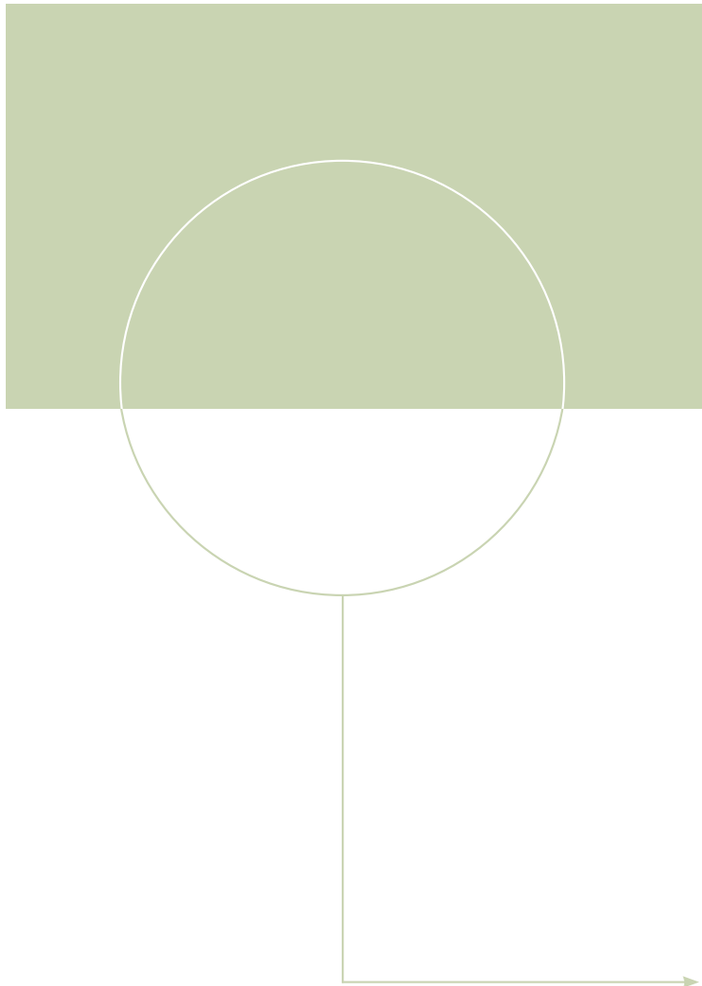

Master's thesis

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Effects of Fermented Primary Sludge Supernatant as Carbon Source in an Enhanced Biological Phosphorous Removal Process

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Civil and environmental engineering
Submission date: June 2019
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Abstract

The purpose with this master thesis is investigation and optimization of the enhanced biological phosphorous removal (EBPR) process in a continuous moving bed biofilm reactor (CMBBR). With different wastewater compositions (diluted, semi-diluted and concentrated) and conditions. Especially in cold conditions and different dissolved oxygen (DO) levels. Main focus is to check the effect of fermented supernatant as carbon source in the pilot plant due to low concentrations of chemical oxygen demand (COD) in wastewaters. Especially in periods with diluted wastewater due to intrusion of rainwater to sewers to avoid high cost and environmental footprint by adding other carbon sources. Fermentation is done on primary sludge to find the optimal fermentation conditions. The supernatant extracted are used in kinetics jar-test with carriers from CMBBR. With a goal of producing a lot of volatile fatty acids (VFAs) since those are well known as carbon sources for EBPR.

Methodology used is literature research and experimental work. State of the art for EBPR process and fermentation is presented. Literature is first of all about activated sludge (AS) EBPR. This CMBBR is a new process developed at Hias WWTP. EBPR is a process removing phosphorous (P) as an excess amount of what is usual for ordinary heterotrophic organisms (OHOs). By exposing the carriers for fluctuating anaerobic and aerobic conditions; polyphosphate accumulating organisms (PAOs) grows and take up P by utilization of carbon storage in cell. In laboratory a lot of kinetics mimicking the pilot plant are carried out on different conditions in beakers. Fermentation reactor have been used to produce batches of fermentation supernatant for use in kinetics experiments and to see how fermentation process work. Both batch and sequence-batch reactor has been investigated for the fermentation.

The production of fermented primary sludge supernatant(FPSS) is more effective at 24-29 °C and highest total solids(TS) in a completely mixed reactor. Hydraulic retention time (HRT) of 4 days was used in fermentation number seven and resulted in 89% VFA-COD of soluble COD(SCOD) measured at day 4. Out of this, 51% was propionic acid and 29% was acetic acid. Addition of the fermented supernatant doesn’t always give better removal of phosphorous in the kinetics batch experiments, sometimes actually worse removal. This could be because VFA is not the preferred carbon source for the larger part of Bio-P bacteria in the pilot. Most of them probably like other carbon sources better.

Todays knowledge and configurations are possible to develop and improve. Fermentation as carbon source for the EBPR process seems to be as good as acetate, but in this specific process at NTNU the wastewater seems to do better without having extra addition even if the inlet SCOD is low. Except for conditions were the organisms is exposed for diluted wastewater over time and are having a rough time, addition of fermented supernatant would be useful.

Sammendrag

Formålet med denne masteroppgaven er å undersøke og optimalisere biologisk fosforfjerning i en reaktor med biofilm bærere. Ved ulike konsentrasjoner i avløpsvannet og ulike forhold. Spesielt ved kalde temperaturer (11-14°C) og ulike oksygen konsentrasjoner. Hovedfokuset er å teste fermentert primærslam som karbonkilde. Bakgrunnen for dette er at biologisk fosforfjerning er avhengig av karbon, og avløpet er ofte veldig utvannet. Spesielt i regntunge perioder når overvann trenger inn i rørene, er det fordelaktig å ha en kostnadsbesparende og bærekraftig karbonkilde. Fermenteringen gjøres på primærslam siden dette inneholder mest organsik materie og lite næringsstoffer. Produktet fra fermenteringen brukes i jartester med bærere fra reaktoren. Målet med fermenteringen er å produsere lett nedbrytbar karbon som flyktige fettsyrer, VFA, siden dette er beste karbon for biologisk fosforfjerning.

Det er utført literatur studie og forsøk. Dagens situasjon og kunnskap er presentert for både fermentering og biologisk fosforfjerning. Prosessen som kombinerer biologisk fosforfjerning og MBBR er utviklet på Hias avløpsrenseanlegg. Det er en prosess som fjerner fosfor utover hva som er vanlig for ordinære heterotrofe bakterier. Ved å ha vekslende anaerobe og aerobe forhold dannes det en fosfor akkumulerende organisme. Denne slipper fosfor i anaerob sone når den tar opp karbon og lagrer for så å bruke energien fra dette når den igjen tar opp fosfor i aerob sone. Jartester er utført for å teste fermentert produkt og andre parametere som kan innvirke på prosessen. Fermenterings forsøk er utført på ulike måter i en oppvarmet reaktor og med ulike oppholdstider. Forsøket har blitt kjørt som en batch reaktor og en hvor 50% av reaktorens innhold har blitt byttet ut med jevne mellomrom.

Ser at fermenterings effektiviteten er høyest ved 24-29°C og så høyt som mulig innhold av tørrstøff. I forsøk nummer 4 på fermentering ble VFA-KOF innholdet målt til å være 89% av målt løst KOF på dag 4. Av dette var det 51% propionsyre og 29% eddiksyre. Forsøkene har vist at biologisk fosforfjerning har fungert vel så bra uten tilsetning av fermentert produkt. Den biologiske fosforfjerningen har i hovedsak vært best uten tilsetting av fermentert produkt, bortsett fra når avløpet var veldig tynt over en lengre periode. Da kom det fermenterte produktet til hjelp. Forsøkene viser at bakteriene i prosessen ikke er avhengige av VFA slik som antatt og at de trolig liker andre lett tilgjengelige karbonkilder bedre.

Dagens prosesser og utforminger er mulig å utvikle og forbedre. Fermentert produkt ser ut til å fungere like godt som tilsetting av acetat, men i denne prosessen klarer bakteriene seg i hovedsak uten tilsetting av noe ekstern karbon kilde. Bortsett fra i lange perioder med tynt avløp.

Preface

This master thesis is written at NTNU – Water and wastewater institute. The experiments are performed in the wastewater laboratory at Valgrinda near Lerkendal in Trondheim. The thesis is a follow-up from the master project written in the period from September 2018 to January 2019, which gave 7.5 credits. This thesis is written and worked on in the period from January to June 2019, with a lot of effort in the lab from January to April. The course gives 30 credits, and is the last part of a master´s degree program in civil and environmental engineering.

RECOVER project on NTNU is the basis for the thesis. RECOVER is focusing on nutrient and energy recovery in wastewater treatment with attention to keep removal requirements with low environmental footprint.

I would like to thank my supervisor Stein W. Østerhus for giving me inspiration and knowledge enough to choose this project, a whole amount of expertise in the guidance during the experiments and discussion of results. I also want to thank co-supervisor Blanca Magdalena Gonzalez Silva for helping me out in the laboratory giving great support, guidance, inspiration and new knowledge in accordance to being a great discussion partner and support for writing.

Most of the time was spent in the laboratory this semester. The other lab workers there have been great as support by questions and by socializing. Johanne Prestvik have been there whenever I have needed an extra hand. Also the support from my cohabitant at home, roommate in Trondheim and other friends and family have been great to have during this semester. Thank you!

Kine Hagelund Svendby

Trondheim, 7th June 2019

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Abbreviations

AS	Activated Sludge
BOD	Biological Oxygen Demand
BPR	Biological Phosphorous Removal
bsCOD	Bioavailable COD
CMBBR	Continuous Moving Bed Biofilm Reactor
COD	Chemical Oxygen demand
DNPAO	De-nitrifying Polyphosphate Accumulating Organism
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorous Removal
FCOD	Filtrated Chemical Oxygen Demand
GAO	Glycogen Accumulating Organism
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor
N	Nitrogen
NH_4-N	Ammonium
OHO	Ordinary Heterotrophic Organism
P	Phosphorous
PAO	Polyphosphate Accumulating Organism
PCOD	Particulate Chemical Oxygen Demand
PHA	Poly-ß-hydroxyalkanoates
PO_4-P	Phosphate
PolyP	Intracellular Polyphosphates
rbCOD	Readily Biodegradable COD
SCFA	Short-chain fatty acid
SCOD	Soluble Chemical Oxygen Demand
SS	Suspended Solids
TCOD	Total Chemical Oxygen Demand
TOD	Theoretical Oxygen Demand
TS	Total solids
TVS	Total Volatile Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
WW	Wastewater

1 Introduction

1.1 State of the art

Nutrients in wastewaters are causing eutrophication by changing the condition in ecosystems. That's why it's important to remove nutrients from wastewater before discharge to sources to avoid eutrophication. There are treatment requirements regarding phosphorous (P) and nitrogen (N) removal, mostly P-removal with discharging to sources of freshwater and N removal with discharging to sources of saltwater. Life on earth is dependent on P. Every living plant and organism demand phosphorous, and are very important for food production. The world is limited on nutrients, and P sources declines [1]. To keep life on earth its crucial to reuse what we waste and removes from the wastewater.

United Nations have developed a set of goals to achieve a better and more sustainable future for all. There are 17 goals. They are all connected in one way. Every goal have sub goals to achieve the main goal [2]. Goal number 6 is about water and sanitation, and shows the focus and importance of wastewater treatment. Goal number 11 is about making cities sustainable and shows the focus and importance of being sustainable in every step we take. This together shows the importance of having sustainable wastewater treatment.

Traditionally eutrophication problem has been the focus when removing nutrients from wastewater. These days being sustainable is equally important. A way to be more sustainable is by recover from wastewater. NTNU has an ongoing project that is called RECOVER, focusing on nutrient recovery in wastewater [3]. The main process, the continuous moving bed biofilm reactor (CMBBR), is developed at Hias and is called the Hias-process® [4]. This process is based on the theory of the traditionally activated sludge (AS) enhanced biological phosphorous removal (EBPR) process and combined with moving bed biofilm reactor (MBBR) concept. The RECOVER project is working on further research of this and complementary processes for having a even more sustainable process.

N is removed by biological treatment processes and P has traditionally been removed by chemical precipitation as the main process. Precipitation results in chemically bound P and are therefor hard to recover. Addition of chemicals are not sustainable and will give more sludge production. P-removal in biological treatment processes makes the P more available for recovery. The efficiency of the EBPR process is very dependent on the carbon source. If the inlet concentration to the treatment plant has low values of readily biodegradable chemical oxygen demand (rbCOD), especially volatile fatty acids (VFAs), carbon addition is crucial and demanding. An alternative is to add external substrate such as acetate. This leads to addition of chemicals in EBPR process too and is not as sustainable as wanted. Another option, a cheaper and more environmental friendly method is to produce carbon source at site by fermentation of sludge. A process that is performed in anaerobic conditions with simultaneously hydrolysis and fermentation breaking down carbohydrates, proteins and fats and making rbCOD. For EBPR purposes primary sludge is most suitable with high content of organic compounds and low nutrient content. Another positive effect from using fermented sludge is the reduction of sludge

volume and solids content, reducing disposal costs [5] [6]. The efficiency of the EBPR is also dependent on other factors than carbon source, as dissolved oxygen level, temperature and wastewater composition.

1.2 Research questions (R.Q.)

This project is carried on with a goal to see how fermentation products can be made in this lab and try those on experiments mimicking the CMBBR. In addition to fermented product, other parameters are also investigated in experiments to optimize the process in the CMBBR. This led to the following R.Q.

- How should the setup for fermentation be to have the most effective method to generate soluble organic compounds?
- Does the fermentation produce VFA that can be used as carbon source for the CMMBR?
- Are there other factors that can help improve the efficiency of the CMMBR?
- How does the CMMBR work in different conditions as cold temperature and different wastewater composition?

1.3 Build up of the text

R.Q. are tried to be answered by theoretical research and laboratory work. This work is first presented by a theoretical background for the fermentation and EBPR process to have an understanding of the fermentation process and what kind of products the EBPR need from the fermentation. Also looking after other factors than carbon source that decides the efficiency of the CMBBR. Further the methods for lab work and analyses is presented. Then the results are presented and discussed. At last the conclusion is presented and further work on the topic is proposed.

Major part of the theoretical background was written in my master project work Autumn 2018 at NTNU [7]. Some sections are directly copied from my own work, some are modified and some are new. Also other parts of the project work have inspired and been used. The difference from the project work to the master thesis is more focus on EBPR and lab work. Having more abundance results.

2 Theoretical background

2.1 Fermentation

Fermentation happens in two processes; the first is the hydrolysis and the second is the acidogenesis, also called fermentation. Hydrolysis breaks down the larger molecules into soluble molecules. Further conversion to organic acids happens in the fermentation by acid formers. [8]

2.1.1 Primary treatment

Figure 2.1 Salsnes filter placed in the laboratory

Primary treatment of wastewater is often also called mechanical treatment, and are frequently used as pre-treatment. Sludge from this process is called primary sludge. Purpose with this treatment step is particle removal. Required removal is 20% reduction of biological oxygen demand (BOD) with a maximum concentration of 40 mg/l in the outlet. Suspended solids (SS) needs to be reduced by minimum 50 % and maximum concentration in the outlet is 60 mg/l [9]. In this project the particle separation is conducted by a salsnes filter shown in Figure 2.1. This is a rotating belt sieve and is the most common sieve for primary treatment.

2.1.1.1 Salsnes filter

Salsnes filter is a space-saving option compared to conventional sedimentation for primary treatment and are performing both solids separation, sludge thickening and dewatering [10]. Required removal for primary treatment are reached in average. Best performance of the filter will be when composition of inlet wastewater is suitable. To be suitable the ratio between filtered chemical oxygen demand (FCOD) and total chemical oxygen demand (TCOD) should be below 4 and more than 20% of SS should have a diameter bigger than 350 µm [11]. The sludge from the filter will also have higher energy value than conventional and would have 20 – 30% total solids (TS) [10].

2.1.1.2 Characteristics of primary sludge

Removal of SS and organic materials are the goal in primary treatment and are also what's found in the sludge. Therefor primary sludge fits great for fermentation when the goal is to use it as a carbon source and achieve as much soluble chemical oxygen demand (SCOD) as possible. A low concentration of nutrients in primary sludge is also positive to avoid addition of N and P. Cellulose from toilet paper is an important factor among the organics since it represent approximately 30-50% of primary sludge in Western European countries [12]. Expected amount of sludge in fine sieving is shown in Table 2.1.

Sludge production [g SS/pe*d]	SS [%]	Amount of VSS of SS [%]
40	2-4	70-90

Table 2.1 Expected amount of sludge production [9]

Sludge production in g SS/m^3 from primary treatment can be calculated by Equation (1).

$$SP = SS_{in} - SS_{out} \quad \textbf{(1)}$$

2.1.2 Methods for sludge hydrolysis

There are three different ways to hydrolyze the sludge; biological, chemical and thermal. Retention time and chemical oxygen demand (COD) release differ, comparison is shown in Table 2.2. Methods are presented in the next subchapters.

Hydrolysis	HRT	Max COD release [%]
Biological	>1d	15-20
Chemical	<1h	20-25
Thermal	<0,5h	30-35

Table 2.2 Sludge hydrolysis information [13]

2.1.2.1 Biological hydrolysis

In biological hydrolysis microbes are converting particulate organic matter into soluble compounds. This is an extracellular process, since microbes cannot accept particles. Carbohydrates, proteins and fats are the substrates that breaks down into sugars and amino acids in the hydrolysis. Different groups of fermentative bacteria are extracting enzymes that is needed to breaking down different substrates by different bacteria. For example, the carbohydrate cellulose is broken down to glucose by the enzyme cellulase produced by cellulytic bacteria [14]. Since the process is producing acids the pH decrease when hydrolysis is ongoing.

2.1.2.2 Chemical hydrolysis

Chemical hydrolysis is performed by addition of chemicals, for example Sulfuric acid (H_2SO_4). An example of how a break down of cellulose to glucose happens in chemical hydrolysis is when the fibres are dissolved in H_2SO_4, then diluted with water and also heated. This is a complex process since the concentrated acids needs to be handled afterwards and this is a hazardous process [15].

2.1.2.3 Thermal hydrolysis

Thermal hydrolysis is a high pressure and high temperature process. Cambi delivers a patented process for thermal hydrolysis [16]. Sludge is feed continuously into Pulper where the sludge is pre heated before batches is feed into the reactors. In the reactor sludge is heated up by steam under pressure at 120-160 ºC. Next step is a flash release of pressure into the flash tank. At this stage a vapor explosion happens and the larger organic molecules breaks down by cell explosion.

2.1.3 Fermentation products

Fermentation (Acidogenesis) produce organic acids as rbCOD, such as alcohols and VFAs. As the process also release N and P.

2.1.3.1 VFA

VFA is another name for short-chain fatty acids(SCFAs), having less than 6 carbon atoms [17]. List of some VFAs are shown in Table 2.3. Acetic acid, propionic acid and butyric acid can be made out of fermentation of carbohydrates, proteins and lipids. Valeric acids are made mainly trough proteins. Acetic acid for example is formed by bacterial fermentation via bacteria converting sugars directly to acetic acid. Acetate is the salt of acetic acid, depending of the pH which is present.

Name	Structural formula	Salt	Mass (g/mol)
Formic acid	$HCOOH$	Formate	46.03
Acetic acid	CH_3COOH	Acetate	60.05
Propionic acid	CH_3CH_2COOH	Propionate	74.08
Butyric acid	$CH_3(CH_2)_2COOH$	Butyrate	88.11
Iso-butyric acid	$(CH_3)_2CHCOOH$	Isobutyrate	88.11
Valeric acid	$CH_3(CH_2)_3COOH$	Valerate	102.13
Iso-valeric acid	$(CH_3)_2CHCH_2COOH$	Isovalerate	102.13

Table 2.3 Overview of VFA with salt and molar mass

2.1.3.2 N and P

Not only organics are produced in the fermentation, also nutrients are being released in the process. N is dissolved to ammonium (NH_4-N) and P as phosphate (PO_4-P). N and P are present in the cells and therefore release happens trough hydrolysis of organic N and P [18]. Recycling of N and P may be a problem when the supernatant is used in other processes.

2.1.4 Reactor configuration for biological hydrolysis

There are many ways to configure the reactor for fermentation. Reactor tank can have different design; square or cylindrical shapes are two possibilities. To have an anaerobic tank an airtight closing mechanism are demanded. Reactor can be working with or without mixing, mixing can for example happen by magnetic stirring or rotating blades driven by a motor. The reactor can be running as a batch reactor, semi-continuous or continuous reactor. For heating one possibility is to have the reactor in a heated water bath. The reactor system can be driven automatically by monitoring sensors or it can be

done manually. Monitoring sensors need to be chosen after what the system need to be monitored by, examples could be pH, temperature or time. A separation system also needs to be included to separate liquid and solids for use in further processes.

2.1.5 Effect of retention time, temperature, pH, dilution and mixing

Anaerobic fermentation is a slow process. Temperature, pH, dilution and mixing will influence the retention time.

2.1.5.1 Retention time

Particulate organic matter are gradually hydrolysed into soluble substances [19]. Literature shows that the first 5 days are the most effective ones. This can be seen on experiments that show a flatten curve of SCOD when reaching 5 days, and it seems to be the optimal hydraulic retention time (HRT) [19] [20] [21]. Longer HRT demand bigger tanks, therefore 5 days is optimal since the major amount is produced within these days. Its also positive to avoid the methanogenesis face that follows the acidogenesis. Since methanogenesis use VFAs. Sludge blanket height is another parameter that can be a design parameter for fermentation when other parameters are difficult to monitor [22].

2.1.5.2 Temperature

Temperature is an important factor for fermentation. A lot of experiments are done, showing that temperature rise gives higher VFA release, the biggest difference are from 10°C to 20°C and is in the scale of 20%. Temperature raise from 20°C to 24°C give almost no change [23]. Further temperature rise to mesophilic conditions are not recommended since this will give a more unstable process [12]. Hydrolysis constant is also higher at higher temperatures [20]. Seasonal variations in fermentation rate can therefore be expected if the temperature is not controlled. The quality of the VFA products is not influenced by the temperature [23].

2.1.5.3 pH

Composition of the fermented products, VFA, are not affected by the temperature, but pH will affect composition since hydrolysis is affected. Stock solutions could be used to control the pH level. A range of pH between 5.6 and 7.3 is reported to be optimum for fermentation, but pH higher than 9, alkaline conditions, promote fermentation and stops the process before methanogenesis happens [12]. Crutchnik et al. [12] showed that for cellulosic primary treatment pH at initial value between 7.5 and 8 was best when the temperature where in mesophilic conditions, and propionate was the wanted product. pH can also be used to check if the hydrolysis has started since the pH will decrease when acids are produced.

2.1.5.4 Composition of sludge

Dilution of the sludge affect the fermentation. TS content will give information about how much reactants that is in the reactor. High concentration of microbes will give higher rates for reaction. Both the hydrolysis and acidogenesis processes depend on the available volatile suspended solids (VSS) in the sludge that is fermented. Amount VSS and the rate of fermentation is proportional [23]. In rainy seasons sewage sludge is often very diluted due to intrusion of rainwater in sewage systems and have therefore less TS

and total volatile solids (TVS) present. VFA yields are also found by Bouzas et al. [24] to be higher with higher TVS concentrations in the feed.

2.1.5.5 Mixing

Mixing would affect the reaction time. With effective mixing the contact would be better and therefore the reaction faster and better than without mixing. With adjustable mixing intensity, batch experiments show that highest intensity with G =233s^{-1} gave best carbon source recovery, and was also about 50% higher than without mixing [19].

2.2 Moving Bed Biofilm Reactor (MBBR)

MBBR is a process based on biofilm carriers for microbial growth on them. On the carrier, a lot of different bacteria's can possible be there. MBBR is a way to do biologically wastewater treatment. Instead of having AS where the bacteria are suspended in the water, the MBBR are having both the carriers and biofilm suspended in the water together permanently. Meaning that when the slow growth of microorganisms has happened, they stay in the reactor. This is a process that is space saving and therefor gives less footprint. MBBR can be configured to remove BOD easily by having aeration in a tank. Nitrogen removal is more complex and water return is needed between different tanks with both aeration and anaerobic conditions. [25]

Removal of phosphorous is a tertiary treatment goal. At this level 90% of P should be removed and 70% of N should be removed based on a yearly basis [26]. Some places have only P requirements and others only N in addition to secondary treatment requirements.

2.2.1 Diffusion

When having a carrier with biofilm there are some limitations with diffusion. Diffusion will in many cases not go trough the whole biofilm so that the diffusion depth is an important factor of reaction rates and performance of a process. Having a thick layer of biomass would not be favorable if the biomass isn't fed with substrates or oxygen because they can´t reach into the deepest layer. The diffusion depth is maximum 0,5 mm, if the layer is thicker fouling and H2S generation may happen and disturb the active biomass [27]. This is the reason why MBBR often needs high dissolved oxygen (DO) levels.

2.3 Enhanced Biological Phosphorous Removal (EBPR)

The definition of EBPR is stated as followed:

> *Wastewater treatment biomass removes phosphorous beyond its anabolic requirements by accumulating intracellular polyphosphates (polyP) reserves. [28]*

This is a very sustainable end economical process for phosphorous removal compared to chemical precipitation. EBPR also gives less sludge and phosphorous is available for recover, not chemically bound as in precipitation. There are a lot of different configurations and combinations of this process that also can remove nitrogen. The most used process is the UCT process [29]. Traditionally EBPR has been an activated sludge process or batch reactor for moving bed biofilm reactor (MBBR), new research has found a possibility to use EBPR also with continuous MBBR. The Hias-Process® developed at Hias WWTP.

2.3.1 Principle of EBPR

In regular aerated activated sludge processes a small amount of phosphorous is removed by ordinary heterotrophic organisms (OHOs). To have EBPR the key is to have changing conditions from aerobic and anaerobic conditions to generate the wanted microorganisms, polyphosphate accumulating organisms (PAO).

PAO and OHO are removing P in the EBPR process. Together with the de-nitrification PAO (DNPAO). PAO is much more effective than OHO since OHO is removing P with assimilation and PAO removes by accumulation. Figure 2.2 sums up how the concentrations changes over time in the anaerobic and aerobic phase of EBPR. Showing the P release in the anaerobic part when carbon source is utilized and P uptake in aerobic part when stored carbon is used.

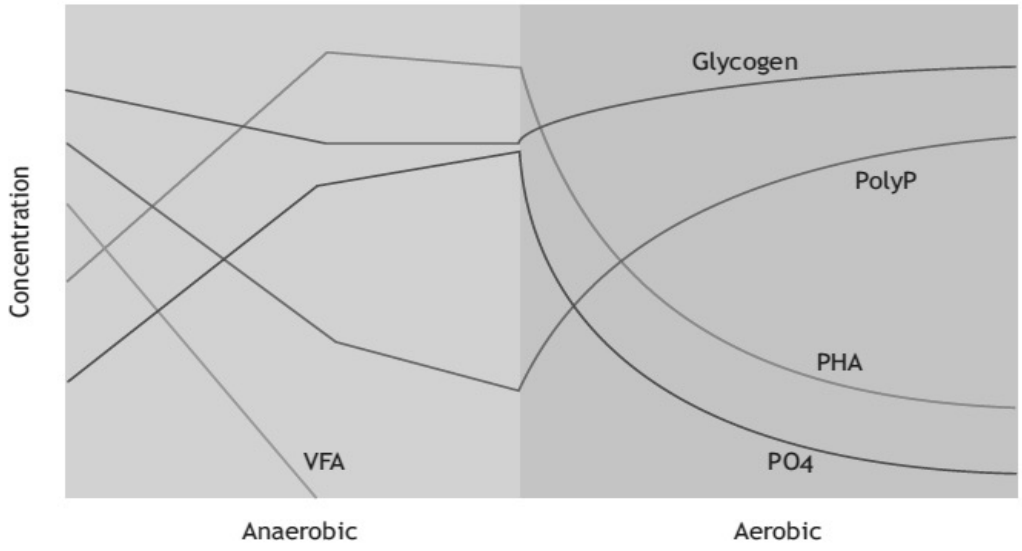


Figure 2.2 Concentration change of VFA, Glycogen, PolyP, PHA and PO4 in EBPR Process [28]

2.3.1.1 PAO

PAO works as seen in Figure 2.3. In anaerobic conditions rbCOD is taken up and P is released. PAOs stores food from the accumulated rbCOD into poly-ß-hydroxyalkanoates (PHA) with the help of energy from hydrolysis of intracellular polyphosphates (PolyP) and Glycogen. If rbCOD isn't available, fermenting microorganism's converts fermentable COD to rbCOD.

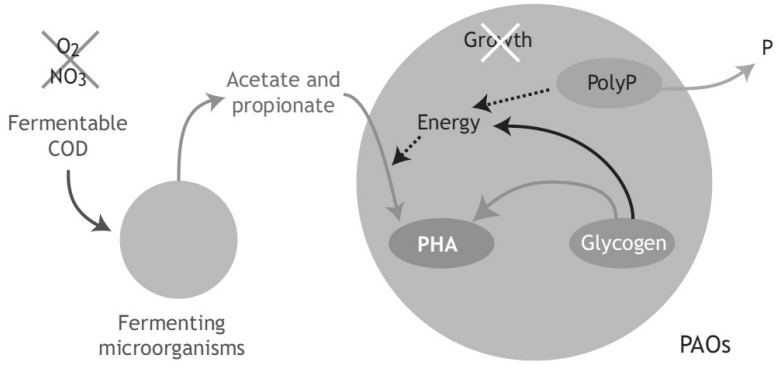


Figure 2.3 PAO cell in anaerobic conditions [28]

In aerobic conditions PAOs uptake P by use of the energy stored in PHA by oxidizing [5]. P is stored into PolyP. The simplified biochemical model for this is shown in Figure 2.4. Together with the uptake the energy stored is also used for microbial growth in the aerobic zone.

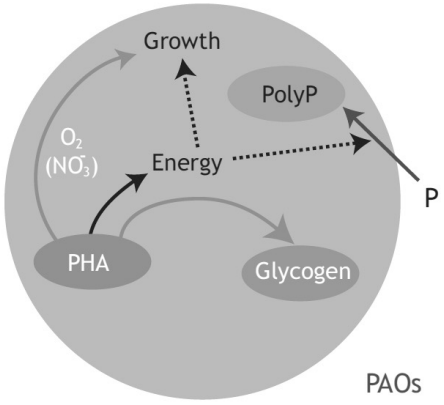


Figure 2.4 PAO cell in aerobic conditions [28]

2.3.1.2 DNPAO

De-nitrifying PAO (DNPAO) uptakes carbon source in the anaerobic zone as PAO. Different from PAO, DNPAO use the carbon stored to uptake P in the aerobic zone by using nitrate and nitrite instead of oxygen. This process reduce the need of carbon compared to traditional N- and P-removal since the same carbon is used for removal of both [30]. De-nitrifying is possible if the biofilm is thick enough to have an anoxic zone on the carrier where DNPAO lives. The aerobic zone must be long enough to have good N removal [31]. Both nitrification and de-nitrification happens in the aerobe zone.

2.3.1.3 GAO

Glycogen accumulating organisms (GAO), coexists with PAO. GAOs are organisms that also like the alternating conditions. GAO is not wanted in the process since they use carbon source, but does not remove phosphorous. If a high share in the process is GAO, then the EBPR process could stop working. But they are found in processes working well, especially full-scale plants. They could use the excess rbCOD in anaerobic zone if PAO have what they need [32].

2.3.2 Parameter influence

2.3.2.1.1 Inlet wastewater composition

Stable inflows with stable concentrations of substrates are favourable for BPR [33]. With high differences, recovery of the system takes time [33]. The COD/P ratio should be above the requirement to work good [31]. Helness et al. [31] found the requirements of >95% P-removal and >70% N-removal to appear when SCOD/PO4-P ratio were 40. D. Mulkerrins et al. [33] also reported that COD/P >40 and BOD/P >20 should be in the influent.

For the N-removal a COD/N ratio above 4 should be available [23].

Jabari et al. [34] found the particulate COD (PCOD) in influent to be important because of hydrolysation when rbCOD is limiting. FCOD/TP influent around 37 show very good P-removal results compared to 20 when retention time was prolonged.

2.3.2.2 Carbon sources

As mentioned in the introduction, the EBPR process is dependent on the available carbon source in the inlet wastewater. Both the concentration and the composition is important factors. rbCOD availability decides how big the fraction of PAO will be in the process, and therefor also how much EBPR.

Good P and N removal is best achieved if the inlet rbCOD is used in the anaerobe zone. But it is important to have enough rbCOD to have microbial growth and to produce enough PHA for uptake in aerobe zone [31]. The PHA composition is also dependent on which carbon source used [35]. When dosing acetate, PAOs mainly produce PHB [36].

rbCOD as VFA is preferred as carbon source in BPR. Acetic acid is preferred and is followed by propionic acid [23]. Some carbon sources can affect which organisms that is found in the EBPR [5]. Low acetate/propionate ratio favour PAOs over GAOs and is what is wanted [12] [32]. Pijuan, M. t al. [35] reported propionate to give the highest uptake rates, but acetate to have the highest carbon recovery ratio. Biomass performing was developed with propionate as the carbon source. This gave a PAO rich biomass culture.

Its shown that P-removal efficiency is higher with fermented supernatant as external carbon source than with acetate [5]. Results shows that 20 mg of VFA-COD is required to remove 1 mg of soluble phosphate [37]. Other reported a need of 7-9 mg VFA for removal of 1 mg P. Its important to not have any VFA left after anaerobic zone, since this can lead to P-release in anoxic and aerobe zone [38].

2.3.2.3 pH

pH could be a part of the decision of which organisms occur. pH of 7.4-8.4 favours PAOs and 6.4-7.0 favour GAOs [32].

2.3.2.4 Dissolved oxygen(DO)

There are many different organisms and requirements in a BPR. In the anaerobe zone, oxygen should be avoided, at least less than 0,2 mg/l [33]. Mulkerrins et al. [33] also reports a DO concentration of 2 mg/l as required and higher, 3-4 mg/l, if nitrification also is demanded. Above 4 mg/l would not increase effectiveness.

2.3.2.5 Temperature

In general, biological wastewater treatment performance is decreased by low temperatures and lead to non fulfilled discharge requirements. Microorganisms in biological wastewater treatment have optimum temperatures of 20-35°C [39]. PAOs have optimal temperature at 20°C, and is observed to work better in lower (5-15°C) temperatures than in higher (20-37°C) which GAOs likes better [33].

The growth rate for microorganisms and microbial activity are in general decreased by 50% for every 10 °C [39]. PAOs can grow in temperatures from 5°C-30°C [39].

Biofilm technology have a positive effect of temperature on biomass. In fact, decreasing the sensitivity of temperature change [39].

2.3.2.6 Filling degree

Since MBBR are designed by the area of the carrier and gives the amount of biomass that can be produced, filling degree could design the biomass concentration for MBBR as SA for AS.

2.3.3 P-release and P-uptake

P-release is affected by a lot of parameters. P-release to acetate uptake rate is varying with pH and also temperature. PAO/GAO ratio also affect P/acetate-rate [36].

PAOs uptake phosphorous more than whats usual for microorganisms. OHO removes P with by assimilation. They are usually found in AS systems as showed in Table 2.4 [28].

mgP/mgTSS(TS in biofilm)	Releated to
0,015	OHO – Removal by assimilation
0,05-0,10 (up to 0,17)	PAO – Removal by excess uptake

Table 2.4 Organisms related to different sludge mass concentrations

P-uptake is controlled by PHA and shows high correlation with the P-release [31]. But as temperature sinks, the correlation also sinks. At 5 °C there are no correlation [33].

2.4 EBPR in CMBBR

A new continuous process combining MBBR with EBPR and simultaneous nitrogen removal is developed at Hias [38]. The process concept is shown in Figure 2.5. First the inlet wastewater is blended with carriers in chamber 1 and continue trough the anaerobic zones, with mixing, into the aerobic zones. In the last zone a conveyor belt moves carriers over to chamber 1 again without water and discharged treated wastewater

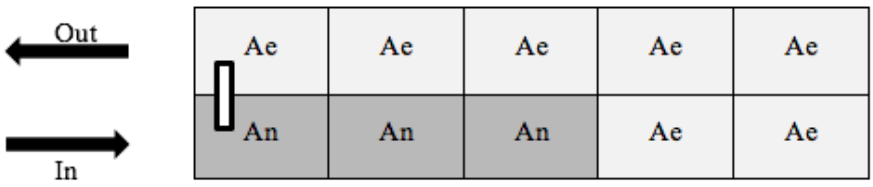


Figure 2.5 Concept of the HIAS-process

In the aerobic step a quite high DO level is demanded to diffuse into the biofilm [38]. Both denitrification and nitrification takes place in the aerobic zone, this is a space-saving process compared to activated sludge and less resource demanding since pumping back is avoided for N-removal.

3 Methods

The following chapter presents the methods for the experiments first before the analytical methods used for analysing samples.

3.1 Location and experimental setup

Wastewater laboratory used is located at NTNU Valgrinda. The schematic overview of the lab is seen in Figure 3.1. An inlet pump from a sewer collects wastewater from the nearby housing. Lerkendal wastewater have low COD concentrations. Once an hour it is pumped into Tank A for storage before the wastewater is filtered trough a Salsnes filter four times a day and stored after filtration in Tank B for feeding into the CMBBR pilot.

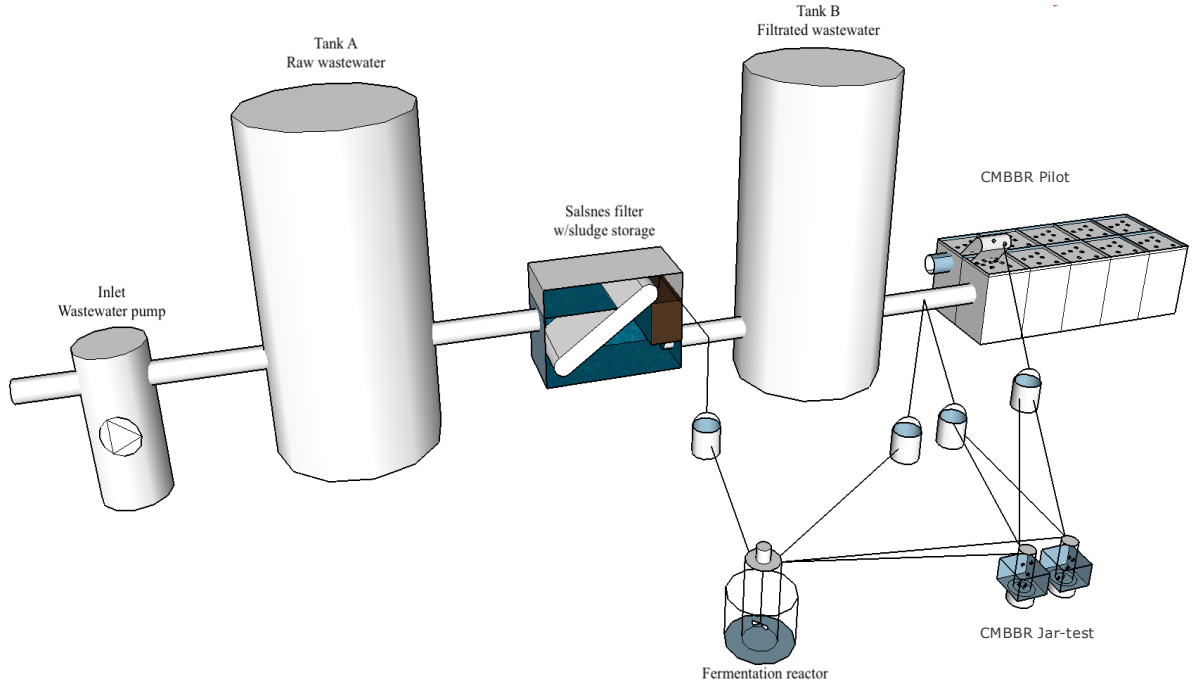


Figure 3.1 Schematic view of the laboratory at NTNU Valgrinda

CMBBR pilot in this laboratory is based on the Hias-process®. The pilot has 10 chambers, the first 3 anaerobic with mixing and number 4-10 with aeration. Carriers in the pilot is floating trough all chambers before they are moved from chamber 10 to 1 with a conveyor belt giving fluctuating aerobic and anaerobic conditions for the microorganisms on the carriers. Carriers are held in suspension by mixers in chamber 1-3 and with aeration in chamber 4-10. K1 from Kruger Kaldnes is the carriers used in the pilot. They are shown in Figure 3.2, with specific surface area of 500m^2/m^3. The CMBBR pilot had been running for about a year when the experiments in this thesis started, so the system and microorganisms were well established.

From the wastewater treatment line in the lab, fermentation is conducted and used in the jar-tests mimicking the CMBBR pilot. Carriers are taken from the pilot and filtrated wastewater from Tank B. All fermentation experiments have been named by an F#. Having experiments from F1-F7. The same goes for kinetics experiments in jar-test which

is named by K#. Having experiments from K1-K8. The numbering is chronologically after date the experiment was run.

Figure 3.2 K1 carriers from Kruger Kaldnes used in the CMBBR

3.1.1 Experimental setup of sequence batch fermentation reactor (SBFR)

SBFR is a manually driven air tight cylinder shaped reactor with a mixer. The mixer motor gives maximum 100 rpm with blades rotating against the watch making the sludge go upwards for complete mixing. The rotation of the blades makes it suitable for thick solutions. The blades are shown in Figure 3.3. The cylinder reactor is placed in a water bath for temperature control. One of the reactors used is shown in Figure 3.4. Other configurations have also been used. They have been based on the same principle with difference in size. Anaerobic conditions are obtained by an air tight reactor, closing some holes with plastic and to open the reactor for as short period of time as possible when sampling. Sampling is trough the valve in Figure 3.4 that was open for about a minute for each sample. Sampling was done by a syringe with a long tube and filled into centrifuge tubes. For the reactors that was small enough, temperature and pH sensor was held into the reactor with the lid open. pH and temperature was measured at the same time as sampling for other analyses, at time zero and then at decides time steps.

Figure 3.3 Mixer blade in fermentation reactor

Experiments was running with different conditions, time and solutions. To keep another temperature than room temperature in the reactor, the reactor was placed in a water bath warmed up by aquarium heaters that was having maximum heating of 30°C.

Retention time was changed from time to time, also the tactic for removing supernatant from the reactor and add fresh solution when it was run as sequence batch.

K2 was added a second batch by settling the reactor for 2 hours before removing the supernatant from the top and after that removing also half the biomass remaining in the bottom. New solution was filled in with the same TS and to make the same volume in the reactor as before.

When doing experiment in room temperature, biomass didn't settle. Therefor the tactic was changed and started to remove half the reactor when it was complete mixed. Also adding new batch with same TS and half amount as started the fermentation experiment.

The solution was made by primary sludge from the salsnes filter and filtrated wastewater from Tank B as shown in Figure 3.1. Making the solution the total solids content was the decided target. To do this as effective as possible for this specific sludge, an experiment in section 3.1.1.1 was executed.

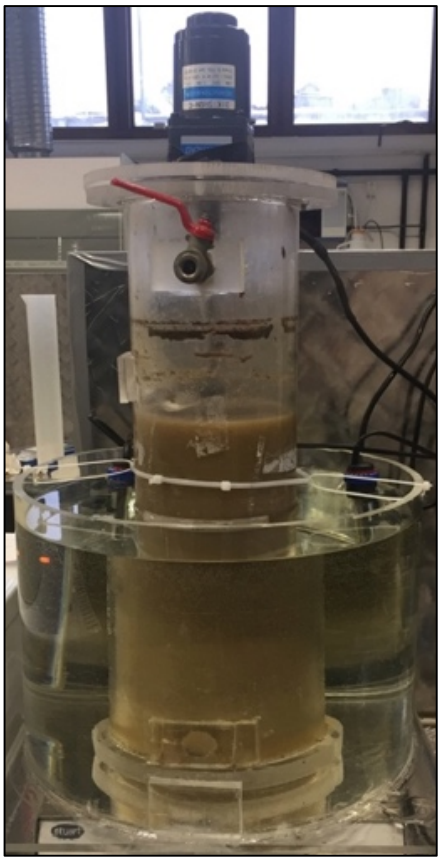


Figure 3.4 Fermentation batch reactor setup

3.1.1.1 Target TS

First the sludge storage in the Salsnes filter was mixed to ensure homogeneous sludge before extracting sludge. The wastewater tap was running for about a minute before extracting sample. Pre decided amount of PS and WW was mixed to check the TS according to section 3.3.1 for each sample. Making a regression line out of these samples to know how to target a specific TS when the wanted volume is known. Sludge from salsnes filter and filtrated WW was used here to. Assuming the sludge to be homogeneous at all time during the semester.

3.1.2 Kinetics jar-test with Bio-P carriers from CMBBR

Jar-tests are conducted with carriers from the CMBBR pilot in the wastewater laboratory. Both as it is and compared with addition of fermented products in parallel as seen in Figure 3.5. The temperature has been held between 11-14 degrees. Temperature regulation is done by use of snow and tap water in a bucket around the beaker.

Figure 3.5 Jar-test kinetics in cold temperature

Beakers of 1 liter are filled with filtrated wastewater or a mix of filtrated wastewater and fermented supernatant together with carriers. The carriers are taken from the conveyor belt in the CMBBR pilot. To have 1 liter, 600 ml with carriers are added together with 794 ml of wastewater or a blend of fermented supernatant and wastewater of 794 ml. Finstad, I.K. et al. [40] reported the volume to be 794 ml found from experiments. This gives a filling degree of 60% as it is in the pilot. In experiments with fermented supernatant an increase of 100 mg SCOD/l is reached for. Volume of this addition is different for each fermented supernatant since 100mg SCOD/l is added each time. An example of the calculations is shown in Appendix 1. Fermented supernatant was extracted from the fermentation reactor the day before the kinetics experiment in jars. Filtrated trough a fruit filter bag before being stored for experiment and analyzed to be able to calculate wanted volume. The wastewater was exposed to nitrogen gas before time zero to remove the oxygen content in inlet wastewater before start.

In the anaerobic part, the mixing happens at 150 rpm with magnetic stirrer. A lid is placed on the top to ensure anaerobic conditions. When the aerobic chambers are mimicked an aerator is started, in addition to magnetic mixing, with tubes into the beakers. The amount and size of holes in the tubes is changed due to which oxygen level goal is set.

Temperature, pH and oxygen level are monitored during the experiments at the same time as sampling. Some experiments have computer loggings of these parameters. A sampling plan as in Appendix 2 was made to mimic the chamber in the pilot. Samples were taken with syringes and putted into centrifuge tubes, 10 ml each sample. Samples was analyzed following the methods presented in the sections about analyses coming up.

Initial values were sampled when everything was blended into the beakers. Also sampling throughout the experiments was done. In addition, inlet wastewater and fermented supernatant was analyzed. Needed for the calculations for the supernatant addition and to calculate removal efficiency in the process.

3.1.2.1 Carrier characterisation

The carriers in the CMMBR was characterized to know how much biomass there are on the carriers when jar-tests was conducted. Carriers from conveyor belt from the carriers with biomass are seen in Figure 3.6. Tried to do one test each week a CMBBR jar-test was conducted. In the beginning of the semester this was not done in the same pattern since there were some limitations. Carriers was cached from the conveyor belt to have the same characterization as the ones in the experiments. Using numbers from this analyses for calculations, they were first adjusted to be the results of one carrier. And by knowing that there are 236 carriers per 200 ml of carriers, a number of 708 carriers are expected to be in a jar of 1 liter. Therefor to have numbers in liter, the number characterizing 1 carrier are multiplied with 708.

Figure 3.6 Carriers in CMBBR with biofilm attached

3.1.2.1.1TP, TSS and VSS in detached biomass on carriers

First method used was to add 20 carriers into 100 ml of distilled water. Shaking the tube until the biomass was detached. Then TP was analyzed as in section 3.2. TSS was analyzed as in section 3.3.3 and VSS as in section 3.3.4.

Second method was to add 5 carriers into 25 ml of distilled water and shaken in a big centrifuge tube to get the biomass off the carriers. Then TP was analyzed as in section 3.2. TS was analyzed as in section 3.3.1 and volatile solids (VS) as in section 3.3.2.

Changed method to avoid using filters because of the small weight and uncertainties seen in results from analyses.

3.1.2.1.2TS in detached biomass on carriers

15 carriers were putted in a pre dried and weighted dish before putting carriers in the oven holding 105 degrees for 24 hours. Then the dish with dried carriers was weighted. Carriers was putted into a solution of distilled water and chlorine for cleaning. When all biomass was detached the carriers was flushed with distilled water and then putted in a pre dried and weighted porcelain dish for drying in 105 degrees for 24 hours. Then the dish was weighted and the TS on the carriers was calculated with Equation (2).

$$TS = \frac{(W_{dish1+carriers\ 105} - W_{dish1}) - (W_{dish2+cleanedcarriers\ 105} - W_{dish2})}{15\ carriers} * \frac{1000mg}{1g} \qquad [\frac{mg\ TS}{carrier}] \quad \textbf{(2)}$$

With:

$W_{dish+carriers\ 105°C}$ =Weight of dish and carriers with biomass after drying in 24h in 105°C [g]

W_{dish1} = Weight of the first dish after evaporated for 1h in 105°C [g]

$W_{dish2+cleanedcarriers\ 105°C}$ =Weight of dish and cleaned carriers after drying in 24h in 105°C [g]

W_{dish2} = Weight of the second dish after evaporated for 1h in 105°C [g]

3.2 Analyses of totals and solubles

To analyze a soluble parameter, sample are taken in a centrifuge tube and then centrifuged before being filtrated trough a 0,45 μm filter. This was done immediately to ensure no more reactions to happen after sampling. Then the sample were ready for cuvette test. HACH supplies the cuvettes used in this thesis. The Cuvette used are presented in Table 3.1.

Parameter	Cuvette product number
SCOD, TCOD	LCI 400; LCK 314, 614
PO_4-P, TP	LCK 350, 348, 349
NH_4-N	LCK 303, 304
NO_3	LCK 339, 340
Total Nitrogen	LCK 138

Table 3.1 Cuvette tests

Analyzing totals was done by using cuvettes as for soluble without filtration first. Nitrogen has its own cuvette stated in Table 3.1.

For heating of the samples HACH LANGE LT200 is used. For reading the samples HACH DR900 is used. If the parameter is out of range, samples are diluted with distilled water.

3.3 Analyses of solids

Solids are suspended or dissolved in wastewater. Total solids (TS) are defined as the solids remaining after evaporation of a sample at a certain temperature [41]. The weight used is Sartorius analytic A 210 P with 4 decimals. With an error of <±0.001.

3.3.1 TS

For TS sampling a dish is evaporated in 105°C for 1 hour and stored and cooled in a desiccator afterwards. The dish is weighted immediately before use. The sample volume is chosen and the sample is weighted after it is poured into the dish. Next step is to dry in the oven for 24 hours before cooling in the desiccator and weight the sample again. Then the TS can be calculated according to Equation (3) or (4) for having respectively the answer in percentage or weight per volume.

$$TS = \frac{W_{dish+sample,after\ 105℃} - W_{dish}}{W_{sample}}\ [\%] \quad \textbf{(3)}$$

With:

$W_{dish+sample,after105°C}$ = The weight of the dish and sample after drying in 24h in 105°C [g]

W_{dish} = The weight of the dish after evaporated in 105°C for 1h [g]

W_{sample} = Weight of the sample [g]

$$TS = \frac{W_{dish+sample,after\ 105℃} - W_{dish}}{V_{sample}}\ [g/l] \quad \textbf{(4)}$$

With:

$W_{dish+sample,after\ 105°C}$ = Weight of dish and sample after drying in 24h in 105°C [g]

W_{dish} = Weight of the dish after evaporated for 1h in 105°C [g]

V_{sample} = Volume of the sample [l]

3.3.2 VS

For the VS, also called TVS, same procedure as for TS is proceeded and in addition the sample is incinerated for 30 minutes in 550°C. Then the VS is calculated according to Equation (5) giving the amount VS of TS.

$$VS = \frac{W_{dish+sample,after\ 105℃} - W_{dish+sample,after\ 550℃}}{W_{dish+sample,after\ 105℃} - W_{dish}}\ [\%] \quad \textbf{(5)}$$

With:

$W_{dish+sample,after\ 105°C}$ = Weight of dish and sample after drying for 24h in 105°C [g]

$W_{dish+sample,after\ 550°C}$ = Weight of dish and sample after incineration for 0,5h in 550°C [g]

W_{dish} = Weight of the dish after evaporated for 1h in 105°C [g]

3.3.3 TSS

TSS is found by putting a filter into a dish, evaporate 0,5 hour in 105°C, cool in desiccator and weigh the filter. Measuring a decided volume of sample, in this thesis 25 ml is used. Filtrate trough the filter that is placed in a vacuum pump. Placing the filter back in the aluminum dish before 1 hour evaporating again. Cooling in desiccator before weighted again. Then the TSS can be calculated with Equation (6).

$$\boldsymbol{TSS} = \frac{\boldsymbol{W_{filter\ with\ biomass,\ after\ 105℃}} - \boldsymbol{W_{clean\ filter,\ after\ 105℃}}}{\mathrm{V_{sample}}}\ [\mathrm{g/l}] \qquad \textbf{(6)}$$

With:

$W_{\text{filter with biomass,after 105°C}}$ = Weight of filter and sample after drying for 1h in 105°C [g]

$W_{\text{clean filter, after 105°C}}$ = Weight of clean filter after evaporating for 0,5h in 105°C [g]

V_{sample} = Volume of the sample filtrated [g]

3.3.4 VSS

VSS is found by taking the filter that gave the TSS and incinerate for 30 minutes in 550°C. Then cooling in desiccator before weighing the filter again. VSS is calculated by the Equation (7).

$$\boldsymbol{VSS} = \frac{\boldsymbol{W_{filter\ with\ biomass,\ after\ 105℃}} - \boldsymbol{W_{filter\ with\ biomass,\ after\ 550℃}}}{\mathrm{V_{sample}}}\ [\mathrm{g/l}] \qquad \textbf{(7)}$$

With:

$W_{\text{filter with biomass,after 105°C}}$ = Weight of filter and sample after drying for 1h in 105°C [g]

$W_{\text{filter with biomass, after 550°C}}$ = Weight of filter + sample after incinerated for 0,5h in 550°C [g]

V_{sample} = Volume of the sample filtrated [g]

3.4 Other analyses

3.4.1 pH and temperature

pH and temperature is measured with a portable meter named ProfiLine pH3110 in the fermentation experiments. The error in this equipment are ±0.005 pH and ±0.1 °C. For the kinetics in jar-test WTW Multi 3630 IDS is used.

3.4.2 Dissolved oxygen

Dissolved oxygen is measured with the same equipment as pH and temperature, WTW Multi 3630 IDS.

3.4.3 Determination of VFA

Possibilities for VFA measurement are Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Titration method.

In this case VFA-analyses are sent to NMBU. At NMBU they have used GC to analyze the samples. The samples was taken in the lab as normal and centrifuged then filtrated with 0,45 μm filter. 2 ml of this sample was putted into a freezable container and then added 5% of formic acid before sent with postal service. Results was then given in mmol/l.

3.4.4 Theoretical oxygen demand (TOD) calculation

VFA results is given in mmol/l. Multiplying with the molecular weight in Table 2.3 gives the amount in mg/l.

The acids as a theoretical oxygen demand is found by finding the formula of the acid, balancing the equation, finding the molecular weight and oxygen need for each acid. See Appendix 6 for calculation. TOD is also stated as "acid as COD" in this thesis.

3.5 Limitations

In every experiments and analyses there could be some error sources limiting the results.

3.5.1 Experimental setup

In the setup conditions can be hard to held stable. For example, temperature and oxygen levels. In parts that should be anaerobic, it can be hard to keep them totally anaerobic. Sampling procedures and methods can be different from time to time even if they are tried to be similar because of personal mistakes.

3.5.2 Analyses

Uncertainties in the cuvette tests comes from error in equipment, personal mistakes and interrupting marks on cuvette. Error in equipment could be amount from pipettes – can give wrong amount in pipettes, and therefor wrong dilution. The spectrometer can read wrong, both because of error in equipment but also scratches and marks on the cuvettes.

Totals are affected by homogeneousity of the samples. Large particles are hard to separate and get homogeneous into pipettes, even if the pipettes are cut to have larger particles into it than usual.

For solids, the limitations in homogeneousity also goes. In this case, duplicates are taken to avoid some uncertainty. With sometimes large particles its hard to have everything correct all time. There are uncertainties in VS and VSS samples for solids because the dishes isn`t incinerated before incineration with samples.

4 Results and discussion

The goal with the experiments was to optimize the CMBBR by doing kinetics jar-tests with carriers from the pilot. The main focus was to find the best way to produce fermented supernatant in this lab and see if the supernatant can give better results in jar-tests. In this chapter the fermentation results are presented and discussed first before the results from the kinetics jar-tests are presented and discussed. Both the use of fermented supernatant and other parameters influencing the kinetics in the jar-tests are discussed.

4.1 Production of fermented supernatant

All raw data from the fermentation experiments are in Appendix 5. The first fermentation experiment, F1, run for a long period. Trend in SCOD production shows steeper curve for SCOD the first 3-4 days and then a slower production before the production stops as seen in Figure 4.1. Literature shows the same pattern; the curve flattens after 5 days, the yield reduces [24]. In this case there were some days without sampling and therefor the slope could have been different between 4-9 days. A reason for production stop could be limitations of available fermentable products. pH pattern also shows stop in the fermentation since the pH sinks from 6.5 to 5 at day 10 and stabilized afterwards. Since pH decreasing is a sign for hydrolyzing, this also shows no hydrolysis process as the stop in SCOD production also shows. In this case it is an uncertainty with too few points to conclude with the exact development curve of fermentation, but it seems to be very similar with what earlier experiments show [21].

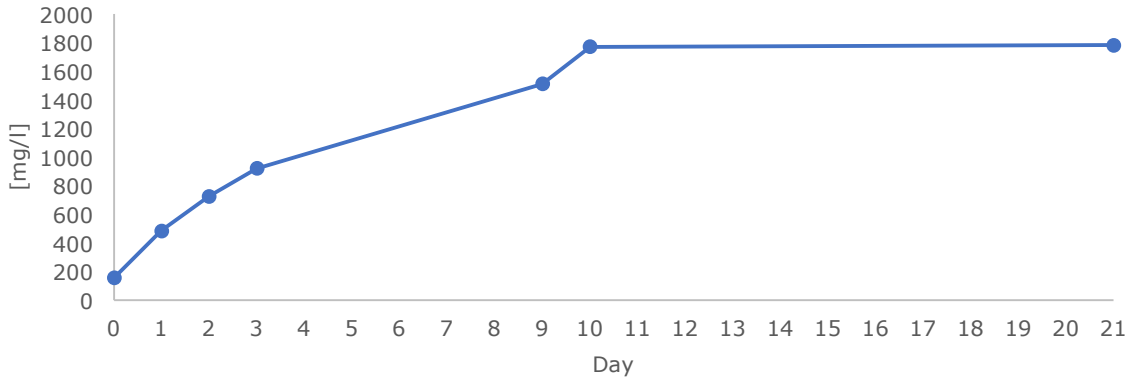


Figure 4.1 SCOD production in F1

Further fermentation experiments were driven as a sequence batch reactor with a goal and a thought of having higher yields for each batch. The idea was to have build-up of biomass in the reactor that would give higher yields and rates. Each fermentation experiment was started at zero, by cleaning reactor between, for easier comparison with each other. Table 4.1 shows an overview of the experiments, important parameters and results. All experiments had the same mixing velocity, meaning this parameter was not investigated.

Experiment	Batch	Volume	TS	Days	Volume add	HRT	Temp	SCOD Production	TVS	Yield
[#]	[#]	[L]	[%]	[d]	[%]	[d]	[◦C]	[mg SCOD hydrolysed]	[g/l]	[mg SCOD hydrolysed / g TVS_{fed}]
F1	1	7	0,93	21	100	21	20-24	1625	8,69	187,05
F2	1	6	1,30	2	100	4	24-28	1463	11,62	125,91
	2	6	1,09	2	50			969	8,98	79,92
F3	1	7	1,46	5	100	5	24-28	3449	13,06	264,14
F4	1	3	1,77	2	100	4	17-21	438	16,60	26,39
	2	3	1,63	2	50			139	15,12	18,39
	3	3	1,97	2	50			514	17,72	58,03
	4	3	1,76	2	50			453	15,98	56,69
F5	1	1,5	2,25	2	100	4	24-28	1116	21,00	53,13
	2	1,5	1,97	2	50			900	18,04	129,39
	3	1,5	2,28	2	50			1092	21,57	101,23
	4	1,5	1,91	2	50			588	17,72	66,36
F6	1	2	2,12	4	100	8	24-28	2266	19,96	113,53
	2	2	2,40	4	50			1969	22,42	175,67
F7	1	2	1,64	4	100	8	27-29	1123	15,31	66,75

Table 4.1 Overview of fermentation experiments with characteristics, SCOD production and yield of SCOD production to total volatile solids (TVS) fed

4.1.1 Target TS

The preparation of a new fermentation experiment or a change for a new batch was made by the help from the graph in Figure 4.2 produced after method in section 3.1.1.1. Giving a relationship between the amount of sludge and filtrated wastewater for addition by knowing wanted TS. F2-F7 had a target of 2% TS. Therefore, addition of 300 ml primary sludge from the container in the salsnes filter per liter of volume in the reactor was used. This was found by following the red lines in Figure 4.2.

An important factor to have in mind in this case is the sludge. Production of sludge in the lab happens from the Salsnes filter which filtrates real time wastewater. The sludge will therefore be different from time to time since the inlet wastewater are not exactly the same at all time. This shows off in experiment F6 and F7 that is prepared and run exactly the same way for the first batch and produced 2266 mg/l SCOD in F6 and 1123 mg/l for the F7. Visually the sludge looked different too. This might have been different if the assumption of equal sludge at all time wasn't assumed. And then prepared solutions different. The reason for the assumption is the time-consuming analyze of TS and TVS in the sludge if this was to be done individually for each experiment.

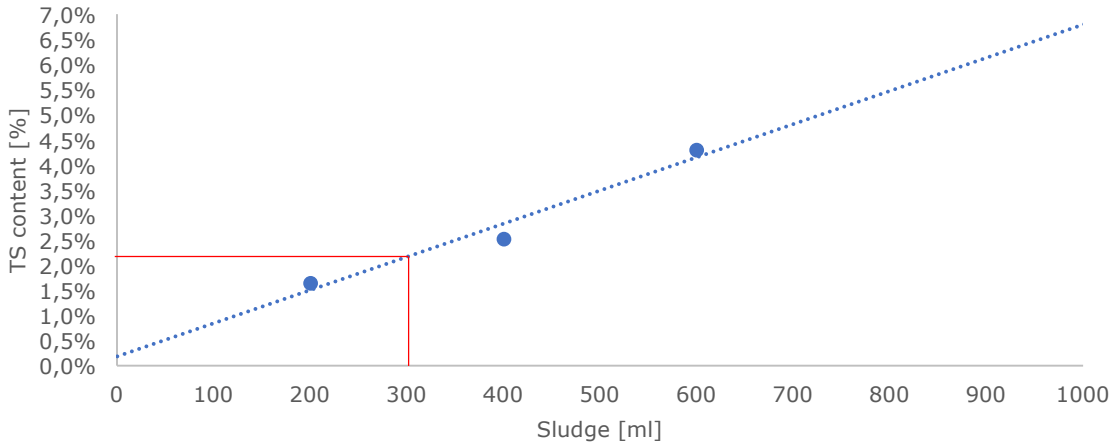


Figure 4.2 Regression line to target a specific TS for start up of fermentation batch. Decide TS content and read what amount of sludge is needed to mix with filtrated wastewater to have 1 L of solution in decided TS.

4.1.2 Retention time

Since the fermentation produces SCOD different over time, the experiments has been running for different retention time. Only F1 was run for very long time. In addition to produce best in the beginning of the fermentation, another reason for having HRT shorter than 5-6 days are to avoid methanogenesis. Looking at SCOD production, F3 with 5 days HRT, this was the experiments that produced highest concentration.

4.1.3 Temperature

F3 that produced most SCOD was in a water bath holding temperatures between 24-28 ◦C. F4 was held in room temperature, 20-24 ◦C; this one shows the lowest concentrations of SCOD giving no reasons for continuing with room temperature for the experiments that followed. Also because temperature doesn't affect composition, only the rate. Room temperature also gave bad settleability. One parameter discussed to influence poorer settling in biological systems is low temperature [33] [39]. Therefore, strategy of exchanging sludge was swapped at this time because settling was not possible.

4.1.4 Yield and Rate

Yield in Table 4.1 is giving the generation of SCOD over the amount of food as TVS fed. TVS in the volume of primary sludge and wastewater added. Rate and yield calculations are adjusted for different amount of added fresh primary sludge. Production is in the whole volume of the reactor, but the added sludge is half the volume.

Reasonable thought as discussed was to have higher yields with having more batches running to build-up biomass to do the hydrolysis. As presented in other papers about fermentation, a mixture of digested sludge and feed sludge is used in the beginning to have a quick start since fermentative bacteria already are present [42]. In this case there are no clear pattern showing this. Maybe this could have happened with more batches by having the reactor running for a longer period with sequence batch. D.Crutchik et al. [12] were running a fermentation sequence batch reactor with 4 days HRT by daily exchange of 25%. They reported production of specially propionate and they found the yield to gradually increase and reached a stable production in the 36-96 day.

Rate is giving the generation of SCOD over time to TVS fed. Calculated as a rate from day to day, graph as in Figure 4.3 is made. Looking at the development in the rate there doesn't seem to be any clear pattern for higher rates during batches here either. But indeed there seems to be a drop in the rate after 20 hours or up to 40 hours. After that the rate rises again. A pattern that can be seen in all graphs in Figure 4.3.

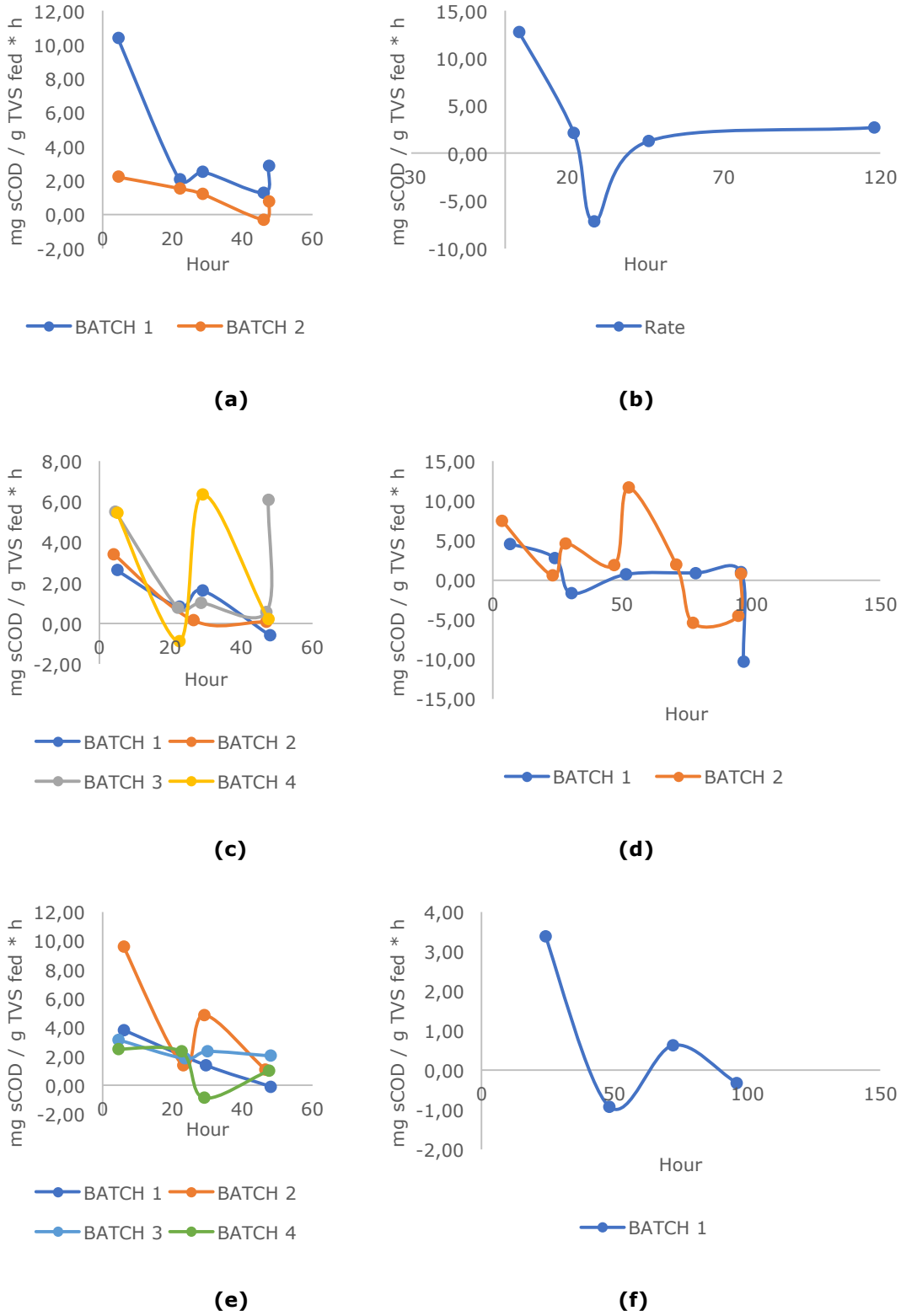


Figure 4.3 Rates from day to day for (a) F2, (b) F3, (c) F4, (d) F6, (e) F5, (f) F7

TCOD was measured in the beginning of each batch for F4-F7. Results from this analyses shows a big difference from time to time that sometimes could be a bit suspicious. Probably the samples haven't been homogeneous. Since the sludge have a lot of cellulosic fibers due to toilet paper, big parts haven't been easy to avoid. Looking at the SCOD/TCOD yield gives an average of solubilisation degree of 17% ± 17%. With STD showing the fluctuating numbers with uncertainties. Ucisik et al. [42] reports COD-yield of 8.1-12,6% in comparison. And the theory about biological hydrolysis says that the max COD release is 15-20% and therefor shows the release in this reactor good results.

4.1.5 Phosphorous and nitrogen release

Phosphorous and nitrogen releases as NH4-N and PO4-P, and release seems to happen in almost same pattern most of the time. Also following the same development as SCOD. Sometimes N and P is actually decreasing and not released. Looking at F1 again in Figure 4.4, nitrogen is totally removed after 21 days, and are actually decreasing almost every day. Phosphorous starts to release in the beginning in the same pattern as SCOD but development stops some days before SCOD production stops. A probable reason for nutrition decrease is assimilation. When other food sources are limited they probably start using nutrients.

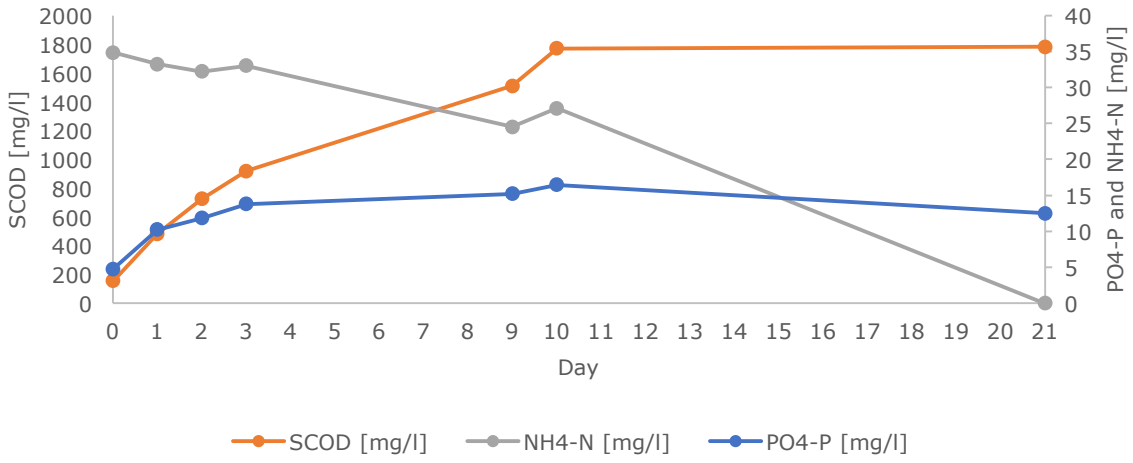


Figure 4.4 Phosphorous and nitrogen development compared to SCOD development in experiment F1

Table 4.2 shows P- and N-release values. PO4-P have more the same pattern for release as SCOD since the average at 0,01 mg PO_4-P/ mg $SCOD_{hydrolysed}$ have a STD of 0,00. For nitrogen the yield is also 0,01 mg NH_4-N/ mg $SCOD_{hydrolysed}$ in average but with STD at 0,02. For P-release there are no negative values even if there is decrease, the net-value haven't been negative.

Experiment	Batch	PO4-P released [mg/l]	NH4-N released [mg/l]	TVS [g/l]	mg PO_4-$P_{released}$/ g TVS_{fed}	mg NH_4-$N_{released}$/ g TVS_{fed}	mg PO_4-$P_{released}$/ mg $sCOD_{hy}$	mg NH_4-$N_{released}$/ mg $sCOD_{hy}$
F1	1	7,72	-34,84	8,69	0,89	-4,01	0,00	-0,02
F2	1	18,33	35,47	11,62	1,58	3,05	0,01	0,02
	2	5,91	-10,60	8,98	1,32	-2,36	0,02	-0,03
F3	1	28,42	59,36	13,06	2,18	4,55	0,01	0,02
F4	1	5,51	3,39	16,60	0,33	0,20	0,01	0,01
	2	0,08	-4,76	15,12	0,01	-0,63	0,00	-0,03
	3	5,07	5,31	17,72	0,57	0,60	0,01	0,01
	4	3,16	3,61	15,98	0,40	0,45	0,01	0,01
F5	1	16,70	32,20	21,00	0,80	1,53	0,01	0,03
	2	11,17	30,50	18,04	1,24	3,38	0,01	0,03
	3	8,54	17,54	21,57	0,79	1,63	0,01	0,02
	4	7,86	7,46	17,72	0,89	0,84	0,01	0,01
F6	1	31,42	71,31	19,96	1,57	3,57	0,01	0,03
	2	5,63	8,87	22,42	0,50	0,79	0,00	0,00
F7	1	11,84	24,84	15,31	0,77	1,62	0,01	0,02
Average					0,92	1,01	0,01	0,01
STD					0,57	2,24	0,00	0,02

Table 4.2 P- and N-released in fermentation experiment F1-F7.

Fermentation experiments shows overall low P-release compared to how much TVS fed. Total N and P analyses have also shown low part of the inlet TVS with respectively 1,17% mg TN/mg TVS and 0,29% mg TP/mg TVS in average. D. Crutchik et al. [12] reported release of 1,6±0,5 mg P/g TVS_{fed}*d and 6,1±1,4 mg N/g TVS_{fed}*d, with concentrations of 130±23 mg P/l and 430±29 mg N/l . These numbers are way much higher than both concentrations in the fermentation liquid and yield than in this thesis. This could be due to concentration differences in the sludge used. Ucisik et al. [42] reported big differences in N- and P-releases within same fermentation method but with sludge from different treatment plants.

4.1.6 VFA production

Samples from experiment F7 was sent to VFA-analyses. The development of the production of VFA is shown in Figure 4.5 in concentration of mg/l, converted from the given results of mmol/l by multiplying with the molar mass in Table 2.3. As the graph and calculated number shows, the fermentation gives most propionic acid in total at the end. Other studies shows similar results like Ucisik et al. [42] found the composition in a batch fermentation study to be 26-31% acetate and 43-49% propionate, with primary sludge from different origin. They also found butyric acid to be the third largest compound produced as also can be seen for F7 in Figure 4.5.

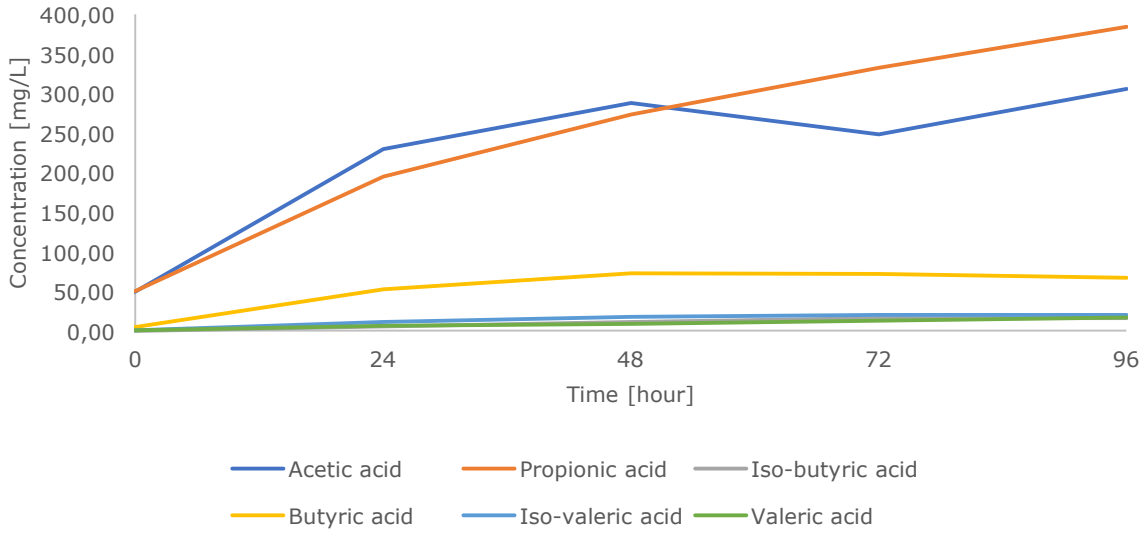


Figure 4.5 VFA-development in fermentation of 4 days in F7

Looking at Figure 4.6 the sum of acids are converted to a TOC and shows a total sum lower than measured SCOD in the lab. In average VFA-COD is 68% of SCOD. Meaning that there must be a quite big amount of other COD than VFA. This can show that the hydrolysis and acidification happens simultaneously. Another thing emphasize this is the drop in SCOD when the VFA still rises. At this point the acidification seems to be more rapid than the hydrolysis. After 1 day VFA-COD is 45% of SCOD, at day 4, VFA-COD is 89 % of SCOD.

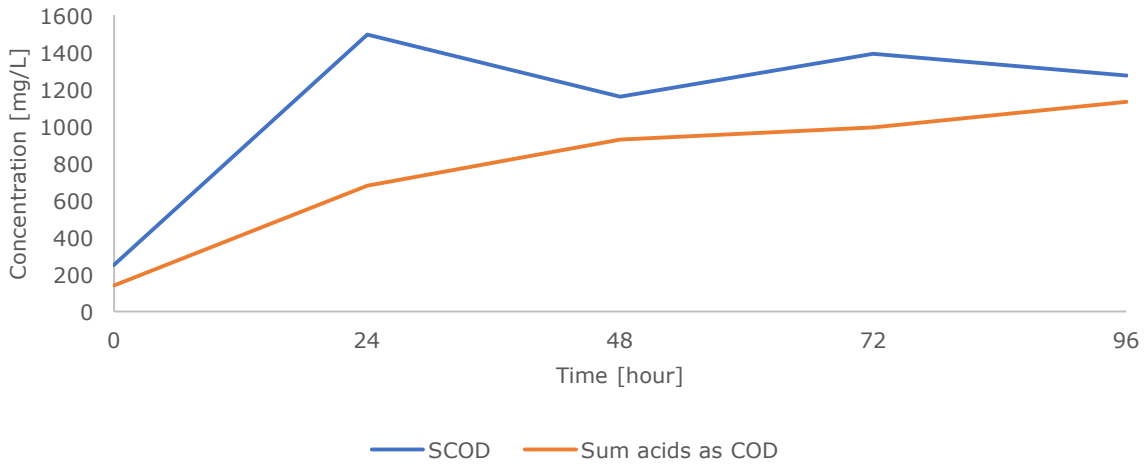


Figure 4.6 SCOD-development and sum of VFA-development as COD in F7

Results shows that acetic acid was in average 32% of the total VFA production as COD over the fermentation period of 4 days and propionic acid was 49%. Figure 4.7 shows the percentage composition of VFA from time zero to production stop at day 4. Showing that there always is more propionic acid than acetic acid. And Butyric acid is the third largest.

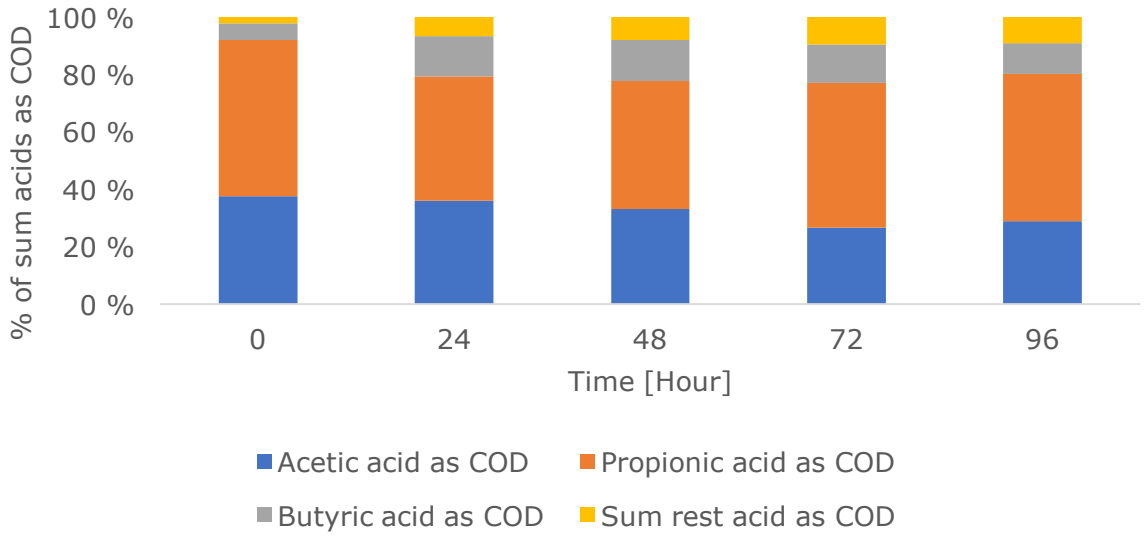


Figure 4.7 VFA fraction development in F7 over time converted to theoretical oxygen demand

4.1.7 pH

pH can affect the VFA composition due to influence of the hydrolysis process. In F7 that has been analyzed for VFAs the pH development is as seen in Figure 4.8. Starting at 6,9 and decreasing to 5,3. The primary sludge has a lot of cellulosic fibers due to toilet paper, and to hydrolyze this pH between 5,6 and 7,3 is preferred [12]. At this case the pH is in this range almost whole time, so it's a reasonable thought that cellulosic fibers are being hydrolyzed and fermented in this process.

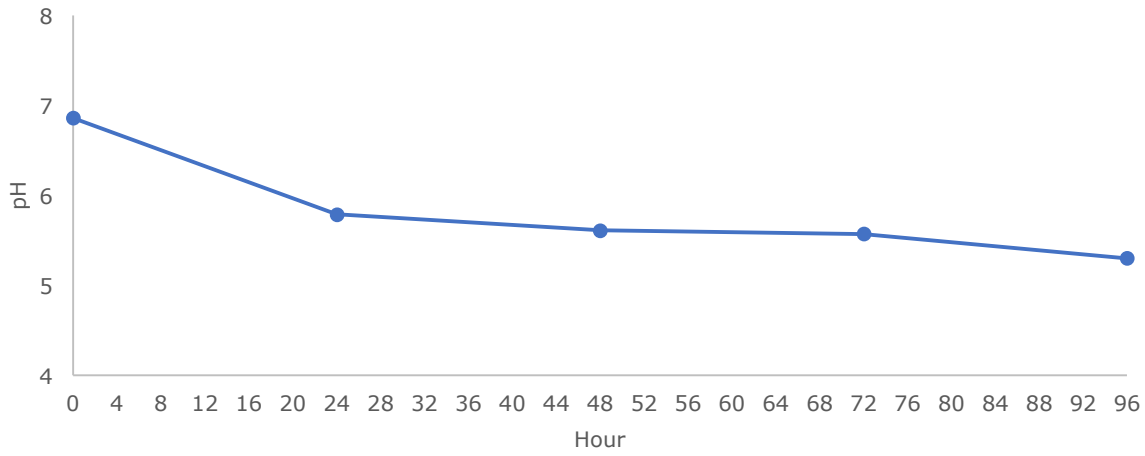


Figure 4.8 pH development in F7

4.1.8 Effect of biomass concentration

As mentioned, F6 and F7 was prepared and run similar, but showed results that was different. Both SCOD as seen in Figure 4.9 and NH4-N and PO4-P showed in Figure 4.10 are clearly higher for F6 than F7. F6 had in batch 1 19,96 g TVS/l and F7 had 15,31 g TVS/l and show a big difference.

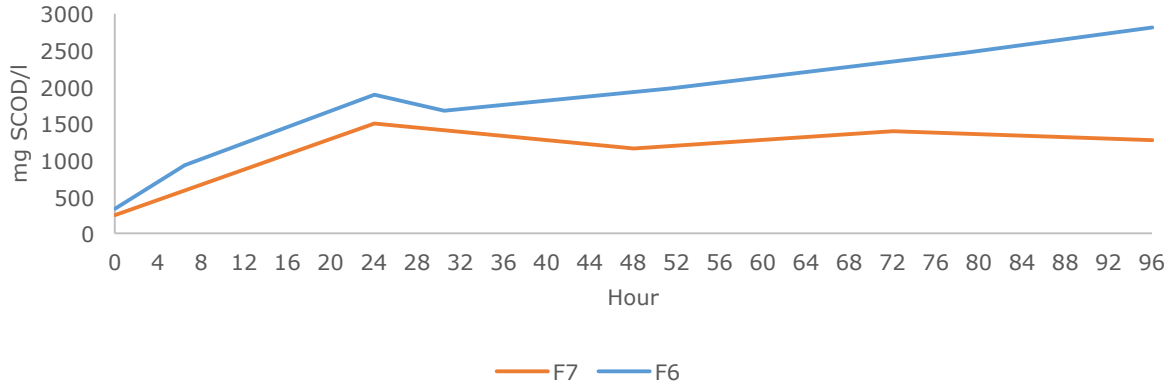


Figure 4.9 SCOD development in F6 and F7

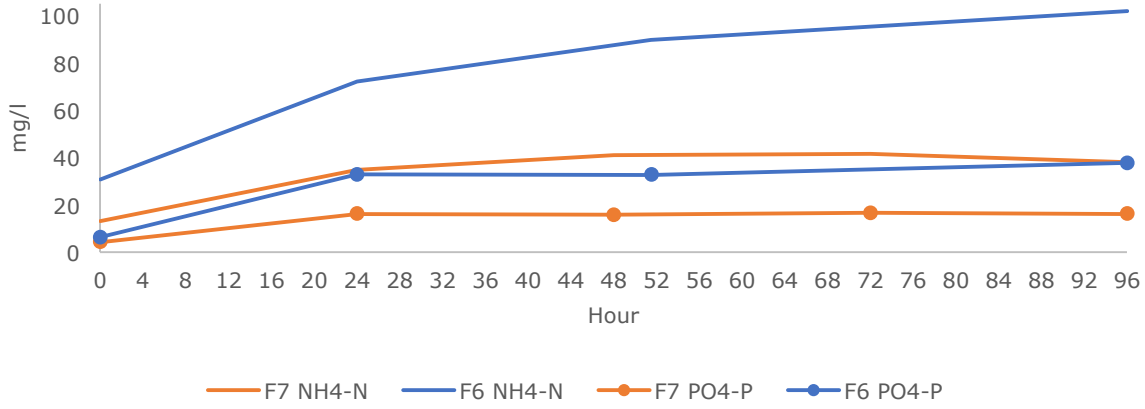


Figure 4.10 NH4-N and PO4-P development in F6 and F7

Simultaneous as some of the fermentation experiments run in the lab, Prestvik [43], run similar experiments. The biomass added was half the amount, but with similar volume and reactor configuration. Biomass was extracted and added at the same time giving equal conditions and therefore comparable results. Table 4.3 shows comparable results of SCOD production. Results from this thesis is from F7. The numbers from Prestvik [43], shows a fermentation with 0,84% TS that is almost 51% of the TS in F7. In average the SCOD production in Prestvik [43] is 46% of F7. This gives a pattern of a linear relationship between TS content and SCOD production. Meaning that with the same volume, a high TS content would be preferred. Then the mixer and the reactor configuration will be the limiting factor of how much SCOD that is possible to produce. In this case the reactor configuration and the mixer had problems to have complete mixing when the TS content was around 2,5%. This could be optimized to be higher.

Time [hour]	SCOD in 0.84% TS [mg/l] [43]	SCOD in 1,64% TS [mg/l]	Fraction[%] of SCOD in 0.84% TS compared to 1,64% TS
0	161	252	64
24	452	1497	30
48	526	1160	40
62	648	1392	47
86	584	1274	46

Table 4.3 Results from F7 compared to numbers from J. Prestvik [43] with lower total solid concentrations

Also VFA-analyses was done with the same method as in this thesis. A quick look at Figure 4.11 shows approximately half the amount of production in total from Prestvik [43] compared to F7.

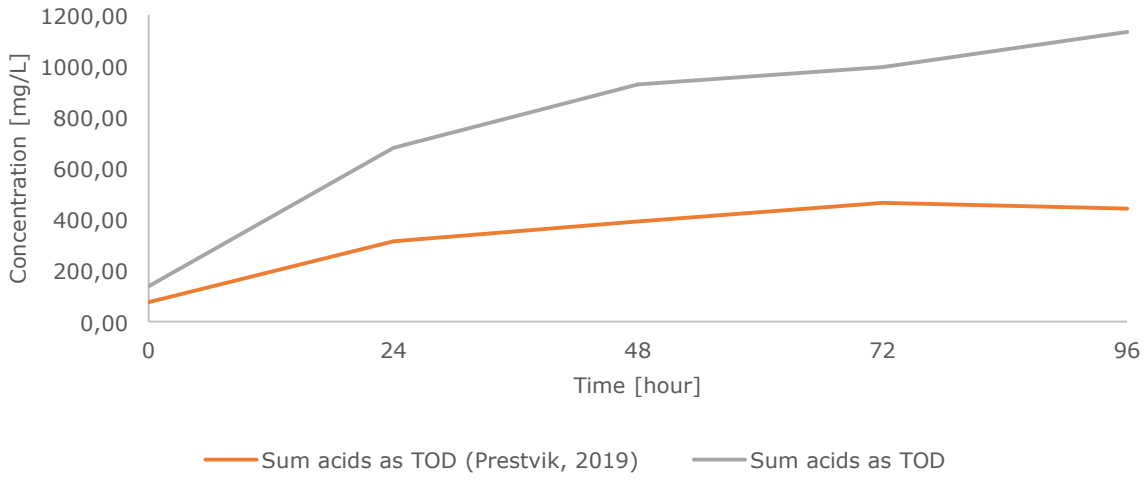


Figure 4.11 Sum of VFA production as TOD compared to lower amount of biomass in Master thesis of Johanne Prestvik [43].

Looking closer to the VFA production also composition of the VFA is different in Prestvik [43]. As seen in Figure 4.12 compared to Figure 4.7 acetic acid part in 1,64 % TS is higher than in 0,84 % TS after both 1 and 4 days. When it comes to propionic acid the part is the same in the two. Butyric acid is clearly increasing from day 1 to 4.

Ucisik et al. [42] found the VSS and TSS concentration to be important in the result of the composition. They looked at four different types of primary sludge, and the one with lowest TSS and VSS got higher percentage of acetic acid. Meaning that fermentation produced for biological nutrient removal with PAOs as the most important bacteria, high concentrations in the sludge is favorable. Cokgor et al. [23] reported the same, acetic acid concentration decrease with increased VSS. Even tough PAOs is favorable and is favored by high propionic/acetic acid ratio, BPR works best with acetic acid [23]. Propionic acid is the second best VFA source.

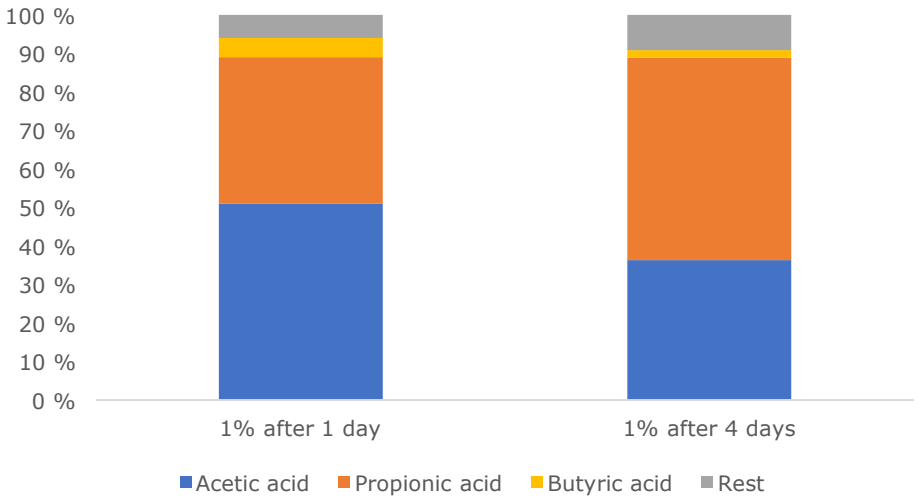


Figure 4.12 VFA distribution after 1 and 4 days for 1% TS [43]

Looking at phosphorous release and nitrogen release, there are also a difference. Phosphorous and nitrogen seems to be removed more frequently in fermentation with lower TS concentrations. This could be due to limited food ass discussed.

4.2 Use of fermented supernatant in kinetics experiments with Bio-P carriers from CMBBR

The jar-test with biofilm carriers from the CMBBR reactor has been performed with and without addition of fermented products. An overview of kinetics experiments with date, what kind of addition and initial values for SCOD and P is shown in Table 4.4. Table 4.5 shows initial values and characteristics of the fermented supernatant added. And Table 4.6 shows the retention times in the experiment. As a note, experiment K1 was performed in room temperature and all the other with cold temperature(11-14°C)

Experiment	Date	Beaker	Addition	Initial SCOD	Initial PO4-P	SCOD in /PO4-P in	bSCOD/ PO4-P in
[#]	[-]	[#]	[-]	[mg/l]	[mg/l]	[-]	[-]
K1	27/11/2018	1	Fermented	260	4,99	52,10	42,48
K2	29/01/2019	1	None	130	7,23	17,98	12,86
		2	Fermented	230	7,96	28,89	23,62
K3	05/02/2019	1	None	179	5,45	32,84	26,06
		2	Fermented	262	5,83	44,94	35,85
K4	14/02/2019	1	None	50	1,551	32,24	22,57
		2	None	56	1,554	36,04	23,17
K5	20/02/2019	1	None	77	2,44	31,56	18,85
		2	Fermented	142	2,8	50,71	38,21
K6	27/02/2019	1	None	66,7	2,08	32,07	18,51
		2	Fermented	177,3	3,29	53,89	44,19
K7	12/03/2019	1	None	143	4,61	31,02	21,04
		2	Fermented	249	5,65	44,07	35,75
K8	02/04/2019	1	None	74	2,73	27,11	18,68
		2	Fermented	164	3,51	46,72	38,18

Table 4.4 Overview of kinetics experiments with Bio-P carriers from CMBBR in jar-test

Kinetics experiment	Fermen-tation experiment	Batch nr.	Retention time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	Days in fridge
K1	F1	1	21	1780	12,47	00,00	1
K2	F2	1	5	1410	13,79	46,30	1
K3	F2	1	5	1410	13,79	46,30	8
K5	F4	3	2	918	10,40	31,69	1
K6	F5	2	2	1704	24,56	68,82	1
K7	F6	1	4	2605	35,94	96,00	1
K8	F7	1	4	1375	17,40	40,86	1

Table 4.5 Overview of characteristics of fermented supernatant added to kinetics and from which fermentation experiments the supernatant is extracted from

Experiment [#]	Beaker [#]	Anaerobic HRT [h:min]	Aerobic HRT [h:min]	HRT [h:min]
K1	**1**	03:10	04:10	07:20
K2	**1**	03:10	04:10	07:20
	2	03:10	04:10	07:20
K3	**1**	02:30	04:50	07:20
	2	02:30	04:50	07:20
K4	**1**	02:30	04:50	07:20
	2	03:10	04:10	07:20
K5	**1**	02:30	04:50	07:20
	2	02:30	04:50	07:20
K6	**1**	02:30	03:50	06:20
	2	02:30	03:50	06:20
K7	**1**	02:30	03:50	06:20
	2	02:30	03:50	06:20
K8	**1**	02:30	03:50	06:20
	2	02:30	03:50	06:20

Table 4.6 Overview of retention times in kinetics experiments

A typical SCOD and PO4-P concentration development is shown in Figure 4.13. When SCOD consumes in the anaerobic zone, P concentration is increasing due to P-release from bacteria exactly like the theory says. Not every experiment shows this pattern, but the majority. The ones not showing this pattern, haven´t worked as well either. All raw data results for each experiment are in Appendix 3.

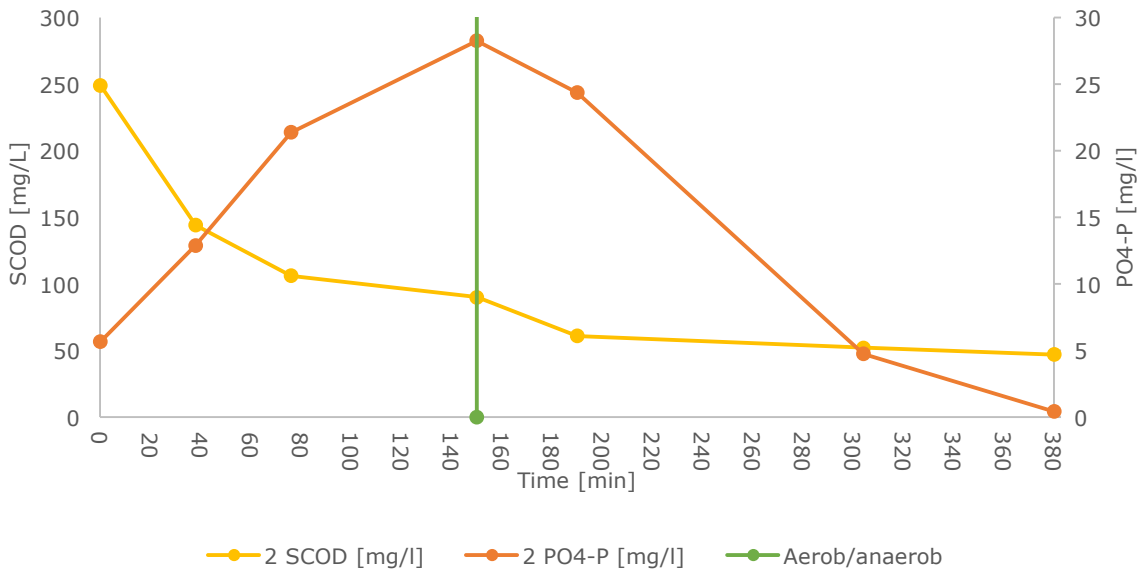


Figure 4.13 Typical SCOD and PO4-P development during jar-test illustrated by experiment K7 with fermented supernatant. Vertical line devide anaerobic and aerobic zone.

4.2.1 Inlet composition of wastewater

Not only SCOD and PO4-P have been investigated in these experiments. Table 4.7 shows average values of also other parameters with standard deviation. TCOD and TP is calculated with respectively 5 and 3 values since the first experiments don't have these analyses.

Time zero values for wastewater	Average value [mg/l]	STD
tCOD	309	±135
sCOD	97	±47
TP	9	±2
PO4-P	3	±2
NH4-N	30	±13
pH	8,2	±0,3

(a)

Time zero values for fermented	Average value [mg/l]	STD
tCOD	641	±132
sCOD	212	±50
TP	10	±2
PO4-P	5	±2
NH4-N	36	±11
pH	7,8	±0,2

(b)

Table 4.7 (a) Influent average parameters for CMBBR wastewater experiments. (b) Time zero values for wastewater experiments with fermented supernatant.

Theoretical influent value of the ratio COD/P is stated to be more than 40 to have a good working biological phosphorous removal (BPR) [33]. In Figure 4.14 all jar-tests are included showing the removal efficiency, calculated with the inlet PO4-P concentrations, against COD/P initial values. Looking at this there are experiments under the required SCOD/P ratio that achieve P-removal requirements. And also with values above requirements that doesn't reach the removal requirement.

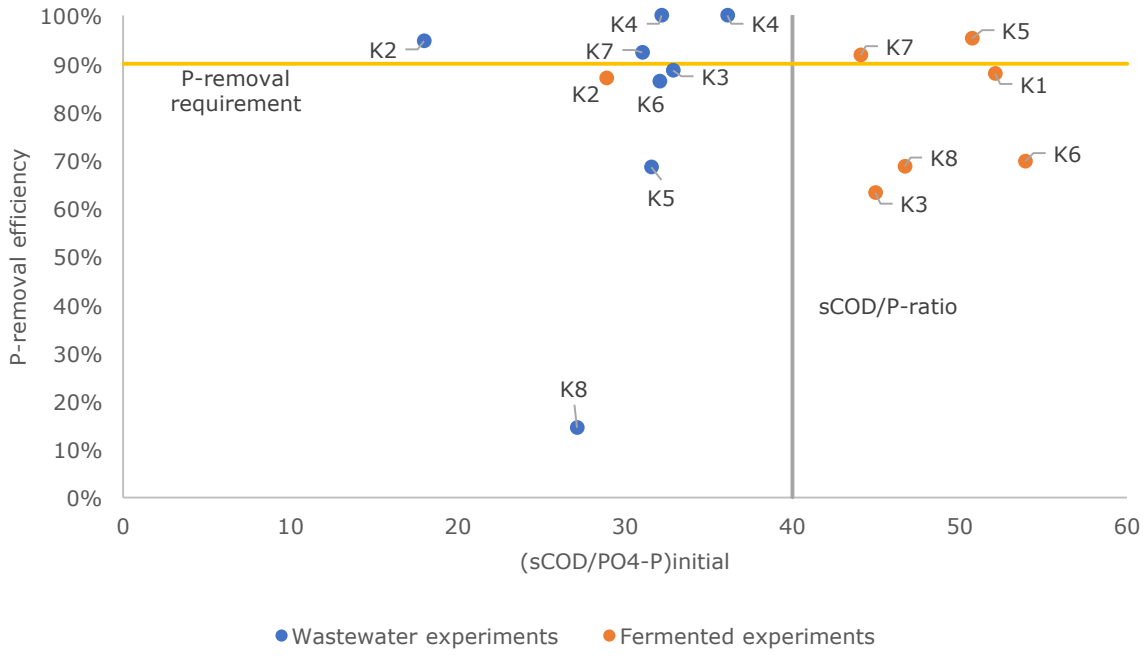


Figure 4.14 SCOD/PO4-P-ratio in inlet concentration against %P-removal. With theoretical value for a good working process.

Figure 4.15 shows bSCOD /PO4-P initial against P-removal efficiency. bSCOD is the bioavailable SCOD, the difference between time zero concentration of SCOD and the end point. bSCOD is not the complete same as BOD, but a BOD/P-ratio of 20 [30] is required to have a functioning BPR. Helness [44] reported the bSCOD/PO4-P in to also be 20 for having a good process. A lot of experiments shows bad potential for the process, even if they achieve high removal efficiency.

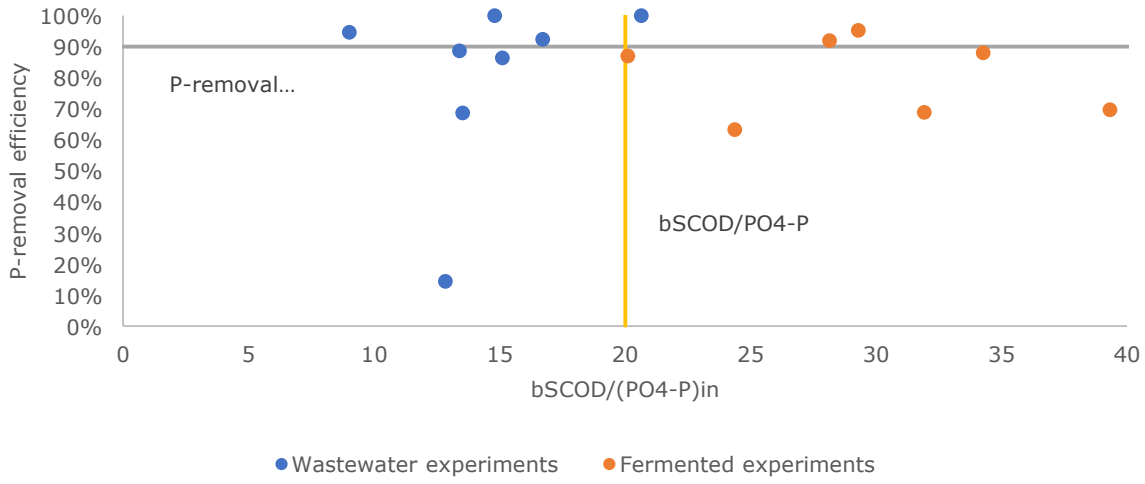


Figure 4.15 bSCOD/(PO4-P)in against P-removal efficiency.

In general, it looks like the theoretical values given doesn't give any hint of having a good working process or not since a lot are both higher and don't work and lower and does work well.

According to inlet concentration of phosphorous, 3 categories has been used following Table 4.8 (a) to divide the experiments into logical differences to try to see any patterns. Also dissolved oxygen level was a parameter divided into three levels in Table 4.8 (b). Table 4.9 show what category the experiments are in.

Category	Concentration of PO4-P in mg/l
Diluted	<2
Semi-diluted	2-3
Concentrated	>3

(a)

Category	Concentration of DO in mg/l
Low	2-4
Medium	4-6
High	6-8

(b)

Table 4.8 (a) Dilution category according to concentration of phosphorous in start condition. (b) Oxygen level category.

Aeration \ Dilution	Diluted	Semi-diluted	Concentrated
Low		K6	
Medium	K8	K5	K1,K2, K7
High	K4		K3

Table 4.9 Dilution and aeration categories in kinetics experiments

4.2.2 Removal efficiency in experiments

Figure 4.16 shows the removal efficiency of PO4-P and P-removal requirement. Can see that the removal of P is very good, but doesn't reach the percentage removal requirements all the time. But the effluent limit is as low as 0,4 mg P/l [38]. Which is easier to reach. In only wastewater experiments this has happened in all except for K8, K5 and K3 which only K8 has been far away. Table 4.10 shows TP analyses which not are as effective as removal of soluble P, but this doesn't matter that much since requirements are on the soluble P.

Experiment	TP in [mg/l]	TP out [mg/l]	Removed [mg/l]	% Removed
K6_WW	9,25	8,6	0,65	7 %
K6_F	11,5	9,06	2,44	21 %
K7_WW	10,68	5,24	5,44	51 %
K7_F	12,26	6,17	6,09	50 %
K8_WW	6,56	5,63	0,93	14 %
K8_F	7,73	4,75	2,98	39 %

Table 4.10 TP analyses from K6, K7 and K8

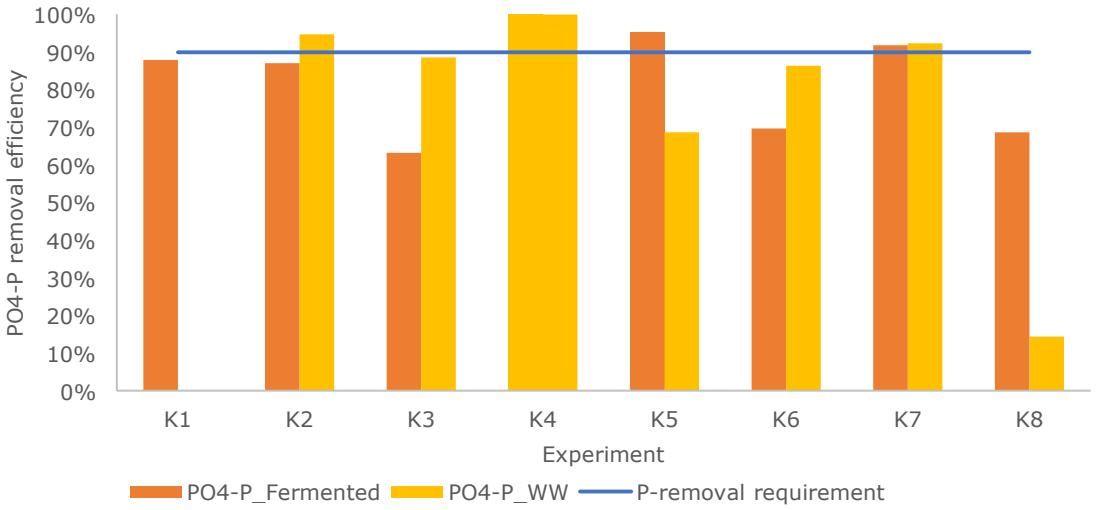


Figure 4.16 Removal efficiency(%) of PO4-P for kinetics wastewater experiments and fermented experiments

SCOD removal requirements are 75% for secondary treatment requirements or less than 125 mg/l in the outlet [9]. In average 68% is removed from all kinetics, 66% with fermented added and 70% with only wastewater. 80% of SCOD is removed in anaerobic zone for fermented experiments and 72% for only wastewater. Every kinetics experiments have final concentrations of SCOD below 53 mg/l and therefor meet the requirement of less than 125 mg/l.

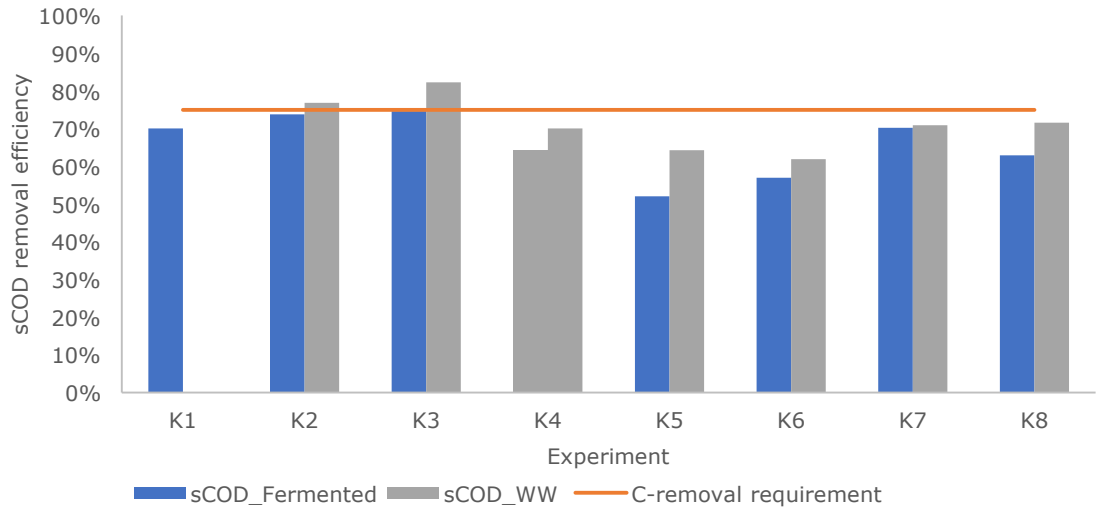


Figure 4.17 Removal efficiency(%) of sCOD for kinetics wastewater experiments and fermented experiments

4.2.3 P-release and P-uptake

P-release happens in the anaerobic part of the process as described in theory after carbon uptake and converted to glycogen, PHA and PHB. The reactant (SCOD) consumption rate will affect the P rate, not the P it self. Both the concentration of COD, the type and the amount of PAO in biomass. In theory the bSCOD/P-release is constant, but this seems to depend on the carbon source. Theoretical value of bSCOD is presented in Figure 4.18 having only one experiment using more bSCOD for P-release than theory says is normal. In the anaerobic part of the process its assumed to be some potential fermentation going on in the process by fermenting organisms. So if bSCOD isn't enough in the inlet, a possible solution could be to extend the anaerobic phase. In this case the release rates will decrease if carbon source requires conversion. The reason for slow P-release rates could also be different organisms using different carbon sources, some use VFA and work fast and others use different C-source and work slower. Therefor more P-release if they get the C-source they like.

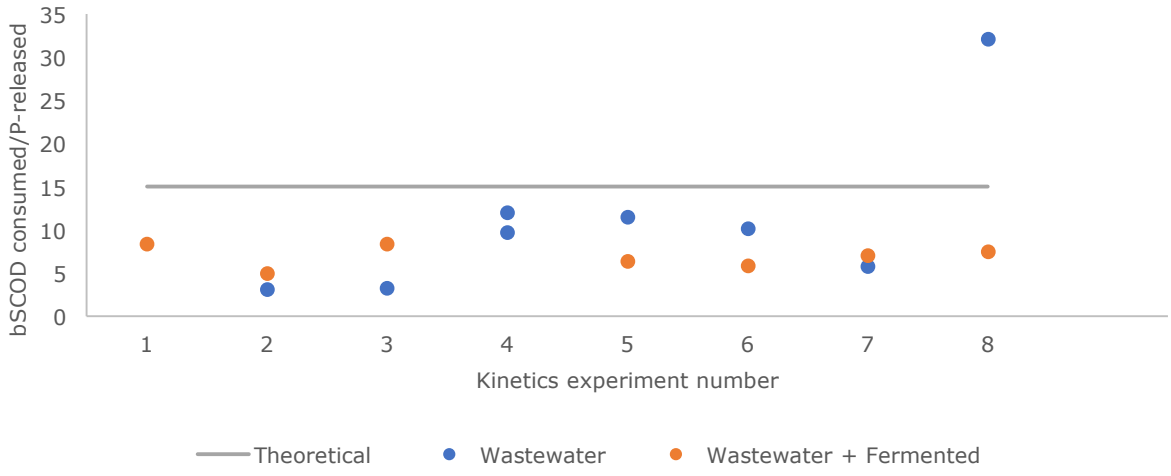


Figure 4.18 (bsCOD)consumed/(PO4-P)released in each kinetics compared to theoretical value

Saltnes et al. [38] presents an release and uptake test in lab scale that shows 10.7 SCOD uptake/P release for anerobic zone and 50.1 Net SCOD uptake/P uptake. In average results from similar tests gave in this thesis average 9.1 SCOD uptake/P release and 39.2 Net SCOD uptake/P uptake. Showing similar anaerobic SCOD/release, but a much larger Net value.

High P-release doesn't need to be favorable, but an amount that is needed to produce enough PAO for wanted uptake. Figure 4.19 shows the P development in all kinetics experiments. Showing that for only wastewater the release is often low, but as seen in Figure 4.16 and Figure 4.17 removal of PO4-P and SCOD is often better with only wastewater. Therefor a limited amount of COD seems to be beneficial to uptake better. This could give a more compact process. But a buffer capacity with plenty of PAOs available could be good to have if not all are utilized at all time. This is against common sense. Theory is based on having a lot of PAOs and a lot of VFAs. But then they need to have a lot of time to take up the P. Variations in inlet concentrations is a challenge. VFA can seem to be like drugs, this process is built-up with low concentrations with very low VFA, but tend to work anyway. Therefor a theory could be that if bacteria are built-up with high VFA concentrations they will not work with little, and if they have low concentrations from the beginning they get use to it and work.

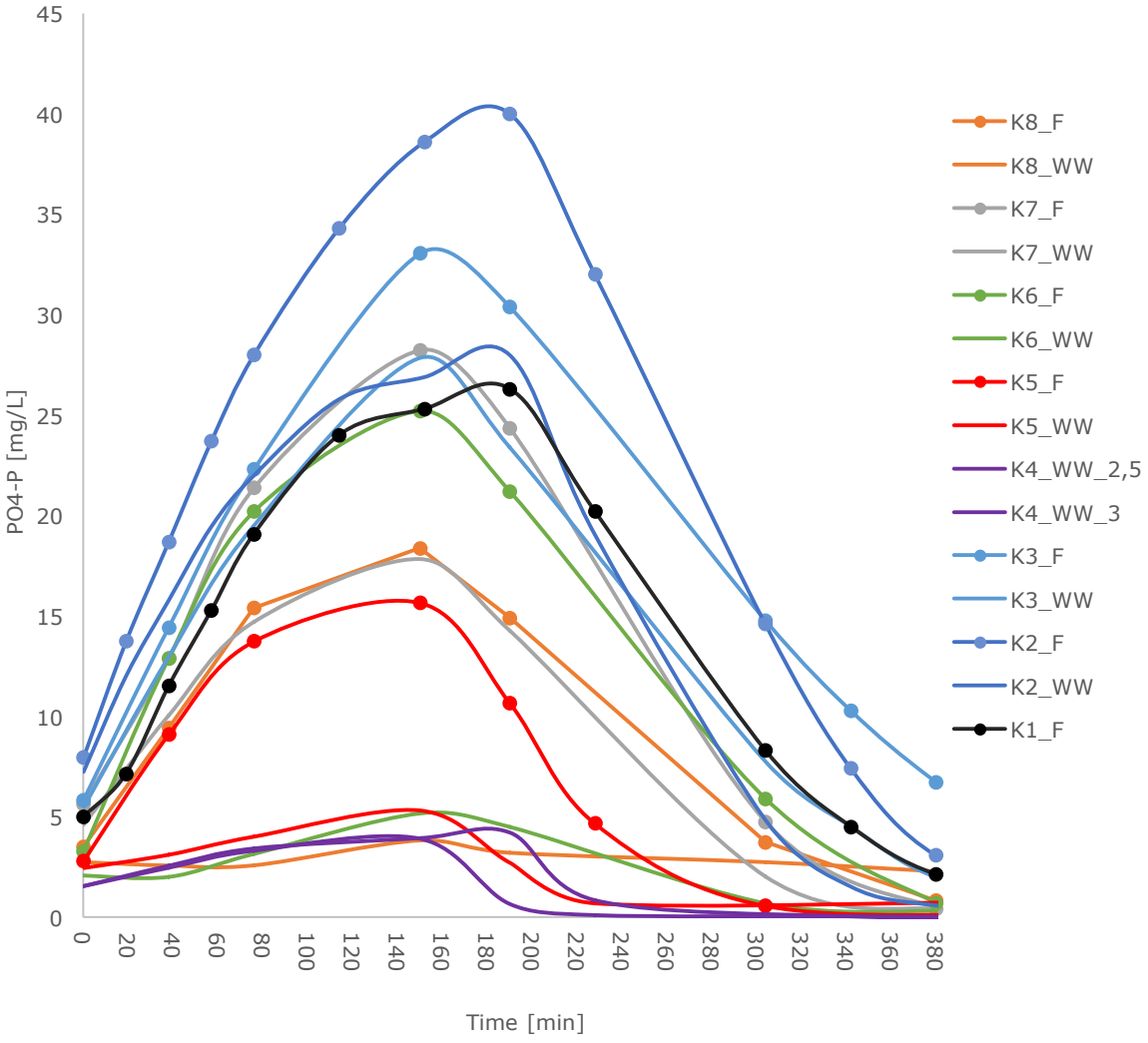


Figure 4.19 P development in kinetics experiments

K8 shows a difference from the other experiments. Since the one with only wastewater doesn't work, but it works very good with fermented supernatant. K6 have used similar fermented supernatant and works like K8, but much better without addition. This shows that there are plenty of PAOs present, but the carbon source seems to be the problem. The amount of SCOD in inlet is also quite similar, but before experiment K8 the wastewater had been diluted for several days. This could show that the organisms are on a boarder line. Having a pool with C-storage for reproduction that in limited times are used to work. The pool is enough in low inlet concentrations if they are fed with good and a lot of C between low periods. But in long low periods they don´t have anything left in the pool.

Uptake rates in Figure 4.20 is in average 7.3 anaerobic consumed bSCOD/P-uptake for only wastewater experiments and 5.4 bSCOD/P-uptake with added fermented supernatant. For wastewater only there were one extreme case in K4 as seen in Figure 4.20, so without this out layer it would be 5.7. In general, it seems like the most diluted experiments as K8 and K4 use more bSCOD/P uptaken.

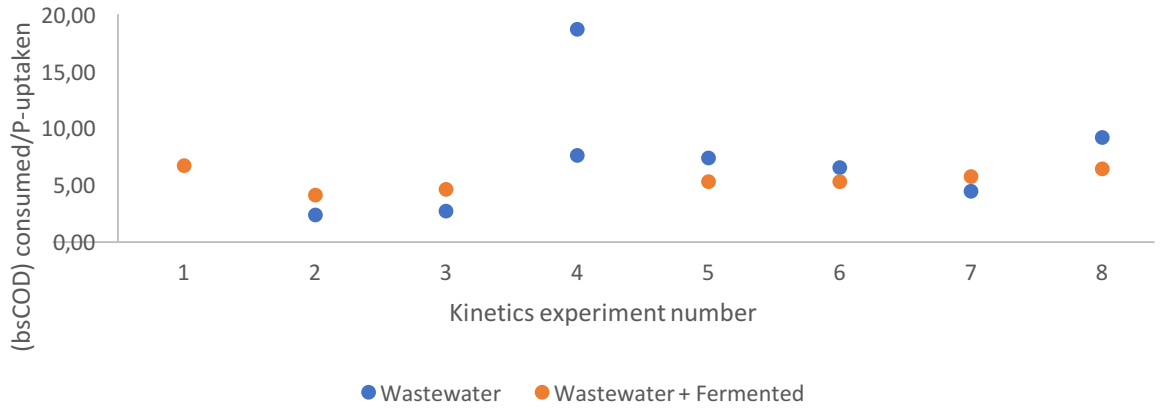


Figure 4.20 (bsCOD)consumed/(PO4-P)uptaken in each kinetics

4.2.4 Biomass characteristics on the carriers

PAO can be related to the TP on the carriers. TP on the carriers have been measured together with the TS and VS. In average the biomass on the carriers have 0.049 ± 0.023 mg TP/ mg TS and 0.054±0.025 mg TP/ mg VS. Typical value of the biomass is 0.02 mg TP/ mg TS [45].

Analyses shows fluctuating results. All results can be seen in Appendix 7. VS analyses is done, but an error in the method made some numbers unusable and therefor an average from the rest are used. Also TS results gave very different results in different analyzing method. The most correct method will be the one evaporating directly from the carriers. But this analyze is limited due to only TS results, but a ratio as mg TP/ mg TS will give more correct answer even if not all the biomass has been removed from the carrier before the analyses.

4.2.5 pH

High pH (>7,5) will favoure PAOs over GAOs and are preferred. In this case inlet pH is higher (Table 4.7). The pH development was logged on a computer in some experiments. In K3, shown in Figure 4.21, pH decreased in the anaerobic zone before increasing again

in the aerobic zone, claerly following the DO level in the beaker. Graphs for the other computer logged experiments are in Appendix 4.

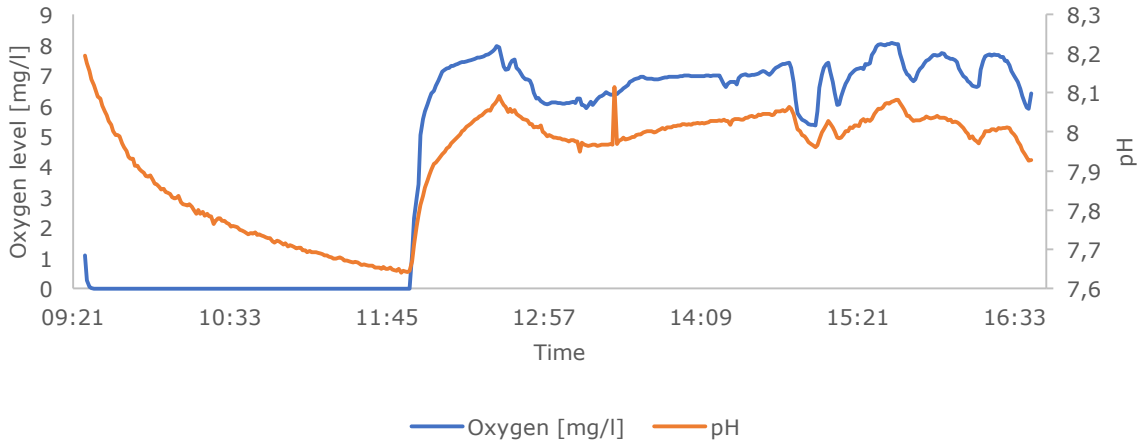


Figure 4.21 pH and DO development in K3

4.2.6 DO

Looking at Figure 4.16 and Table 4.8 together there doesn't seem to be any consistent pattern between the concentration and aeration level. Looking at for example K6 and K5 that is very similar experiments, both is semi-diluted. K5 has actually lower P-removal than K6 which have higher DO level and longer aerobic zone with only wastewater. Development of jar-test are seen in Figure 4.22.

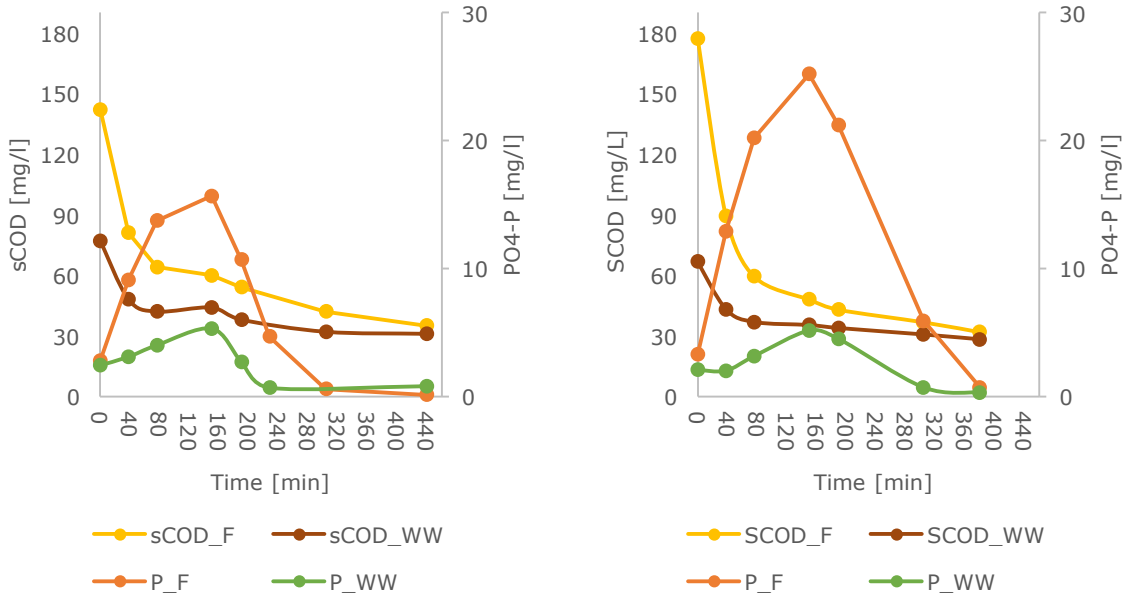


Figure 4.22 SCOD and PO4-P development in (a) K5 and (b) K6

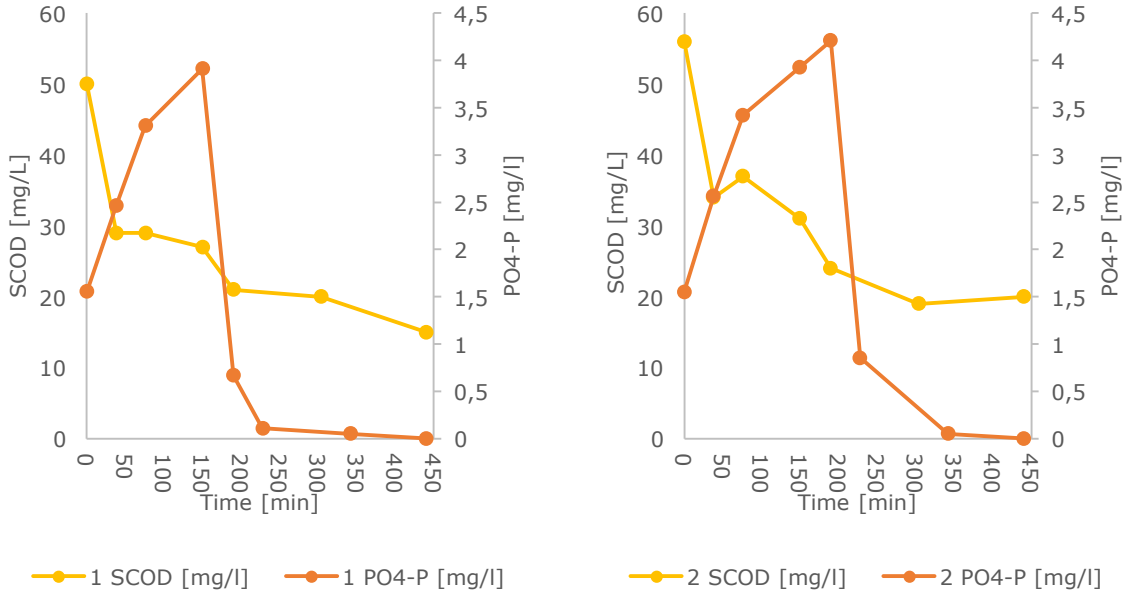
4.2.7 Temperature

The temperature was held to test how the process works in cold temperatures, but also since it was the usual conditions for the carriers at this time it seems logic that they work like they usually do.

Microorganisms in biological wastewater treatment have optimum temperatures of 20-35°C [39]. With experiments running in low temperatures, in the range of 11-14°C, they still seem to work very well in this system. PAOs have optimal temperature at 20°C, and is observed to work better in lower (5-15°C) temperatures than in higher (20-37°C) [33]. This is against normal logic on microorganisms. For Norwegian wastewaters this is promising results due to large period with winter season giving low temperatures and still same requirements of removal.

4.2.8 Retention time

K1 and K2 was run with anaerobic retention time of 3h 10min. Before changing to 2h 30min anaerobic retention time in K3, to be more similar to the retention time in the pilot. K4 was run with only wastewater to compare these two retention times, results in Figure 4.23. They gave almost the same net removal, but the release was higher with longer anaerobic retention, and therefor the uptake rate was higher in the one with highest an HRT.



(a) **(b)**

Figure 4.23 SCOD and PO4-P development in K4 with different retention time. (a) 3h 10min (b) 2h 30min.

4.2.9 Carbon source

As presented in theory and discussed earlier the effectiveness of the EBPR process is dependent on the carbon source available. VFAs are said to be the preferred source.

4.2.9.1 VFA analyses

The levels of VFA in the samples from K8 with only wastewater was too low to be detected. VFA samples from time zero in K8 with fermented supernatant are seen in Figure 4.24. 7-9 (or 20) mg of VFA are said to be needed to remove 1 mg of phosphorous [33]. In this kinetics 77,25 mg of VFA is present in the beginning, and the influent phosphorous value 3,51 mg. This gives a needed VFA amount of 24,57-31,59 (70,20) mg. So in theory this process should work, but the requirements are not

reached. Effluent level was 0,83 mg. In the kinetics with only wastewater the effluent level was 2,27 mg, only 17% removal versus 76% with fermented. One reason could be the VFA level since its no VFA and therefore under theoretical level for having removal. This is a one-time case since VFA analyses only have been performed once, so can´t conclude with this.

Fermented products added have more propionic acid than acetic acid. A large propionic/acetic acid ratio is preferred since this will favor the PAOs over GAOs. The end points in Figure 4.5 shows the composition of the fermented product added in the K8.

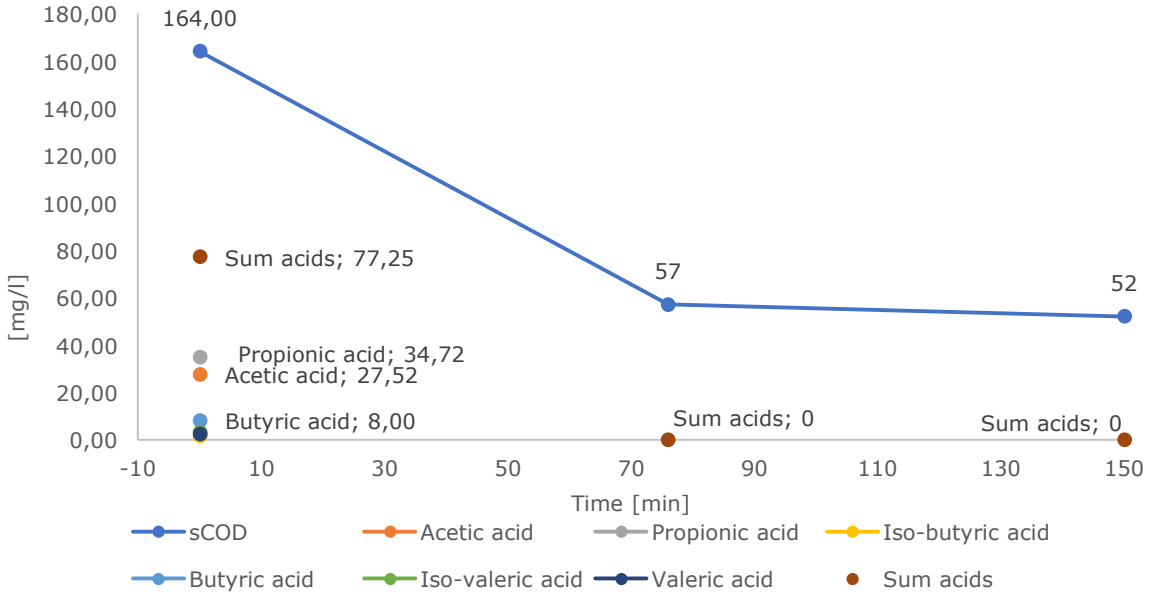


Figure 4.24 VFA in K8 with fermented products

For the fermented experiment in K8 it can be observed that SCOD still decreases even if the VFA concentration is gone after 76 minutes. The same for the wastewater experiments which have no VFA at point zero, but the SCOD decreases. This indicates that its not only VFA they are using as carbon source but as well other SCOD. Based on kinetics the metabolism indicates that there are a Bio-P microbial community that not necessarily depend on VFA. Other bacteria than PAO in the Bio-P community that use glucose and amino acids instead of VFA [46]. If this is the case the fermentation product after 1 day could be even more suitable for use in these kinetics since the SCOD fraction that not are VFA is a lot higher than in day 4 as seen in Figure 4.6

4.2.10 Nitrification

In the kinetics there have been some nitrification and de-nitrification(DN) going on. DNPAO is hidden in the biofilm when the DO is high to have anoxic conditions. DNPAO is also demanded to have some P left in the process to work. Nitrification can be limited when its too much. Nitrification in the kinetics are in average 21,09 mg/l or 73%. Further the de-nitrification isn't complete, and the nitrite isn´t tested, but by extracting the nitrate and ammonium in the outlet from the inlet ammonia concentration, removal of 38% is achieved in average.

K8 has been stated out to be different than the others since with only wastewater removal was very bad. But actually the NH4-N is almost totally removed, but DN haven't happened.

5 Conclusion

5.1 Fermentation

Production of fermented primary sludge supernatant have been produced with biological hydrolysis in anaerobic conditions. In this case highest amount of SCOD is produced at high temperature(24-29°C). Theory says that the temperature should not be high to avoid unstable process, but that doesn't happen before mesophilic conditions. Primary sludge produces large amount of SCOD and not too much P and N, actually sometimes decreasing or keep stable N and P values during fermentation. HRT at 5 days gave highest SCOD production. After this the rate of production sinks and is a good retention time to avoid methanogenesis. Running as a sequence batch did not produce higher yields. For this specific case, a batch reactor seems to be the best option. Reactor shape and mixer are not checked, but the reactor used, cylindrical and with mixing at 100 rpm, worked. The TS and TVS should be as high as possible to produce as much SCOD as possible. Composition of VFA produced was favorable with higher TVS. VFA produced was mostly propionic acid, acetic acid and butyric acid. These are the VFAs that are traditionally reported to be preferred by the Bio-P bacteria in EBPR.

5.2 Kinetics with Bio-P carriers from CMBBR

The kinetics experiments show results that is in harmony with theory about how PAOs work by looking at results for SCOD consumption, and P-release and P-uptake pattern. Also having some nitrogen removal. The amount of carbon source the Bio-P bacteria use is not in harmony with theory. In this case they need a very low amount of carbon to perform. VFA analyses show SCOD consumption even if no VFA is available. Since this is the case Bio-P bacteria doesn't seem to be dependent on VFA as expected. This is also supported by results from parallel experiments. Wastewater without addition work best most of the time. Except for conditions where the inlet wastewater had been diluted for a longer period, then the process worked better with addition of fermented supernatant.

Another theory could be to high availability of carbon with added fermented supernatant. Which could result in no complete removal of carbon before the aerobic zone, and therefor inhibit the process. If this is the case, addition of supernatant giving exactly what they need could give a more compact and effective process.

A reason for having different results than other studies could be the differences in carbon source and microbial community. Many papers present experiments with addition of acetate instead of fermented supernatant as used in this thesis.

There are other factors influencing the EBPR process. The retention time could be a parameter that also can increase the efficiency since its observed different uptake rates at different retention times. Temperature doesn't seem to extremely affect the bacteria. They work well in cold conditions(11-14°C). Looking at different inlet concentrations and DO levels, no clear pattern for what is working best have been detected. And therefore low DO is preferred for energy cost savings to be sustainable.

Overall the process works good without addition of fermented supernatant and meet removal requirements.

6 Further research

Due to limited duration for this thesis, further and extended work on the topic is needed.

For fermentation, further research with longer duration on experiments to check if its possible to get higher yield and rates could be interesting. And to try even higher TS fractions to see if this gives even higher production rates. Also more analyses on the SCOD fractions that is produced could be interesting. Both to see what the fermenter actually produce and to continue and investigate what rbCOD the Bio-P bacteria in the CMBBR actually prefer. Also more experiments on retention time to optimize is needed to further investigate. Together with differences in wastewater inlet composition, temperature and DO level.

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Appendix

Appendix 1: Calculation of addition of fermented supernatant in jar-test

Appendix 2: Sampling procedure in jar-test

Appendix 3: Raw data from jar-test

Appendix 4: Online measurements of pH and DO

Appendix 5: Raw data from fermentation

Appendix 6: Theoretical chemical oxygen demand calculations

Appendix 7: Carrier characterization

Appendix 1: Calculation of addition of fermented supernatant in jar-test

Goal: C_1 = 100 mg SCOD/L in addition into wastewater

Know: V_1 = Total volume of liquid in beaker

C_2 = Concentration of SCOD in fermented supernatant

Unknown: V_2 = Volume of fermented supernatant to have 100 mg SCOD/L in addition

$$C_1 * V_1 = C_2 * V_2$$

$$\Longrightarrow V_2 = \frac{C_1 * V_1}{C_2}$$

$$\Longrightarrow V_2 = \frac{100\ mg/l * 0{,}794\ l}{C_2}$$

Appendix 2: Sampling procedure in jar-test

Time label	Time [min]
t0	0
t1	19
t2	38
t3	57
t4	76
t5	114
t6	150
t7	190
t8	228
t9	304
t10	342
t11	380
t1	440

Appendix 3: Raw data from jar-test

K1:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	5,63	160	43,02
Fermented sludge	12,47	1780	Under range

Experiment

	Sample number	Time [min]	Time [h:min]	PO4-P [mg/l]	SCOD [mg/l]	NH4-N [mg/l]	DO [mg/l]	pH
Anaerobic	**t0**	0	00:00	4,99	260	35,32	0,14	
	t1	19	00:19	7,14	215			
	t2	38	00:38	11,51	170			
	t3	57	00:57	15,27	160		0,15	
	t4	76	01:16	19,08	121			
	t5	114	01:54	24	93			7,7
	t6	152	02:32	25,3	89		0,09	
	t7	190	03:10	26,3	85		5,4	
Aerobic	**t8**	228	03:48	20,2	67		5,5	
	t9	304	05:04	8,3	51		5,77	
	t10	342	05:42	4,51	51		5,82	
	t11	380	06:20	2,13	49		6,23	
	t12	440	07:20	0,678	48	Under range	7,5	
	t13	460	07:40	0,522				

K2:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	4,95	160	45,8
Fermented sludge	13,74	1410	46,3

Experiment

				Kinetics 1 WW cold temperature					Kinetics 2 Fermented cold temperature				
	Sample number	**Time [min]**	**Time [h:min]**	**1 PO4-P [mg/l]**	**1 SCOD [mg/l]**	**1 NH4-N [mg/l]**	**1 DO [mg/l]**	**1 T [◦C]**	**2 PO4-P [mg/l]**	**2 SCOD [mg/l]**	**2 NH4-N [mg/l]**	**2 DO [mg/l]**	**2 T [◦C]**
Anaerobic	**t0**	0	00:00	7,23	130	40,87	0,23	12	7,96	230	43,18	0,21	12
	t1	19	00:19	12,01	150			10,8	13,74	210			12,1
	t2	38	00:38	15,78	90			11,4	18,69	141			12,4
	t3	57	00:57	19,45	82			12,1	23,7	116			13
	t4	76	01:16	22	76			12,2	28	108			12,7
	t5	114	01:54	25,8	67			12,8	34,3	88			13,2
	t6	152	02:32	26,9	67			11,9	38,6	77			11,8
	t7	190	03:10	28	65	43,6	3	13,1	40	70	42,31	2,5	13
Aerobic	**t8**	228	03:48	19,05	47		3,37	12,7	32	55		3,11	12,6
	t9	304	05:04	4,84	38	33,63	3,51	12,6	14,6	47	33,22	3,38	12,4
	t10	342	05:42	1,515	43		3,29	12,8	7,43	45		3,49	12,8
	t11	380	06:20	0,579	40	26,17	3,78	12,8	3,09	42	25,87	3,55	12,2
	t12	440	07:20	0,264	37	19,76	3,59	13	0,644	42	19,56	3,96	13,1

K3:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	5,66	209	47
Fermented sludge	13,74	1410	46,3
Wastewater + Fermented	5,54	332	45,78

Experiment

				Kinetics 1 WW cold temperature						Kinetics 2 Fermented cold temperature					
	Sample number	Time [min]	Time [h:min]	1 PO4-P [mg/l]	1 SCOD [mg/l]	1 NH4-N [mg/l]	1 NO3 [mg/l]	1 DO [mg/l]	1 T [°C]	2 PO4-P [mg/l]	2 SCOD [mg/l]	2 NH4-N [mg/l]	3 NO3 [mg/l]	2 DO [mg/l]	2 T [°C]
Anaerobic	**t0**	0	00:00	5,45	179	47			13,2	5,83	262	45,78		0,26	13,1
	t1	19	00:19		141				13,3		197				13,3
	t2	38	00:38	12,97	121				13	14,43	175				12,5
	t3	57	00:57		110				12,5		151				12
	t4	76	01:16	19,5	101				11,9	22,3	142				12,2
	t5	114	01:54						12,7						13
	t6	150	02:30	27,85	106				12,7	33,05	120				12,7
Aerobic	**t7**	190	03:10	23,4	55				13	30,4	63				12,9
	t8	228	03:48						12,4						12,1
	t9	304	05:04	7,75	46			7,04	14,9	14,78	59			5,7	14,6
	t10	342	05:42	4,52				7,05	13,6	10,29				6,7	13,3
	t11	380	06:20	1,99	42			7,19	13,5	6,73	53			6,68	13
	t12	440	07:20	0,648	37	34,7	2,51	6,11	13	2,084		24,24	2,14	6,1	13,2

Totals

	Total COD	
	1 TCOD [mg/l]	**2 TCOD [mg/l]**
t0	590	740
t6	815	1250
t12	905	780

K4:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	-	-	-

Experiment

					Kinetics 1 WW cold temperature 2,5 h								Kinetics 2 WW cold temperature 3h				
	Sample number	Time [min]	Time [h:min]	1 PO4-P [mg/l]	1 SCOD [mg/l]	1 NH4-N [mg/l]	1 NO3 [mg/l]	1 DO [mg/l]	1 pH	1 T [°C]	2 PO4-P [mg/l]	2 SCOD [mg/l]	2 NH4-N [mg/l]	2 NO3 [mg/l]	2 DO [mg/l]	2 pH	2 T [°C]
Anaerobic	t0	0	00:00	1,554	50	16,38		0,24	8,272	13,4	1,551	56	15,66		0,24	8,444	13,4
	t1	19	00:19							12,4							11,9
	t2	38	00:38	2,466	29				7,79	12,9	2,562	34				7,85	12,6
	t3	57	00:57						7,685	13,6						7,67	13,4
	t4	76	01:16	3,31	29				7,706	12,6	3,42	37				7,739	12,5
	t5	114	01:54						7,652	12,4						7,656	12,8
	t6	150	02:30	3,91	27				7,455	11,9	3,93	31				7,451	12,4
Aerobic	t7	190	03:10	0,667	21			7,74	7,737	13,5	4,21	24				7,425	13,5
	t8	228	03:48	0,11				8,74	7,72	13	0,854				8,21	7,798	12,9
	t9	304	05:04		20	3,16		8,42	7,654	13,7		19	6,6		8,5	7,701	13,6
	t10	342	05:42	0,051				8,33	7,271	13,9	0,052				8,19	7,445	13,6
	t11	380	06:20					8,64		13,2					8,59	7,505	13,6
	t12	440	07:20	UR (<0,05)	15	UR	4,55	8,42	7,7	14,2	UR	20	UR	5,56	8,41	7,675	14,5

Totals

	Total COD	
	1 TCOD [mg/l]	2 TCOD [mg/l]
t0	157	157
t6/t7	524	521

K5:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	2,58	73	24,66
Fermented supernatant	10,4	918	31,69
Wastewater + Fermented	2,96	153	25,21

Experiment

		Kinetics 1 WW cold temperature									Kinetics 2 WW + Fermented cold temperature						
	Sample number	Time [min]	Time [h:min]	1 PO4-P [mg/l]	1 SCOD [mg/l]	1 NH4-N [mg/l]	1 NO3 [mg/l]	1 DO [mg/l]	1 pH	1 T [°C]	2 PO4-P [mg/l]	2 SCOD [mg/l]	2 NH4-N [mg/l]	2 NO3 [mg/l]	2 DO [mg/l]	2 pH	2 T [°C]
Anaerobic	t0	0	00:00	2,44	77	26,24			8,022	13	2,8	142	25,89			7,854	13,1
	t1	19	00:19							12,6							12,7
	t2	38	00:38	3,10	48				7,818	13,2	9,1	81				7,615	13,2
	t3	57	00:57						7,79	12						7,704	12,4
	t4	76	01:16	4,00	42				7,773	13,1	13,74	64				7,684	12,7
	t5	114	01:54						7,747	12,3						7,66	12,1
	t6	150	02:30	5,30	44				7,708	12,3	15,66	60				7,637	12,2
Aerobic	t7	190	03:10	2,71	38			5,48	7,825	12,5	10,68	54			5,2	7,803	12,6
	t8	228	03:48	0,69				4,91	7,748	12,8	4,67				4,28	7,794	12,9
	t9	304	05:04		32	12,28		4,31	7,606	13,8	0,573	42	14,88		3,42	7,695	14
	t10	342	05:42					5,79	7,549	12,4					4,74	7,664	12,5
	t11	380	06:20					5,8	7,495	12,7					4,64	7,606	12,9
	t12	440	07:20	0,81	31	1,375	18,42	5,2	7,395	12,6	0,124	35	4,62		6,01	7,526	12,6

Totals

	Total COD	
	1 TCOD [mg/l]	2 TCOD [mg/l]
t0	411	797
t6	423	778

K6:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	2,29	74,1	
Fermented supernatant	24,56	1704	68,82
Wastewater + Fermented	4,18	252,4	

Experiment

		Kinetics 1 WW cold temperature									Kinetics 2 WW + Fermented cold temperature						
	Sample number	Time [min]	Time [h:min]	1 PO4-P [mg/l]	1 SCOD [mg/l]	1 NH4-N [mg/l]	1 NO3 [mg/l]	1 DO [mg/l]	1 pH	1 T [°C]	2 PO4-P [mg/l]	2 SCOD [mg/l]	2 NH4-N [mg/l]	2 NO3 [mg/l]	2 DO [mg/l]	2 pH	2 T [°C]
Anaerobic	t0	0	00:00	2,080	66,7	21,27		0			3,29	177,3	24,84		0,1		14,4
	t1	19	00:19					0	7,88	12					0	7,631	12,6
	t2	38	00:38	2,010	43,2			0	7,86	12,1	12,89	89,4			0	7,595	11,6
	t3	57	00:57					0	7,825	12,9					0	7,591	12,4
	t4	76	01:16	3,150	36,8			0	7,787	12,2	20,2	59,4			0	0,582	11,7
	t5	114	01:54					0	7,72	12,3					0	7,545	11,9
	t6	150	02:30	5,160	35,3			0	7,697	12	25,2	48			0	7,542	11,9
Aerobic	t7	190	03:10	4,490	33,8			2,26	7,665	12,7	21,2	43			2,75	7,614	12,3
	t8	228	03:48					2,02	7,6	12,6					3,16	7,654	12,2
	t9	304	05:04	0,698	30,6	12,93		2,41	7,579	13,2	5,9	36,8	13,06		3,1	7,658	13,3
	t10	342	05:42					2,52	7,53	12,4					3,05	7,653	11,9
	t11	380	06:20	0,314	28,2	7,3	6,76	2,71	7,491	12,5	0,694	31,9	6,41	3,43	3,27	7,618	11,6
	t12	440	07:20					1,98	7,488	13,4					2,78	7,537	12,9

Totals

	Total COD		Total P	
	1 TCOD [mg/l]	2 TCOD [mg/l]	1 TP [mg/l]	2 TP [mg/l]
t0	357	642,5	9,25	11,5
t6	310,8	411	12,66	33,9
t11	161	272,4	8,6	9,06

K7:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	5,2	158	53,3
Fermented supernatant	35,94	2605	96
Wastewater + Fermented	6,21	265	56,6

Experiment

		Kinetics 1 WW cold temperature									Kinetics 2 WW + Fermented cold temperature						
	Sample number	Time [min]	Time [h:min]	1 PO4-P [mg/l]	1 SCOD [mg/l]	1 NH4-N [mg/l]	1 NO3 [mg/l]	1 DO [mg/l]	1 pH	1 T [∘C]	2 PO4-P [mg/l]	2 SCOD [mg/l]	2 NH4-N [mg/l]	2 NO3 [mg/l]	2 DO [mg/l]	2 pH	2 T [∘C]
Anaerobic	t0	0	00:00	4,61	143	46,54		0,15	8,474	12,6	5,65	249	50,7		0	7,959	12,7
	t1	19	00:19					0	8,318	12,1					0	7,948	12,2
	t2	38	00:38	10,05	90			0	8,204	12	12,9	144			0	7,905	12,4
	t3	57	00:57					0	8,113	12,5					0	7,855	12,6
	t4	76	01:16	14,70	86			0	8,042	12,4	21,38	106			0	7,83	12,5
	t5	114	01:54					0	7,952	12,1					0	7,776	11,9
	t6	150	02:30	17,82	66			0	7,759	12,5	28,24	90			0	7,631	12,3
Aerobic	t7	190	03:10	14,26	53			5,55	7,831	12,4	24,36	61			4,22	7,801	12,3
	t8	228	03:48					4,8	7,856	11,6					5,97	7,904	11,7
	t9	304	05:04	2,03	41			4,18	7,768	12,6	4,74	52			4,88	7,827	12,8
	t10	342	05:42					4,25	7,756	11,1					5,23	7,815	10,8
	t11	380	06:20	0,40	46	26,55	6,39	5,51	7,747	12,8	0,425	47	24,08	6,19	5,15	7,79	12,5
	t12	440	07:20														

Totals

	Total COD		Total P	
	1 TCOD [mg/l]	2 TCOD [mg/l]	1 TP [mg/l]	2 TP [mg/l]
t0	486	650	10,68	12,26
t6	454	470	25,5	38,04
t12	313	340	5,24	6,17

K8:

Initial conditions/characterization

	PO4-P [mg/l]	sCOD [mg/l]	NH4-N [mg/l]
Wastewater	2,65	81	25,97
Fermented supernatant	17,4	1375	40,86
Wastewater + Fermented	3,11	171	25,65

Experiment

				Kinetics 1 WW cold temperature							Kinetics 2 WW + Fermented cold temperature						
	Sample number	**Time [min]**	**Time [h:min]**	**1 PO4-P [mg/l]**	**1 SCOD [mg/l]**	**1 NH4-N [mg/l]**	**1 NO3 [mg/l]**	**1 DO [mg/l]**	**1 pH**	**1 T [°C]**	**2 PO4-P [mg/l]**	**2 SCOD [mg/l]**	**2 NH4-N [mg/l]**	**2 NO3 [mg/l]**	**2 DO [mg/l]**	**2 pH**	**2 T [°C]**
Anaerobic	**t0**	0	00:00	2,73	74	22,74		0,05	7,843	13	3,51	164	23,72		0,05	7,567	13
	t1	19	00:19														
	t2	38	00:38	2,56	42	20,29		0	7,821	12,4	9,41	79	22,03		0	7,744	12
	t3	57	00:57														
	t4	76	01:16	2,57	36			0	7,708	12,6	15,4	57			0	7,6	12,3
	t5	114	01:54														
	t6	150	02:30	3,82	39			0	7,605	12,9	18,38	52			0	7,555	12,6
Aerobic	**t7**	190	03:10	3,20	38	18,99		4,5	7,529	13,7	14,9	37	20,57		4,1	7,62	14,1
	t8	228	03:48														
	t9	304	05:04	2,73	27			5,02	7,308	12,9	3,74	33			4,63	7,443	12,2
	t10	342	05:42														
	t11	380	06:20	2,27	23	0,106	22,13	6	7,302	12,9	0,829	30	1,068	16,03	5,09	7,198	13,3
	t12	440	07:20														

Totals

	Total COD		Total P	
	1 TCOD [mg/l]	**2 TCOD [mg/l]**	**1 TP [mg/l]**	**2 TP [mg/l]**
t0	283	473	6,56	7,73
t6	229	319		
t11	176	28	5,63	4,75

Appendix 4: Online measurements of pH and DO

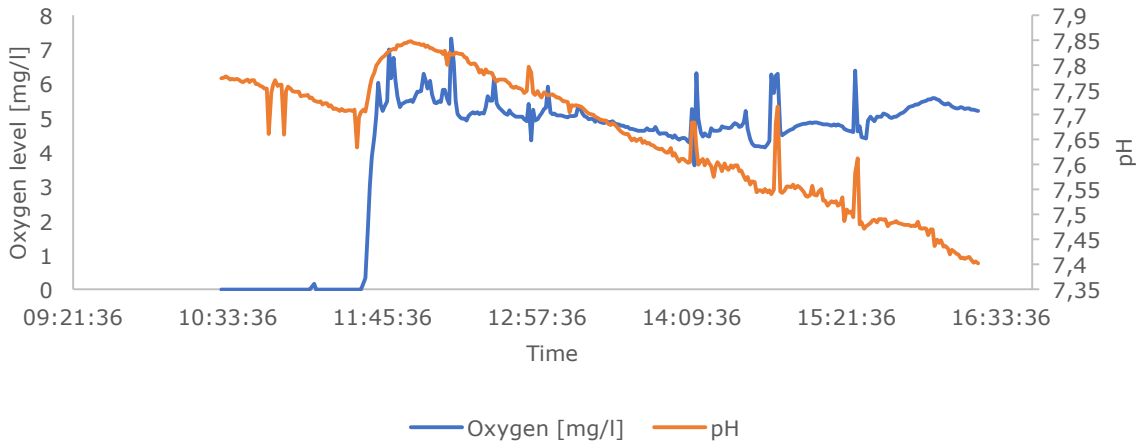

Figure shows DO and pH in K5

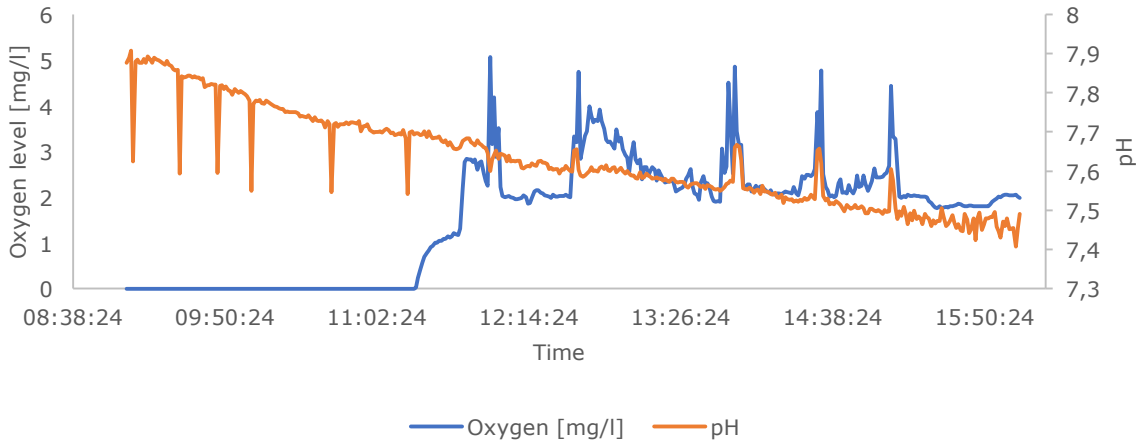

Figure shows DO and pH in K6

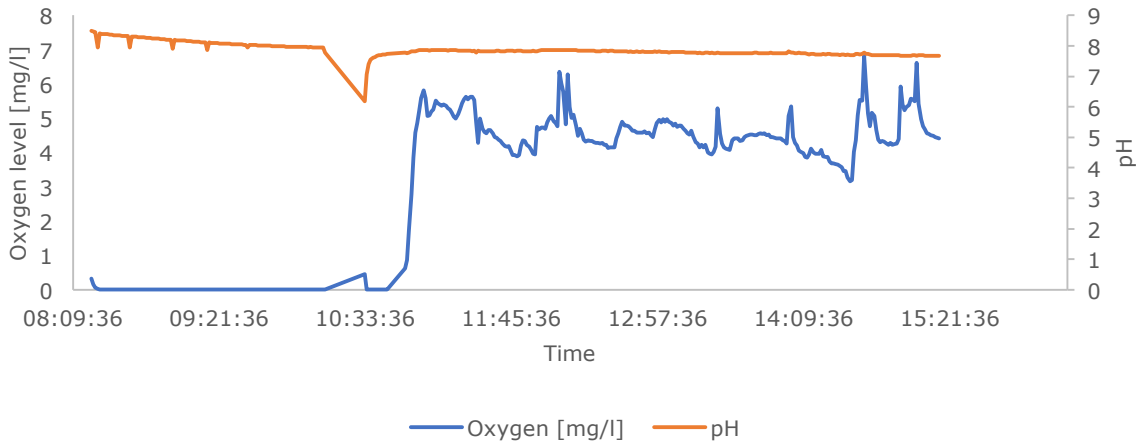

Figure shows DO and pH in K7

Appendix 5: Raw data from fermentation

F1:

	Time zero			
	TS		VS	
	[%]	[g/L]	[%]	[g/L]
Test 1	0,8964	9,085	0,8308	8,42
Test 2	0,9561	9,585	0,8933	8,955
Average	0,92625	9,335	0,86205	8,6875

Date	Day	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	pH	Temp. sample [◦C]	Temp. water [◦C]
06/11/2018	0	155	4,75	34,84			
07/11/2018	1	483	10,23	33,2		22,4	24,8
08/11/2018	2	725	11,82	32,2	6,178	22	25,2
09/11/2018	3	919	13,76	33	5,736	23,9	26,2
10/11/2018	4						
11/11/2018	5						
12/11/2018	6						
13/11/2018	7						
14/11/2018	8						
15/11/2018	9	1510	15,2	24,5	4,964		
16/11/2018	10	1770	16,45	27,1	4,974	24,3	27,8
17/11/2018	11						
18/11/2018	12						
19/11/2018	13						
20/11/2018	14						
21/11/2018	15						
22/11/2018	16						
23/11/2018	17						
24/11/2018	18						
25/11/2018	19						
26/11/2018	20						
27/11/2018	21	1780	12,47	0	4,972	21,5	24,4

F2:

	BATCH 1			BATCH 2		
	1	*2*	*Avg.*	*1*	*2*	*Avg.*
W_dish [g]	2,1768	2,1848		2,1814	2,1596	
W_sample [g]	36,6417	38,3337		35,2519	33,6243	
V_sample [ml]	40,0000	40,0000		40,0000	40,0000	
W_after105 [g]	2,6775	2,6539		2,5368	2,5558	
TS [g/l]	12,5175	11,7275	**12,12**	8,8850	9,9050	**9,40**
TS	1,37 %	1,22 %	**1,30 %**	1,01 %	1,18 %	**1,09 %**
W_after550 [g]	2,1973	2,2045	**2,2009**	2,1976	2,1762	**2,1869**
VS	95,91 %	95,80 %	**95,9 %**	95,44 %	95,81 %	**95,6 %**

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	pH	Temp. [◦C]
21/01/2019	0	10:30	397	8,07	43,43	7,121	16,5
21/01/2019	4,5	15:00	940	15,29	44,25	6,303	26
22/01/2019	22	08:30	1360	22,2	47,3	5,824	26,3
22/01/2019	28,5	15:00	1550	24,5	61,3	5,808	25,9
23/01/2019	46	08:30	1810	25,5	71,8	5,633	26
23/01/2019	47,5	10:00	1860	26,4	78,9	5,675	24,5

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	pH	Temp. [◦C]
23/01/2019	0	10:30	441	8,9	49	6,896	19,9
23/01/2019	4,5	15:00	530	10,25	48	6,718	25,3
24/01/2019	22	08:30	770	13,35	52,2	6,222	25,1
24/01/2019	28,5	15:00	840	13,96	49,7	6,083	26,3
25/01/2019	46	08:30	790	14,56	46,2	5,88	25,3
25/01/2019	47,5	10:00	800	14,81	38,4	5,83	26,2
Supernatant:							
28/01/2019	**119**	**09:30**	**1410**	**13,79**	**46,3**		

F3:

	1	*2*	*Avg.*
W_dish [g]	93,9580	91,6688	
W_sample [g]	38,5620	37,4568	
V_sample [ml]	40,0000	40,0000	
W_after105 [g]	94,4784	92,2538	
TS [g/l]	13,0100	14,6250	**13,82**
TS	1,35 %	1,56 %	**1,46 %**
W_after550 [g]	93,9882	91,6992	**92,8437**
VS	94,20 %	94,80 %	**94,5 %**

Date	**Hour**	**Time**	**SCOD [mg/l]**	**PO4-P [mg/l]**	**NH4-N [mg/l]**	**pH**	**Temp. [◦C]**
30/01/2019	0	10:30	471	10,38	45,34	6,73	16,7
30/01/2019	4,5	15:00	1220	21,9		6,169	25,6
31/01/2019	22	08:30	1710	29,1	60,5	5,599	26,2
31/01/2019	28,5	15:00	1100	21,5		5,696	26,2
01/02/2019	46	08:30	1390	24,3	56,8	5,555	24,3
04/01/2019	118	08:30	3920	38,8	104,7	4,962	28

F4:

	Duplicate	PS BATCH 1	BATCH 1	BATCH 2	BATCH 3	BATCH 4
W_dish [g]	1	96,3246	86,368	93,9628	95,8052	86,37
	2	93,9636	81,0142	96,3254	86,368	91,3554
W_sample [g]	1	38,8083	39,789	39,5643	39,212	38,3979
	2	40,1287	39,8468	40,1436	37,8705	39,0748
V-Sample [ml]	1	40	40	40	40	40
	2	40	40	40	40	40
W_105 [g]	1	98,6822	87,062	94,538	96,4924	87,0156
	2	96,5604	81,7274	97,0528	87,1946	92,0706
TS [g/l]	1	58,9400	17,3500	14,3800	17,1800	16,1400
	2	64,9200	17,8300	18,1850	20,6650	17,8800
TS snitt [g/l]		61,9300	17,5900	16,2825	18,9225	17,0100
TS	1	6,07 %	1,74 %	1,45 %	1,75 %	1,68 %
	2	6,47 %	1,79 %	1,81 %	2,18 %	1,83 %
TS snitt		6,27 %	1,77 %	1,63 %	1,97 %	1,76 %
W_550 [g]	1	96,4514	86,4094	94,006	95,8518	86,4042
	2	94,0973	81,052	96,375	86,4174	91,404
VS	1	94,62 %	94,03 %	92,49 %	93,22 %	94,70 %
	2	94,85 %	94,70 %	93,18 %	94,02 %	93,20 %
VS snitt		94,74 %	94,37 %	92,84 %	93,62 %	93,95 %

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
13/02/19	0	10:00	222	5,9	37,56	26800			7,26	17,3
	5	15:00	440	8,25	37,95				7,054	19,8
14/02/19	22,5	08:30	675	11,35	42,15				6,573	20,1
	29	15:00	850	13,83	49,4				6,391	21,2
15/02/19	48	10:00	660	11,41	40,95				6,269	19,4

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
15/02/2019	0	11:00	455	7,53	35,07	16900	29,05	137	6,882	17,7
15/02/2019	4	15:00	558	8,42					6,729	20,9
16/02/2019	26,5	13:30	582	8,15	33,67				6,421	20,5
17/02/2019	47	10:00	594	7,61	30,31				6,509	19,8

BATCH 3

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
17/02/2019	0,00	10:30	404	5,57	26,87	12800	46,8	162	6,949	17,4
17/02/2019	4,50	15:00	624	7,71					6,705	19,6
18/02/2019	22,00	08:30	742	9,45	31,85				6,31	20,3
18/02/2019	28,50	15:00	800	10,39					6,25	21
19/02/2019	47,00	09:30	891	10,64	32,18				6,289	19,6
Supernatant:										
19/02/2019	47,50	10:00	918	10,4	31,69					

BATCH 4

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
19/02/2019	0	10:00	506	6,94	30,93	2700	57,2	147	6,919	18,2
19/02/2019	5	15:00	724	8,97					6,662	20,2
20/02/2019	22,5	08:30	600	8,69	27,67				6,353	19,7
20/02/2019	29	15:00	930	10,99					6,338	20,1
21/02/2019	47,5	09:30	959	10,10	34,54				6,48	19,6

F5:

		BATCH 1	BATCH 2	BATCH 3	BATCH 4
W_dish [g]	**1**	95,9582	96,108	86,3668	94,98
	2	91,172	96,1028	85,5828	80,635
W_sample [g]	**1**	39,9052	38,9945	40,3806	39,2859
	2	39,3375	39,0665	40,3449	39,4782
V-Sample [ml]	**1**	40	40	40	40
	2	40	40	40	40
W_105 [g]	**1**	96,7484	96,8488	87,2164	95,718
	2	92,1594	96,897	86,5734	81,403
TS [g/l]	**1**	19,7550	18,5200	21,2400	18,4500
	2	24,6850	19,8550	24,7650	19,2000
	Snitt	**22,2200**	**19,1875**	**23,0025**	**18,8250**
TS	**1**	1,98 %	1,90 %	2,10 %	1,88 %
	2	2,51 %	2,03 %	2,46 %	1,95 %
	Snitt	**2,25 %**	**1,97 %**	**2,28 %**	**1,91 %**
W_550 [g]	**1**	96,0034	96,152	86,422	95,0254
	2	91,2236	96,1508	85,6414	80,6778
VS	**1**	94,28 %	94,06 %	93,50 %	93,85 %
	2	94,77 %	93,96 %	94,08 %	94,43 %
	Snitt	**94,53 %**	**94,01 %**	**93,79 %**	**94,14 %**

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
22/02/2019	0	09:00	300	6,4	32,04	7300	43,2	226	6,967	16,7
22/02/2019	6	15:00	782	15,1	39,5				6,367	26,5
23/02/2019	29,5	14:30	1458	25	64,42				5,701	26,9
24/02/2019	48	09:00	1416	23,1	64,24				5,453	28

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
24/02/2019	0	10:00	804	15,69	44,16	5540	63	290	5,943	22,6
24/02/2019	6	16:00	1324	21,3					5,699	27,9
25/02/2019	23	09:00	1536	25,34	67,1				5,45	28,2
25/02/2019	29	15:00	1799	27,04					5,395	28,1
26/02/2019	46,5	08:30	1971	26,86	74,66				5,2	25,7
Supernatant:										
26/02/2019		**09:00**	**1704**	**24,56**	**68,82**					

BATCH 3

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
26/02/2019	0	09:00	1005	16,82	48,38	4300	37,8	252	5,895	24,6
26/02/2019	4,5	13:30	1158	19,1					5,556	28
27/02/2019	23,5	08:30	1538	23,02	60,44				5,308	26,6
27/02/2019	30	15:00	1702	24,38					5,351	26
28/02/2019	48	09:00	2097	25,36	65,92				5,206	26,5

BATCH 4

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
28/02/2019	0	10:00	950	15,2	39,52	7020	47,8	260	5,67	21,6
28/02/2019	4,5	14:30	1050	18,82	40,72				5,481	27,5
01/03/2019	22,5	08:30	1424	23,42	51,1				5,349	26,3
01/03/2019	29	15:00	1374	22,86					5,282	27,7
02/03/2019	47,5	09:30	1538	23,06	46,98				5,182	26,6

F6:

		BATCH 1	BATCH 2
W_dish [g]	**1**	85,5832	99,9538
	2	96,3254	95,9562
W_sample [g]	**1**	39,85	39,4501
	2	39,551	39,5754
V-Sample [ml]	**1**	40	40
	2	40	40
W_105 [g]	**1**	86,395	100,9174
	2	97,194	96,8898
TS [g/l]	**1**	20,2950	24,0900
	2	21,7150	23,3400
TS snitt [g/l]		**21,0050**	**23,7150**
TS	**1**	2,04 %	2,44 %
	2	2,20 %	2,36 %
TS snitt		**2,12 %**	**2,40 %**
W_550 [g]	**1**	85,6242	100,0064
	2	96,368	96,0074
VS	**1**	94,95 %	94,54 %
	2	95,10 %	94,52 %
VS snitt		**95,02 %**	**94,53 %**

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
07/03/2019	0	08:30	339	6,28	30,69	6510	77,9	207	6,775	17,2
	6,5	15:00	930						6,273	27,4
08/03/2019	24	08:30	1896	32,82	72,26				5,942	26,1
	30,5	15:00	1677						5,91	27
09/03/2019	51,5	12:00	1977	32,66	89,78				5,653	26,5
10/03/2019	78,5	15:00	2457						5,374	26,7
11/03/2019	96	08:30	2811	37,7	102				5,249	26,5

Supernatant:

11/03/2019	**97**	**09:30**	**2605**	**35,94**	**96**

BATCH 2

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
11/03/2019	0	09:30	1416	23,02	67,48	12290	100	460	5,983	20,7
	3,5	13:00	1710						5,55	27,2
12/03/2019	23	08:30	1834	28,84	62,4				5,438	25,7
	28,15	13:45	2100						5,335	27
13/03/2019	47	08:30	2496	31,29	84				5,23	26,2
	52,5	14:00	3215						5,185	26,8
14/03/2019	71	08:30	3615	39,09	105,75				5,137	26
	77,5	15:00	3220						5,094	26,9
15/03/2019	95	08:30	2330	28,65	76,35				4,991	25,3
Supernatant:										
15/03/2019	96		3385	36,6	98,31					

F7:

		BATCH 1 Add
W_dish [g]	**1**	91,6834
	2	91,3626
W_sample [g]	**1**	39,8028
	2	38,8703
V-Sample [ml]	**1**	40
	2	40
W_105 [g]	**1**	92,3332
	2	92,0044
TS [g/l]	**1**	16,2450
	2	16,0450
TS snitt [g/l]		**16,1450**
TS	**1**	1,63 %
	2	1,65 %
TS snitt		**1,64 %**
W_550 [g]	**1**	91,717
	2	91,381
VS	**1**	94,83 %
	2	
VS snitt		**94,83 %**

BATCH 1

Date	Hour	Time	SCOD [mg/l]	PO4-P [mg/l]	NH4-N [mg/l]	TCOD [mg/l]	TP [mg/l]	TN [mg/l]	pH	Temp. [◦C]
25/03/2019	0	08:30	252	4,27	13,23	2100	35,2	90,5	6,861	15,2
26/03/2019	24	08:30	1497	16,18	34,75				5,788	27,3
27/03/2019	48	08:30	1160	15,78	41,12				5,61	28,1
28/03/2019	72	08:30	1392	16,6	41,68				5,571	28,5
29/03/2019	96	08:30	1274	16,11	38,07	3700			5,303	28,5
Supernatant:										
29/03/2019			**1375**	**17,4**	**40,86**					

Appendix 6: Theoretical chemical oxygen demand

Example of calculations of acetic acid after 4 days in F7:

At day 4, acetic acid was analyzed to be 5.09 mmol/l at NMBU.

Acetic acid

Chemical formula: CH3COOH

Balanced equation: C2H4O2+2O2->2CO2+2H2O

Molar mass: 60.05 g/mol

Oxygen need: 2*(16+16) = 64

g COD/g acid: 64 * 60.05 = 1.07

Acetic acid = 5.09 mmol/l * 60.05 mg/mmol = 305.66 mg/l

Acetic acid as TOD = 305.66 mg/l * 1.07 = 325.76 mg/l

Appendix 7: Carrier characterization

			Total Solids (TS) or TSS						
Date	Carriers	Distilled water	Dish [g]	Clean filter [g]	Volume [ml]	Filter + dish after 1h in 105 [g]	TS [g/l]	mg TS/ carrier	mg TS average
			2,3720	0,1220	25	2,3925	0,8200	4,1000	
19/02/19	20	100	2,3504	0,1222	25	2,3711	0,8280	4,1400	4,1200
			2,1904	0,1231	25	2,3296	0,6440	3,2200	
26/02/19	20	100	2,2262	0,1233	25	2,3642	0,5880	2,9400	3,0800
			2,1897	0,1212	25	2,3320	0,8440	4,2200	
14/03/19	20	100	2,2260	0,1222	25	2,3682	0,8000	4,0000	4,1100
			2,1520		25	2,1734	0,8560	4,2800	
26/03/19	5	25							4,2800
			2,1575		25	2,1735	0,6400	3,2000	
02/04/19	5	25							3,2000

Volatile Solids (VS) or VSS							
Filter + dish after 0,5h in 550 [g]	VS [g/l]	mg VS/ carrier	mg VS average	g VS/ carrier	g VSS/L	g VS/l average	% VS
2,3145	0,6040	3,0200		0,0030	2,1382		
2,3506	0,5440	2,7200	2,8700	0,0027	1,9258	2,0320	0,9318
2,3135	0,7400	3,7000		0,0037	2,6196		
2,3500	0,7280	3,6400	3,6700	0,0036	2,5771	2,5984	0,8929
2,1508	0,0226	0,1130		0,0001	0,0800		
			0,1130			0,0800	
2,1551	0,7360	3,6800		0,0037	2,6054		
			3,6800			2,6054	

*ERROR!! Dish has lower weight after burning and give wrong results…

Total Phosphorous (TP)				Comment
TP [mg/l]	**mg TP/ carrier**	**mg TP/ mg TS**	**mg TP/ mg TVS**	
22,0000	0,1100	0,0267	0,030	Filter (TSS)
51,3000	0,2565	0,0833	0,093	Filter (TSS)
28,7500	0,1438	0,0350	0,039	Filter (TSS)
33,4500	0,1673	0,0391	0,043	No filter
38,7500	0,1938	0,0605	0,067	No filter

Total Solids (TS)									
Carriers	**Date**	**Dish for carriers with biomass**	**Biomass After 24h in 105**	**Dish for cleaned carriers**	**Cleaned After 24h in 105**	**TS [g]**	**mg TS/ Carrier**	**g TS/l**	**g VS/l**
15	19/02/19	91,1620	93,2994	96,1094	98,1234	0,1234	8,2267	5,8245	5,2420
15	26/03/19	90,1908	92,2928	85,8314	87,8128	0,1206	8,0400	5,6923	5,1231
15	02/04/19	90,1972	92,2518	85,8270	87,7780	0,1036	6,9067	4,8899	4,4009