Different treatment methods have been used to remove phosphorus. One of these most widely used methods is the Enhanced biological phosphorus removal (EBPR) method, which can remove high levels of phosphorus. This method has been implemented in the Oceanhamnen project for greywater in Helsingborg. In this master thesis, the potential for phosphorus removal by using the EBPR process was investigated.

This was done by using one-year laboratory data provided by NSVA company along with two laboratory tests, P-release and 5-point titration experiments. In the early period of this master thesis, the concentration of sludge in aeration basin was significantly reduced and therefore re-inoculation process was performed by adding sludge from Öresundverket to accelerate the recovery operation. The experiments were done before and after the re-inoculation. Based on the results, despite the relatively high rate of phosphorus removal in the effluent, there was limited Bio-P activity in the system due to low sludge concentration as well as operational condition. Therefore, phosphorus removal might also occur by other mechanisms such as assimilation. Finally, by assuming that the sludge in Reco-Lab would be of the same quality of Öresundverket, the maximum potential for phosphorus removal was calculated and considered as maximum capacity of the EBPR system. However, this potential can only be considered for the Reco-Lab project with its specific condition as well as greywater characteristics.

Sammanfattning

I takt med att världens befolkning växer har oron för vattenbrist och ökande föroreningar som fosfor och kväve ökat väsentligt. Gråvatten har ansetts av forskare som ett idealiskt alternativ till sötvatten i decennier, och flera projekt har genomförts för att behandla och återvinna det för olika återanvändningsändamål. Gråvatten, som utgör cirka 80 % av hushållens vattenförbrukning, behandlas och återanvänds med olika behandlingsmetoder. En av parametrarna som i stort sett bör elimineras är fosfor, vars stora mängd orsakar många problem för människors hälsa och miljön, inklusive övergödning. Olika reningsmetoder har använts för att avlägsna fosfor. En av dessa mest använda är Enhanced Biological phosphorus removal (EBPR), som kan ta bort höga halter av fosfor. Denna metod har implementerats i Oceanhamnen-projektet för gråvatten i Helsingborg. I det här mastersarbetet undersöktes potentialen för borttagning av fosfor genom att använda EBPR-processen.

Detta gjordes genom att använda ettåriga laboratoriedata från NSVA-företaget tillsammans med två laboratorietester, P-release och 5-punkts titreringsexperiment. Under den tidiga perioden av detta examensarbete reducerades mängden slam i luftningsbassängen avsevärt och därför genomfördes åter inokulering processen genom att tillsätta slam från Öresundverket för att påskynda återvinningsoperationen. Experimenten gjordes före och efter åter inokuleringen. Baserat på resultaten, trots den relativt höga hastigheten av fosforavskiljning i avloppsvattnet, fanns det begränsad Bio-P-aktivitet i systemet på grund av dålig slamkoncentration såväl som drifttillstånd. Därför kan fosforavlägsnande ske genom andra mekanismer såsom assimilering. Slutligen, genom att anta att slam i Reco-Lab skulle vara av samma kvalitet som Öresundverket, beräknades maximal potential för fosforavskiljning och betraktades som maximal kapacitet för EBPR-systemet. Denna potential kan dock endast beaktas för Reco-Lab-projekt med dess specifika tillstånd såväl som gråvattenegenskaper.

Greywater is gold

How greywater could save our planet from drought!

What if mankind no longer had to worry about water shortages and a drought crisis? Along with population growth and increasing water consumption, the amount of wastewater produced has also increased significantly, which if not controlled will create many challenges such as water shortages as well as environment problems. For instance, if the presence of nutrients such as phosphorus and nitrogen is not sufficiently removed from wastewater, it can lead to many problems including eutrophication and groundwater pollution that affect our lives. Hence, source separation as a solution has been proposed in the last two decades. The part of sewage that comes from the washing, kitchen and laundry sections is called greywater, which plays an important role since it constitutes about 80% of the household water consumption. Greywater pollution is far less than that of toilet water, which is called blackwater. Greywater as an exceptional opportunity can be treated and reused with easier and more cost-effective methods for different reuse applications, including agriculture, car wash, land washing and even as drinking water!

However, in order to reuse this part, different types of pollution such as phosphorus, nitrogen as well as micropollutants which comes mostly from chemical products as well as contaminants that are produced in the kitchen should be greatly removed. A unique project in the city of Helsingborg, which is called Oceanhamnen, has carried out a full-scale separation process, so that the wastewater produced in this area is transported to the treatment facility through three separate pipes. These three pipes include greywater, black water, as well as food waste provide a good opportunity for nutrients recovery, energy production as well as recycling water. To fulfil the existing reuse water standards, greywater should be treated properly through different steps which is called treatment process. Phosphorus as an important parameter should be largely separated from greywater. Instead of adding different chemicals to remove the contaminants, biological treatment methods can be successfully used to achieve high quality water in the effluent. One of the suitable methods in this section is Enhanced biological phosphorus removal (EBPR) which can remove phosphorus in greywater to a great extent.

The aim of this study was to investigate the potential for phosphorus removal from greywater by using this method. This was done through several laboratory experiments. By having high quality treated greywater, high portion of consumed water by people can be returned to the urban system and it is hoped to reduce water scarcity throughout the world. Therefore, the treatment processes should be selected carefully to meet the requirements. In the end, it can be said that greywater is the key to achieving this great goal!

Gråvatten är guld

Hur gråvatten kan rädda vår planet från torka!

Tänk om mänskligheten inte längre behövde oroa sig för vattenbrist och torka? I kombination med befolkningsökning och ökad vattenförbrukning, har mängden producerat avloppsvatten också ökat markant. Om detta inte kontrolleras kommer många utmaningar skapas såsom vattenbrist och miljöproblem. Till exempel, om näringsämnen som fosfor och kväve inte avlägsnas tillräckligt från avloppsvattnet, kan det leda till många problem, inklusive övergödning och grundvattenföroreningar som i sin tur påverkar våra liv. Därför har källseparering föreslagits som en lösning under de senaste två decennierna. Den del av avloppsvattnet som kommer från bad-, disk- och tvättvattenavdelningarna kallas gråvatten, och utgör cirka 80 % av hushållens vattenförbrukning. Gråvattenföroreningarna är mycket mindre än i toalettvatten, som kallas svartvatten. Gråvatten har en exceptionell möjlighet att behandlas och återanvändas med enklare och mer kostnadseffektiva metoder för olika återanvändningsapplikationer, inklusive jordbruk, biltvätt och marktvätt men även som dricksvatten!

Men för att återanvända gråvatten bör olika typer av föroreningar som fosfor, kväve, mikroföroreningar, som till största delen kommer från kemiska produkter, samt föroreningar som produceras i köket reduceras kraftigt. Ett unikt projekt i Helsingborgs stad, som kallas Oceanhamnen, har genomfört en fullskalig separeringsprocess, så att avloppsvattnet som produceras i detta område transporteras till reningsanläggningen genom tre separata rör. Dessa tre rör inkluderar gråvatten, svartvatten, samt matavfall vilket ger goda möjligheter till näringsåtervinning, energiproduktion samt återvinning av vatten. För att uppfylla befintliga återanvändningsvattenstandarder bör gråvatten behandlas ordentligt genom olika steg i en reningsprocess. Fosfor, som en viktig parameter, bör till stor del separeras från gråvatten. I stället för att tillsätta olika kemikalier för att avlägsna föroreningarna, kan biologiska reningsmetoder framgångsrikt användas för att uppnå vatten av hög kvalitet. En av de lämpliga metoderna för detta är Enhanced Biological phosphorus removal (EBPR) som kan ta bort fosfor i gråvatten i stor utsträckning.

Syftet med denna studie var att undersöka potentialen för fosforavskiljning från gråvatten genom att använda EBPR. Detta gjordes genom flera laboratorieförsök. Genom att få fram högkvalitativt renat gråvatten kan en stor del av det förbrukade vattnet av människor återföras till stadssystemet. Förhoppningen med detta är att man kan minska vattenbristen i hela världen. Därför bör behandlingsprocesserna väljas noggrant för att uppfylla kraven. I slutändan kan man säga att gråvatten är nyckeln till att nå det stora målet med en värld utan vattenbrist!

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List of Abbreviations

BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
CW	Constructed wetland
DO	Oxygen concentration
EBPR	Enhanced Biological Phosphorus removal
fCOD	filtered COD
GAO	Glycogen accumulating organism
GW	Greywater
HRT	Hydraulic retention time
MBR	Membrane bio reactor
N	Nitrogen
NSVA	Nordvästra Skåne Vatten och Avlopp AB
NSR	Nordvästra Skånes Renhållnings AB
P	Phosphorus
PAO	Phosphate accumulating organism
PHA	Polyhydroxyalkanoates
RBC	Rotating biological contactor
rbCOD	Readily biodegradable COD
SCST	Sanitation concept for separate treatment
SRT	Sludge retention time (Sludge age)
VFA	Volatile fatty acid

1 Introduction

Along with the increase in the world's population and increasing water consumption by people, it has led to various challenges for human beings such as water scarcity as well as problems with wastewater management. These challenges can appear in different ways in different parts of the world including drought, flooding, public health issues, and environmental problems. One of the possible solutions is to treat the wastewater and remove contaminants to maintain the ecosystem cycle. Beside treating wastewater, many beneficial outcomes could be achieved such as nutrient recovery as fertilizers for agricultural industry and water reuse for different potable/non-potable applications. Greywater as a main part of domestic wastewater which is separated from toilet wastewater, black water, is considered as a promising alternative to reuse in different purposes. Greywater contains about 70-90% of domestic sewage and comes from different parts of a house including kitchen, laundry, showering and washing sections. Hence, recently the study on greywater is of great importance to researchers and several projects have been done in this field. Although greywater contains much lower contaminants and pathogens compared to wastewater from toilets that contains higher amount of nutrients, there are still many organic micropollutants which come from several chemical products that are used by inhabitants for cleaning and hygiene purposes. As a result to fulfil the different standards for reuse purposes such as irrigation or drinking water, suitable treatment methods should be used to remove nutrients and micropollutants to a great extent (Turner *et al.*, 2013). The idea of source-separation has been one of the fascinating issues among researcher during the last two decades. The main purpose of source separation is to achieve higher resource recovery as well as energy production. From the beginning of 2021, a unique project that is called Oceanhamnen has been carried out by Nordvästra Skåne Vatten och Avlopp AB (NSVA) company in cooperation with the municipality of Helsingborg. The separation process is done with the aim of achieving higher rate of resource recovery from sewage system. This is done by separating the sewage into three separate pipes that transfer blackwater, greywater and food wastes to the Reco-Lab treatment facility which is located near Öresundverket treatment plant. In Reco-Lab, each of these streams will be conveyed through suitable treatment methods to enhance the recycling of water, nutrients as well as producing biogas (NSVA, 2020). However, it remains to be solved how to most efficiently treat such source separated wastewater, especially the greywater which constitutes the largest volume of domestic wastewater. According to the study done by Arinaitwe (2018), different treatment methods such as moving bed biofilm reactor (MBBR), sequencing batch reactor (RBC) and constructed wetlands have been carried out to evaluate the efficiency of phosphorus removal from greywater. The results showed that only constructed wetland had an acceptable phosphorus removal while this method will require a lot of space, which is not applicable for every project.

1.1 Problem description

For three decades, Enhanced Biological Phosphorus Removal (EBPR) method has been used widely to remove phosphorus from wastewater and showed promising results that has led to more research in this field (Kobylinski et al., 2008). However, there is not much academic research on the effect of EBPR for greywater and therefore the efficiency of this method according to greywater characteristics is still unknown. Due to the high concentration of organic material in greywater which is mostly washed down in the sinks and dishwashers as well as low amount of nitrogen and phosphorus due to the separation of blackwater, the ratio of chemical oxygen demand (COD) to nutrients are relatively high. This high ratio could be beneficial for the EBPR process. Thus, more research is needed in order to gain further knowledge on how to more efficiently treat greywater with EBPR method.

1.2 Aim and objectives

The main aim in this master thesis is to assess the potential for enhanced biological phosphorus removal (EBPR) for greywater treatment. However, the aim is only focused on the Reco-Lab facilities that have used an EBPR system as a part of treatment for greywater. To make the overall aim more understandable, it is broken down to following specific questions as objectives:

- Is the sludge biomass working properly in the system?
- What sludge age should be used?
- What exposure time in anaerobic and aerobic tanks should be utilized?
- What would be the maximum phosphorus removal?

Answering these questions would help to answer the aim of this thesis, while also helping NSVA company to have a better understanding of the EBPR process.

1.3 Limitations

One of the challenges to find a proper solution to treat greywater is that the characteristics of greywater can vary significantly based on several factors such as geographical location, households' income, lifestyle, rate of water consumption, water supply quality, distribution system as well as the types of chemical products that are used. In this master project, only the Reco-Lab installation has been used as a specific case to evaluate the EBPR performance for greywater.

2 Literature overview

In this section, the information about greywater and its characteristics along with different treatment methods are provided. In addition, phosphorus removal as well as EBPR method are explained in detail and key parameters for the optimal design of this process are stated.

2.1 Greywater characteristics

Greywater is a suitable alternative for various reuse applications such as irrigation, car wash, firefighting, toilet flushing and land washing purposes due to its lower pollutants. In addition, there is also the possibility for recycling greywater as drinking water by adopting extra treatment processes to meet the requirements in drinking water standards. Greywater consists of wastewater from different parts of a household such as kitchen, laundry and bathroom that is separated from toilet wastewater which is called black water. Therefore, due to the lack of urine and faecal contaminants which contain high concentration of nutrients and pathogens, the concentration of phosphorus, nitrogen and pathogens are considerably low in comparison with black water. Greywater can be divided into two groups based on rate of pollutants: light greywater and dark greywater. The wastewater from shower and washing sections are considered as light greywater and the portion which comes from kitchen and laundry are called dark greywater due to higher contaminations from consumed chemical and cleaning products. In the dark greywater group, there are higher turbidity, nutrients, suspended solids as well as organic compounds that mostly come from detergents, food preservatives, oil and fats (Shaikh and Ahammed, 2020). Due to the presence of phosphorus in various chemical products which are used for cleaning purposes, it will lead to several problems specially if greywater would reuse for irrigation section. Phosphate in greywater can be absorbed by soil and subsequently enter to plants' body or groundwater in aquifers. This would cause future health issue as well as environmental problems such as eutrophication (Turner *et al.*, 2013). Phosphates are normally added to chemical products to mitigate the hardness and enhance the washing efficiency. The amount of phosphorus depends on the rate and type of chemical products that each household use in their daily life. In addition, some countries like Sweden have banned using phosphorus in dishwashing and laundry detergents (Arinaitwe, 2018).

One of the essential factors is to understand the characteristics of greywater. This would help to select a proper treatment method and achieve better effluent quality for further reuse purposes. Greywater characteristics can be varied by several factors such as households' lifestyle, income, their everyday life habit that led to different flow and strength in greywater. This will specifically alter the efficiency of biological treatment methods. By comparing greywater characteristics according to different studies, which is shown in Table 1, it can be said that average concentration of phosphorus in greywater can be varied between 0.1 and 74 mg/l depends on the geographical location and other mentioned factors. However, according to the study done by Shaikh and Ahammed (2020), this value can be as high as 187 in the kitchen. Nevertheless, this high value of phosphorus might be unrealistic and should be controlled again (Kasak *et al.*, 2011). In addition, by comparing the values in different sources of greywater, the amount of BOD and COD in the dark greywater which comes from kitchen and laundry are much higher than the light greywater sections. This is because of the presence of organic matter as well as organic micro-pollutants in the kitchen sinks and laundry section *(Shaikh and Ahammed, 2020).*

Table 1. Results from literature review on average characteristics of greywater

Location of Greywater	Recent Studies	Eriksson & Henze, 2002	Jefferson et al., 2004	Ridderstolp, 2004	Abu Ghunmi, 2009	Li et. Al, 2009	Ghaitidak & Yadav, 2013	Edwin et. al, 2013	Oteng-Peprah et. al, 2018 High income countries	Oteng-Peprah et. al, 2018 Low income countries	Arinaitwe, 2018	8Gnanaraj et. al, 2019	Shaikh & Ahammed, 2020
Kitchen	pH	6.3-7.4	-	-	6.83	5.9-7.4	6.5-8.3	6.9	-	-	-	-	5.58-10
	BOD (mg/l)	5-676	-	-	1850	536-1460	40.8-4450	932.4	-	-	47-1460	-	185-2460
	COD (mg/l)	936-1380	675	-	8071	26-2050	58-1340	1122.8	-	-	-	-	411-8071
	Total P (mg/l)	0.06-74	2.37	-	4.3	2.9-74	0.69	48.3	-	-	68-74	-	2.7-187
	Total N (mg/l)	40-74	25.31	-	25.9	11.4-74	6.44	31.2	-	-	40-74	-	0.5-65
Laundry	pH	8.1-10	-	-	9.6	7.1-10	8.3-9.3	9.1	-	-	-	-	5-10.33
	BOD (mg/l)	282-474	-	-	1266	48-472	44.3-462	186.5		-	48-380		44-3330
	COD (mg/l)	725	675	-	2500	231-2950	58-1338	1545.8	-	-	-	-	58-4155
	Total P (mg/l)	0.062-57	2.37	-	9	0-171	51.58	19	-	-	0.1-101	-	0.2-51.6
	Total N (mg/l)	6-21	25.31	-	2.8	1.1-40.3	14.25	18.9	-	-	6-21	-	2.8-31
Bathroom	pH	6.4-8	-	-	7.15	6.4-8.1	7-7.6	7.4	-	-	-	-	5.94-8.4
	BOD (mg/l)	192-252	120	-	120	50-300	129-205	135	-		26-300	-	20-673
	COD (mg/l)	282-433	420	-	537	100-633	230-587	357.9		-			64-903
	Total P (mg/l)	0,11-2	0.3	-	1.2	0.11-48.8	-	1.2	-	-	0.1-49	-	0.1-60
	Total N (mg/l)	5-17	8.7	-	2.4	3.6-19.4	6.6-10.4	11.3	-	-	3.6-17	-	2.7-148
Mixed greywater	pH	6.5-8.7	-	-	7.6	6.3-8.1	-	7.6	6.4-7.6	6-8.1	-	6.6-8.7	4.9-10.33
	BOD (mg/l)	119-360	123	150-400	149	47-466	-	149	39-155	56-518	41-500	90-290	20-3330
	COD (mg/l)	240-549	246.63	-	551	100-700	-	551	96-587	146-2000	-	280-800	23-8071
	Total P (mg/l)	0.16-27.3	1-12.1	1-10	7	0.11-22.8	-	7	0.4-4	-	0.6-68	0.6-27.3	0.1-187
	Total N (mg/l)	0.54-18.1	2.81	0.5-15	10	1.7-34.3	-	10	4.6-15.2	-	0.6-11	2.1-31.5	0.5-148

2.1.1 Quantitative characteristics

As mentioned earlier, the amount of greywater depends on several parameters that cause different volumes from each of sources. This amount can be as high as 70-90% of mixed wastewater that is generated by consumers. Higher amount occur when a vacuum toilet are installed for toilets and hence there would be less portion of black water (Ghaitidak and Yadav, 2013). One of the factors is the level of income in each household that can affect the rate of water consumption. Hence, two groups of high-income and low-income countries have been introduced to analyze greywater generation. In low-income countries or rural areas, the rate of water consumption is much lower and will produce less amount of greywater. As shown in Table 2, average portion of greywater generation can be varied in different sources in a household. Greywater in kitchen and laundry are about the same for both types of countries. The higher volume of greywater in bathroom for high-income countries comes from having more bathtub and more daily water consumption. Moreover, greywater volume in the kitchen part is little higher for low-income countries. This might be because in high-income countries use more dishwasher that consume less water and normally, they cook less food in the house and sometimes eat in restaurants. Thus, it can be realized that despite the fact that amount of greywater would be different for other countries with different level of income, the way of distribution could be the same (Shaikh and Ahammed, 2020).

Table 2. Average distribution of greywater from each source

Countries	Laundry (%)	Kitchen (%)	Hand basin (%)	Bathroom (%)
High-income	27	23	10	40
Low-income	19	30	23	28

* Data is taken from *(Shaikh and Ahammed, 2020).*

2.1.2 Qualitative characteristics

Like quantity aspect, qualitative characteristics of greywater can be widely varied depend on mentioned factors. There is a quality variation in different sources of greywater in a household. For instance, laundry and kitchen have much higher bacteria, turbidity, and suspended solids. To choose a proper treatment method for greywater, identifying and analyzing quantity and quality of incoming greywater will be needed. This can be done by proper sampling and controlling different parameters. It should be noted that quality parameters are less varied for different days of a week (Ghaitidak and Yadav, 2013).

Physical parameters

Turbidity, number of suspended solids and temperature are the important physical parameters that should be monitored. The amount of turbidity and suspended solids are considerably higher in laundry and kitchen than bathroom due to using more chemical products in dishwashers and laundry as well as pollutants such as oil and fats that enter into sinks (Samayamanthula *et al.*, 2019). Generally, temperature in greywater is higher than mixed wastewater due to higher temperature which comes from bathroom and kitchen for cleaning and cooking purposes. This can be varied between 18- 35 ºC (Oteng-Peprah *et al.*, 2018).

Chemical parameters

Due to separating toilet wastewater from greywater, the amount of nutrients such as nitrogen and phosphorus in the greywater are significantly decreased. The concentration of nitrogen varies according to the flowrate pattern and can be as low as 7 mg/l while this could be 20-80 mg/l for mixed wastewater. The amount of phosphorus depends mainly on the rate of phosphorus in laundry, dishwashing detergents and other cleaning products that are used in everyday life. As shown in Figure 1, the distribution of nitrogen and phosphorus in different sources of greywater have been compared. According to the figure, dark greywater which includes kitchen and laundry contain more portion of phosphorus. However, due to some reasons such as washing babies, trace of urine, and sweating of the body, the amount of nitrogen can also be high in the bathroom (Shaikh and Ahammed, 2020). pH in greywater as an important parameter depends on different factors such as pH of water supplied as well as types of chemical products used in a household. In general, the pH level in greywater is close to neutral except for the laundry section where the pH level is higher than in other sections due to the presence of chemicals used (Oteng-Peprah *et al.*, 2018).

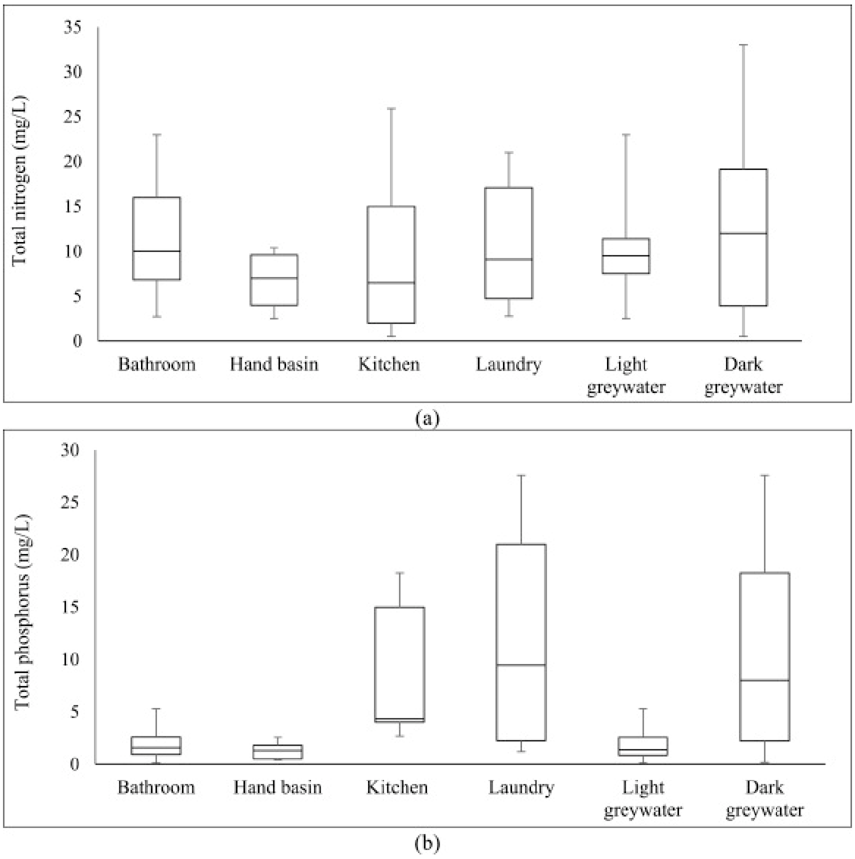

Figure 1. Distribution of nutrients in different sources of greywater (Shaikh and Ahammed, 2020). (Published with permission from Elsevier)

Organic micropollutants and heavy metals

Micropollutants such as pathogenic viruses, protozoa and bacteria can come from different activities like changing baby diapers, washing hands after using toilets, cleaning materials as well as washing uncooked vegetables and raw meat. According to the study done by Eriksson *et al.* (2002) there are approximately 900 micropollutants that come from bathroom, kitchen and laundry sections. These substances can be categorized as biocides, UV-filters, preservatives, plasticizer, fragrances and surfactants that are harmful for environment and human health and should be removed from greywater (Leal, 2010). In addition, metals such as magnesium, sodium, and potassium which mostly come from detergents and personal care products should be also removed from greywater according to the effluent requirements as well as the purpose of reuse (Shaikh and Ahammed, 2020). Beside all the existing contaminants, there is a possibility of generating by-products due to reaction of various chemicals that could cause further risk to the treatment stage (Eriksson *et al.*, 2002).

2.2 Greywater treatment methods

A proper treatment method should be adopted to different factors such as the temperature of the area, living conditions of households and other parameters that were mentioned earlier (in section 1.3). For instance, In Sweden , temperature plays an important role for selecting proper treatment method since cold and warm weather can significantly affect the greywater temperature in winters and summers respectively (Arinaitwe, 2018). During last three decades several research studies have been done on treatment of greywater to achieve an opportunity for recycling in different applications. Most of treatment methods such as constructed wetland (CW), different types of filtrations, coagulation, and flocculation, rotating biological contactor (RBC), membrane bioreactor (MBR), sanitation concept for separate treatment (SCST) and sequencing batch reactor (SBR) have been tested to remove contaminants especially phosphorus and organic matter from greywater. However to meet the standards' requirements for drinking water as well as none-potable reuse purposes like irrigation, these treatment methods should be combined with other methods and therefore further studies will be needed in this field (Ghaitidak and Yadav, 2013).

2.2.1 Physical treatment

Physical treatment methods such as filtration are frequently used for treating greywater due to its cost efficiency and being uncomplicated for utilizing. Sand filters are one of the suitable options to remove contaminants through different layer of various media. It can simply consist of sand and gravel filter or compacted sand filters. According to the study done by Li *et al.* (2009), Sand filter normally can reduce COD, turbidity and suspended solids by about 50%. Therefore, it cannot remove all the micropollutants such as pathogens alone and needs to be combined with chemical or biological treatment methods (Edwin *et al.*, 2014).

2.2.2 Chemical treatment

Another treatment method that are widely used in different treatment plants is chemical precipitation that is done by adding coagulant in different steps of treatment process in greywater to remove wide range of phosphorus as well as other contaminants. Different chemicals might be used for achieving an acceptable phosphorus removal such as aluminum, iron or calcium. By adding the coagulants, suspended and dissolved compounds can be removed by flocculation and separated from the greywater. Another common chemical method that degrades contaminants such as phosphorus by reacting with hydroxyl radicals is the Advanced oxidation process (AOP). This method is usually used in the final stage of the treatment process and is also suitable for removing non-degradable and toxic materials. However, the high cost of operation in chemical methods as well as higher sludge production are the disadvantages of these methods (Edwin *et al.*, 2014).

2.2.3 Biological treatment

One of the suitable methods for greywater treatment is biological treatment, by which organic pollutants are broken down with the help of microorganisms. This method has been promising in different pilot and full-scale projects and is more cost-effective than the chemical and physical methods. This can be used as the secondary or the main step of a treatment system. Biological treatment is normally divided into two parts, aerobic and anaerobic. About 74% of greywater contaminants are biodegradable during the anaerobic conditions (Elmitwalli and Otterpohl, 2007). In addition, during this period, no energy is needed to produce oxygen and less sludge is produced. For these reasons, using the anaerobic period as the first step in the biological treatment of greywater is recommended. This can lead to lower operation and maintenance cost in the system. To design and operate a biological treatment plant, the rate of degradability of organic matter should be evaluated. The ratio of BOD_5/COD represents the biodegradability of greywater in a biological treatment method. This value can be changed due to living habits of a household. Oteng-Peprah *et al.* (2018) compared different studies and they could find that the ratio is normally between 0.31 to 0.71 in general greywater (*Halalsheh et al. 2008).*

Several biological methods such as RBC, MBBR, MBR, SCST, CW and combination of biofilters and constructed wetlands have been studied/tested in the last three decades to analyze the efficiency of contaminants removal in greywater. The aim is to enhance the possibility of recycling the greywater as a suitable alternative for drinking water and other reuse purposes. Although these methods could remove a wide range of pollutants during biological process, as it is shown in Table 3, each process didn't show promising result for removing the phosphorus in an acceptable level. Therefore, combination with other treatment processes is needed to increase the rate of removal. For instance, according to the table, phosphorus could not be removed in MBBR process and just 14% of phosphorus was removed in this biological method while this value could be as high as 94% by using the Constructed Wetlands combined with biofilters. However, this method needs large footprint that cannot be used in every project (Arinaitwe, 2018). Normally, the average BOD/COD ratio in greywater is about 0.5, which shows good potential for biological treatment. However, the presence of micro-pollutants in greywater such as surfactants can be resistant and cause negative effects during the anaerobic conditions (Leal *et al.*, 2007).

Chemical oxygen demand (COD) as a key parameter is often assumed to reflect the rate of materials which contain carbon in the influent of the plant. This amount is important for designing the activated sludge process. Some COD are biodegradable while some are non-biodegradable. Each of these two categories are divided into two parts, soluble and particulate. The non-biodegradable group has no role in the biological activity and therefore only the biodegradable part is of great importance. The soluble fraction is rapidly consumed by the sludge, while the particulate fraction needs to be absorbed and stored in the organism and broken down into smaller chemical units for metabolism. This step is much slower than soluble part with a ratio of 0.1. Calculating the degradable part helps a lot in designing the activated sludge process (Ekama *et al.*, 1986).

Table 3. Phosphorus removal in different biological treatment methods for greywater (data is taken from (Arinaitwe, 2018)).

Methods	Phosphorus removal (%)
RBC + sand filtration	58
MBBR	14
SCST	53
MBR	53
Constructed Wetland	78
Constructed Wetland +Biofilter	94
Biofilter	67

In the biological process, phosphate can be removed through three mechanisms:

- Enhance phosphate uptake (Bio-P)

- Phosphate assimilation (cell-P)

- Flocculation of phosphate containing particulate (floc-P)

The Bio-P method, which is also known as EBPR, is based on the presence of readily biodegradable organic matter or volatile fatty acid (VFA). When the VFA concentration in the incoming wastewater is low, two other mechanisms are expected to play greater roles in phosphorus removal. According to the study done by Ødegaard (1999) on different treatment plants in Sweden, the assimilation process plays an important role to remove soluble part of Phosphorus.

2.3 Phosphorus removal

Phosphorus, as an important nutrient, plays an important role in the quality of wastewater as well as greywater. The presence of phosphorus in the effluent can lead to environmental issues like eutrophication, algae blooms that lead to reduction of dissolved oxygen in recipients. Normally, phosphorus in greywater mostly comes from detergents from laundry and dishwashing (Kasak *et al.*, 2011). However, in some developed countries, like European countries, the phosphorus concentration would be much lower due to the ban of using phosphorus in dishwashing and laundry chemical products since 2017. Norway and Sweden were among first countries in Europe that started banning phosphorus in laundry detergents in 2011 (Arinaitwe, 2018). Therefore the amount of phosphorus in wastewater is normally as low as 6 mg/l and this value in greywater would be much lower (Ødegaard, 1999). In Sweden, phosphorus in greywater comes from food waste and other cleaning products. Since greywater might be used for different purposes, the amount of phosphorus should be reduced as much as possible according to different standards and requirements that are available for each country. For instance, Öresundverket treatment plant that is located beside the Reco-Lab facility, has specific discharge requirement for the effluent wastewater and the amount of phosphorus should be as low as 0.5 mg/l. In addition, to reuse the treated greywater of Reco-Lab, stricter requirements would be needed. For example, to discharge the effluent of Öresundverket treated wastewater to Öresund, phosphorus should be removed to a concentration as low as 0.1 mg/l that is according to the standard range for lakes and water course (SEPA, 2000).

Table 4. Average quality in WWTPs in Sweden. Data is taken from (Arinaitwe, 2018; Jönsson et al., 2005; Ødegaard, 1999)

Parameters	Unit	Ave. value
Total N	(mg/l)	16
Total P	(mg/l)	9
SS	(mg/l)	243
COD	(mg/l)	477
fCOD	(mg/l)	157
BOD	(mg/l)	329
fBOD	(mg/l)	63
BOD/COD	Tot.	0.32
	Filtr.	0.38
COD/TN	-	29.8
COD/TP	-	53

Phosphorus in wastewater is usually found in two forms: orthophosphate, which is soluble, and organically bound phosphorus, which can be found both in soluble and in particulate form. Analyzing the characteristics of the incoming wastewater is of great importance. These characteristics have a great impact on how the process in an activated sludge system is designed as well as the amount of nutrient removal. Normally, average COD, influent flow rate, amount of readily biodegradable COD (rbCOD), Total phosphorus/COD ratio and temperature are the important parameters that are measured as design parameters (WRC, 1984). According to the data different wastewater treatment plants in Sweden by Ødegaard (1999), Jönsson *et al.* (2005) and Arinaitwe (2018) , the average quality such as BOD, COD and nutrients are shown in Table 4. fCOD and fBOD are the filtrated values that are passed through a 1 μm filter. In addition, according to the table, the ratio of BOD / COD and fBOD / fCOD for wastewater in Sweden is approximately equal to 0.35 and 0.4, respectively that is considered a good ratio for biological treatment. However higher ratio would be favorable for biological treatments.

2.3.1 Enhanced Biological Phosphorus Removal

Since chemical precipitation for phosphorus removal will require considerable cost as well as cause further chemical substances in sludge, biological treatment methods are a suitable alternative to remove an acceptable amount of phosphorus by using microorganisms. As mentioned earlier, several biological treatment methods have been tested to remove phosphorus from wastewater as well as greywater. However, the results were not promising (Zuthi *et al.*, 2013). One of the promising biological methods for phosphorus removal is the enhanced biological phosphorus removal (EBPR) method. In this method, phosphorus is removed by storing polyphosphate by polyphosphate accumulating organisms (PAOs) that will be removed with sludge later in the system. Since 1950, different adjustments of enhanced biological phosphorus removal (EBPR) have been used for removing phosphorus from wastewater due to its sustainability and low cost of operation. Organic matters are considered a suitable source of food for Bio-P bacteria due to their degradability. Unlike conventional biological treatment that can remove 15 to 25% of phosphorus, EBPR method can remove an acceptable amount of phosphorus from the system, up to 90%, through a simple anaerobic and aerobic sequence (Pitt *et al.*, 2008). EBPR method is simple and cost-effective and existing treatment plants can easily be upgraded to this system with some modifications. In this method PAOs as well as Volatile fatty acid (VFA) are the key factors to enhance the efficiency of the process (Curtin *et al.*, 2011). It should be noted that adding chemical as a complementary treatment during EBPR process will interfere with the biological mechanisms and reduce the efficiency of this method (Izadi *et al.*, 2020).

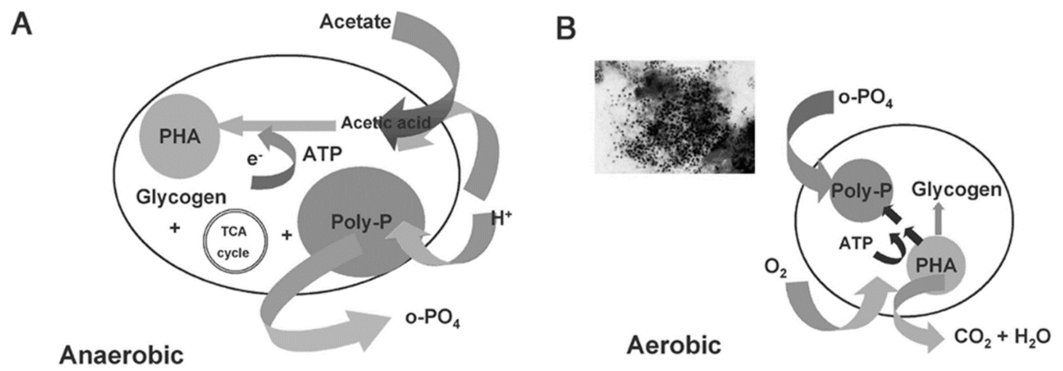

Figure 2. EBPR process during anaerobic and aerobic condition (Seviour et al., 2003)
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Phosphate Accumulating Organisms (PAOs)

The two steps of anaerobic and aerobic condition of EBPR process shown in Figure 2. VFAs are consumed by PAOs as polyhydroxyalkanoates (PHAs) through the conversion into energy rich products during the anaerobic condition which is marked as step A in the figure. In order to this conversion to the energy rich products, there is need of energy that is provided by breaking down of polyphosphate in the PAOs. The polyphosphate breaks down result in phosphate release into mixed liquor in the anaerobic condition. Since polyphosphates are used during this period, the organisms have a depletion of polyphosphate. Therefore, in the aerobic condition, which is marked as step B in the figure, due to the presence of oxygen, it takes the energy rich products that are in the organisms and converts released phosphate to polyphosphate. The amount of phosphate removed in the aerobic condition is much more than the amount of released phosphate during the anaerobic condition and the organisms take up more phosphate that they originally contain and result in high phosphorus removal. However, the performance of PAOs depend on the availability of volatile fatty acids (VFA) and there should be enough VFA in the anaerobic condition to have high efficiency of EBPR process (Izadi *et al.*, 2020).

Glycogen Accumulating Organisms (GAOs)

According to the Figure 2, GAOs with similar metabolism to PAOs, consume available VFAs to reduce COD levels without phosphate release and to form glycogen in the aerobic section (Izadi *et al.*, 2020). GAOs use glycogen the same way that PAOs use phosphorus but do not absorb any phosphorus and therefore have a negative effect on phosphorus removal in the anaerobic period. If chemicals are added at the same time with biological process, the amount of phosphate will be reduced and the activity of GAOs overcomes the PAOs, and therefore the Bio-P process will need more chemicals to be added (Tykesson *et al.*, 2005).

Volatile Fatty Acids (VFAs)

As mentioned earlier, VFAs as an important factor in EBPR process can be found in form of acetic or propionic and other acids. PAOs are directly dependent on the amount of VFA to have an acceptable removal rate. Generally, VFA can be found in the influent wastewater as well as produced by fermentation process. The ratio of VFA to phosphate in the influent can show the efficiency of the EBPR process, and for wastewater it should be between 20 to 30 mg COD-VFA/mg P. This means that 20-30 mg COD of VFA will be required to remove 1 mg of phosphate. Generally, the amount of VFA in municipal wastewater is between 50 to 100 mg COD VFA / l which is about 15 to 25% of the total COD (Lie, 1996).

Hydraulic retention time (HRT)

One of the essential parameters in EBPR method to have a reliable and stable system is hydraulic retention time (HRT) which has a direct impact on design configuration and operating conditions. HRT indicates the amount of time that greywater passes from each section. It is especially important for the anaerobic basin to provide sufficient time for carbon and polyphosphate metabolism that result in consuming all the VFAs. According to different studies, HRT between 1 to 3 hours in the anaerobic zone is suitable to provide proper metabolism. However, this value can be changed depends on the amount of VFAs which enter into the system (Coats *et al.*, 2011; Izadi *et al.*, 2020). According to the study done by Izadi *et al.* (2021), in the aerobic condition, increasing the amount of HRT and DO level reduce the efficiency of the EBPR process. This leads to increasing the activity of GAOs compared to PAOs. For this reason, the amount of HRT of 120-200 minutes was introduced as the optimal value for aeration section. The detention time for the collection tank could be between 1 to 2 hours to improve the VFA concentration by hydrolysis and fermentation process (Jansen *et al.*, 2019). However, the detention time can be zero if there is enough carbon source in the influent. The HRT in the sedimentation tank depends on the design parameters such as sludge settling velocity, depth, and area of the settling basin. However, this detention time should be selected sufficiently to provide enough time for sludge to settle in the basin. On the other hand, selecting high HRT can lead to anaerobic condition in the settling basin and cause unfavorable secondary phosphate release and high phosphorus value in the effluent (Lilleland, 2019).

Sludge age (SRT)

Sludge retention time (SRT), also known as sludge age, is the length of time that an activated sludge particle is present in the aerobic tank and is expressed in days. Usually in conventional activated sludge systems, this amount is between 3 to 15 days, and in some conditions, such as winter, this amount can be increased up to 30 days to maintain the appropriate biological mass. Choosing a short SRT usually causes several challenges in the EBPR system that need to be considered in the long run (Lie, 1996). For example, according to the study done by Ward and Moor (2018) different SRT between 3 to 12 days were tested in the EBPR process. In this study, reducing SRT from 12 days to 6 days doesn't affect or change the efficiency of the EBPR process. However, low SRT, like 3 days lead to failure in the system over time. Nevertheless, according to different studies, the SRT of 4 days is suitable for achieving high phosphorus removal. In addition, in another study done by Lie (1996), the 5-day SRT showed promising efficiency in the EBPR system provide that only the anaerobic and aerobic sections are used in the process and nitrogen removal effects is not considered. Anaerobic HRT and SRT in the aerobic section are provided in Table 5 according to different studies.

Table 5. Optimal SRT and HRT values according to different studies.

Study	Aerobic SRT	Anaerobic HRT
Christensson 1997	8	-
Tyksson 2005	-	0.65-0.75
Zheng et al 2019	5	-
Barat and Loosdrech 2006	-	3.8
Valverde Prez et al 2015	4	2
Izadi 2021	4	2
Urdalen 2015	4	3.2

Other parameters

Other design parameters that can affect the EBPR process are pH, temperature, oxygen level in the aeration basin. Increasing the pH will increase the rate of phosphate release in the anaerobic period and higher phosphorus removal will be obtained in the aerobic period. According to different studies optimal pH for proper performance of PAOs is about 7.5 to provide a dominance of PAOs over GAOs (Izadi *et al.*, 2020; Metcalf and Eddy, 2014). Temperature variations have also an effect on different parts of the Bio-P process. At lower temperatures the production of sludge increases and as a result the ability of the microorganism to store polyphosphate increases. According to research done by Tian *et al.* (2013), PAOs dominate

GOA at temperature between 10-14 °C. However, lower temperatures under 10 °C will reduce the release of phosphate in the anaerobic period and as a result the phosphate uptake in the aerobic stages will decrease. The next parameter that is important in the aerobic tank is the oxygen concentration (DO). According to Izadi *et al.* (2020) poor aeration in the aerobic stage prevents PAOs from absorbing phosphorus. On the other hand, over-aeration will oxidize stored PHA and lead to smaller phosphorus uptake. For this reason, the rate of aeration should be reduced when we have lower load of organic matter. Otherwise, the amount of phosphorus will be increased in the effluent. The optimum DO level can be varied between 2-5 mg/l based on the load of organic matter (Izadi *et al.*, 2021).

3 Materials and methods

In this chapter, Oceanhamnen as the study area is introduced, and different parts of treatment process are explained. Along with the laboratory data, P-release batch test and 5-points titration method were performed as the experimental section in order to estimate the amount of phosphate release and volatile fatty acids (VFAs) to analyze the performance of the EBPR process in Reco-Lab. Due to the low sludge concentration which occurred during this master thesis project, re-inoculation was done with Öresundverket's sludge and is explained in detail later in this chapter. Thus, the experiments were done before and after the re-inoculation to evaluate the effect of sludge recovery process beside the objectives.

3.1 Study area

A full-scale pilot project called Oceanhamnen (Ocean harbor) has been carried out, by cooperation of NSVA and the municipality, in the city center of Helsingborg next to the harbor to evaluate the effectiveness of a source separation system and its effects on enhancement of nutrient recovery as well as environmental benefits in the future. This project is part of a renewal plan of the central part of Helsingborg which is called H+ aiming of having modern and environment friendly living area by 2035. So far, about 1,000 people, including residential, restaurants and official units have settled in the area for the first phase. The ultimate goal will be to serve 2 500 residents in the area. As it shown in Figure 3, greywater, black water, and organic waste from food in the kitchen are separated into three pipes that be transferred from each unit to the Reco-Lab facilities which is located beside the Öresundverket treatment plant to treat and recover nutrients, energy, and recycled water. The main purpose for treating greywater is to reuse it in a recreational water park and swimming pool that will be built in this area. Since many people will be in touch with the water, the effluent quality should satisfy the drinking water or bathing water standards to ensure human health (Arinaitwe, 2018; NSVA, 2020).

At the Reco-Lab facility, the three pipes enter into individual collection tanks. Food waste and black water, which are not evaluated further in this thesis, are treated in two separated anaerobic mesophilic digestion reactors with hydraulic retention time of 7 hours. Sludge is collected and transferred to Öresundverket wastewater treatment plant (Kjerstadius, 2022).

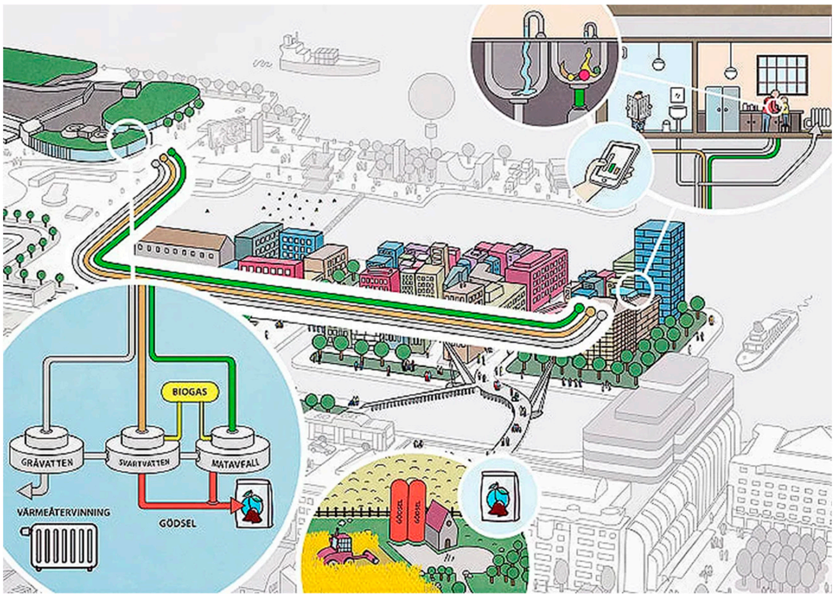

Figure 3. Graphical illustration of Oceanhamnen project.
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In greywater section, as shown in Figure 4, greywater passes through different steps to get purified and have the possibility to be reused in different applications. Due to use of vacuum toilets in all the houses and official units in the Oceanhamnen, and according to the given flowrate data, the proportion of greywater is 81% of total wastewater of the three pipes (Kjerstadius, 2022). The influent greywater enters to a collection tank before entering the enhanced biological phosphorus removal process. In the EBPR step, phosphorus will be removed by passing through anaerobic, aerobic and sedimentation process. The anaerobic and aerobic basins are divided into two parts with proportions of one-third and two-thirds. This is due to the gradual entry of residents into the area, which the smaller part of basins was used and is switched to the larger parts with increasing the inhabitants during this thesis. Due to the non-disclosure agreement between the EBPR system supplier and NSVA, the volumes of each part of this system cannot be published. In the aeration section, dissolved oxygen is set at 3.8 mg O_2 / l and the aerator starts working when it reaches 2 mg O_2 / l. In the sedimentation basin, part of return sludge will go back to the collection tank and the rest will be transferred directly to the Öresundverket treatment plant (Kjerstadius, 2022; NSVA, 2020).

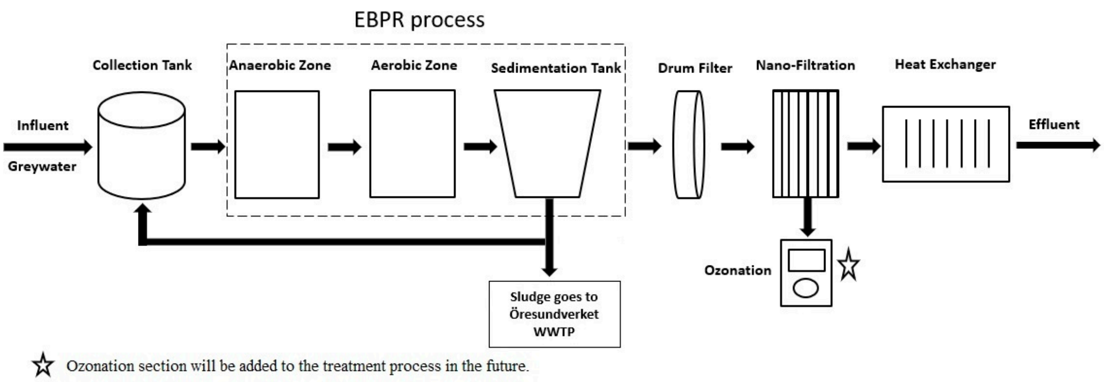


Figure 4. Greywater treatment processes including EBPR.

According to the figure, the treated water which has passed through the EBPR process continues to pass a drum filter and nano-filtration steps to remove organic micropollutants such as pharmaceutical contaminants to reach high quality water for reuse purposes. According to the development plan of greywater treatment processes, ozonation steps will be added for removing residual contaminants from the portion that cannot pass the nanofiltration step. However, this step has not been applied in the treatment processes at the time of writing this thesis. Although the temperature of influent greywater is about 17 ºC before the EBPR process, the temperature increases during the process that will be removed by heat exchanger after the nano-filtration process and will be used for increasing the temperature of digesters in black water and food waste treatment systems. Although the greywater effluent has high quality, it is pumped to Öresundverket for treatment. This is because NSVA tends to ensure that the operational condition will be stable over time (Kjerstadius, 2022; NSVA, 2020).

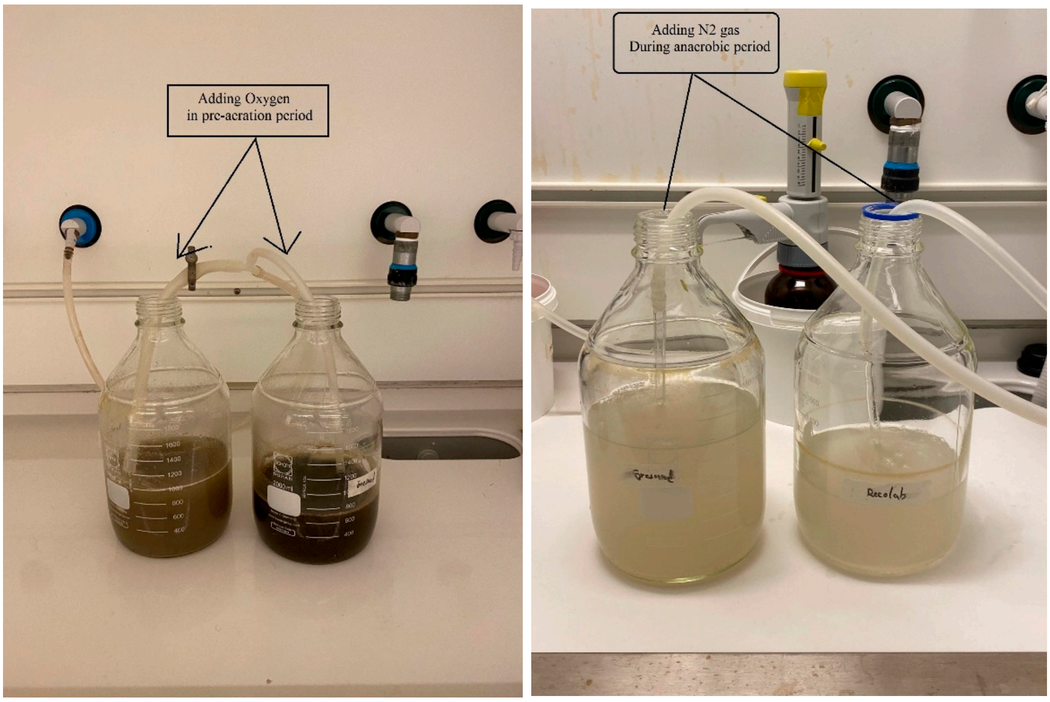
3.2 P-release batch test

The P-release test is a method to evaluate the performance of the EBPR process. This test can be used to evaluate the activity of PAOs. In this experiment, after adding a carbon source to the sludge sample, the release and uptake rate of phosphate are measured during anaerobic and aerobic steps, respectively. Although this experiment is easy to perform and does not require special equipment, various parameters must be considered (Tykesson and Jansen, 2005). In this project, this experiment is used for Reco-Lab sludge and sludge from Öresundverket as a reference and adding the influent greywater as carbon source. The main purpose of this experiment is to compare the performance of the EBPR system in Reco-Lab before and after the re-inoculation. In addition, the effect of carbon source rates on phosphate release rates has been compared by adding different proportions of greywater to sludge samples. To estimate the VFA concentration, a 5-points titration test as described by Moosbrugger *et al.* (1992) was performed simultaneously with P-release test and has been described in detail in the next section.

3.2.1 Experimental procedure

Sludge samples from Reco-Lab and Öresundverket were taken from the return sludge section along with an influent greywater sample as a carbon source and transferred from treatment facilities in Helsingborg to the university lab in Lund on the same day of the experiment. To perform P-release test relatively simple equipment will be needed such as bottles, filters, funnels, syringes, hoses, glasses, pipette, stopwatch, and the possibility to measure the oxygen, pH and temperature. In addition, it should be possible to add oxygen and nitrogen during the pre-aeration and anaerobic periods respectively. According to similar projects which have performed the P-release test, in order to evaluate the effect of carbon source on phosphate release, the experiment environment should be performed under standard conditions at a temperature of 20 °C and pH = 7 (Tykesson and Jansen, 2005; van Loosdrecht *et al.*, 2016; Wang *et al.*, 2018).

According to the instruction of P-release test, it includes three steps Pre-aeration (if needed), anaerobic and aerobic period. In this project, due to the time limit for the experiments only the pre-aeration and anaerobic periods have been performed. It is suggested to perform one and three hours for pre-aeration and anaerobic period respectively. Since some phosphorus may be released during the transport of samples from the treatment plant to the laboratory, Pre-aeration period would be needed at the start of the experiment. Normally pre-aeration is done for one hour to increase phosphorus storage as well as oxidize the existing VFAs in the sludge, which lead to better understanding of relationship between phosphate release and the amount of carbon source. The concentration of COD in greywater has been measured and compared with the data from Reco-Lab. COD measurement was done by using cuvettes (Hach-LCK214) that were provided from Hach-Lange company with the specific instruction with the product. To measure the amount of released phosphate at different times, Ion Chromatograph device (IC) was used to measure phosphate concentrations, and the results were recorded.



(a) *(b)*

Figure 5. Greywater treatment processes including EBPR method.

As it is shown in Figure 5a, the first step to perform the experiment was to pre-aerate the sludge samples for one hour by adding oxygen with diffusers. In addition, to reduce the possibility of oxygen exposure during the anaerobic period, the greywater samples were subjected to anaerobic conditions by adding nitrogen gas at the same time of pre-aeration period which is shown is Figure 5b and were added to the sludge samples at the end of pre-aeration period. Nitrogen gas was added to the mixture throughout the anaerobic time to provide anaerobic conditions. Due to adding of the nitrogen gas by the diffusers, the samples were constantly mixed during the experiment, and therefore no stirrer was used. Instead of using beakers as reactors, in this experiment bottles with narrow caps have been used to reduce the possibility of entering oxygen during anaerobic period. At the beginning of the anaerobic period, after adding greywater as well as nitrogen gas, the oxygen level of the mixture was measured by oxygen meter to ensure anaerobic conditions.

According to the projects done in different articles, different ratios of carbon source have been proposed. In this project, to provide an appropriate amount of carbon source common rate of 0.3 (mg COD- substrate /mg VSS-sludge) was used for adding greywater to the samples (van Loosdrecht *et al.*, 2016). Moreover, to evaluate the influence of changing the carbon source rate on the amount of phosphate release, the two ratios of 0.6 and 0.18 were used for Reco-Lab and Öresundverket respectively in another p-release experiment and were compared with the rate of 0.3. These ratios were selected to see the effect of higher and lower carbon source ratio on the amount of phosphate release. However, in real EBPR process, the excess carbon source that will leak to the aeration period would cause negative effects on up taking phosphates. For this reason, estimating an optimal rate of carbon source that would be completely used in the anaerobic period is of great importance.

In this experiment sampling was done at specific times of the pre-aeration and anaerobic period. To provide better understanding of phosphate variation, samples were taken from the first and end of the pre-aeration period and every 30 minutes in anaerobic period and labeled. Due to the limitation of capacity for the bottle, which was 1.8 liters, the following volumes in Table 7 were selected to provide the stated ratio. Since three samples of 300 ml for 5-point titration method and eight samples of 20 ml during the P-release test were used, these volumes were sufficient for the experiment.

Table 7. Selected volumes of sludge and greywater for the Batch phosphate release tests.

Sample volume & ratio				
Samples		**Return Sludge Vol. ml**	**Greywater Vol. ml**	**Ratio mg COD/mg VSS**
Reco-Lab	Before re-inoculation	700	700	0.3
	After re-inoculation	400	1200	0.3
Öresunds-verket	-	400	1320	0.3
Test round For diff. ratio	Reco-Lab	600	1200	0.6
	Öresund	600	1200	0.18

According to the table, after the re-inoculation more greywater was needed to add to Reco-Lab sludge sample due to increasing the VSS of the sludge. The ratios in the table were calculated according to given data from Reco-Lab laboratory which the values of COD, the amount of VSS for Reco-Lab before re-inoculation, after re-inoculation and Öresundverket sludge were 500, 1500, 5000 and 5500 mg/l respectively. It should be noted that due to the required sludge sampling of 300 ml at the end of pre-aeration period for titration experiment, the selected volume for Reco-Lab and Öresundverket sludge were 300 ml more than the mentioned volumes in the table. As it can be seen in Figure 6, after sampling, the samples were immediately filtered by the filters placed inside the funnel. Filtration was done through Munktell filter paper. Then, at the end of the experiment, the amount of released phosphate in the filtered samples was measured and recorded by the IC Machine. It should be noted that another filtering step using PTFE syringe filters was also required before measuring phosphate with the IC machine.

Figure 6. Filtering samples from batch P-release tests immediately for measuring released phosphate.

3.2.2 P-release rate

The maximum phosphate release can be calculated by the phosphate difference at the beginning and end of the anaerobic period. To calculate the release rate of phosphate, after measuring phosphate at different times and drawing its graph relative to time, release rate can be calculated with the help of the linear part of the diagram and following equation: (Hansson, 2016)

$$Release\ rate = \frac{P2-P1}{(t2-t1)VSS} \quad \text{(Eq. 1)}$$

P1: is the amount of phosphate at the beginning of the anaerobic phase (mg/l)
P2: is the amount of phosphate at the end of the linear part of the anaerobic part (mg/l)
t1: time at point P1 (h)
t2: time at point P2 (h)
VSS: Sample sludge rate to (g VSS / l)

In addition, in Table 8 Bio-P classification is shown according to the phosphate release rate *(Janssen, 2002).*

Table 8. Bio-P classification (Janssen, 2002)

Classification of Bio-P potential	
mg PO_4-P/g VSS x h > 7	Very good
7> mg PO_4-P/g VSS x h > 3	Good
mg PO_4-P/g VSS x h < 3	mediocre

3.3 VFA analysis

VFAs and Alkalinity parameters are two indicators for evaluating the performance of the Bio-P process. Moreover, according to the different research, VFA is a crucial indicator to measure the efficiency of the anaerobic period (Elmitwalli and Otterpohl, 2007). In a well-functioning EBPR process, it is expected to find less VFAs at the end of the anaerobic period, zero in the best condition, that means VFAs are consumed as carbon source by PAOs (Hey et al., 2012). The main purpose of this experiment was to estimate the variation of VFAs before and after re-inoculation and evaluate the Bio-P performance.

3.3.1 5-points titration method

In this project, 5 pH points titration method was used to calculate the amount of VFAs in Reco-Lab and Öresund samples. This method was first introduced by *Kapp (1984)* under the name 4 pH point titration and developed by Moosbrugger *et al.* (1992) as 5 pH point titration method. In this method, the sample is titrated by using Hydrochloric acid (Hcl) as a titrant from the initial pH to 4 selected pH_1, pH_2, pH_3 and pH_4. According to the studies, optimal values for pH to achieve an acceptable result are $pH_1 = 6.7$, $pH_2 = 5.9$, $pH_3 = 5.2$ and $pH_4 = 4.3$. Due to its simplicity, cost-effectiveness, accuracy of results as well as robustness, this method has been considered as an acceptable method to estimate VFA concentration. In addition, in this method, alkalinity and VFAs also can be obtained at the same time by a user-friendly excel program that will require less skill to use. One of the disadvantages of this method is the lack of separation of different types of acids in VFA, which can be done by gas chromatography (GC) method (Hey et al., 2012). Since VFA can disappear quickly by contact with oxygen, two types of experiments were designed: two experiments in the university laboratory at the same time with P-release test to compare the amount of consumed VFAs during phosphate release before and after re-inoculation and two experiments in Reco-Lab laboratory in Helsingborg to perform the experiment immediately after taking the sample from sampling points (Vannecke *et al.*, 2015).

This method assumes that the amounts of ammonium, phosphate and sulfide are known. In order to achieve an accurate and optimal results, the amounts of ammonia, phosphate and sulfide on the undiluted sample should be measured at the beginning of the experiment and entered into the software as input data. If these parameters are not measured, value of zero can be replaced. It should be noted that the effect of ammonia and sulfide parameters in estimating VFA and alkalinity will be negligible. However, phosphate has a considerable effect on the results and should be measured at the beginning (Moosbrugger *et al.*, 1992). In this project, these amounts have been measured and are given in Table 9.

Table 9. Required input values for calculating VFA/Alkalinity in 5-point titration method

Input Data					
	Conductivity m.s/m	**Phosphate mg P/l**	**FSA mg N/l**	**Sulfide mg S/l**	**Acid normality**
Influent greywater	52.3	*	2.2	0	0.05
Reco-Lab before re-inoculation	52.3	*	2.2	0	0.05
Reco-Lab after re-inoculation	62.3	*	2.2	0	0.05
Öresundverket	93.2	*	2.2	0	0.05

** Phosphate values are different according to the sample's time and location (Appendix C)*

According to the table, in this experiment, the amount of sulfide was assumed zero as input data. In addition, based on the provided data from Reco-Lab, the value of 2.2 was selected for FSA parameter. Titration method is relatively simple. however, it requires special attention to achieve the correct results. Equipment needed in this experiment are (Moosbrugger *et al.*, 1992):

- Titration burette
- pH and temperature meter
- pH prob
- magnetic stirrer
- pipette
- beaker
- Stopwatch
- hydrochloric acid 0,05
- distilled water
- pH buffer solution

As shown in Figure 7, the initial pH is recorded and then the sample is titrated with acid until it reaches the desired pH. At this point, the corresponding volume is recorded. This step continues until pH_4 is reached and the obtained volumes are entered in the Excel file to calculate VFA and alkalinity. In this method, the pH_0 should be higher than 6.7, otherwise it can be increased by adding a strong base. To achieve an acceptable result, pH should be reached with accuracy of ±0.1. According to the instruction, it is recommended to filter the sample before titration in order to separate the solid part from the sample and extract CO_2 from the solution that may affect the result. This will help a lot in controlling the pH during the test. Moreover, the sample volume should be large enough to control the pH fluctuation and Mixing was provided continuously during the test (Buchauer, 1998).

Figure 7. Required equipment for performing 5 points titration method.

To increase the accuracy of the test, some important preparation should be done to ensure the correct results would be achieved including calibration of the pH probe, mixing conditions during the experiment and accuracy in determining the normality of the titrant are essential. In addition, to control pH changes during the test, it has been suggested that the titrant be incremented by 0.01 ml in each step (Buchauer, 1998). According to the given calculation excel file in 5 points titration method, the VFA is obtained in mg/l as acetate. However, to compare the obtained results with the available data in the literature, it has been converted to mg COD/l by using the conversion factor of 1.07 (Bedaso, 2019). It should be noted that since the sample point for the influent greywater in the Reco-Lab is placed after the collection tank, the VFA concentration consists of VFA in greywater as well as the VFA produced due to hydrolysis and fermentation process in the collection tank.

3.3.2 Experimental procedure

3.3.2.1 University laboratory

The 5-points titration test was performed at the same time with the P-release test to evaluate variation and consumption of VFAs during the anaerobic period. Three 300 ml samples were taken at the end of the pre-aeration section, the beginning of the anaerobic section after adding greywater as a carbon source, and the end of the anaerobic period from the both Reco-Lab and Öresundverket samples, respectively. This test was done before and after re-inoculation to compare the concentration and variation of VFAs before and during anaerobic period. According to the proposed instructions, samples were filtered to remove solid part. This was done by using centrifuge device to settle solid part and then filtering by using glass microfibers filter (No.691- 55 mm). To control the pH and achieve acceptable results, a volume of 250 ml was selected for performing the experiment. The sample was mixed continuously in a beaker and on a magnetic stirrer at an average speed of about 400-500 rpm during the experiment. For the first step, initial pH (pH_0), and temperature are recorded. Next, after adding titrant gradually according to the instructions, the related volumes of pH_1 to pH_4 are recorded to calculate the concentration of VFA and alkalinity. The input values such as conductivity, phosphate, ammonia, and sulfide were taken from Table 9.

3.3.2.2 Helsingborg laboratory

Since VFAs are rapidly oxidized when exposed to oxygen during transportation, a 5-point titration experiment was also performed to investigate VFA variation at the Reco-Lab facility in Helsingborg. This was done by taking samples from the greywater influent as well as the end of the anaerobic section where taking sample was possible. Again, this test was performed once before re-inoculation process and one time after that to compare the changes in VFA concentration before and after the anaerobic period. For this purpose, samples were taken from each of the mentioned parts at 30-minute intervals and 5 pH points titration test was performed immediately. The steps of this test were similar to the steps performed in the university laboratory as well as the mentioned referenced. It should be noted that during the experiment, the amount of phosphate was measured at the beginning of the experiment by using cuvettes of Hach company. In addition, due to lack of access to the centrifuge machine, samples were placed stationary for about 10 minutes until the solid part was settled and 250 ml from the upper part of the sample was used for testing. The results of this section and previous sections have been brought in result section.

3.3.3 VFA concentration from 2 points titration method

Beside the stated VFA measurement by using 5-points titration method, there is possibility to calculate the VFA from Total Alkalinity (TA) and Bicarbonate Alkalinity (BA) by using following equation from 2 points titration method (Brovko and Chen, 1977):

$$BA = TA - 0.83 \times 0.85 \times VFA \qquad \text{(Eq. 2)}$$

Where the unit of BA and TA is mg/l $CaCO_3$ and VFA is based on mg/l Hac. The factor 0.83 convert the volatile acids from mg/Hac to mg/$CaCO_3$. 0.85 is a correction factor because approximately 85% salts from organic acids convert to acid form. According to the equation and having TA and BA values, VFA concentration can be roughly calculated from the one-year lab data from Reco-Lab which is given by NSVA company and be compared with experiments' results and literature data.

4 Results and discussion

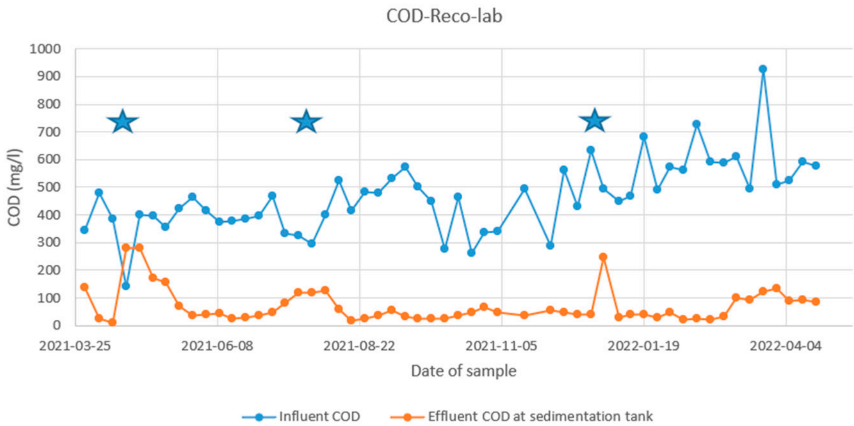
In this chapter, the provided laboratory data which contain different parameters were analyzed and compared with the data in the literature and optimal values such as hydraulic retention time, sludge age as well as preferred pH and temperature were proposed to have an efficient EBPR process. Moreover, the results of P-release batch test and 5-points titration method were presented and discussed. Finally, maximum potential of phosphorus removal for EBPR process was estimated by considering the sludge quality equivalent to Öresundverket sample.

4.1 Data analysis and optimal design values

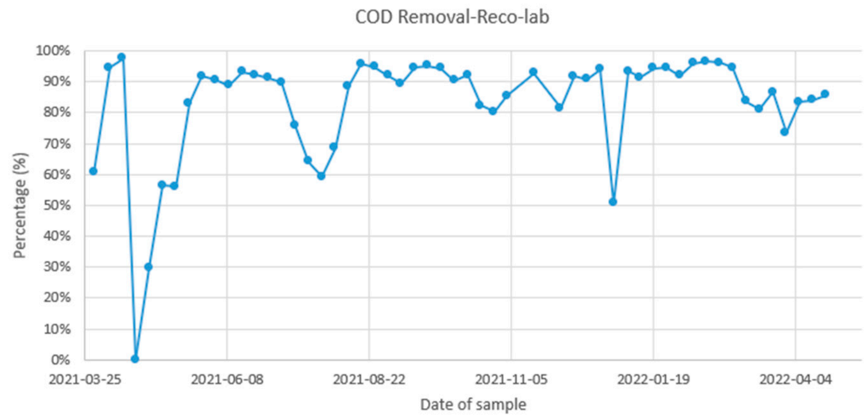
One year sampling data was provided by NSVA performed by the Reco-Lab laboratory. From these data, several useful information can be obtained and used to evaluate the performance of EBPR process. In this part different aspects of these information were explained.

4.1.1 COD

The influent and effluent of COD as well as COD removal in the EBPR system are shown in Figure 8(a) and 8(b) respectively during the operation period. According to the Figure 8(a), the average COD concentration is between 500-600 mg/l. Higher COD concentration from December 2021 might be due to starting a restaurant of the hotel in the area that led to more concentration of organic material and COD. Moreover, the high COD concentration in March 2022 is due to the re-inoculation process. In addition, according to the Figure 8(b), except the periods that the system did not work properly due to operational issues which are marked by starts, about 90% of COD is removed through the EBPR process. The operational problems can come from different issues. For instance, due to the sludge washed out during July 2021, the system could not remove sufficient COD through the process.



(a)



(b)

Figure 8. (a) COD concentration for influent and effluent of EBPR process (b) COD removal.

According to the laboratory data, COD concentration after being centrifuged is also measured and shown by fCOD in Figure 9 This value will be compared with the amount of VFA in the result section. However, it should be noted that this value is not filtrated COD and might cause possible error in the comparison. According to the figure, the average of fCOD concentration is about 300 mg/l. The high value in October 2022 which is marked by star was an operational problem when black water was accidently mixed into the greywater.

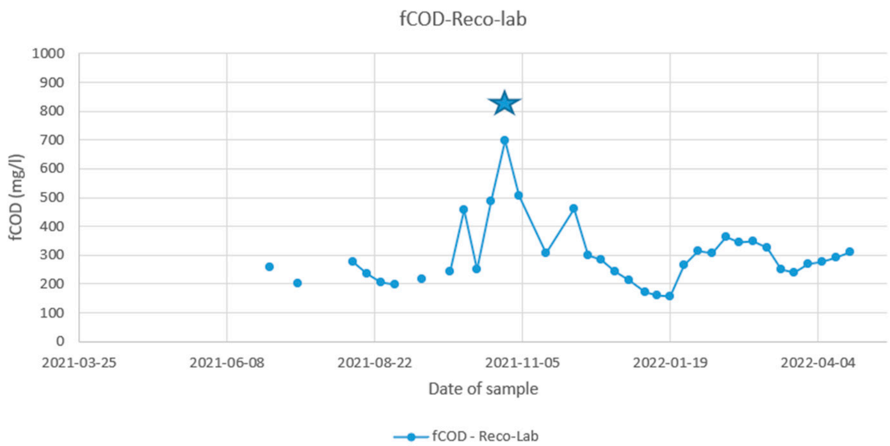


Figure 9. centrifuged COD concentration in the influent greywater of EBPR process.

4.1.2 Sludge concentration in aerated zone

According to Figure 10, the sludge concentration in the aerobic basin has been measured and recorded by online system. In addition, in Figure 11, the concentration of suspended solids in the returned sludge during the same period has been recorded. As it can be seen in the Figure 7, the sludge concentration has fluctuated significantly during the implementation of EBPR process due to various reasons. According to the calculation of correlation between sludge concentration in aeration zone and suspended solids concentration in return sludge that is given in Appendix D, Figures 10 and 11 are correlated, with a factor of 0.97, so that when the sludge was removed out from the aerobic section, there would be also less sludge in the return sludge part at the same period.

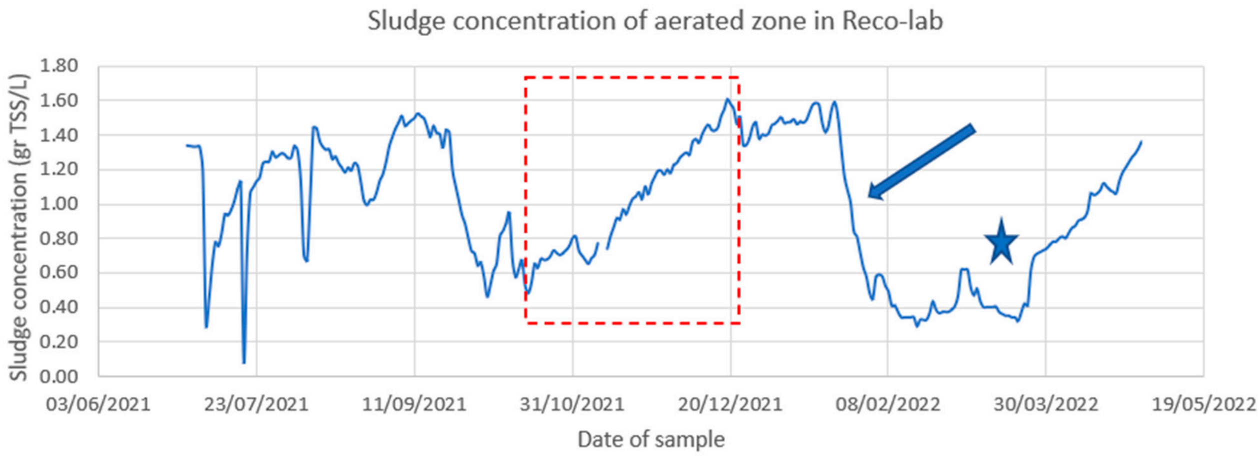


Figure 10. Sludge concentration variation in the aeration tank during operational period (measured using an online turbidity meter located in the aerated zone)

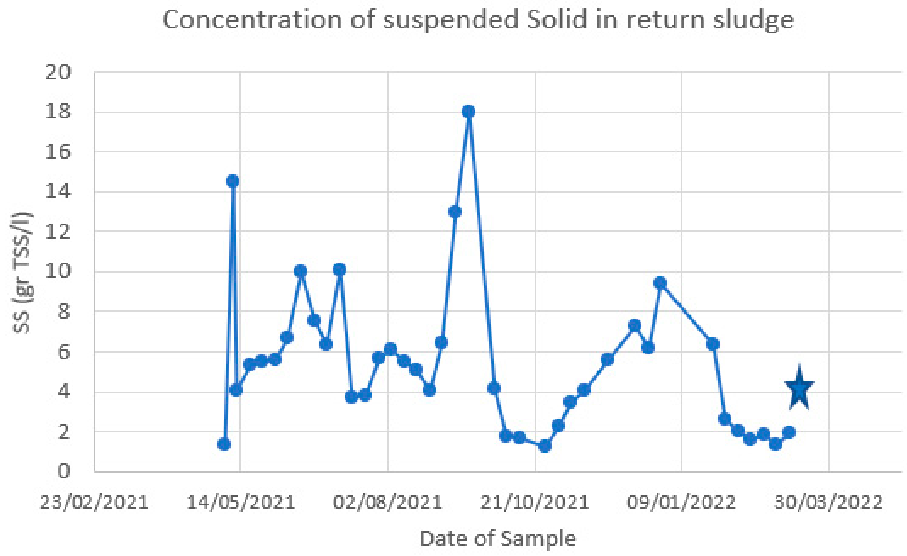


Figure 11. Concentration of Suspended solid in return sludge during operational period (Measured weekly grab samples). The added star indicates the date of re-inoculation.

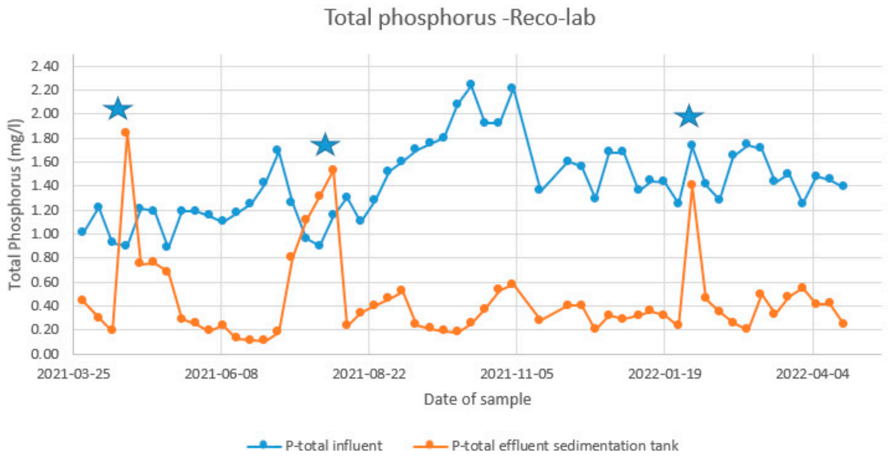
At the beginning of this master project, in January 2022, the online measuring system detected a considerable decrease in the sludge concentration that is shown with blue arrow in Figure 10. Hence, sludge washed out in February 2022 and subsequently they experienced poor settleability in the settling tank. At the same time, since more people moved into the area in January 2022, it is decided to use the larger part of the basins that lead to higher hydraulic retention time in anaerobic and aerobic sections. According to the Figure 10, and the history of sludge recovery, which is marked by red square, it is estimated that it would take about 3 months to restore the sludge concentration. Therefore, to recover the sludge concentration, it was decided to do re-inoculation with Öresundverket sludge. This was done on 23 March 2022 by adding 5 m^3 of Öresundverket sludge to the aerobic zone. This re-inoculation is indicated by a star in the Figure 10. The sludge concentration successfully recovered to the previous range after the re-inoculation. One of the objectives of this master thesis was to perform P-release batch test and 5 points titration experiments, which were explained in section 3.2 and 3.3, to evaluate the effect of the re-inoculation by doing one time before and one time after mixing with Öresundverket sludge. According to the provided data, other parameters that are not used directly in this thesis are shown in Table 6. More information of these parameters was provided in Appendix A.

Table 6. average BOD, N and P concentration of influent and effluent in the EBPR process

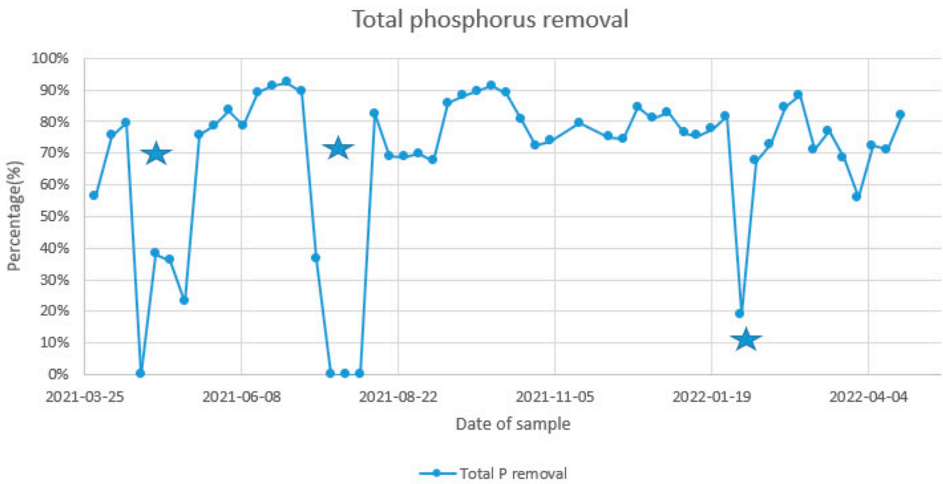
Parameter	Unit	Value
Ave. BOD_{in}	mg/l	200
Ave. N_{in}	mg/l	16
Ave. No_{ut}	mg/l	6
Ave. P_{in}	mg/l	1.4
Ave. P_{out}	mg/l	0.4
Flowrate	m^3/h	4.83
Temperature	°C	17
pH	-	7-7.5

4.1.3 Total phosphorus

As mentioned earlier, the amount of phosphorus and nitrogen in greywater are considerably lower than blackwater that contain most of nutrients. However, there are still different sources of phosphorus in greywater such as phosphorus in chemical products, cooking material and track of urine in bathrooms. The concentrations of influent phosphorus as shown in Figures 12 are quite low. According to the Figure 12(b), except in some operational issues in some periods that mentioned earlier, high phosphorus removal, 75-90% has been achieved. However, the operational conditions will affect the effluent concentration. For instance, washing out the sludge in July 2021 and February 2022 caused high effluent concentration. The concentration and removal of phosphate is also shown in Figure V in Appendix A.



(a)



(b)

Figure 12. (a) Total phosphorus in the influent and effluent of the EBPR system. (b) phosphorus removal. Added stars indicate low removal rate due to operational issues.

According to a study done by Barnard and Abraham (2006), the minimum ratio for COD/total phosphorus in wastewater is 40, and below this value the removal of phosphorus in the biological treatment would be difficult. In addition, according to a study done by Ødegaard (1999), the average ratio of COD/total phosphorus for wastewater in 17 treatment plants in Sweden is about 80 (Table 4). As shown in Figure 13, except some period when the system did not work properly, the average ratio for COD/total phosphorus for greywater in Reco-Lab is between 300 to 400 that shows great condition to remove phosphorus in biological treatment. According to the figure, the high ratios in February and March 2022 might be due to the opening of a few restaurants in the area that cause higher COD concentration. This increase can also be seen on similar dates in Figure 5(a). According to the Table 4, the average phosphorus concentration in wastewater in Sweden is 9 mg/l while these values in greywater in Reco-Lab are 1.4 and 0.3 mg/l. These low values would be beneficial to achieve lower effluent concentration after the treatment process.

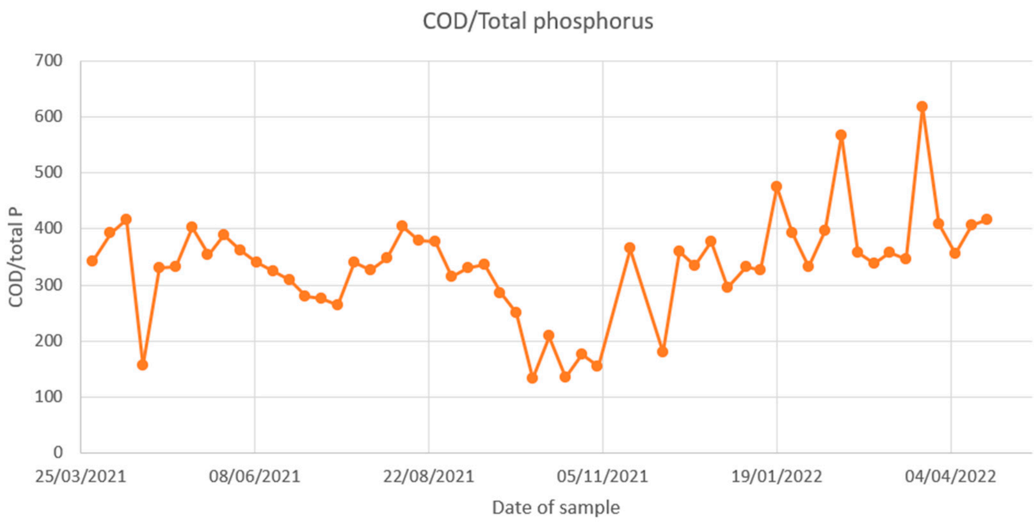


Figure 13. Ratio of COD/total phosphorus according to laboratory data.

4.1.4 Hydraulic retention time and sludge age

As shown in Table 10, optimum values of HRT and SRT are proposed according to different studies which mentioned in chapter 2. These values can be used by NSVA to evaluate the performance of each section in the future. Although in EBPR process no detention time would be needed for collection tank and influent can be transferred directly to the anaerobic basin, the suggested value for this section is due to providing hydrolysis and fermentation to improve the VFA concentration before the anaerobic period. The HRT in the sedimentation basin depends on different factors such as flowrate and the settling velocity of the sludge. However, this value should provide sufficient time for sludge to settle the bottom of the basin. High HRT leads to anaerobic condition and secondary phosphate release that is unfavorable (Lilleland, 2019).

Table 10. Design parameters to have an efficient EBPR system.

Stage	HRT (hours)	SRT (days)
Collection tank	1-2	-
Anaerobic basin	1-3	-
Aerobic basin	2-3	4-5

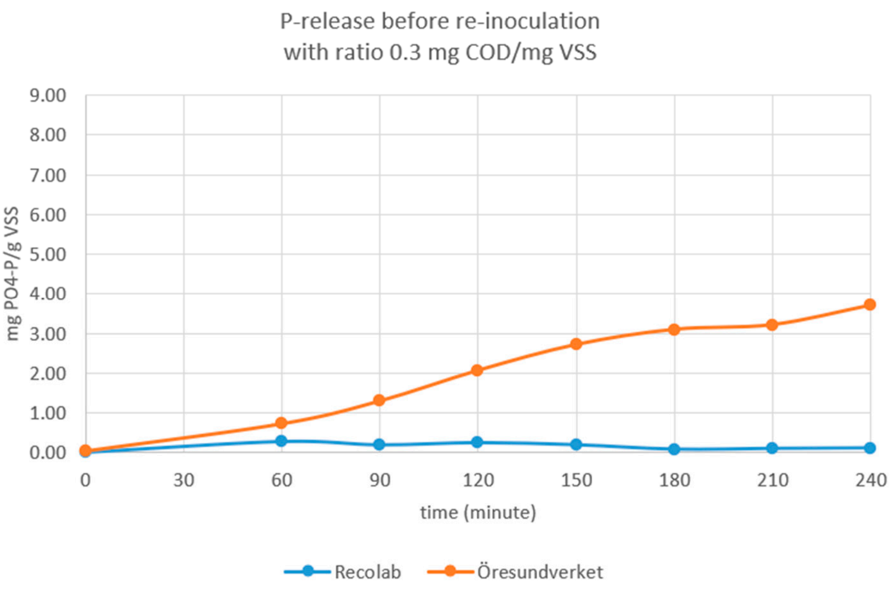
* Data is taken from (Coats *et al.*, 2011; Izadi *et al.*, 2021; Jansen *et al.*, 2019; Lie, 1996)

4.1.5 Temperature, pH and oxygen level

As mentioned in Table 6, the average temperature of influent greywater at Reco-Lab is about 17 ºC. According to the literature study, this temperature would be suitable for EBPR process. As mentioned in literature review, according to Tian *et al.* (2013) lower temperature between 10-14 ºC could increase the dominance of POAs over GAOs and lead to higher performance of EBPR process. However, since only one article has proposed this condition for temperature, more investigation needs to be done before any recommendation can be made. In addition, according to the suggested pH values during the EBPR process, 7-7.5, the pH value in Reco-Lab seems to be in the appropriate range and would not have negative effects on the results. Finally, the optimum DO level for the aeration basin can be varied between 2 to 5 mg/l based on the load of organic matter. As mentioned in section 3.1, the DO level at the Reco-Lab is 3.8 mg/l.

4.2 Batch test results

As mentioned in the previous section, three batch tests were done to evaluate the performance of EBPR process. Two experiments were done before and after the re-inoculation with the same ratio of 0.3 (mg COD substrate/mg VSS sludge) to understand the effect of re-inoculation process in Bio-P activity. According to the Figure 14(a) which is done before the re-inoculation, there is almost zero activity in the Reco-Lab sample and the phosphate release during the anaerobic period was about zero. The reason of this result, as mentioned before, was due to low sludge concentration during the sampling period that is shown in Figure 10. The batch test was also done for Öresundverket sample as a reference which showed an acceptable activity in releasing phosphate of 8 mg PO_4-P/g VSS. For the next step, P-release test was done for both Reco-Lab and Öresundverket samples two weeks after the re-inoculation process and results are shown in Figure 14(b). According to the figure, the increased phosphate release showed that the re-inoculation helped the sludge recovery process and increased the Bio-P activity. However, the maximum value and the pattern of phosphate release is still not following the results in Öresundverket sample. Like the previous test, Öresundverket sample as a reference, shows a promising phosphate release and it can be stated that the amount of carbon source in greywater would be sufficient to reach the maximum phosphate release during anaerobic period.



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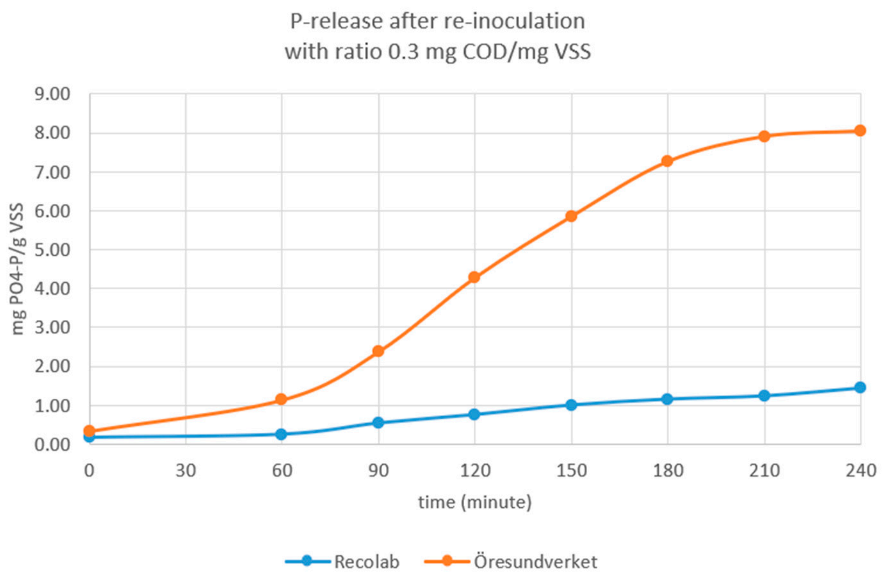
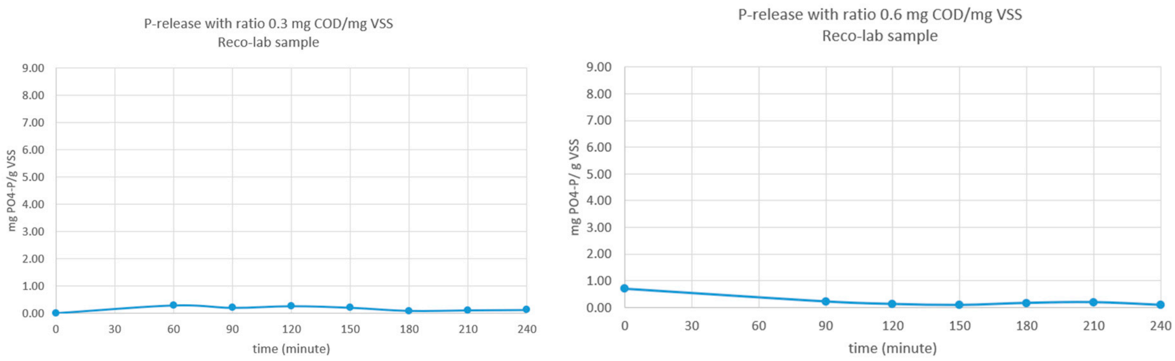


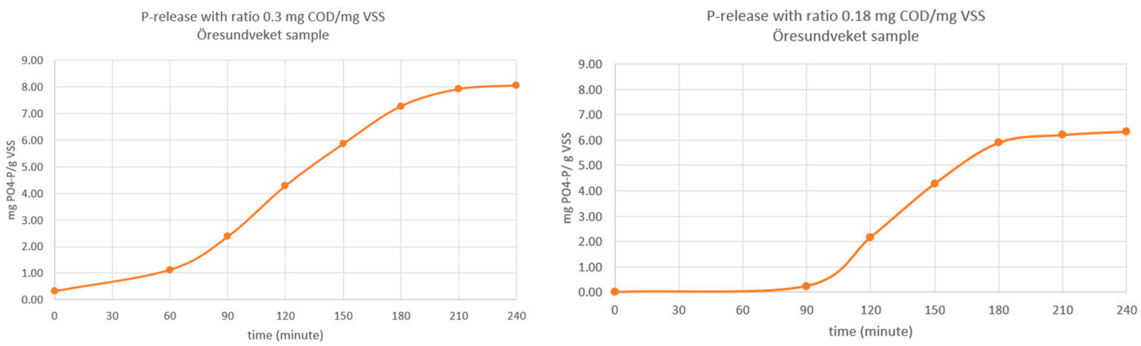
(b)

Figure 14. Results of P-release batch test before and after the re-inoculation.

Another batch test was also done before the re-inoculation to compare the effect of different greywater ratio on the phosphate release during the anaerobic period. Hence, different ratio of 0.6 and 0.18 were selected for Reco-Lab and Öresundverket samples respectively. According to the results, as shown in Figure 15 (a), due to having low sludge concentration in Reco-Lab, increasing the ratio does not affect the amount of phosphate release and still there is almost zero P-release in this sample. Hence, it might be said that there was no Bio-P in Reco-Lab during this period. On the other hand, as shown in Figure 15(b), as it is expected, the phosphate release in Öresundverket sample with ratio of 0.18 is lower than results from ratio of 0.3. However, according to the obtained phosphate released, 6.36 mg PO_4-P/g VSS, this ratio could be sufficient. Since only two different ratios have been tested in this project, optimum ratio cannot be specified according to these experiments. However, according to the results of Öresundverket samples, it is expected that the ratio between 0.18 and 0.3 would lead to an acceptable result and provide enough carbon source for PAOs to release phosphate during the anaerobic period which result in more phosphorus removal in the next step. By comparing the obtained results of three Öresundverket samples, it seems that the maximum released phosphate of 4 PO_4-P/g VSS is lower than the other two. This might come from possible error from limited condition during the experiments.



(a)



(b)

Figure15. Results of P-release batch test for different ratios, (a) Reco-Lab (b) Öresundverket

According to a similar experiment done by Tykesson *et al.* (2005) on Öresundverket many years ago, as shown in Figure 16, the phosphate release was obtained with a maximum of 35 mg/l (6.36 mg PO_4-P/g VSS). However, in this experiment, acetate was used as a carbon source with a ratio of 300 mg COD/l, and by assuming the same VSS value of sludge before the re-inoculation (1500 mg/l), the ratio would be 0.2 mg COD/mg VSS that is the same unit used in this master thesis. By comparing the maximum phosphate release with this ratio and the experiment done in the laboratory with ratio of 0.18 that is given in the first row of Table 11, it can be said that the historical value is consistent with the obtained experimental result. The maximum released phosphate with the two ratios will be used as an ideal condition for phosphorus removal potential in Reco-Lab later in this thesis in section 4.4.2.

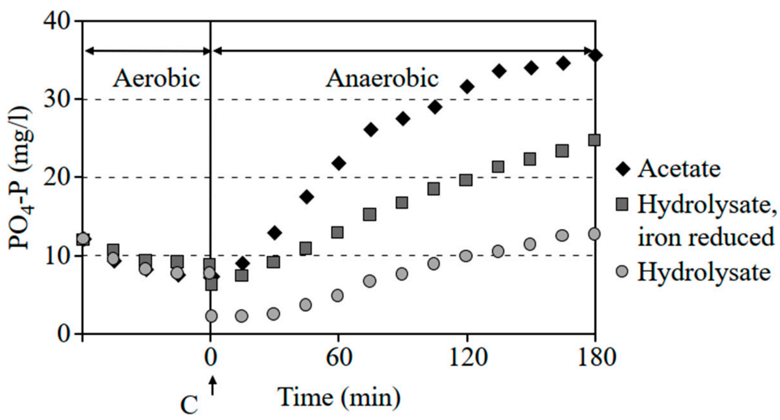


Figure 16. Historical Result of P-release batch test for Öresundverket (Tykesson et al., 2005) (Published with permission from Water Science and Technology (IWA))

4.2.1 P-release rate & maximum release

As mentioned earlier, maximum phosphate release can be obtained by subtracting the phosphate release at the end of anaerobic period from the start point. These values are given in Table 11 for Reco-Lab and Öresundverket samples. As mentioned earlier, the value of 16.38 mg/l in Öresundverket sample seems to be wrong in comparison to the other experiment with the same ratio and therefore is not mentioned in the table.

Table 11 Maximum phosphate release for Reco-Lab and Öresundverket samples

Experiment	Reco-Lab (mg PO_4-P/l)	Öresundverket (mg PO_4-P/l)
Before re-inoculation (Variable ratio)	0	33.6
Before re-inoculation (ratio= 0.3)	0	-
After re-inoculation (ratio= 0.3)	6	38.1
Historical result (ratio= 0.2)	-	35

*The selected ratio is based on mg COD- substrate /mg VSS-sludge

In addition, according to the formula provided in chapter 3, P-release rate can be calculated by using linear part of the graph in Figures 14 and 15 and the amount of VSS samples which are presented earlier which is given in Table 9. According to the releasing rate and the classification, which mentioned in Table 8, potential of both samples with ratio of 0.3 are classified as mediocre. According to the table, the releasing rate in Öresundverket sample with ratio of 0.18 (mg COD/mg VSS) is higher than ratio of 0.3 and classified as good. By assuming the same value of VSS for historical sludge of Öresundverket, P-release rate can also be calculated for this sample. The historical phosphate release of Öresundverket has an acceptable P-release rate that is placed in the good category.

Table 12 P-release rate for Reco-Lab and Öresundverket samples (mg PO_4/g VSS x h)

Experiment	Reco-Lab	Classification	Öresundverket	Classification
Before re-inoculation (Variable ratio)	0	Mediocre	3.63	Good
Before re-inoculation (ratio= 0.3)	0	Mediocre	1.19	Mediocre
After re-inoculation (ratio= 0.3)	0.4	Mediocre	2.76	Mediocre
Historical result	-	-	3.18	Good

4.3 VFA analysis

4.3.1 5 points titration results

5 points titration analysis was done to evaluate the concentration of VFA. The results are shown in following graphs and show different aspects of VFA and Bio-P efficiency. According to the Figure 17, VFA concentration has been measured for Reco-Lab in three sample points during the p-release test: end of pre-aeration period before adding greywater as substrate, after adding greywater and end of anaerobic period. Pre-aeration was also used to oxidize existing VFA in the sludge sample. This was done to evaluate only the effect of VFA in the greywater as substrate Hence, the VFA concentration is also measured at the end of pre-aeration period to control this value. In addition, to find the consumption rate of VFA during the anaerobic condition, sampling for VFA experiment was done at the first and last point of anaerobic period. During the anaerobic period it was expected that all the VFAs are consumed by PAOs to release phosphate during the anaerobic period. However, as it is shown in Figure 17, due to poor activity of Bio-P in Reco-Lab sample, not all the VFAs are consumed during the anaerobic period and will leak to aerobic period. This could have negative effect on the rate of phosphate uptake during aeration period and VFA will be oxidized with oxygen. This result is completely consistent with the result obtained in the p-release test.

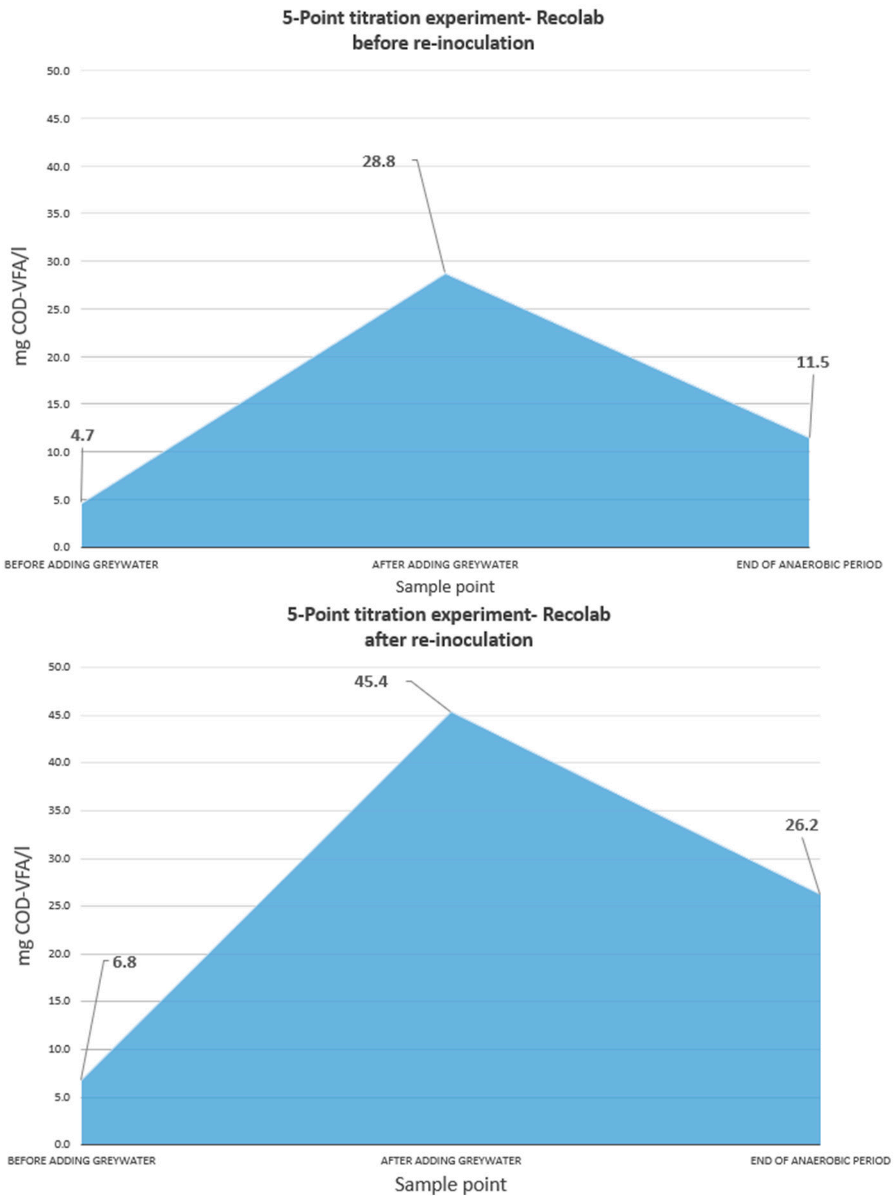


Figure 17. VFA concentration for Reco-Lab samples during P-release tests

For Öresundverket samples as a reference, the results are successful and all the VFAs as are shown in Figure 18, are consumed during the anaerobic period that led to an acceptable phosphate release in P-release test. In addition, the amount of VFA for two experiments are similar which confirms the accuracy of the experiments. According to the results of p-release test and VFA graphs, it can be said that Bio-P is working well for Öresundverket samples. 5-points titration method is also done for the experiment with different ratios of greywater. However, since the purpose of this experiment was only to compare the effect of ratios on phosphate release, the VFA test was done 48 hours after p-release test and filtrated samples were kept in fridge with 4 ºC. As mentioned earlier, since VFAs are consumed rapidly in fresh samples when they have contact with air, the obtained VFA concentrations were very low, 10.7 mg COD/l, and not valid for the comparison. However, the results are available in the Appendix C.

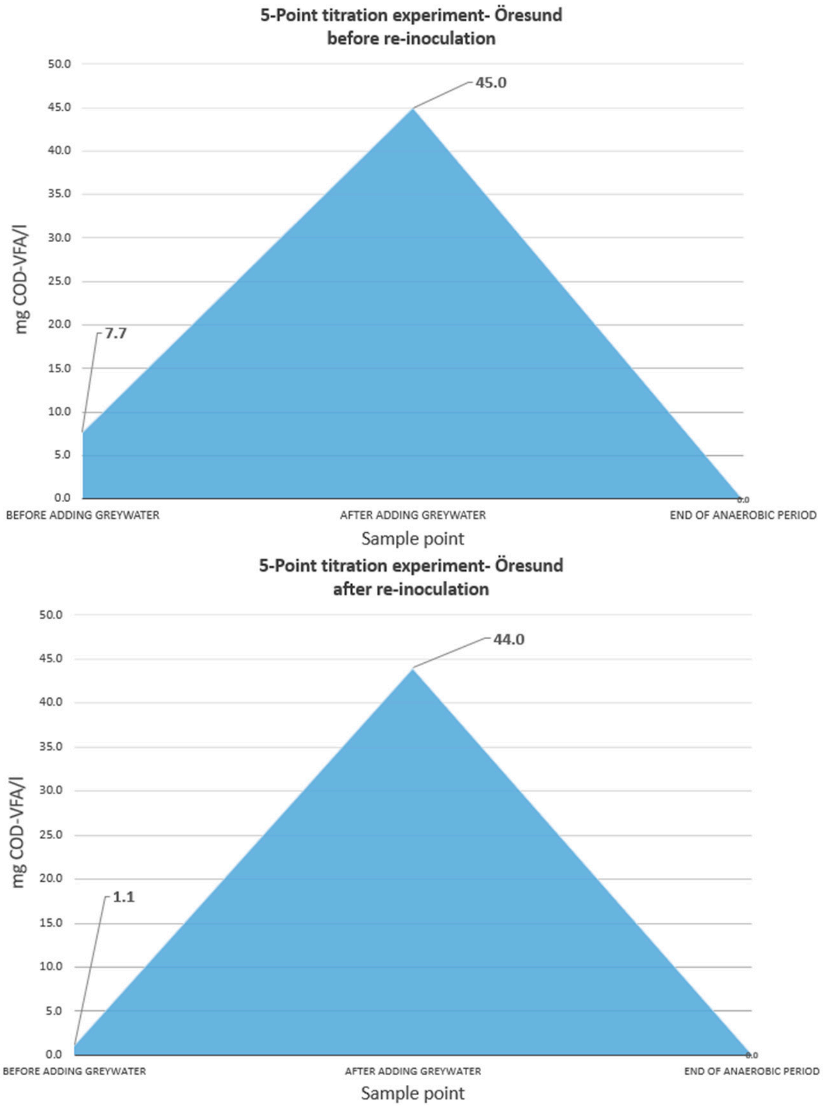


Figure 18. VFA concentration for Öresundverket samples during P-release tests

In Reco-Lab laboratory in Helsingborg, there was a possibility to measure VFAs more accurately immediately after taking samples from sampling points. This is done for greywater influent to estimate amount of carbon source in greywater and at the end of anaerobic basin to control if all the VFAs are consumed by PAOs to release phosphate. It should be noted that the influent greywater sample point is placed after the collection tank. According to the results which are shown is Figure 19, VFA concentration in the influent greywater changes between 65 to 85 mg COD/l. This value is the same VFA concentration range in influent wastewater in Sweden, 50 to 100 mg COD-VFA/l, that is mentioned earlier. As it is expected from previous experiments, the VFA concentration at the end of anaerobic zone is not zero that means all the VFAs have not been consumed by PAOs during releasing phosphate and is consistent with previous results. However, VFA concentration is higher in compare with university laboratory's results and since is done immediately after sampling would be more reliable. A possible reason is that some VFAs will be consumed during transportation of samples to university laboratory and therefore lower concentrations have been obtain during p-release test.

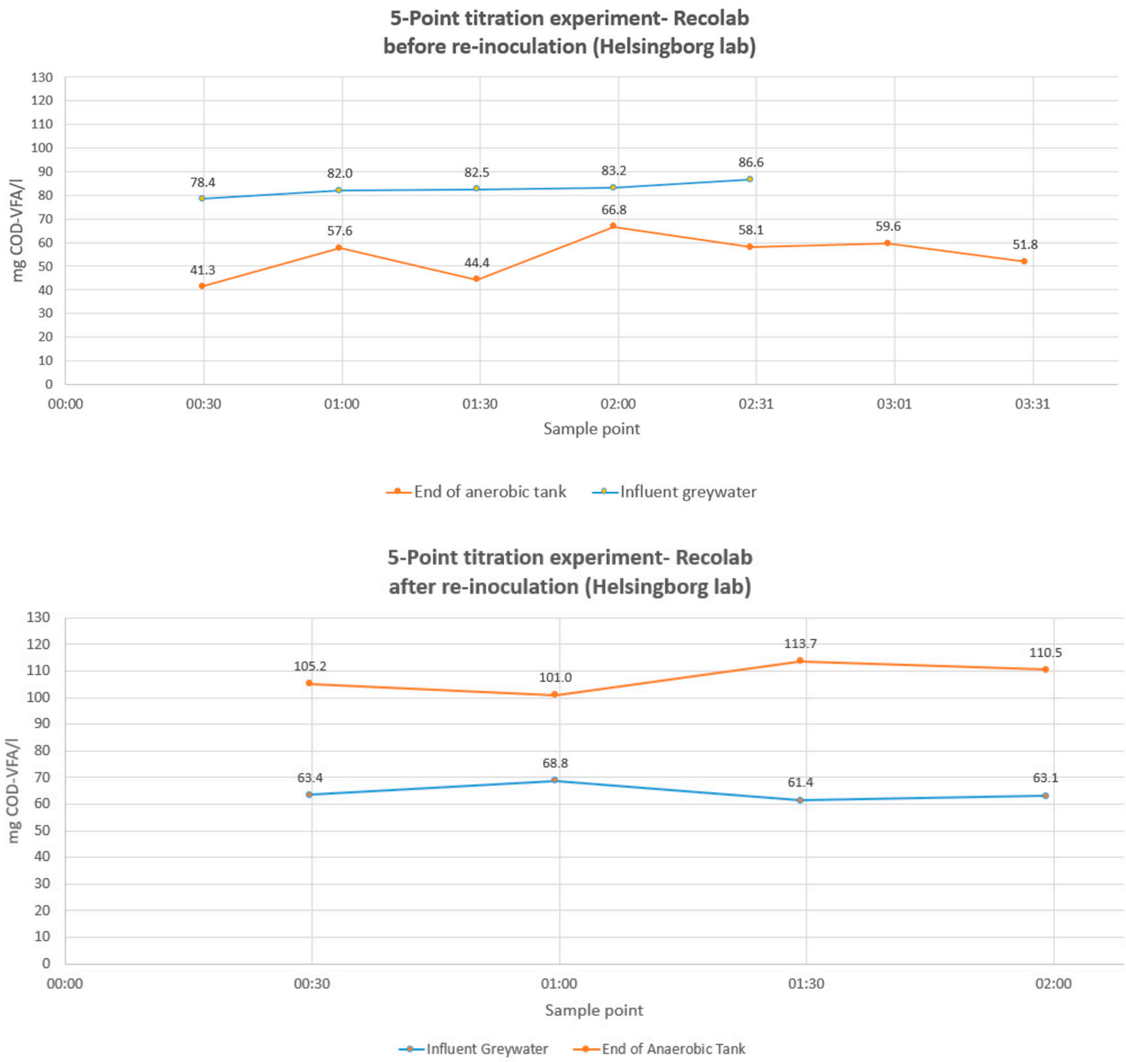


Figure 19. VFA concentration at different sample points

The average VFA concentration before the re-inoculation at the end of anaerobic period is 54 mg COD-VFA/l. Surprisingly, this value has been increased considerably after the re-inoculation process while it was expected to have less VFA concentration at the end of anaerobic period. The results are shown in Figure 19 with average of 107 mg COD-VFA/l. This might be due to high VFA concentration in added sludge from Öresundverket. In addition, there might be some hydrolysis process during the anaerobic period which lead to VFA production. In the Reco-Lab facility, there are four sampling points for greywater system: Influent greywater, end of anaerobic zone, end of aerobic zone and return sludge outlet. The five-points titration method also was done for end of aerate zone to control the amount of VFA. It is expected that no VFAs are present in the aerobic period. However, according to the results which is shown in Figure 20, there are still high VFA values in aerated zone that show there might be problem in the EBPR process and needs to be fixed. Nevertheless, it is believed that according to the obtained results, the re-inoculation could help the process to recover sludge concentration and release more phosphate during anaerobic period. However, it is believed that it will take time to have a better Bio-P activity in Reco-Lab again.

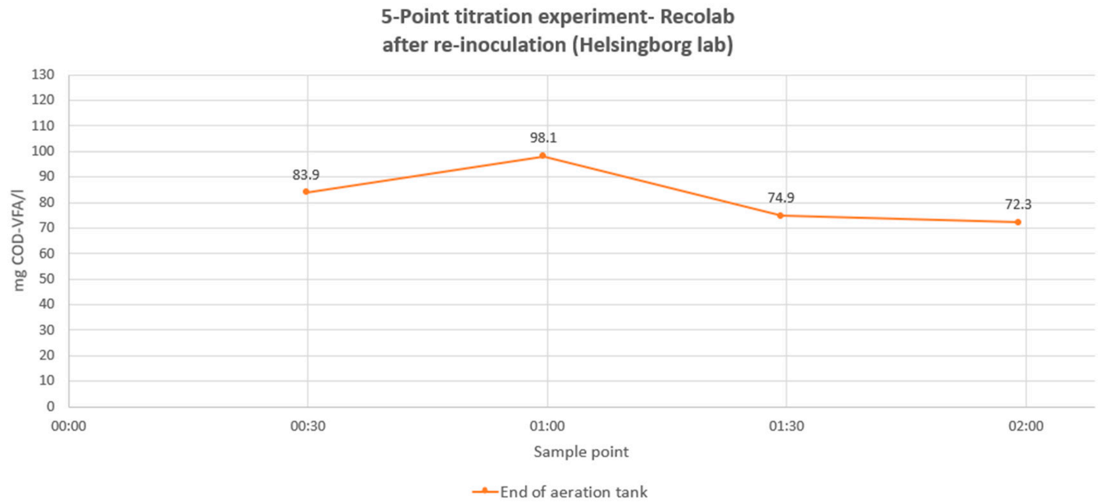


Figure 20. VFA concentration at the end of aeration tank in Helsingborg laboratory

4.3.2 2 points titration results

By using the equation 2, VFA can be calculated and converted as mg COD/l which is shown in Figure 21. Here, it is assumed all of the VFA is acetate and is converted to COD equivalent to compare with centrifuged COD. According to the figure, there is high VFA concentration in the influent greywater that shows a good carbon source for EBPR process. In addition, by comparing the ratio of obtained VFA to centrifuged COD for 2points and 5points titration, as it is shown in Figure 22, we can see that this ratio for 2 points titration and 5-points titration are approximately 65% and 30% respectively. However, more investigation should be done about the accuracy of the obtained result from the 2-point titration equation as it is calculated by using total alkalinity and bicarbonate alkalinity and not from the experiment.

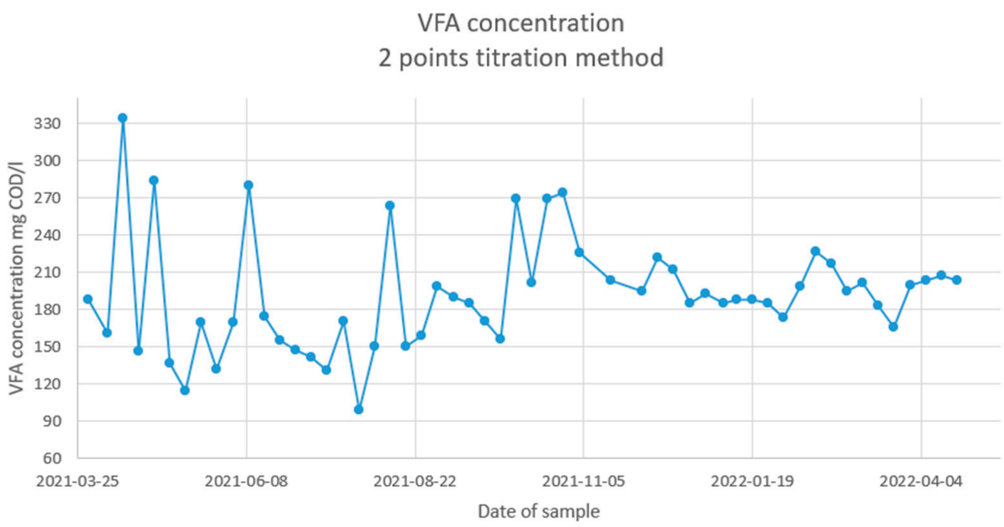


Figure 21. VFA concentration of influent greywater from one year laboratory data

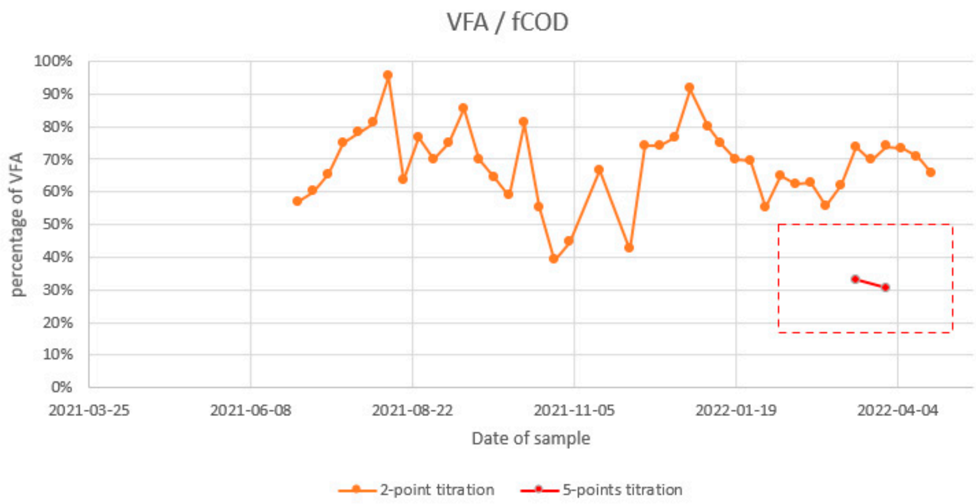


Figure 22. VFA to fCOD ratio for 2-points and 5-points titration method

As it can be seen in the results, the average concentration of VFA in the influent greywater by using 5-points titration method and 2-point titration calculation are 75 mg COD-VFA/l (Figure 19) and 190 mg COD-VFA/l (Figure 21) that by comparing these values with common values in other reported studies, these values are in a high range and show a good capacity of greywater for this method. In addition, as mentioned in section 4.1.1, the ratio of VFA/phosphate in both results are much higher than the values for wastewater in other studies and show a high capacity for the EBPR process. According to the results of chapters 4.3.1 and 4.3.2, by comparing the VFA concentration of the same dates with the performed experiments in Helsingborg, the values of 2-pints titration method are about 3 times higher than experimental results. Moreover, the ratio of VFA/PO_4-P as an important parameter to evaluate the efficiency of EBPR process is compared with literature data. As mentioned earlier, according to Lie (1996), this ratio for wastewater is normally between 20-30 mg COD-VFA/mg P which means about 20-30 mg COD-VFA would be required to remove 1 mg phosphate. However, according to the average VFA/Phosphate in the laboratory data, this ratio can be as high as 477 that provide a great opportunity to have an efficient EBPR process. In addition, by calculating this ratio from the obtained experimental results that is explained in section 4.3.1, this value can be 233 which also provides suitable condition for EBPR process.

4.4 Maximum phosphorus removal

By having the values of VSS in the sludge from the return sludge, aeration basing and assuming that all the released phosphate will be removed in the aeration period, maximum theoretical phosphorus that can be removed due to Bio-P activity was calculated for different scenarios.

4.4.1 Existing condition

According to the calculations that are provided in Appendix E, the maximum phosphorus removal before and after the re-inoculation in the Reco-Lab sample were 0.3 and 0.97 mg PO_4-P/l respectively. Based on the data provided in Table 6, approximately 1 mg/l phosphorus is removed through the EBPR process while based on the calculations only 3% of phosphorus removal occurred through Bio-P process before the re-inoculation. As mentioned in chapter 2 (2.2.3), other mechanisms such as assimilation can play main role in this condition.

On the other hand, by assuming that all the released phosphate is removed in the aeration period, the phosphorus removal increased to 0.97 mg PO_4-P/l that showed promising result after the re-inoculation. However, according to the obtained removal before the re-inoculation, we can say that not all of the phosphorus was removed through Bio-P activity and still other mechanisms play role in the removal.

4.4.2 Hypothetical condition

In addition, to estimate the maximum phosphorus removal potential for EBPR system in the Reco-Lab, it is assumed that the sludge quality in Reco-Lab is similar to Öresundverket experiment samples that shown earlier. Maximum potential can be calculated the same way that was done in the existing condition. Based on the calculations in Appendix E, the maximum phosphorus removal potential for the two different carbon source ratios that are used in the experiments were 7.3 and 6.4 mg PO_4-P/l respectively. It can be seen that the EBPR process will have high potential for removing phosphorus by using Öresundverket sample as an ideal condition, 6.4-7.3 mg/l. These results are promising especially for the countries like Sweden that the influent phosphorus is quite low due to the ban of phosphorus in detergents.

5 Conclusions

The water shortage crisis in recent years has prompted researchers to think of alternative solutions. The source separation in the Oceanhamnen area is considered as a successful process in the field of energy and nutrient recovery, as well as recycling water. Greywater as a main part of domestic wastewater is a suitable alternative for fresh water and can be biologically treated due to its characteristics. In this master thesis, the potential for phosphorus removal through the EBPR process was investigated. This was done by analyzing laboratory data as well as performing P-release tests and 5-point titration analyses to measure the phosphate release as well as VFA concentration.

According to the obtained results from the P-release tests, no initial Bio-P activity could be detected in the treatment of greywater at Reco-Lab. Since about 75% of phosphorus has been removed through the EBPR process at that period, it can be concluded that other mechanisms such as assimilation had the main role for removing phosphorus from greywater. The maximum phosphate release in the Öresundverket's sample as a reference was acceptable that showed good Bio-P activity during the anaerobic period. After re-inoculation, released phosphate increased in the Reco-lab sample which showed a promising result. However, the maximum value was still low in compared with the Öresundverket's sample. In addition, based on the results of Öresundverket's samples, the HRT value of 3 hours would be sufficient for VFAs to be consumed by PAOs in the anaerobic period.

The results from 5-points titration analysis showed that the VFA concentration in the influent greywater is acceptable. In addition, the ratio of VFA/PO_4-P which represents the EBPR efficiency in greywater is much more than wastewater that shows the high capacity of greywater to use this method. Moreover, according to the calculations for the maximum phosphorus removal potential through EBPR process, by assuming the ideal condition in the system, phosphorus can be removed to a great extent. Therefore, EBPR method seems to be a suitable option for greywater to remove phosphorus provided that the operational condition is stable and mentioned parameters are selected properly. However, to maintain the required phosphorus concentration in the effluent, chemical method can be used after the EBPR process as a back-up when the system has operational issues.

6 Future study

In this master thesis, the effect of nitrogen is not investigated and could be considered as possible error and therefore further study should consider this parameter during the process. In addition, the presence of micropollutant such as surfactants can have negative effects on the efficiency of the EBPR process and these parameters should be investigated during this method. According to the high phosphorus removal during the poor results of the EBPR system and the possibility of removing phosphorus with other mechanisms such as assimilation, more study on determining the exact rate of assimilation and other possible mechanism would help to have a better understanding for this method.

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Appendix

Appendix A – Data analysis

According to the Figure II, average flow rate that enter to the EBPR system is 2.5 m^3/h. There is also a flowrate which is pumped from return sludge to the collection tank. This flowrate is not continuous and is set to be off and on for four and two minutes respectively. The average flow from return sludge is 2.33 m^3/h for sake of simplicity and will be added to the feeding rate to the EBPR process. The flowrate can be calculated as below:

Flowrate through the EBPR process = Influent from GW collection tank + Return sludge flow

$$\text{Flowrate through the EBPR process} = 2.5\ m^3/h + 2.33\ m^3/h = \mathbf{4.83\ m^3/h}$$

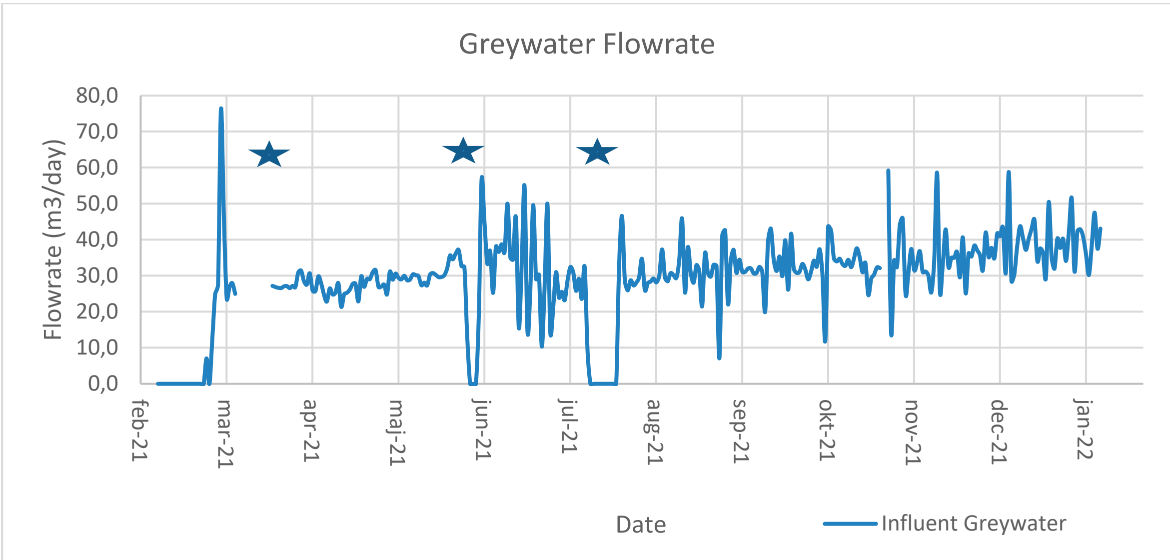


Figure I. Measured Flowrate of greywater between the collection tank and the EBPR process. Stars indicate zero value due to operational issues in the system

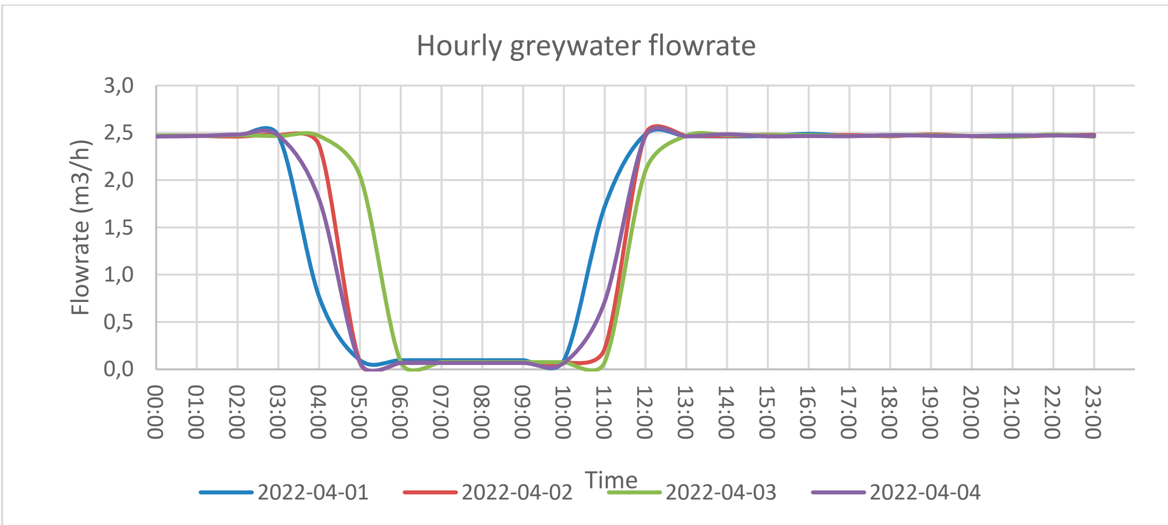


Figure II. Example of Hourly flowrate over 24h of greywater to the EBPR process.

pH variation is also recorded during the operation period and shown in Figure III in Appendix A. Except the periods that the system had operational issues, the average pH in the influent and effluent of EBPR system is between 7-7.5. Normally, as shown in Figure IV, the average of temperature in the system is about 17 ºC. However, according to the figure since April 2022, the system has experienced a higher temperature, which is likely due to temperature variation of changing seasons.

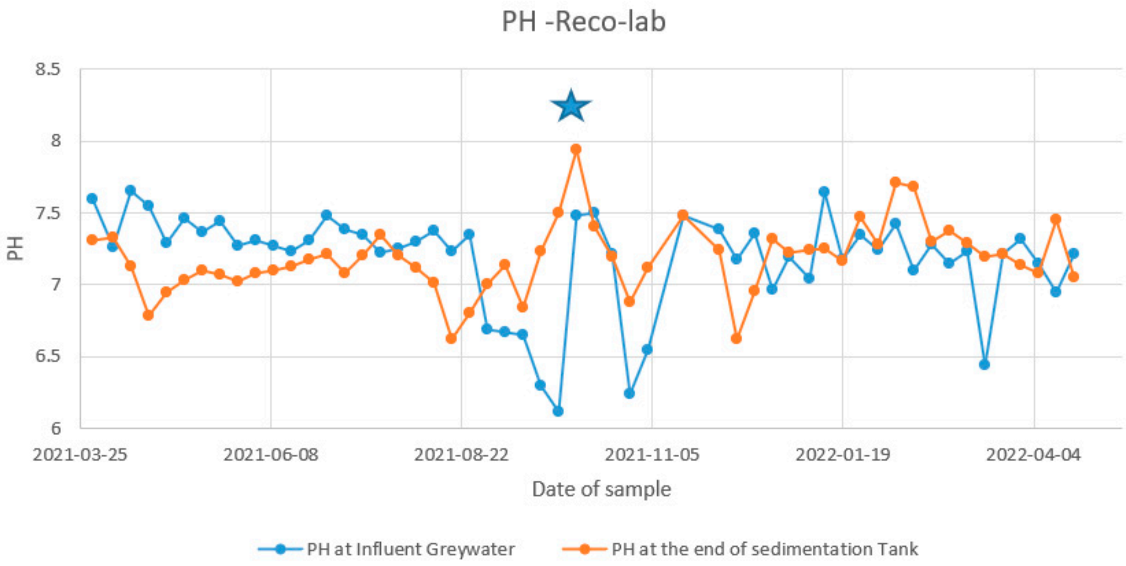


Figure III. PH variation in influent greywater.

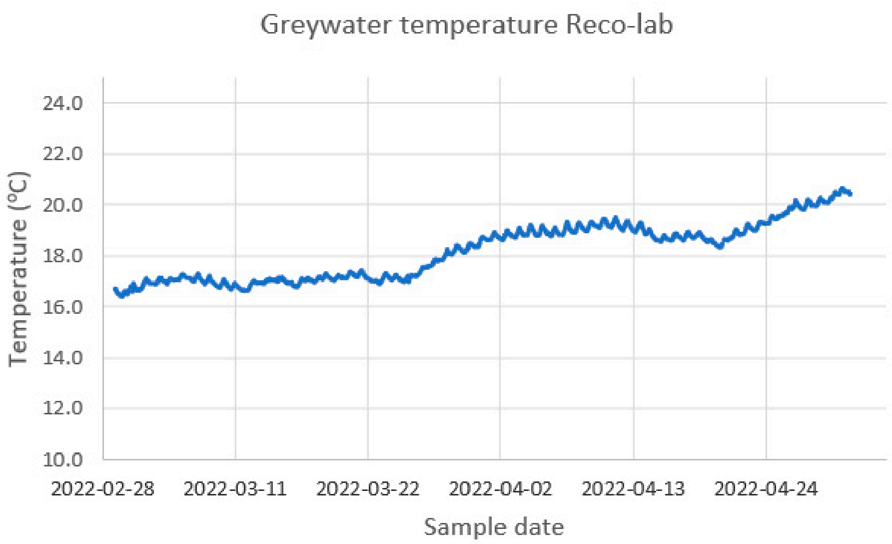
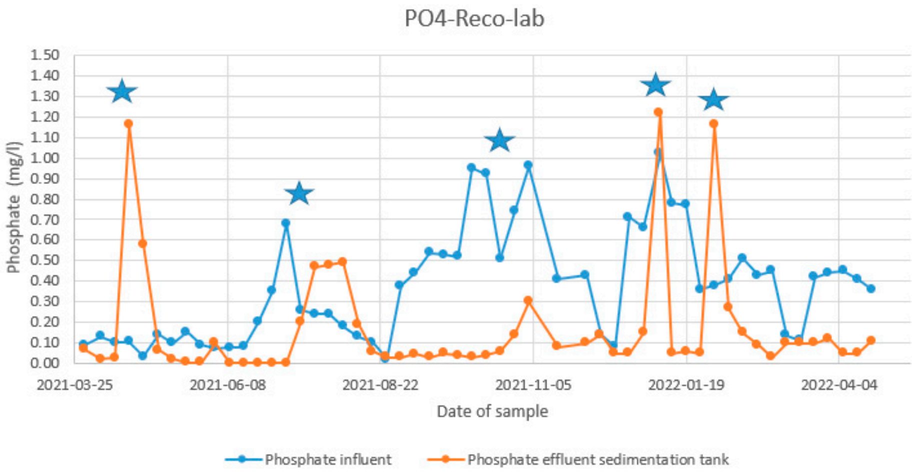
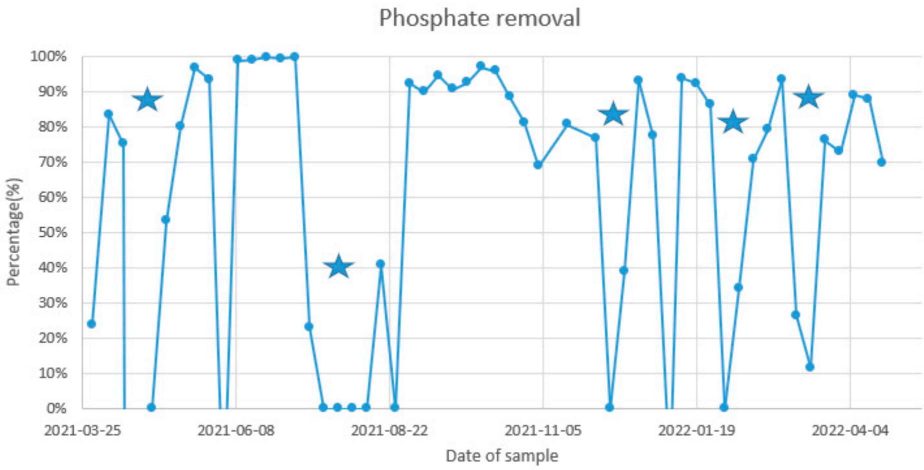


Figure IV. Temperature variation in influent greywater.



(a)



(b)

Figure V. Phosphate concertation in the influent and effluent of EBPR system. Added stars indicate low removal rate due to operational issues.

Appendix B – P-release results

Results of P-release test (before re-inoculation)				
Test period	time (min)	experiment time (min)	P- Recolab (mg/l)	P- öresundverket (mg/l)
Pre-aeration	0	0	0	0.21
Anaerobic period	0	60	0.422	3.98
	30	90	0.291	7.14
	60	120	0.381	11.37
	90	150	0.297	14.938
	120	180	0.124	17.025
	150	210	0.154	17.675
	180	240	0.174	20.362

Results of P-release test (after re-inoculation)				
Test period	time (min)	experiment time	P- Recolab	P- öresundverket
Pre-aeration	0	0	0.809	1.777
Anaerobic	0	60	1.22	6.203
	30	90	2.674	13.038
	60	120	3.76	23.538
	90	150	5.003	32.187
	120	180	5.762	39.964
	150	210	6.18	43.504
	180	240	7.196	44.26

Results of P-release test (Test for other ratios)				
	time (min)	experiment time	P- Recolab	P- öresundverket
Pre-aeration	60	0	0.25	0
Anaerobic period	30	30	0.316	1.313
	60	60	0.174	11.95
	90	90	0.117	23.56
	120	120	0.236	32.479
	150	150	0.277	34.17
	180	180	0.121	34.9

Appendix C – VFA analysis test Results

University Laboratory: (before re-inoculation)

Recolab samples

At the begining of Anaerobic period (Before adding greywater)

PH	Vx	Phosphate	Temperature
PH0=7.5	-	0.01	21
6.7	4.12	Sulphide	FSA
5.9	10.27	0	2.2
5.2	12.91	Volume	conductivity
4.3	14.09	250 ml	52.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
4.359		137.27	

Öresundverket samples

At the begining of Anaerobic period (Before adding greywater)

PH	Vx	Phosphate	Temperature
PH0=7.48	-	0.651	20.8
6.7	3.83	Sulphide	FSA
5.9	9.68	0	2.2
5.2	12.44	Volume	conductivity
4.3	13.75	250 ml	93.2
VFA mg/l as acetate		Alkalinity mg caco3/l	
7.216		132.53	

Recolab samples

At the begining of Anaerobic period

PH	Vx	Phosphate	Temperature
PH0=7.52	-	0.422	20.4
6.7	3.07	Sulphide	FSA
5.9	7.19	0	2.2
5.2	10.39	Volume	conductivity
4.3	12.16	200 ml	52.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
26.9		138.54	

Öresundverket samples

At the begining of Anaerobic period

PH	Vx	Phosphate	Temperature
PH0=7.28	-	3.98	20.5
6.7	2.31	Sulphide	FSA
5.9	8.34	0	2.2
5.2	12.35	Volume	conductivity
4.3	15.28	250 ml	93.2
VFA mg/l as acetate		Alkalinity mg caco3/l	
42.042		165.95	

Recolab samples

At the end of Anaerobic period

PH	Vx	Phosphate	Temperature
PH0=7.7	-	0.174	21.4
6.7	3.81	Sulphide	FSA
5.9	9.12	0	2.2
5.2	13.12	Volume	conductivity
4.3	14.78	250 ml	52.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
10.75		132.12	

Öresundverket samples

At the end of Anaerobic period

PH	Vx	Phosphate	Temperature
PH0=7.25	-	20.362	21.6
6.7	3.03	Sulphide	FSA
5.9	9.69	0	2.2
5.2	13.55	Volume	conductivity
4.3	14.91	250 ml	93.2
VFA mg/l as acetate		Alkalinity mg caco3/l	
0		147.64	

University Laboratory: (after re-inoculation)

Recolab samples			
At the begining of Anaerobic period (Before adding greywater)			
PH	Vx	Phosphate	Temperature
PH0=7.78	-	0.809	20.4
6.7	4.75	Sulphide	FSA
5.9	9.88	0	2.2
5.2	13.23	Volume	conductivity
4.3	14.68	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
6.397		125.26	
Recolab samples			
At the begining of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=7.48	-	1.22	20.2
6.7	2.41	Sulphide	FSA
5.9	7.44	0	2.2
5.2	11.02	Volume	conductivity
4.3	13.78	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
42.396		147.44	
At the end of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=7.83	-	7.196	22
6.7	2.7	Sulphide	FSA
5.9	7.79	0	2.2
5.2	11.36	Volume	conductivity
4.3	13.45	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
24.502		141.25	

Öresundverket samples			
At the begining of Anaerobic period (Before adding greywater)			
PH	Vx	Phosphate	Temperature
PH0=7.53	-	1.777	20.4
6.7	2.83	Sulphide	FSA
5.9	7.6	0	2.2
5.2	10.05	Volume	conductivity
4.3	11.05	250 ml	93.2
VFA mg/l as acetate		Alkalinity mg caco3/l	
1.069		106.81	
Öresundverket samples			
At the begining of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=7.26	-	6.203	20
6.7	1.96	Sulphide	FSA
5.9	6.91	0	2.2
5.2	10.34	Volume	conductivity
4.3	13.01	250 ml	93.2
VFA mg/l as acetate		Alkalinity mg caco3/l	
41.11		141.75	
At the end of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=7.53	-	44.26	22
6.7	3.91	Sulphide	FSA
5.9	9.51	0	2.2
5.2	12.43	Volume	conductivity
4.3	13.4	250 ml	93.2
VFA mg/l as acetate		Alkalinity mg caco3/l	
0		137.05	

University Laboratory: (Test for other ratio)

Recolab samples			
At the begining of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=6.98	-	0.019	14
6.7	2.1	Sulphide	FSA
5.9	7.57	0	3.5
5.2	10.4	Volume	conductivity
4.3	11.76	200 ml	505
VFA mg/l as acetate		Alkalinity mg caco3/l	
10.704		140.17	
At the end of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=7.31	-	0.121	12
6.7	0.76	Sulphide	FSA
5.9	5.94	0	3.5
5.2	8.64	Volume	conductivity
4.3	9.55	200 ml	505
VFA mg/l as acetate		Alkalinity mg caco3/l	
0		135.89	

Öresundverket samples			
At the begining of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=6.9	-	2.07	14
6.7	2.71	Sulphide	FSA
5.9	6.78	0	3.5
5.2	9.65	Volume	conductivity
4.3	10.95	200 ml	505
VFA mg/l as acetate		Alkalinity mg caco3/l	
10.872		111.31	
At the end of Anaerobic period			
PH	Vx	Phosphate	Temperature
PH0=7.46	-	24.4	12
6.7	3.12	Sulphide	FSA
5.9	7.85	0	3.5
5.2	10.02	Volume	conductivity
4.3	10.75	200 ml	505
VFA mg/l as acetate		Alkalinity mg caco3/l	
0		131.81	

Greywater samples			
Round 1			
PH	Vx	Phosphate	Temperature
PH0=6.7	-	0.137	12.8
6.7	0	Sulphide	FSA
5.9	5.12	0	3.5
5.2	8.64	Volume	conductivity
4.3	11.68	200 ml	505
VFA mg/l as acetate		Alkalinity mg caco3/l	
64.33		166.81	
Round 2			
PH	Vx	Phosphate	Temperature
PH0=6.7	-	0.137	12.8
6.7	0	Sulphide	FSA
5.9	5.14	0	3.5
5.2	8.63	Volume	conductivity
4.3	11.67	200 ml	505
VFA mg/l as acetate		Alkalinity mg caco3/l	
64.33		166.81	

Helsingborg Laboratory: (before re-inoculation)

Influent greywater

Round 1 (13:15)

PH	Vx	Phosphate	Temperature
PH0=7.105	-	0.113	20.9
6.7	3.13	Sulphide	FSA
5.9	8.75	0	2.2
5.2	12.401	Volume	conductivity
4.3	16.29	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
73.285	167.05

Round 2 (13.45)

PH	Vx	Phosphate	Temperature
PH0= 7.046	-	0.113	20.9
6.7	2.855	Sulphide	FSA
5.9	8.63	0	2.2
5.2	12.3	Volume	conductivity
4.3	16.293	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
76.59	167.92

Round 3 (14:15)

PH	Vx	Phosphate	Temperature
PH0=7.057	-	0.113	21
6.7	2.712	Sulphide	FSA
5.9	8.416	0	2.2
5.2	12.163	Volume	conductivity
4.3	16.213	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
77.086	169.8

Round 4 (14:45)

PH	Vx	Phosphate	Temperature
PH0= 7.035	-	0.113	21
6.7	2.654	Sulphide	FSA
5.9	8.632	0	2.2
5.2	12.4	Volume	conductivity
4.3	16.46	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
77.74	171.99

Round 5 (15:15)

PH	Vx	Phosphate	Temperature
PH0=7.045	-	0.113	21
6.7	2.746	Sulphide	FSA
5.9	8.402	0	2.2
5.2	12.111	Volume	conductivity
4.3	16.271	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
80.909	169.43

Effluent of Anaerobic zone

Round 1 (7:45)

PH	Vx	Phosphate	Temperature
PH0= 7.25	-	0.2	21.1
6.7	5.81	Sulphide	FSA
5.9	11.15	0	2.2
5.2	14	Volume	conductivity
4.3	16.111	200 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
38.611	166.4

Round 2 (9:00)

PH	Vx	Phosphate	Temperature
PH0=7.305	-	0.2	20.8
6.7	5.969	Sulphide	FSA
5.9	13.159	0	2.2
5.2	16.542	Volume	conductivity
4.3	19.652	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
53.82614	188.99

Round 3 (9:30)

PH	Vx	Phosphate	Temperature
PH0=7.286	-	0.2	21
6.7	5.889	Sulphide	FSA
5.9	12.766	0	2.2
5.2	16.55	Volume	conductivity
4.3	19.36	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
41.45	174.8

Round 4 (10:00)

PH	Vx	Phosphate	Temperature
PH0=7.334	-	0.2	21
6.7	5.84	Sulphide	FSA
5.9	12.975	0	2.2
5.2	15.994	Volume	conductivity
4.3	19.425	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
62.41	195.13

Round 5 (10:30)

PH	Vx	Phosphate	Temperature
PH0=7.32	-	0.2	21.1
6.7	6.125	Sulphide	FSA
5.9	12.872	0	2.2
5.2	16.003	Volume	conductivity
4.3	19.086	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
54.27	180.59

Round 6 (11:00)

PH	Vx	Phosphate	Temperature
PH0=7.355	-	0.2	21.1
6.7	5.844	Sulphide	FSA
5.9	11.98	0	2.2
5.2	15.128	Volume	conductivity
4.3	18.211	250 ml	50.5

VFA mg/l as acetate	Alkalinity mg caco3/l
55.73669	170.03

Helsingborg Laboratory: (after re-inoculation)

Influent greywater			
Round 1 (30)			
PH	Vx	Phosphate	Temperature
PH0=7.22	-	0.59	20.8
6.7	3.769	Sulphide	FSA
5.9	9.568	0	2.2
5.2	13.58	Volume	conductivity
4.3	17.1	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
59.254		180.4	
Round 2 (60)			
PH	Vx	Phosphate	Temperature
PH0= 7.133	-	0.59	20.6
6.7	3.403	Sulphide	FSA
5.9	9.485	0	2.2
5.2	13.485	Volume	conductivity
4.3	17.14	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
64.276		176.27	
Round 3 (90)			
PH	Vx	Phosphate	Temperature
PH0=7.14	-	0.59	20.3
6.7	3.253	Sulphide	FSA
5.9	9.228	0	2.2
5.2	13.28	Volume	conductivity
4.3	16.75	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
57.341		170.79	
Round 4 (120)			
PH	Vx	Phosphate	Temperature
PH0= 7.139	-	0.59	20.4
6.7	3.281	Sulphide	FSA
5.9	9.118	0	2.2
5.2	12.987	Volume	conductivity
4.3	16.45	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
59		166.95	

Effluent of Anaerobic zone			
Round 1 (30)			
PH	Vx	Phosphate	Temperature
PH0= 7.162	-	11.7	21.6
6.7	7.282	Sulphide	FSA
5.9	16.38	0	2.2
5.2	21.64	Volume	conductivity
4.3	26.83	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
98.36		255.37	
Round 2 (60)			
PH	Vx	Phosphate	Temperature
PH0=7.09	-	11.7	21.6
6.7	6.003	Sulphide	FSA
5.9	16.36	0	2.2
5.2	21.98	Volume	conductivity
4.3	26.53	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
94.433		270.2	
Round 3 (90)			
PH	Vx	Phosphate	Temperature
PH0=7.121	-	11.7	21.8
6.7	5.707	Sulphide	FSA
5.9	16.078	0	2.2
5.2	21.12	Volume	conductivity
4.3	27.51	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
106.25		279.02	
Round 4 (120)			
PH	Vx	Phosphate	Temperature
PH0=7.133	-	11.7	21.8
6.7	6.111	Sulphide	FSA
5.9	16.618	0	2.2
5.2	22.17	Volume	conductivity
4.3	27.48	250 ml	62.3
VFA mg/l as acetate		Alkalinity mg caco3/l	
103.238		280.76	

Appendix D – Calculation of Correlation

In this section, according to the section 3.2.6, the correlation of sludge level in aeration tank and suspended solids level in return sludge is examined. This is done by using "Pearson's Correlation" methods which the correlation factor can be calculated by following equation: (Benesty *et al.*, 2009; Pearson, 2008)

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

According to the laboratory data from Reco-Lab, sludge and suspended solids levels are during different period are given that correlation factor can be obtained by using the equation:

Table X: calculation of correlation factor between sludge level and suspended solids

Date	SUSP	sludge	a (ss)	b (sludge)	ab	a2	b2
2021-07-14	3700	0.93	3699.112	0.93	3440.174	13683428.9	0.8649
2021-07-21	3800	1.06	3800	1.06	4028	14440000	1.1236
2021-07-28	5650	1.3	5650	1.3	7345	31922500	1.69
2021-08-04	6060	1.34	6060	1.34	8120.4	36723600	1.7956
2021-08-18	5040	1.24	5040	1.24	6249.6	25401600	1.5376
2021-08-25	4020	1.13	4020	1.13	4542.6	16160400	1.2769
2021-09-01	6400	1.17	6400	1.17	7488	40960000	1.3689
2021-09-29	4120	0.73	4120	0.73	3007.6	16974400	0.5329
2021-10-06	1780	0.61	1780	0.61	1085.8	3168400	0.3721
2021-10-13	1640	0.58	1640	0.58	951.2	2689600	0.3364
2021-10-27	1200	0.7	1200	0.7	840	1440000	0.49
2021-12-15	7300	1.43	7300	1.43	10439	53290000	2.0449
2021-12-22	6140	1.46	6140	1.46	8964.4	37699600	2.1316
2021-12-29	9440	1.38	9440	1.38	13027.2	89113600	1.9044
2022-01-26	6340	1.08	6340	1.08	6847.2	40195600	1.1664
2022-02-02	2560	0.48	2560	0.48	1228.8	6553600	0.2304
2022-02-09	2040	0.41	2040	0.41	836.4	4161600	0.1681
2022-02-16	1560	0.34	1560	0.34	530.4	2433600	0.1156
2022-02-23	1840	0.38	1840	0.38	699.2	3385600	0.1444
2022-03-02	1360	0.47	1360	0.47	639.2	1849600	0.2209
2022-03-09	1960	0.43	1960	0.43	842.8	3841600	0.1849
					91152.97	**446088329**	**19.7005**
					Sum	**Sum**	**Sum**

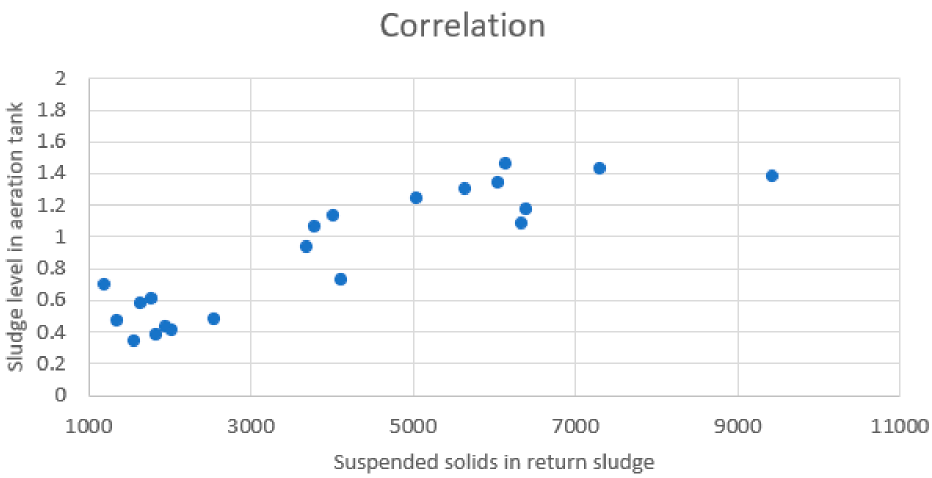


Figure X. Scatterplot to control the correlation

According to the Table X and as it can be seen in Figure X, the correlation factor between sludge level in aeration section and suspended solids in return sludge is 0.97 that shows very good correlation between them.

Appendix E – Calculation of maximum phosphorus removal

In this section, rough calculation is done to estimate the amount of phosphorus that can be removed due to Bio-P activity. In addition, maximum phosphorus removal potential for Reco-lab is estimated by using the released phosphate values of Öresundverket sample by assuming that Reco-Lab will have the same sludge quality in the future. It is assumed that EBPR system operates at optimal condition and all the released phosphate during the anaerobic conditions will be removed during the aeration section.

Existing condition

By having the maximum released phosphate during the anaerobic condition, VSS of the sludge sample in the return sludge as well as VSS in the aeration basin, approximate amount of phosphorus (mg PO_4-P/l) which is removed during the aeration section can be calculated as below:

$$\frac{Maximum\ released\ phosphate\ (\text{mg PO4} - \text{P/l})\ x\ VSS\ in\ the\ aeration\ basin\ (\frac{mg\ VSS}{l})}{VSS\ of\ the\ sludge\ sample\ for\ the\ experiment\ (\frac{mg\ VSS}{l})}$$

As shown in Figure 10, the value of sludge concentration in the aeration tank one week before the re-inoculation and two weeks after the re-inoculation were 0.4 and 0.9 TSS g/l respectively. According to Fan *et al.* (2015), VSS is approximately 75% of TSS. Therefore, based on the equation and given values, the phosphorus removal before and after the re-inoculation can be calculated:

Reco-Lab phosphorus removal before the re-inoculation:

Maximum p-release: 0.174 mg PO_4-P/l

VSS in the test sample = 1500 mg/l VSS in the aeration basin= 300 mg/l

$$\frac{0.17\text{x}\ 300}{1500} = 0.03\ \ (\text{mg PO}_4\text{-P/l})$$

Reco-Lab phosphorus removal after the re-inoculation:

Maximum p-release: 7.2 mg PO_4-P/l

VSS in the test sample = 5000 mg/l VSS in the aeration basin= 675 mg/l

$$\frac{7.2\ \text{x}\ 675}{5000} = 0.97\ (\text{mg PO}_4\text{-P/l})$$

Hypothetical condition

By assuming the same sludge quality of Öresundverket for the Reco-Lab and selecting the average sludge concentration of 1.4 g TSS/l as an ideal condition, the maximum phosphorus removal potential for EBPR system in the Reco-Lab can be calculated as below:

Maximum Phosphorus removal potential: (ratio of substrate: 0.3 mg COD/mg VSS)

Maximum p-release (Öresundverket): 38.1 PO_4-P/l

VSS in the test sample (Öresundverket) = 5500 mg/l

VSS in the aeration basin= 1050 mg/l

$$\frac{38.1 \text{ x } 1050}{5500} = 7.30 \text{ (mg } PO_4\text{-P/l)}$$

Maximum Phosphorus removal potential: (ratio of substrate: 0.18 mg COD/mg VSS)

Maximum p-release (Öresundverket): 33.6 PO_4-P/l

VSS in Test = 5500 mg/l

VSS in the aeration basin= 1050 mg/l

$$\frac{33.6 \text{ x } 1050}{5500} = 6.40 \text{ (mg } PO_4\text{-P/l)}$$

As mentioned in section 4.4.1, the VSS value can be calculated by multiplying this number by 0.75.

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