Biološko uklanjanje fosfora iz komunalne otpadne vode grada Trondheima

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BIOLOGICAL PHOSPHORUS REMOVAL FROM THE MUNICIPAL WASTEWATER OF THE CITY OF TRONDHEIM

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Biological Phosphorus Removal from the Municipal Wastewater of the City of Trondheim

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Summary:
Over the past years the process of the biological removal of nutrients in the wastewater treatment has become more and more scrutinizied in the world. The nutrients like phosphorus and nitrogen cause eutrophication and, consequently, have a detrimental effect on the environment. So, in this thesis the processes of the biological removal of phosphorus in a Moving Bed Biofilm Reactor (MBBR) and the Enhanced Biological Phosphorus Removal (EBPR) were examined. Also, the concentration of dissolved oxygen, temperature, pH, COD and phosphorus in typical Norwegian wastewater obtained from sewage system of city of Trondheim were measured on a daily basis. Moreover, the kinetics of phosphorus removal process was performed in several experiments using a batch bioreactor and the optimal concentration of dissolved oxygen and the activity of bacteria under specific operating conditions were investigated. The results shown that the Polyphosphate-Accumulating Organisms (PAOs) play a major role in the biological removal of phosphorus. The relations among wastewater quality (influent) and the wastewater treatment process parameters on phosphorus biological removal were also determined.

Key words: wastewater treatment, MBBR, EBPR, phosphorus, PAOs

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Biološko uklanjanje fosfora iz komunalne otpadne vode grada Trondheima
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Sažetak:
Posljednjih godina, mnogobrojna znanstvena istraživanja ispituju mogućnost izdvajanja hranjivih tvari iz otpadnih voda u cilju njihove oporabe te smanjenja visokih koncentracija hranjivih tvari u pročišćenoj vodi, poput fosfora i dušika, jer pospješuju pojavu eutrofikacije i štetno utječu na vodne cjeline. Rezultati navedenih istraživanja također pokazuju da hranjive tvari izdvojene biološkim postupkom iz otpadnih voda imaju bolja svojstva biosorpcije u okolišu. Cilj ovog rada je ispitati učinkovitost uklanjanja fosfora iz otpadne vode grada Trondheima primjenom tehnologije bioreaktora s pokretnim filmom (Moving Bed Biofilm Reactor, MBBR) i tehnologijom naprednog biološkog uklanjanja fosfora (EBPR). Svakodnevne analize kakvoće ulazne otpadne vode (koncentracije otopljenog kisika i fosfora, temperatura, pH, KPK) omogućile su određivanje učinkovitosti i kinetike EBPR procesa. Uklanjanje fosfora iz otpadne vode ispitano je u šaržnom bioreaktoru u ovisnosti o koncentraciji otopljenog kisika i aktivnost bakterija u specifičnim radnim uvjetima. Rezultati su pokazali da je za učinkovito uklanjanje fosfora iz otpadne vode MBBR i EBPR procesom najznačajnija aktivnost fosfor akumulirajućih mikroorganizama (PAO). Također su utvrđeni i odnosi između kakvoće ulazne otpadne vode i procesnih parametara obrade otpadnih voda pri biološkom uklanjanju fosfora iz otpadne vode grada Trondheima.

Ključne riječi: obrada otpadne vode, MBBR, EBPR, fosfor, PAOs

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List of Abbreviations

APAOs	Aerobic PAOs
BOD	Biochemical Oxygen Demand
DAF	Dissolved air floatation
DNPAOs	Denitrifying PAOs
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
GAOs	Glycogen Accumulating Organisms
MBBR	Moving Bed Biofilm Reactor
MLSS	Mixed Liqour Suspended Solids
MLVSS	Mixed Liqour Volatile Suspended Solids
NTNU	Norwegian University of Science and Technology
N_2	Nitrogen Gas
OHOs	Ordinary Heterotrophic Organisms
Ortho-P	Orthophosphate
P	Phosphorus
PAOs	Polyphosphate Accumulating Organisms
PHA	poly-β-hydroxy-alkanoates
PHB	poly-β-hydroxy-butyrate
rpm	revolutions per minute
SCOD	Soluble Chemical Oxygen Demand
SVI	Sludge Volume Index
TP	Total Phosphorus
VFAs	Volatile Fatty Acids

WWTP	Wastewater Treatment Plant

Preface

Firstly, I would like to express my sincere gratitude to my dear mentor, Mirna Habuda Stanić, for giving me the opportunity to travel to Norway and do the research needed for this thesis. Special thanks for her guidance, encouragement, and support throughout this research. I cannot imagine a better mentor than her.

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1. INTRODUCTION

The development of settlements and growth of population standards cause pollution in the environment, especially in the water.

The consumption of water has been growing over the past years which, consequently, affects an increase in the amount of wastewater. The wastewater is defined as any water used in homes, factories, businesses, etc., i.e. it is contaminated water which must be collected and treated as wastewater. The characteristics of wastewater vary depending on the source. It contains fats and oils, suspended particles, odors, so it harms all the flora and fauna. In other words, a discharge of wastewater, which contains significant amounts of nutrients such as phosphorus and carbon, into the environment has a negative impact on natural ecosystems. For example, one of the negative outcomes of excessive nutrient discharge is eutrophication, which is the growth of bacteria and algae that consume large amounts of oxygen due to the breakdown of discharged P and N. The process of eutrophication has become a global problem with consequences varying from decrease in the aesthetics of the water to serious medical threats due to toxicity (Seviour et al., 2003).

Phosphorus (P) removal, which can be both chemical and biological, is a key process in preventing eutrophication. It is also important to mention that biological methods of phosphorus removal with activated sludge have been increasingly used in practice.

Therefore, in this paper, the focus is on the biological removal of phosphorus from municipal wastewater using activated sludge and plastic carriers containing Polyphosphate-accumulating organisms.

The research was carried out in Norway at Norwegian University of Science and Technology (NTNU) in a Wastewater Laboratory under various conditions. Phosphorus is an essential nutrient for all life forms, but also for food production. The focus on P as a non-renewable recourse and the necessity for recovering and recycling of P from nutritious wastewater has improved the technology and knowledge related to sustainable use of P. Wastewater could possibly be a major source of P recycling for global P sustainability (Naidu et al., 2012).

2. THEORETICAL PART

2.1. Wastewater

In a simple definition, wastewater is water that is polluted and contaminated in any way. The extent of its pollution is reflected in the amount of harmful substances that water contains. There are few types of wastewater that are distinguished according to the origin of the harmful substances contained in it (**Fig. 1**).

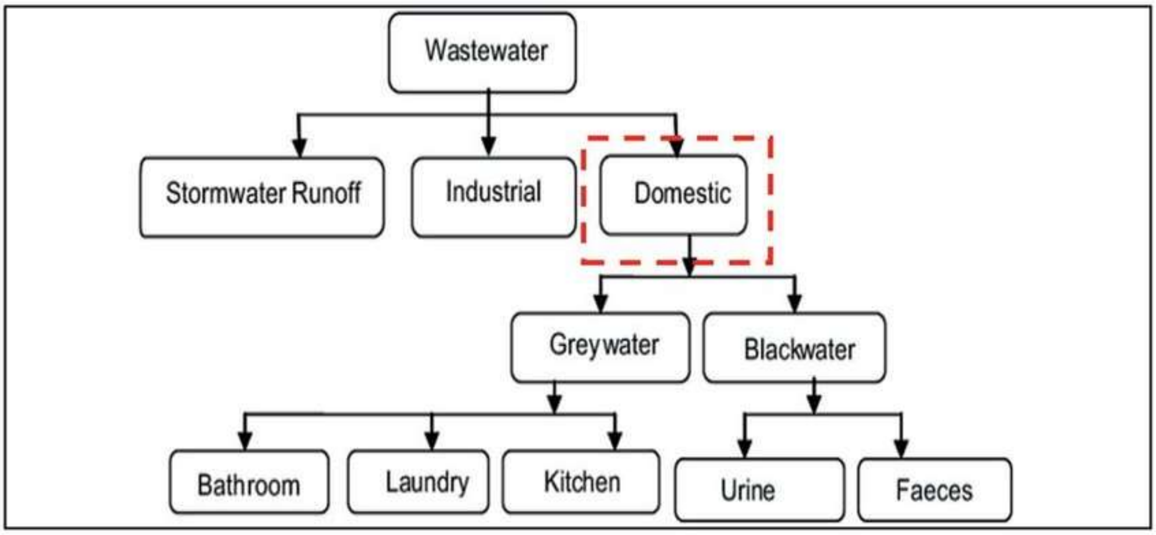

Figure 1 Types of wastewater
(Source: https://www.researchgate.net/figure/Wastewater-types-19_fig1_335218787)

The pollutants reach the water directly and indirectly. On the one hand, direct forms imply the formation of special wastewater into which man injects harmful substances and which flows directly into river flows. On the other hand, water is indirectly polluted in the process of leaching harmful chemicals from the soil. The water contamination with toxic substances and pathogens also has indirect effects on mankind. In other words, highly polluted water cannot be used for drinking nor for irrigating agricultural land (Kitanović et al., 2013).

Furthermore, wastewater reflects the image of social relations and production methods, i.e. where there is a strong water industry, there are different ingredients than in areas where agriculture is prevalent. Also, in areas where a large number of washing machines is used, significantly more phosphate is being released into the water from the laundry detergent than in areas where the laundry is still being washed with only soap and hands (https://www.dw.com/hr/odvajanje-fosfora-iz-otpadnih-voda/a-2778019).

2.2. The Composition of Wastewater

In the chemical context wastewater contains both organic and inorganic components. To be more specific, it contains a large number of microscopic organisms, mostly bacteria that are capable of consuming the organic component as well as different types of solids that present a major challenge for treatment, operation and disposal. The amount of solids in wastewater is expressed as a concentration in milligrams per liter or parts per million. Suspended solids and biological oxygen demand (BOD) are the most frequent measured feautures in wastewater.

The Norwegian wastewater is typically cold and diluted due to the high amount of precipitation and runoff during transportation. The configuration of the transport system affects the degree of dilution as combined transport system allows the wastewater to be diluted by storm water. Moreover, the concentrations found in wastewater are a combination of pollutant load and the amount of water with which the pollutant is mixed (Lagesen, 2017).

2.2.1. Solids In Wastewater

Organic solids

Organic solids occupy 50 % of domestic wastewater. This fraction is generally of animal or plant origin, i.e. dead animal matter, plant tissue or organisms, but it may also include synthetic organic compounds. All of the aforementioned substances contain a carbon, hydrogen and oxygen component and can be combined with nitrogen, sulfur or phosphorus. Fats, proteins and carbohydrates are basic contents in domestic wastewater (Muralikrishna et al., 2017).

Inorganic solids

Inorganic solids are substances that are inert and not subject to decay. These are mineral compounds, such as sulfates, sand, silt and gravel. They also include mineral salts in the water supply that produce the hardness and mineral content of the water.

Suspended solids

Suspended solids, which contain 70 % organic and 30 % inorganic solids, are visible in water and can be removed using physical or mechanical methods, such as sedimentation or filtration. They include the larger floating particles and consist of sand, clay, grit, paper, fecal solids, pieces of wood, food, garbage residues and similar material.

Settleable solids

Settleable solids are the portion of the suspended solids that are of sufficient size and weight to settle in a given period of time, usually one hour (they will be settled in an Imhoff Cone in one hour). The content is expressed in milliliters of settled solids per liter of wastewater. Settleable solids are approximately 75 % organic and 25 % inorganic.

Colloidal suspended solids

Colloidal suspended solids are types of solids that have not completely dissolved and precipitated to the bottom. They are about 65 % organic and 35 % inorganic, and also subject to rapid decay. Nevertheless, they play an important role in the treatment and disposal of wastewater.

Dissolved solids

Unlike suspended and colloidal solids, dissolved solids are smaller in size. About 90 % of the total dissolved solids are in true solution while about 10 % are colloidal. Dissolved solids, as a whole, are about 40 % organic and 60 % inorganic in nature.

Total solids

Total solids, as the term implies, include all of the solid constituents of a wastewater. Furthermore, they are the total of the organic and inorganic solids or the total of the suspended and dissolved solids. In average domestic wastewater, total solids are about half organic and half inorganic, and about two-thirds in solution (dissolved) and one-third in suspension. The organic solids, which are subject to decay, constitute the main problem in wastewater treatment (Muralikrishna et al., 2017).

2.2.2. Determination of total solids

Total solids can be determined by driving off the water fraction whereas suspended solids may be determined by filtering out the solid fraction on a porous pad and drying. On the other hand, settleable solids may be determined by leaving a sample to settle in the Imhoff cone apparatus. It is important to note that any analysis of wastewater composition can provide only an average composition.

2.3. Wastewater Treatment

Wastewater treatment is the process of removing pollutants from water discharged from domestic, industrial and commercial areas as well as a surface runoff.

The treatment typically includes mechanical, biological and chemical processes in order to remove contaminants and is, therefore, necessary before the water discharge.

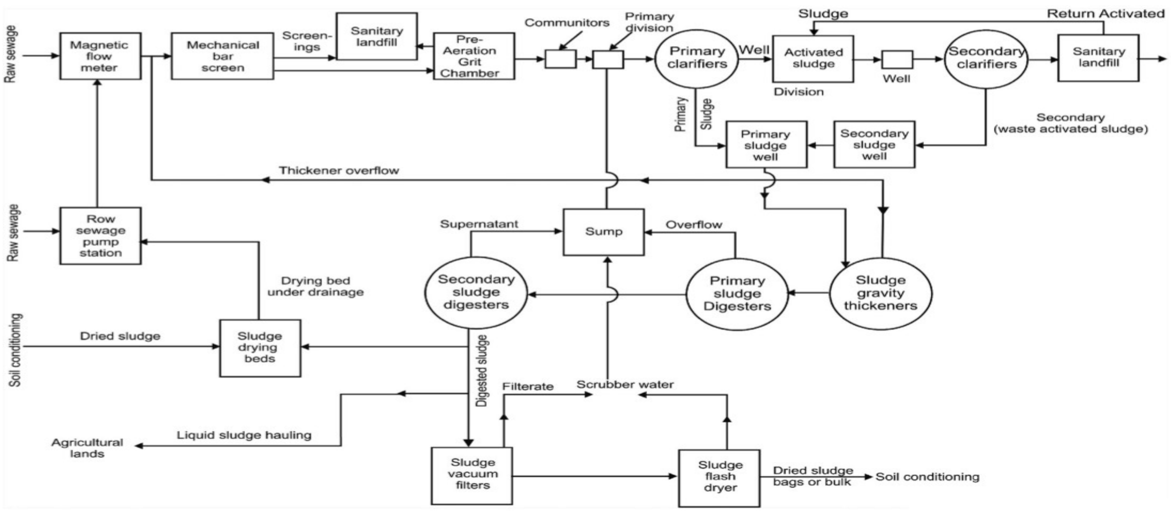

Figure 2 Wastewater treatment plant floc diagram
(Source: Principles of Design and Operations of Wastewater Treatment Pond Systems for Plant Operators, Engineers, and Managers)

2.3.1. Wastewater Collection

The first step in the wastewater treatment process is wastewater collection. The collection systems are located in households, industries and other facilities and go to a central water collection unit. The water goes to the wastewater treatment plant using underground drainage systems or by exhauster tracks. Wastewater transport must be carried out under hygienic conditions (www.conserve-energy-future.com).

What's more, the length of time required for the wastes to reach a treatment facility is important to prevent settling of solids which tend to clog pipes and cause odors. The plant must contain manholes for cleaning and inspection. Pump stations lift the wastewater to a higher elevation, so people who operate the plant must comply with the rules and wear protective clothing (Muralikrishna et al., 2017).

2.3.2. Odor Control

Wastewater contains many odor-causing substances and it is, therefore, very important for the plant to control the odor. To procure that the surrounding areas are free of the odors, treatment processes are initiated at the treatment plant. All odor sources are treated and neutralized with chemicals (www.conserve-energy-future.com).

2.3.3. Screening

The following step in the wastewater treatment is screening. It involves removing large objects such as sanitary items, plastics, cotton buds, nappies, diapers, rags, face wipes, broken bottles or bottle tops which can damage both equipment and plant (www.conserve-energy-future.com).

2.3.4. Preliminary Wastewater Treatment

The most important objective of preliminary treatment process is protecting the pumping equipment and facilitating subsequent treatment processes. In order to achieve that, there are few devices used: screens (rack, bar), comminuting devices (grinders, cutters, and shredders), grit chambers and preaeration tank (Muralikrishn et al., 2017).

In other words, leaving the bar screen, the wastewater flow is slowed down entering the grit tank to concede sand, gravel and other heavy materials which are small enough to hold on the bottom grid. All the collected remains from the grit tank and grid or bar screen is deferred at a sanitary landfill (Xagoraraki, 2016).

2.3.5. Primary Wastewater Treatment

In the primary wastewater treatment process, fats, oils and solids are physically separated. The screened wastewater flows into a primary settling tank where it stays for several hours until all the solid particles settle and the fats and oils float to the surface on the water (Xagoraraki, 2016).

Therefore, the purpose of primary treatment is to reduce the velocity of the wastewater sufficiently to permit solid to settle and floatable material to surface (Muralikrishna et al., 2017).

2.3.5.1. Settling/Sedimentation of Wastewater

The process of solid liquid separation in which the suspension is separated into two phases is called settling.

- Clarified supernatant leaving the top of the sedimentation tank (overflow).
- Concentrated sludge leaving the bottom of the sedimentation tank (underflow).

The purposes of settling are:

- removal of coarse dispersed phase,
- removal of coagulated and flocculated impurities,
- removal of precipitated impurities after chemical treatment,
- settling of sludge (biomass) after activated sludge process/ trickling filters (Xagoraraki, 2016).

2.3.5.2. Dissolved Air Floatation

Dissolved Air floatation (DAF) is a new method used for removing suspended particulates from sewage wastewater. DAF is achieved by blowing air under pressure and then releasing the air at atmospheric pressure into a floatation tank. The contact zone is located at the front end of the DAF tank. The floc-bubble aggregates are then carried by water into the second DAF zone called “separation zone”. Free bubbles and floc-bubble aggregates ascent to the surface of the tank forming a concentrated sludge blanket that can be removed by skimming devices. The DAF method is cost effective and much faster than other classic methods (Gerba et al., 2019).

2.3.6. Secondary Wastewater Treatment

Secondary wastewater treatment involves biological degradation in which the remaining amount of suspended solids is decomposed by microorganisms and the number of pathogens is significantly reduced. At this stage, the effluent from the primary treatment is usually subject to biological treatment in a trickling filter bed, an aeration tank and sewage lagoon (Gerba et al., 2019).

2.3.6.1. Trickling Filters

Trickling filters are wastewater treatment plants made of plastic units or bed of stones through which wastewater drips. It is one of the oldest ways presented as a biological treatment. In addition, the effluent is pumped through an overhead sprayer onto the filter bed, where bacteria and other microorganisms form a biofilm on the filter surfaces. These microorganisms intercept the organic material as it trickles past and decompose it aerobically. The media used in trickling filters may be ceramic material, plastic media, hard coal or stones, when organic matter passes through trickling filter, it is converted into biomass, which forms a thick biofilm on the filter medium. The biofilm that forms on the surface of the filter medium is called zoogleal film. It is composed of bacteria fungi, algae and protozoa. The increase in biofilm thickness leads to limited oxygen diffusion to the deeper layers of the biofilm, creating an anaerobic environment near the filter medium surface. Bod removal is about 85 % for low-rate filters. The effluent from the trickling filter passes into a final clarifier to further separate solids from effluent (Gerba et al., 2019).

Trickling filters can be classified as low-rate, high-rate and super-rate, primarily based on hydraulic and organic loading rates. The hydraulic loading rate is the total flow including recirculation applied on unit area of the filter in a day, while the organic loading rate is the 5-day 20°C BOD, excluding the BOD of the recirculant, applied per unit volume in a day. In high and super rate filters a part of the settled or filter effluent is recycled through the filter which is not the case with low-rate filters. Besides, high-rate filters can be single-stage and two-stage filters. Two-stage filtration will provide a higher degree of treatment than the single-stage for the same total volume of media (Muralikrishna et al*.,* 2017).

2.3.6.2. Conventional Activated Sludge

An activated sludge process is also known as aeration-tank digestion. To explain, the primary effluent is pumped into a tank and then mixed with the activated sludge. Afterwards, the air or pure oxygen is blown into the mixture causing bacteria growth and decomposition of the organic material. After that, it goes to a secondary settling tank where the water is poured on top of the tank and the sludge is removed from the bottom. The sludge known as secondary sludge is added to primary sludge and is subsequently anaerobically digested to produce bio solids. As a result, biomass produced by recycling activated sludge contains a large number of microorganisms that oxidize organic matter in a very short time. The detention time in the aeration basin varies from 4 to 8 hours.

The contents of the aeration tank is called mixed liquor suspended solids (MLSS) whereas the organic part included in MLSS is called mixed liquor volatile suspended solids (MLVSS). The activated sludge process must be regularly and properly controlled to maintain a ratio of substrate to microorganisms or food to microorganism ratio (F/M). This is shown as BOD per kilogram per day.

$$\frac{F}{M} = \frac{Q \cdot BOD}{MLSS \cdot V} \qquad (1)$$

Where:

Q - flow rate of sewage in million gallons per day

BOD_5 - 5 day biochemical oxygen demand (mg/L)

$MLSS$ - mixed liquor suspended solids (mg/L)

V - volume of aeration tank (gallons)

The F/M ratio is controlled by the rate of activated sludge wasting; the higher the wasting rate, the higher the F/M ratio while low F/M ratio indicates that microorganisms are starving in the aeration tank. There are two functions of the final sedimentation tank: clarification and thickening. Sludge deposition capacity is determined using the sludge volume index (SVI).

$$SVI = \frac{V \cdot 1000}{MLSS} \quad (2)$$

Where:

V - volume of settled sludge after 30 minutes (ml/L).

The microbial biomass produced in the aeration tank must settle properly from suspension and a mean cell residence time of 3 − 4 days is necessary for effective settling. However, a common problem in the activated sludge process is filamentous bulking which is usually caused by the excessive growth of filamentous microorganisms consisting of slow settling and poor compaction of solids in the clarifier. A high SVI (>150 ml/mg) indicates bulking conditions, and filamentous bacteria are able to survive under conditions of low dissolved oxygen, low F/M, low nutrient and high sulfide levels. However, these bacteria can be controlled by treating the return sludge with chlorine hydrogen peroxide (Gerba et al., 2019).

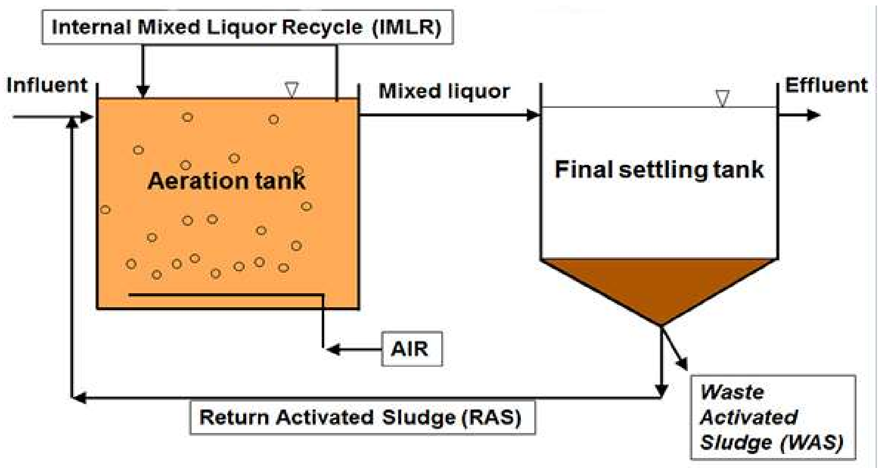

Figure 3 Activated sludge control process
(Source: https://www.ysi.com/ysi-blog/water-blogged-blog/2016/10/activated-sludge-three-steps-to-improve-your-process efficiency)

2.3.6.3. Nitrification

Nitrification is an aerobic process in which autotrophic nitrifying bacteria participate. It is a two-stage biological oxidation process; in the first stage of nitrification, ammonia to nitrite oxidation occurs. The reaction is catalyzed by the enzymes ammonia monooxygenase and hydroxylamine oxidoreductase. The nitrifying bacteria of the genus *Nitrosomonas* are deserving. In the second stage of nitrification, nitrite to nitrate oxidation takes place. The reaction is catalyzed by the enzyme nitrite oxidoreductase with nitrifying bacteria of the genus *Nitrobacter*. An effective nitrification process is when the carbon to wastewater ratio is <0.25 (Velić, 2016).

2.3.6.4. Denitrification

Denitrification is the dissimilatory transformation of nitrate or nitrite into gaseous nitrogen while conserving energy. It involves several reduction steps catalyzed by appropriate enzymes. The bacterial lobes that carry out denitrification are heterotrophic denitrifying bacteria, archebacteria and fungi. The enzymes involved in denitrification are nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. The reduction of nitrite to N_2 is a crucial step as it translates the fixed form of nitrogen into gaseous nitrogen (Velić, 2016).

2.3.6.5. Phosphorus Removal from Wastewater

To avoid the negative effect on ecosystem, as the one eutrophication creates, some measures, as phosphorous removal from wastewater, must be done, so that the load into aquatic ecosystems is not above the ecosystems bearing capacity. There is a two main ways for phosphorous removal from wastewater: (i) biological tretatment and (ii) chemical precipitation. However, within both methods there are several process configurations that operators can use. These differing configurations can all remove phosphorous with different input parameters, operating conditions and at vastly differing costs.

In wastewater phosphorous occurs as soluble, particulate or organically bounded. Soluble form of phosphate present in wastewater are the orthophosphate, while depending on the solution pH, phosphorous can appear in different forms like phosphate ions or phosphoric acid. Polyphosphates are particulate phosphorous fraction which can be converted to phosphate through hydrolysis or by biological activity and they can not be precipitated from the wastewater by chemical precipitation (Al-Rekabi, 2015). The organic phosphorous can be converted are removed from waterwater by microbial decomposition (Minnesota Pollution Control Agency, 2006).

2.3.7. Tertiary Treatment of Wastewater

Tertiary treatment is supplementary to primary and secondary wastewater treatment for the purpose of reducing organics, turbidity, nitrogen, phosphorus, metals and pathogens. In the most cases, processes involve physiochemical treatment such as coagulation, filtration, activated carbon adsorption of organics, reverse osmosis and efluent disinfection (Gerba et al., 2019).

2.3.8. Disinfection of Wastewater

During the primary, secondary and even tertiary wastewater treatment, not all contaminants can be removed, especially microorganisms. Therefore, there are several extra wastewater treatments and methods which can be applied to achieve higher quality of effluent. Disinfection is a treatment of the effluent for the annihilation of all pathogens. Another process used to reduce number of microorganisms is sterilization. Disinfection of wastewater can be conducted by physical methods (heating to boiling, incineration with x-rays, ultraviolet rays) or by chemichal metods (usage of strong acids, alcohols, oxidizing chemicals or surface active agents). During the decades, chlorination was primary wastewater disinfection method with high efficiency in pathogens removal, but scientists revealed its toxicity to fish and plants. Therefore, new trends are set for future wastewater treatments such as usage of ozon and ultraviolet light since their good disinfection characteristics and acceptable cost-effectiveness are founded (Muralikrishna et al*.,* 2017).

2.4. Phosphorus in the aquatic environment

Intensive loading of surface waters by nutrients such as phosphorus (P) and nitrogen (N) from wastewater can cause serious contamination of the receiving waterbodies and occurrence of of water eutrophication, i.e. the growth of bacteria and algae which consume large amounts of oxygen dissolved in water due to breakdown of the discharged P and N. Eutrophication has become a global problem with consequences varying from decrease in the aesthetics of the water to serious medical threats due to occurrence of algal toxins in water (Seviour et al., 2003).

Total phosphorus (TP) in domestic wastewater typically ranges between 4 mg/L and 8 mg/L. Occurrence of higher phosphorus concentrations in surface waters is usually result of wastewater discharge from industrial sources, water conservation, regulations related to detergent usage (Al-Rekabi, 2015). In urine, there is approximately 0.3 kg phosphorus per person per year and in feces an additional 0.2 kg phosphorus per person per year originating from P in the consumed food (Milhelčić et al. 2011). Industry and commercial sources, such as synthetic detergents and other cleaning products, also contribute to the total phosphorus concentration in wastewater. The final phosphorus concentration is determined by the degree of disturbances such as the percentage of wastewater dilution, contribution of industrial waste and the size of the area producing the wastewater. The discharge limit for P will vary depending on the sensitivity of the receiving water. The general limit in Norway is below 1 mg P/L (Lagesen, 2017).

Phosphorus recovery

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

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

Phosphorus recovery rate depends on media in which P is present. In liquid phase, P is present in the range from 10 to 60 % (wastewater treatment plant influent), in sludge from 35 to 70 %, and in sludge ashes percentage of phosphorus is between 70 and 98 %. Technology for phosphorus recovery from wastewater is still challenging since some barriers still occurs between stakeholders and institutions, public policies and regulations as well as public acceptance and economic feasibility. In developing countries, the implementation of nutrient recovery systems is challenging, because the main concern is on the expansion of sanitation coverage. Resource recovery approaches can provide benefits beyond the wastewater treatment sector, not only improving the sustainability of wastewater treatment operations, but generating revenue for the utility provider (Cardoso et al., 2019).

2.5. Phosphorus Removal from Wastewater

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

They all depend on the onset of anaerobic conditions with the complete absence of oxygen and nitrates dissolved in the activated sludge and wastewater suspension. Fermentation products are also essential, especially fatty acids with a short chain of carbon atoms that stimulate the growth and selection of certain types of bacteria that can accumulate them in the cellular structure as a backup food. This process takes place in the anaerobic phase, and as an energy source serves the accumulated polyphosphate which decomposes during that phase. This significantly increases the total concentration of orthophosphates in wastewater suspension (Tušar, 2009).

2.5.1. Phosphorus Removal by Chemical Process

Chemical treatment for phosphorus removal involves the addition of metal salts to react with soluble phosphate and form solid precipitates that are removed by solids separation processes including clarification and filtration. The most used metal salts are alum salt (aluminum sulfate), sodium aluminate, ferric chloride, ferric sulfate, ferrous sulfate, and ferrous chloride.

Chemical addition should be evaluated for two scenarios:

- Effluent polishing in the secondary process: The chemical addition point is in the secondary treatment process to the mixed liquor stream just before the secondary clarifier.
- Two-point chemical addition: Chemical is applied in both the primary clarifier feed and also just before the secondary clarifier. Two-point addition is popular for many applications because it achieves the most efficient use of chemicals for phosphorus precipitation.

The required chemical dose is related to the liquid phosphorus concentration. For target concentrations above 2 mg/L (appropriate for chemical addition to a primary clarifier), a dose of 1.0 mole of aluminum or iron per mole of phosphorus is sufficient. However, for lower phosphorus concentrations (in the range of 0.3 – 1.0 mg/L), the dose can be in the range of 1.2 to 4.0 moles aluminum or iron per mole of phosphorus. The pH value is an important factor for efficient phosphorus removal by alum or other salts, as the solubility of their precipitates vary with pH. Phosphorus removal is most efficient in the pH range of 5 to 7 for alum and of 6.5 to 7.5 for ferric salts since their precipitates will not readily return to solution (Minnesota Pollution Control Agency, 2006).

Commonly used separation methods are sedimentation or flotation. Also, a chemical precipitation is an efficient and easily implemented method where the level of P removed is determined by the amount of chemicals dosed to the system (Morse et al., 1997; Driver et al., 1999).

The efficiency of phosphorus removal by chemical precipitation depends on two factors:

- The chemical equilibrium between the phosphorus containing water and solid.

- The efficiency of the solids removal process. Usually the following process controls the removal efficiency (Sathasivan, 2009).

However, the method is associated with large production of chemical sludge with high metal content, as well as high operating costs as coagulants are expensive. The re-use of the chemical sludge is limited as the high metal content can harm the environment (Morse et al., 1998; Driver et al., 1999).

2.5.2. Phosphorus Removal by Biological Processes

Biological nutrient removal processes remove nitrogen and phosphorus from wastewater through the proper use of microorganisms under different environmental conditions.

Biological phosphorus removal process is more often used than chemical means due to its simplicity, effectiveness and various environmental benefits. What's more, it is the process that relies on enhancing the ability of microorganisms to uptake more phosphorus into their cell. Therefore, these processes are often referred to as enhanced biological phosphorus removal (EBPR) processes. EBPR has been implemented worldwide in many wastewater treatment plants. Despite its promise to provide efficient phosphorus removal performance, at times unreliable performance has been reported.

The phosphorous removal efficiency for biological systems depends on the phosphorus content of the sludge removed and the efficiency of the solids separation process. While this process has been shown to be economical and feasible in many cases, at times phosphorus removal was found to be fluctuating for unknown reasons. The uncertainty has led to intensive research in this field in the past few decades (Sathasivan, 2009).

2.6. Enhanced Biological Phosphorus Removal (EBPR)

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

They have the capability of assimilating phosphorous to a much larger degree than Ordinary Heterotrophic Bacteria (OHO) (Yuan et al., 2012; Mino et al., 1998).

In EBPR, PAOs are exposed to alternating anaerobe and aerobe or anoxic conditions. The biochemical processes occurring inside the PAOs rely on the presence of several compounds, such as glycogen, organic material, carbon source, polyphosphate (poly-P), that during the anaerobic-aerobic or anoxic cycle are accumulated and stored internally in the bacteria cell and subsequently degraded (Helness, 2007).

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

The enhanced biological phosphorus removal (EBPR) process has been implemented in many wastewater treatment plants worldwide. It has shown satisfactory results regarding phosphorous removal from wastewater streams, so there is little doubt that the EBPR process indeed can be capable of efficient phosphorus removal (Al-Rekabi, 2015).

Biochemical anaerobic processes

PAOs require easily biodegradable soluble Chemical Oxygen Demand (BSCOD), such as volatile fatty acids (VFAs) as carbon source for the biochemical processes to occur under anaerobe conditions. The amount of BSCOD available in the wastewater will depend on the concentration of soluble chemical oxygen demand (SCOD). The amount of SCOD is an indirect measure of the BSCOD as a portion of the SCOD is inert and not biologically available (Saltnes et al., 2016).

If there is a substrate available, the Polyphosphate Accumulating Organisms (PAOs) will take it up and store it intracellularly as poly-β-hydroxy-alkanoates (PHA) of which poly-β-hydroxy-butyrate (PHB) is the most common. The PAOs will then release P (orthophosphate) which they have stored intracellularly as poly-P.

Orthophosphate (ortho-P) is released to the liquid phase and this increases the concentration of ortho-P in the liquid phase which is a prerequisite for luxury uptake of P in aerobic phase (Wentzel et al., 2008).

Biochemical aerobic processes

Under aerobic conditions, the PAOs receive the ability to utilize the intercellular stored PHA as a source of energy for growth of new cells, giving them the ability to take up more phosphate than what was released during anaerobe phase (Wentzel et al., 2008).

Also, by oxidizing the carbon reserves built up in the anaerobic phase, PAOs are able to store more phosphate under aerobic conditions than that was released under anaerobic conditions because considerably more energy is produced by aerobic oxidation of the stored carbon compounds than was used to store them under anaerobic conditions (Minnesota Pollution Control Agency, 2006).

Glycogen reserves are replenished due to degradation of PHA. In activated sludge, excess bio-P sludge is removed after aerobe phase as the fraction of PAOs is increasing due to stimulation of cellular growth. The rest of the bio-P sludge is returned to anaerobe phase where it is mixed with fresh influent wastewater (Janssen et al., 2002; Wentzel et al., 2008).

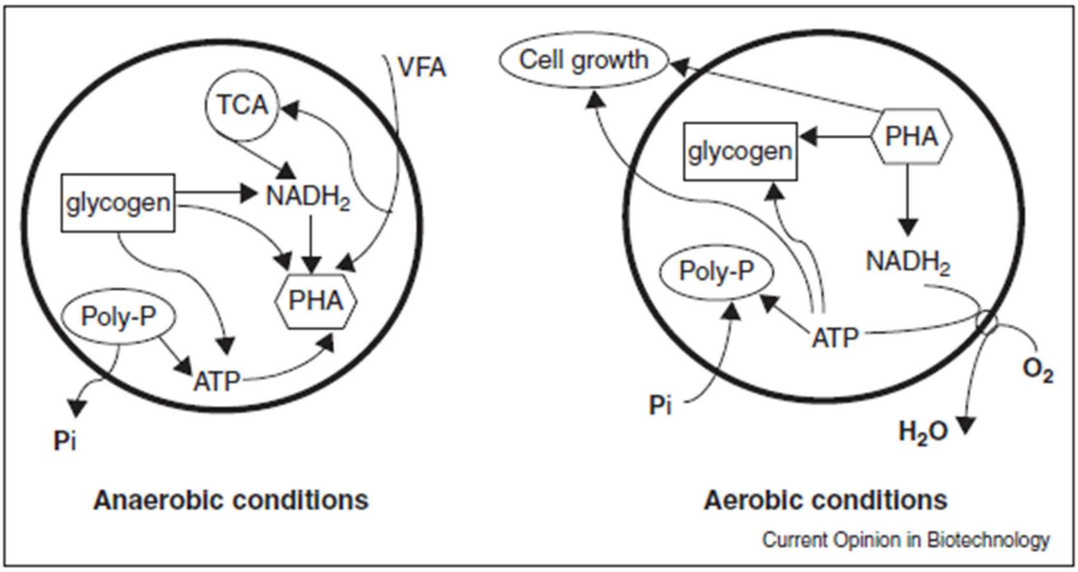

Figure 4 The action of PAOs in the aerobic and anaerobic phases
(Source: www.intechopen.com)

2.6.1. Wastewater Treatment Microbiology

Polyphosphate Accumulating Organisms (PAOs)

Polyphosphate Accumulating Organisms (PAOs) are the group of microorganisms that in essence are responsible for the removal of phosphate and are a community of different strings of bacteria. PAOs can store phosphate above what is required for their growth (Pastorelli et al., 1999).

PAOs are, therefore, referred to as the "real" phosphate bacteria and are the only bacteria able to create the luxury uptake and thus remove large amounts of P from the wastewater. *Candidatus Accumulibacter phosphatis* is one of the most common PAOs known today (Janssen et al., 2002).

What's more, PAOs have strict requirements in the cycling though the anaerobic, aerobic and/or anoxic stages and it is for this reason this process is more complex compared to the regular processes of biological N and COD removal (Zuthi et al., 2013). EBPR process needs to be facilitated by preferable conditions for the bacteria to proliferate (Helness, 2007).

Glycogen Accumulating Organisms (GAOs)

Glycogen non-polyphosphate Accumulating Organisms (GAOs) are defined as "organisms that store glycogen aerobically and consume it anaerobically as their primary source of energy for taking up carbon sources and storing them as PHAs" (Mino et al., 1995).

Unlike PAOs, GAOs rely on energy from glycoses of intracellular glycogen as energy source for the storage of PHA from the VFA uptake. Therefore, the release and uptake effect of phosphorous will not be experienced (Filipe et al., 2001a).

PAOs/GAOs relationship

The challenging part of Enhanced Biological Phosphorus Removal is the reduction of Glycogen Accumulating Organisms (GAOs) accumulation since GAOs are also able to multiply during alternating anaerobic and aerobic conditions (and have a similar metabolism of carbon), but they do not release or uptake phosphate, and thus do not have any phosphorus removal effect. Instead, they are detrimental to the phosphorus removal as they compete with the PAOs for the VFAs, resulting in less PAOs (Yuan et al., 2012).

Lopez-Vazquez et al. (2009) found the PAO/GAO competition to be decided by various factors such as temperature, pH, and the mix of carbon source. To be more specific, PAOs would dominate at low temperatures (<10 °C) regardless of carbon source and pH. At moderate temperatures (20 °C) and only one type of carbon source, the GAOs would be favored, unless at a high pH (7.5). Lastly, at higher temperatures (30 °C) GAOs would dominate, even though a combined carbon source (acetate and propionate) and a high pH (7.5) seemed to help the PAOs. GAOs are less common in full-scale WWTPs since the conditions in WWTPs are not favor for GAOs. However, they are more frequently encountered in lab-studies, as temperatures are closed to room temperature and there often is a single carbon source (Loosdrecht et al., 2016).

Ordinary Heterotrophic Organisms (OHOs)

Ordinary heterotrophic organisms (OHOs) are not able to accumulate poly-P and, therefore, do not remove phosphorus in excess from the wastewater. Under anaerobe conditions OHOs are not able to utilize the available volatile fatty acids as they require external oxygen and/or nitrate as electron acceptor. If the EBPR process is configurated optimal and the return of biomass does not return oxygen and/or nitrate, OHOs do not compete with PAOs for the available VFA. However, if oxygen and/or nitrate is recycled to the anaerobe reactor, OHOs can use the available fermentable COD for energy and growth. The OHOs use 1 mg O_2 to consumes 3 mg fermentable COD (Wentzel et al., 2008).

Denitrifying PAOs (DNPAOs)

DNPAOs have the capacity to use either oxygen or nitrate as an oxygen agent to consume VFA and take up P from the liquid phase (Zeng et al., 2016).

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

2.6.2. Efficiency of EBPR

One of the main factor for efficient phosphorus removal is the presence of Volatile Fatty Acids in the anaerobic phase of the EBPR cycle (Minnesota Pollution Control Agency, 2006). The ratio between organic material and the concentration of phosphorus in the influent assesses the amount of organic material that is required to remove phosphorus by PAOs. Practice shown that a BOD:P ratio above 15 – 20 yield to efficient biological P removal. When the influent ratio between organic material and P is high, it means that it will be easy to biologically remove the phosphorus (Janssen et al., 2002, Saltnes et al., 2016).

Also, the presence of metal ions in influent such as potassium (K), calcium (Ca) and magnesium (Mg) are required for efficient EBPR. These ions affect the operation of PAOs as they act as anti-ions for the negatively loaded phosphate ions (Janssen et al., 2002).

Temperature is a complex operating condition that affects EBPR differently due to the diverse microbial mixture and subsequent optimal growth temperature. Studies have shown contradicting results as to the affect temperature has on the overall EBPR process as it will affect various processes and wastewater characteristics in the system simultaneously (Janssen et al., 2002). Namely, the result of numerous studies indicate that biological P removal is more efficient under low media temperature (5 °C – 15 °C) (Erdal et al., 2003).

Another important parameter for controlling the competition between PAOs and GAOs in the overall process is an optimal pH. The value pH at 6.5 was found to take up P approximately 40 % less efficient compared to P uptake found at pH 7.0, also resulting in a reduced degradation of PHA and growth of biomass (Filipe et al., 2001a; Filipe et al., 2001b).

In an EBPR system the anaerobic zone must not contain oxygen levels above 0.2 mg/l for the process to be efficient (Mulkerrins et al., 2004).

2.7. Moving Bed Biofilm Reactor (MBBR)

Limited water resources and increasing urbanization require more advanced technology to preserve water quality. The Moving Bed Biofilm Reactor (MBBR) has emerged as a compact treatment alternative to conventional activated sludge reactors for the treatment of municipal and industrial wastewater (Kruszelnicka et al., 2018).

Moving bed biofilm reactor (MBBR) is a technology that was first invented by prof. Hallvard Ødegaard at Norwegian University of Science and Technology in the late 1980s.

The main objective for MBBR was to have a continuous, non-leakable, low head loss reactor with a large specific surface area for biofilm. This is achieved by growing biofilm (biomass) on small carriers that move along with the water in the reactor. This biofilm can include differing layers and differing microbial communities that incites removal of substances in the water based on the conditions. The movement is caused by aeration in the aerobic phase and in anaerobic mechanical stirrers (Al-Rekabi, 2015).

The biofilm carriers are made of polyethylene and are small cylinders with a cross inside the cylinder and longitudinal fins on the outside. In order to keep them in the reactor, a sieve is located at the outlet. The main advantage of MBBR is that it takes up very little space compared to conventional activated sludge systems. The entire volume of the reactor is filled. Tests have shown that the biomass on the carriers is very sustainable and shows good results in a high biological activity per kg of attached biomass (Ødegaard et al., 1994).

One of the most important advantages of the MBBR is that the biomass is more specialized and active (Ødegaard et al., 1994). Inactive biomass is continuously washed out of the reactor as it erodes off carriers. In comparison with an activated sludge system with recycled biomass wash-out effect, as previously stated can be experienced, and one will never be able to develop the same specialized biomass as in a MBBR (Ødegaard et al., 1994).

The specialized biomass is a product of having carriers fitted into a single environment full-time making the biomass experts that thrive in the conditions within their reactor (Ødegaard et al, 1994). As PAOs are quite slow growing organisms, this can be very useful. When the thickness of the biofilm increases, biofilm will erode off and the process of separating the sludge from the water will be easier in this process than with activated sludge since it is not pertinent with thickening of the sludge before return to the reactor, as no sludge is returned.

As biofilm is eroded off, the inactive biomass including PAO bacteria will erode off and be lead to the separation stage and phosphorous removal (Ødegaard et al., 2014).A process carried out by H. Helness and H. Ødegaard (2001) showed an excellent phosphate removal. As expected, the aerobic phosphate uptake showed a strong correlation to the anaerobic phosphate release. Since the production of PHA necessary for phosphate uptake is linked to phosphate release, a high phosphate release is an advantage with respect to achieving a high net phosphate removal. The average net phosphate removal in experiments with a phosphate release higher than 30 mg PO4-P/L was 7.7 mg PO4-P/L, demonstrating the phosphate removal capacity of the process.

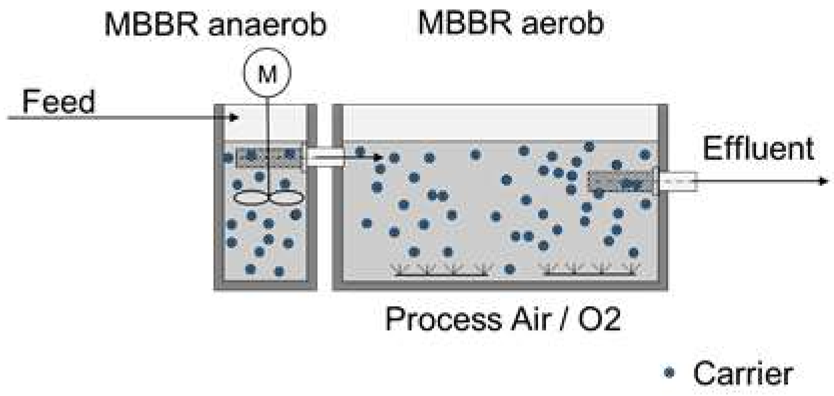

Figure 5 Anaerobic and aerobic phase in Moving Bed Biofilm Reactor
(Source: www.canadianbiomassmagazine.ca)

2.7.1. Carriers in Moving Bed Biofilm Reactor

The MBBR carriers have a slightly smaller specific mass than water, and by growing the attached bio-film, the specific mass becomes larger which allows the carriers to be suspended in water (www.junyue-tech.com).

The most important item in carriers is the surface area. A large surface area enables the development of biofilm and the absorption of substrates from the wastewater (Wang et al., 2019). Carriers can be made of sand, soil, gravels, stones, rubber, wood, agglomerates of the biomass, plastic or any other synthetic materials. Material selection is important to maintain high quantity of active biomass (Wang et al., 2019) while the the following conditions for carriers geometry are required:

- The carrier geometry should protect biofilm produced on it and provide enough area for the proper biofilm development.
- The carrier should not have dead places where oxygen is limited
- The carrier should provide a suitable film thickness (Kruszelnicki et al., 2018).

There are several different sizes and designs of carrier elements used in the MBBR process. The shapes of most used carriers types are shown in **Fig. 6**.

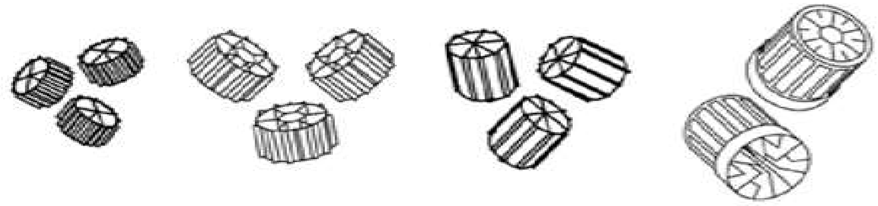

Figure 6 The shapes of most used carriers types
(Source: Al-Rekabi, 2015)

The KMT carrier K1 is the original kindles, mostly used MBBR carriers, is shown in **Fig. 7** and **8**. The effective surface area of the KMT K1 and the AWT carriers were calculated as the whole inner area plus the area of the outer fins. The area between the fins was not included since visual inspection did not show any sign of growth there. For the ANOX carrier, the effective area is calculated as the inner area since there are no fins with outer area (Al-Rekabi, 2015).

All carriers have different characteristics, i.e. different size, shape, surface area, cost and loading as a fraction of total reactor volume. They need to have a large surface area and easily enable complete mixing to be well spaced across the reactor and to ensure homogeneity between the substrate and the biofilm (Helness, 2007).

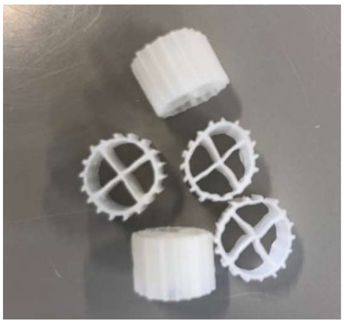

Figure 7 Unused plastic carriers

Figure 8 Used plastic carriers with attached biomass

3. EXPERIMENTAL PART

The aim of this thesis was to examine the biological removal of phosphorus from the wastewater in the city of Trondheim.

During the experiments, concentration of nutrients and organic materials, pH, DO and temperature were measured on a daily basis. The batch experiments were conducted under different conditions in order to determine optimal parameters for the phosphorus removal process. A total of nineteen kinetic experiments were carried out, and the experiments with efficient phosphorus removal were performed in replicates under the same conditions,analyzed and presented in this thesis. The experiments were being carried out every Tuesday and Friday for three months, i.e. in February, March and April of 2019.

3.1. Materials and Methods

During the research, all analyzes were performed to certain standards. Following materials and methods were used during the experimental part od this thesis.

Untreated wastewater

The untreated wastewater (influent) comes from a nearby housing complex with 48 apartments estate Lerkendal (Gnr / Bnr / 64 / 17), Trondheim, Norway, by sewage system directly to tanks in the wastewater laboratory (**Fig. 9**) at the Department of Civil and Environmental Engineering at Norwegian University of Science and Technology in Trondheim.

From the local pump station, wastewater is pumped hourly into two 3.5 m^2 receiving tanks. Since wastewater slowly goes thrue tanks, clarification occurs in tanks. Obtained supernatant contains a high proportion of solids due to the lack of pretreatment of wastewater and activated sludge spread over the water. Outside there is a tank into which the wastewater comes in and goes through coarse grids to reduce solids in the receiving tank inside the lab and in the pilot. The tank is emptied, washed and cleaned 6 times per week to avoid the growth and action of sulfur-reducing bacteria (Fiksdal, 2018).

Figure 9 Wastewater laboratory of the Department of Civil and Environmental Engineering at the Norwegian University of Science and Technology in Trondheim

Wastewater Sampling

A wastewater samples were taken from wastewater storage tanks placed in the Wastewater laboratory of the Department of Civil and Environmental Engineering at the Norwegian University of Science and Technology in Trondheim, Norway. Samples were taken by opening two valves on a wastewater tap, and after the influent was released, it was necessary to wait about one minute to avoid the large amount of biomass remaining when the mixer stopped working inside the tank. The sample was collected in a plastic container or beaker, depending on the amount required for a particular experiment.

Figure 10 Wastewater storage tank in the Wastewater laboratory at Norwegian University of Science and Technology

Analytical methods and wastewater analysis

Samples taken from wastewater storage tanks were filtered through a 0.45 µm cellulose and nitrate filter. Filtration was done bya medical injection needle. Before usage, filters were rinsed three times by distilled water.

Figure 11 Filter and cellulose and nitrate (0.45 µm) filter paper

Hach Dr. Lange cuvette tests were used to measure and determine the total of phosphorus, orthophosphate and soluble chemical oxygen demand (SCOD). In each box there were the cuvettes and required dosing chemicals as well as the instructions for preparation and a measuring process. All cuvette tests contained specification with concentration ranges for measuring individual parameters, and, according to the rank, it depends on whether the sample should be diluted or not.

Figure 12 Hach Dr. Lange cuvette tests for Phosphorus total / Phosphate ortho LCK 348

After dosing the chemicals and placing the sample into the cuvettes, specific color occurs. The intensity of developed color in cuvette is in the coorelation with concentration of measured parameter. Cuvettes with developed color were placed in a spectrophotometer Hach DR 1900^{TM} (**Fig. 13**) to measure the concentrations of each specific parameter.

Figure 13 Spectrophotometer Hach DR 1900 used for measuring concentrations of measured parameters

Soluble Chemical Oxygen Demand (SCOD)

The determination of SCOD was performed by adding 2 mL of filtrated sample in the appropriate cuvette and then putting it in a heating block instrument. Furthermore, tempering SCOD samples was done in an LT 200 (Hach) Thermostat (**Fig. 14**) at 148 °C for 2 hours. After cooling, the concentration of SCOD was measured by inserting the cuvette into the spectrophotometer (**Fig 13.**).

Figure 14 Thermostat Hach LT 200

Orthophosphate (PO4-P)

Determination of PO4-P is performed by adding 2.0 mL of filtrated sample in the cuvette, 0.2 mL reagent B and a content from dosi cap. After 10 min, concentration of PO4-P is measured by inserting the cuvette into the spectrophotometer.

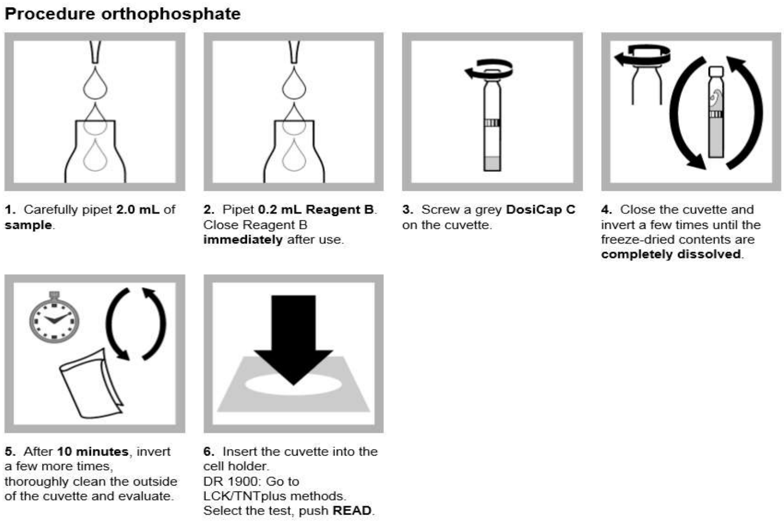

Figure 15 Orthophosphate determination procedure

Dissolved oxygen (DO)

Dissolved oxygen was measured with a suitable Dissolved Oxygen meter according to the following instructions. First, the protective sponge was removed from the electrode of the DO unit, rinsed with distilled water and and then placed in the MBBR. After stabilizing the value on the device screen, the value was recorded and afterwards the electrode was moved from one chamber to another.

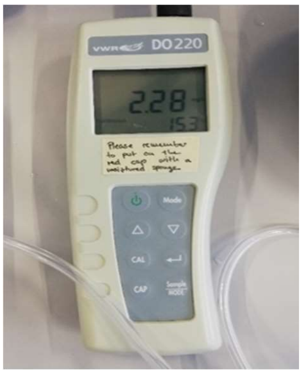

Figure 16 Dissolved Oxygen Meter VWR DO 220

Temperature and pH

The temperature was measured jointly with the pH value using the WTW Oxi 3315 shown in **Fig. 17**. Value reading was done in the same way as for the Dissolved Oxygen.

Figure 17 WTW Oxi 3315

Wastewater Characteristic in city of Trondheim

The samples of Trondheim wastewaterwere being taken every morning at 8 a.m. on Tuesdays and Fridays for 3 months. The sampling was followed by filtration through a 0.45 µm cellulose and nitrate filter. Afterwards, the determination of orthophosphate and SCOD is defined using the cuvette tests. The devices described in the previous sections were used to determine pH, temperature and DO values. Typical Norwegian water during winter and spring is diluted due to a heavy rainfall and has a lower temperature. The average parameter values of Trondehim wastewater obtained during monitored period are presented in **Table 1**.

Table 1 The characteristic of wastewater in the city of Trondheim

Nutrients	Average concentration in influent [mg/L]
PO4-P	3,77
NH4-N	36,42
NO_3	0,60
NO_2	0,03
SCOD	131,47

Batch experiments

The batch experiment monitors the kinetics of phosphorus removal from wastewater. The experiment inside a 1 L bioreactor simulates the process and conditions from MBBR pilot. Several conditions were changed during the experiments to reveal the best and most efficient phosphorus removal rates. The wastewater inside the bioreactor was mixed with a magnetic stirrer with rotational speed of 150 rpm to achieve evenness of the contents inside the bioreactor. Temperature, DO and pH were constantly measured by appropriate devices. A samples from bioreactor were taken every 38 minutes to measure different parameters and phosphorus concentrations at a specific point in time.

Procedure

The amount of 794 mL of the sample was taken from the influent tank. A total of 600 ml of carriers from the conveyor belt were then collected and put together in a bioreactor. Each bioreactor experiment followed the appropriate conditions from the MBBR pilot measured just before sampling from the receiving tank. Although in general related to temperature and pH, in a number of experiments, the DO value in the bioreactor was the same as in the MBBR pilot. The temperature was set below room temperature because the wastewater coming in was cold, so the experiment beaker was immersed in a plastic container filled with ice (**Fig. 18**).

Figure 18 Bioreactor with wastewater and carriers placed in a plastic container with ice

Furthermore, pH of water was always a constant over a certain range. What's more, the water was retained in the MBBR pilot in each chamber for 38 minutes, which means that the overall process took 380 minutes, so aforesaid conditions were applicable to each bioreactor experiment. The anaerobic phase lasted 152 minutes because MBBR contained four anaerobic chambers. However, DO had to be less than 0.05 mg/L in order for the anaerobic phase to occur at all. After that, the aerobic phase started by adding compressed air to the bioreactor using a small electric pump (**Fig. 19**) to which the tubes with holes were connected for dosing the desired amount of air. The aerobic phase lasted for 228 minutes. The measuring process of desired parameters from the sample, specifically phosphorus and SCOD, was carried out using the Dr. Hach Lange cuvette tests.

Figure 19 Electric pump EHEIM 400 for aeration of wastewater

3.2. Protocol No. 1

A total of 794 mL of the wastewater sample was taken from the receiving tank and mixed into the bioreactor with 600 mL carriers from conveyor belt. Once the magnetic stirrer had started operating, the timing started. The anaerobic phase lasted for 152 minutes and the sample was taken at the outset when the time reached zero, and also after 114 and 152 minutes to measure phosphorus and SCOD concentrations. Then the aerobic phase began, so the tube with holes that were connected to the aeration pump was immersed in the bioreactor. Five minutes after adding the air, the sample was taken but also after 266 and 342 minutes. Afterwards, all the samples were filtered and cuvette tests were being prepared. The cuvettes were then placed in a spectrophotometer to measure the exact concentrations. The DO in the aerobic phase was 7 − 8 mg/L and the same conditions as in the MBBR pilot were reached regarding the pH and temperature values. The pH was 7.5 and the temperature ranged from 10 to 13 °C. The experiment was performed to prove the proper conditions in the MBBR pilot necessary for the removal of phosphorus, and the emphasis was on the proper concentration of dissolved oxygen in the wastewater.

3.3. Protocol No. 2

The Protocol No. 2 begins with the same procedure as Protocol No. 1. i.e. the amount of 794 mL of wastewater sample was mixed with 600 mL carriers. Aeration started after 152 minutes and the DO level was between 6 − 8 mg/L. After 228 min, the aeration was stopped and the anoxic phase began and lasted for 76 minutes. After anoxic phase, the aeration started again in the 304th minute. The DO level between 6 and 8 mg/L was achieved. The pH was 7.5 − 8 and the temperature was between 10 − 13 °C. The sample for SCOD measurement was taken initially at zero minute, and finally at 342 minutes. The phosphorus measurement sample was taken at the beginning and after 152 minutes every 38 minutes until the end of a process.

3.4. Protocol No. 3

The experiment included wastewater treatment in three bioreactors. Each of three bioreactors was filled with 794 mL of wastewater sample and 600 mL of carriers (**Fig. 20)**. The anaerobic phase lasted for 152 minutes in each of the three bioreactors, and thereafter, a different concentration of DO was added to them individually. In the first bioreactor the DO level was between 2 and 4, in the second between 4 and 6, while in the third between 6 and 8 mg/L. The samples were taken at the beginning of the process and after 152, 190, 266 and 342 minutes from each bioreactor. Subsequently, the samples were prepared and the measurement was carried out following the aforementioned procedure. In all the three reactors, the temperature was between 13.4 and 14.70 °C, whereas the pH value was around 7.5. This experiment was performed to determine the effect of varying amounts of DO on the removal of phosphorus, in fact, on the action of bacteria.

Figure 20 Three bioreactors with sample and carriers

3.5. Protocol No. 4

This experiment was conducted using two bioreactors with 794 mL of wastewater sample and 600 mL of carriers. The anaerobic phase in both of the bioreactors lasted 152 minutes and samples were taken to measure the concentration of PO4-P two times: at the beginning of the process, and after 38 and 114 minutes. What distinguished this experiment from the others is that after 152 minutes an aerobic phase occurred in one bioreactor by adding oxygen, and in another by adding 0.14375 mg of $NaNO_3$, for calculation see the equation below. The PO4-P measurement samples were taken from both bioreactors at the same time and under the same conditions at 152, 226 and 342 minutes. However, the sample for COD was taken at the beginning and at the end of the process from both bioreactors. The pH value was between 7.5 and 8 and the temperature was around 14 °C. It is important to note that the samples were prepared and measured in the same way as in previous experiments. However, the aim of this experiment in particular was to examine and compare the activity of bacteria in oxygen and when using $NaNO_3$ as an energy source.

$$84.49\, mg\ NaNO_3 \rightarrow 14\, mg\ NO_3 - N$$
$$x \rightarrow 30\, mg\ NO_3 - N$$
$$x = \frac{84.49 \cdot 30}{14} = 181.05\, mg\ NaNO_3$$
$$181.05\, mg\ NaNO_3 \rightarrow 1000\, mL$$
$$x \rightarrow 794\, mL$$

$$x = \frac{181.05 \cdot 794}{1000} = 143{,}75\ mg\ NaNO_3 x = 0.14375\ g\ NaNO_3$$

4. RESULTS AND DISCUSSION

The experiments were performed under different conditions in order to establish the most optimal ones for the phosphorus removal process and obtained results shows that the efficiency of tested biological phosphorus removal is in relation with the characteristics of untreated wastewater, and therefore varies throughout the day. However, possible systematic laboratory errors during the experiment should also be considered.

Wastewater Characteristic in city of Trondheim

The characteristic of Trondheim wastewater was determinated by daily measuring following parameters values: phosphorus concentrations, Soluble Chemical Oxygen Demand (SCOD), ammonia concentration, nitrite and nitrate concentration, pH value and temperature. The values of measured wastewater parameters were vary during the monitored period. The conducted monitoring over listed parameters on a daily and hourly basis gives a good insight into the process optimization capabilities.

Phosphorus concentrations in Trondheim wastewater

In order for Trondheim wastewater to be suitable for carrying out the MBBR or EBPR process, the presence of phosphorus is required. Soluble phosphate present in wastewater is a measure of PO4-P avaliable for uptake by PAOs. Under aerobic conditions, PAOs consume PO4-P and if the concentration of PO4-P in water is low the P uptake rate will be low. If the particulant P is removed from wastewater during pre-treatment, it remains sufficient, PO4-P in the liquid phase for good removal in the pilot (Lagesen, 2017). Measured concentrations of ortophosphates in the wastewater of the citiy of Trondheim shown in **Fig. 21** in the period from January 29 to April 29, 2019 ranged from 6.99 to 0.89 mg/L.

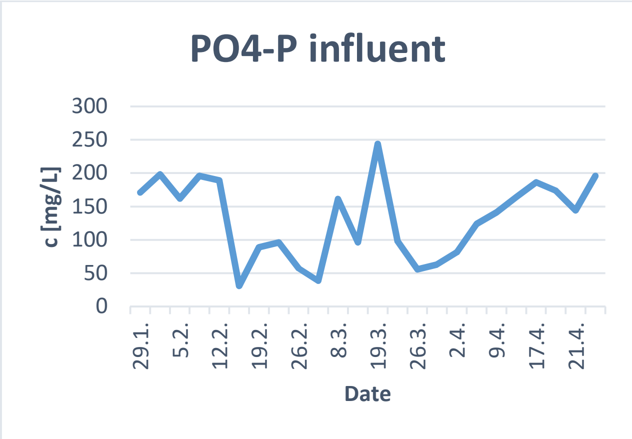

Figure 21 The values of orthophoshate concentrations in the samples of Trondheim wastewater

Soluble Chemical Oxygen Demand in Trondheim wastewater

Efficient phosphorus removal was related to efficient anaerobic COD removal (Ødegaard, 2001). Concentrations of SCOD vary with water dilution but not as much as PO4-P concentrations. The studies have shown that when the concentration of SCOD in wastewater is low, the percentage of phosphorus removal is also low. On the one hand, the anaerobic COD-loading rate should be kept low enough to avoid competition from OHOs, while the COD-loading rate should be so that a sufficient PHA amount is stored for P-uptake and a net growth of biomass (Helness and Ødegaard, 2001).

Previous studies shown that Norwegian wastewaters are usualy diluted and has low initial values of SCOD. Bacteria can utilize carbon in various forms such as VFA, amino acids, glucose and alcohol. Since only acetate and propionate can be used directly, it is necessary to ferment glucose and ethanol to VFA (Finstad, 2018). The value of chemical oxygen demand in the wastewater of the city of Trondheim ranged from 198 to 31 mg/L between January 29 and April 21, 2019 (**Fig. 22**)

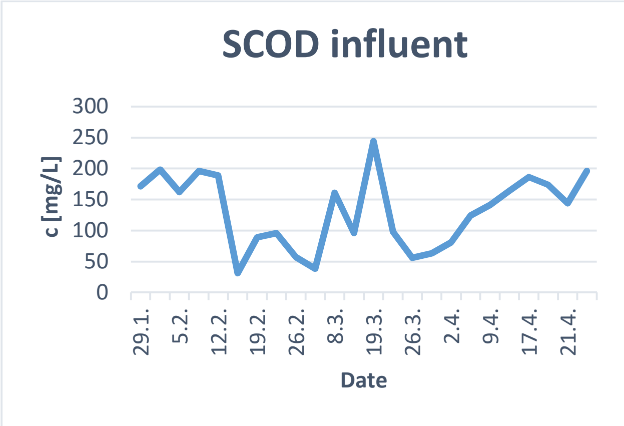

Figure 22 The values of soluble chemical oxygen demand (SCOD) in the samples of Trondheim wastewater

Ammonia concentrations in Trondheim wastewater

In municipal wastewater, 60 % of nitrogen is in the form of ammonia and 40 % is organic form. However, nitrogen occurrence in wastewater is not desirable because it consumes oxygen for oxidation. Heightened nitrogen concentrations in wastewater indicate receant contamination of wastewater with nitrogen compounds. The presence of ammonium ions and ammonia is an indicator of the microbial degradation of nitrogen-containing organics. If nitrifying bacteria are present in the wastewater, the transform of the ammonia into nitrate ions occurs causing dcrease of ammonia content and increase of nitrate content (Tušar, 2009). Between January 29 and April 21, 2019, the measured ammonia concentration in the wastewater of the city of Trondheim ranged from 63.5 to 11.3 mg/L as shown in **Fig. 23**.

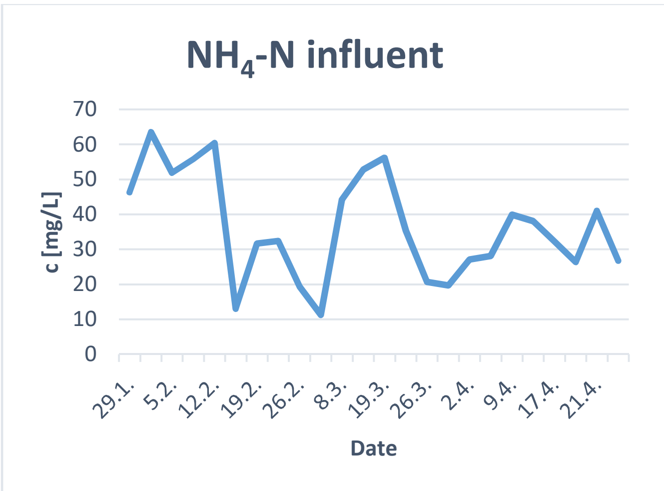

Figure 23 The values of ammonia concentrations in the samples of Trondheim wastewater

Nitrate concentrations in Trondheim wastewater

Occurrence of elevated nitrate concentrations in natural waters cause fatal health effects such as “Baby Blue Sindrome” and different types of cancer. Animals also suffer from nitrate poisoning which in high concentrations cause death and in lower ones various diseases (Kaluđerović, 2008). The high concentration of nitrate in the wastewater is an indicator of the final degree of stabilization of the bio-waste or highly fertilized field (Peternal, 2012). The concentration of nitrate in the wastewater of the city of Trondheim ranged from 1.46 to 0.44 mg/L between January 29 and April 21, 2019 (**Fig. 24**)

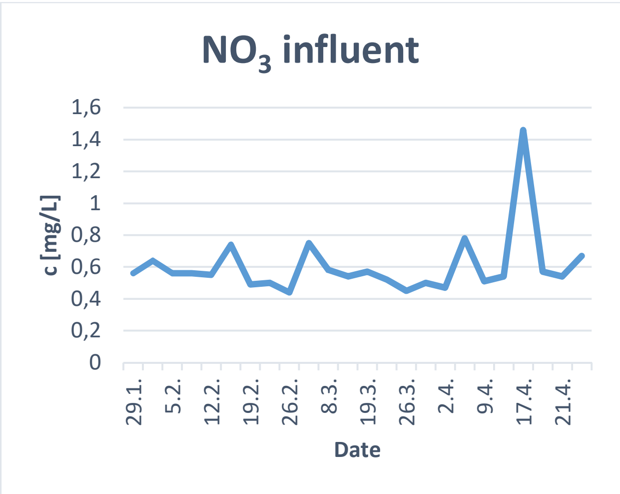

Figure 24 The values of nitrate concentrations in the samples of Trondheim wastewater

Nitrite concentrations in Trondheim wastewater

Nitrites in wastewater are intermediates in the biochemical process of oxidation of ammonia to nitrates. If found in surface water, they oxidize very quickly to nitrate compounds (Tušar, 2009). The concentration of nitrite in the wastewater of the city of Trondheim ranged from 0.271 to 0.005 mg/L between January 29 and April 21, 2019 (**Fig. 25**)

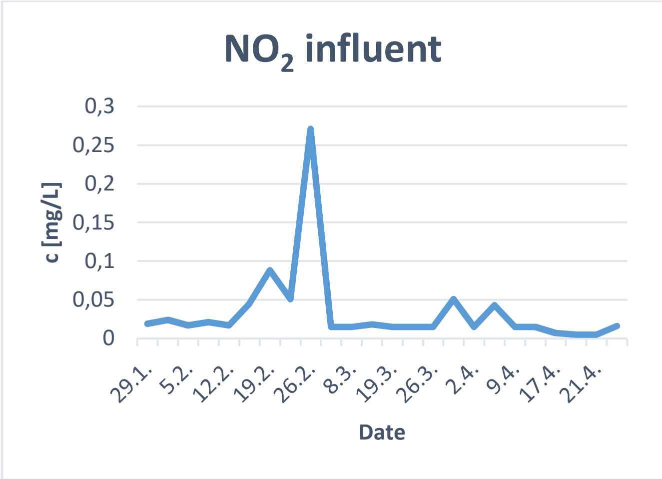

Figure 25 The values of nitrite concentrations in the samples of Trondheim wastewater

pH value and temperature of Trondheim wastewater

The value of pH is one of most important process parameter. Namely, due to the competition between PAOs and GAOs, it is desirable to maintain a high pH in the system, as PAOs inhibiting more energy than GAOs (Filipe et al., 2001a). Biomass produced at high pH concentrations promotes a larger population of PAOs then GAOs (Wang et al.,2013), while a slight change in pH from 7.0 to 6.5 completely alters the microbial composition in biomass and leads to a high reduction in phosphorus removal (Zhang et al. 2005). The average pH value of Trondheim wastewater was 7.87, which is comparable to the average pH in Norwegian wastewater (Odegaard et al., 2014). The pH and temperature values of the city of Trondheim are shown in **Fig. 26** and ranged in a very small range from 8.3 to 7.2 for pH and in terms of temperatures in the range of 11.4 to 15.09 ° C.

Weather oscilations cause the differences and oscilations in wastewater influent temperature as well. Any change that happens at once causes the bacteria to adapt to new conditions.

GAOs and PAOs have their competitive advantage at lower temperatures. As PAOs are more competitive at lower temperatures, this can have led to then outcompeting the GAOs (Finstad, 2018). The results of several studies shown that the process of phosphorus removal is more efficient at lower temperature. While the formation of glycogen from biomass decrease (Oehme et al. 2007; Erdal et al. 2003). Temperature bellows 4 °C cause lower percentage of hydrolysis, which explain the low SCOD concentration is in the Trondheim wastewater as well as in Norwegian wastewater in general (Lagesen, 2017).

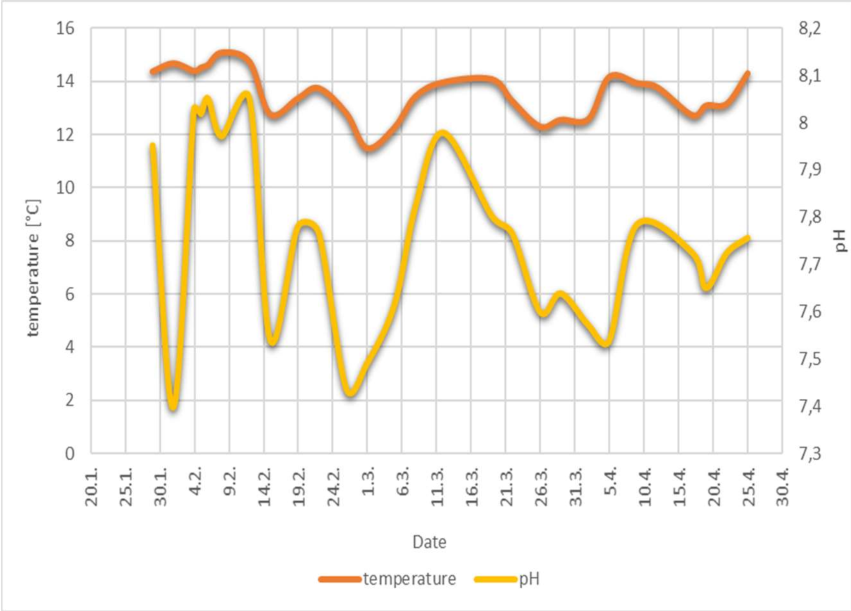

Figure 26 Temperature and pH values of wastewater in MBBR in the monitored period (January 29 –April 24 2019)

Protocol No. 1

The kinetics of phosphorus removal was examined by experiments described in Protocol No. 1. The initial PO4-P concentration in influent was 3.17 mg/L. The anaerobic phase was 152 minutes, and after 38 minutes the PO4-P concentration increased to 5.12 mg/L. After 114 minutes PO4-P concentration was 9.05 mg/L wich demonstrated efficient PAOs activity. PAOs was taken up SCOD as substrate and at t = 0 it was 93 mg/L. Subsequently, SCODs were stored as PHA and PHB, and then the PAOs released orthophosphate, which they stored intracellularly as polyphosphates.Orthophosphate was released in the liquid phase and its concentration increased, which is a prerequisite for so called luxury uptake of P in the aerobic phase (Wentzel et al., 2008).

In the aerobic phase, the concentration of PO4-P was 7.48 mg/L at t = 152 minutes. At the end of the process, 92 % of the phosphorus was removed. At t = 114 minutes, the concentration was 1.17 mg/L and decreased to 0.25 mg/L at t = 342 minutes, which means that PAOs utilized intracellularly stored PHA to grow new cells. This gave them the ability to uptake more phosphate than was released during the anaerobic phase (Wentzel et al., 2008).

The experiment was conducted on March 22, 2019. The wastewater was diluted due to a rainfall that lasted for days. Therefore, at the beginning of the experiment there was a low concentration of PO4-P and SCOD in the influent.

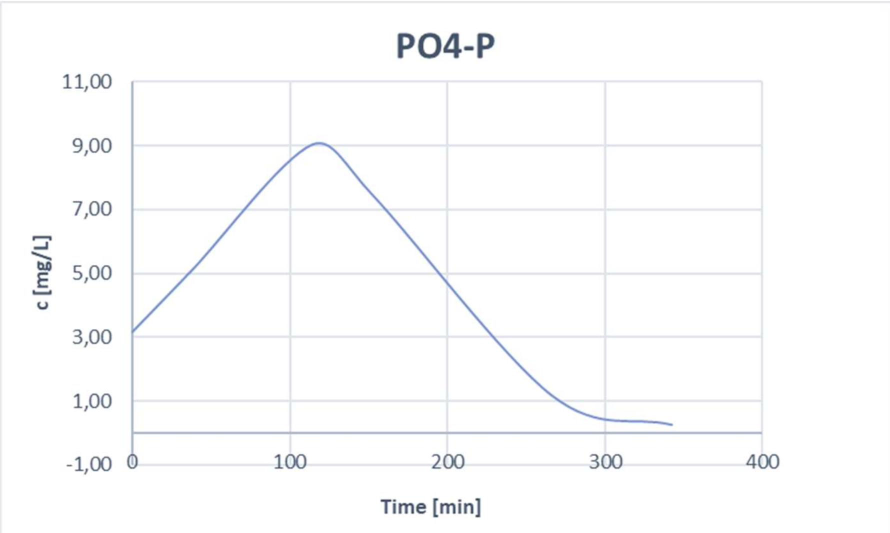

Figure 27 Orthophosphate concentrations as a function of time during the Protocol No. 1

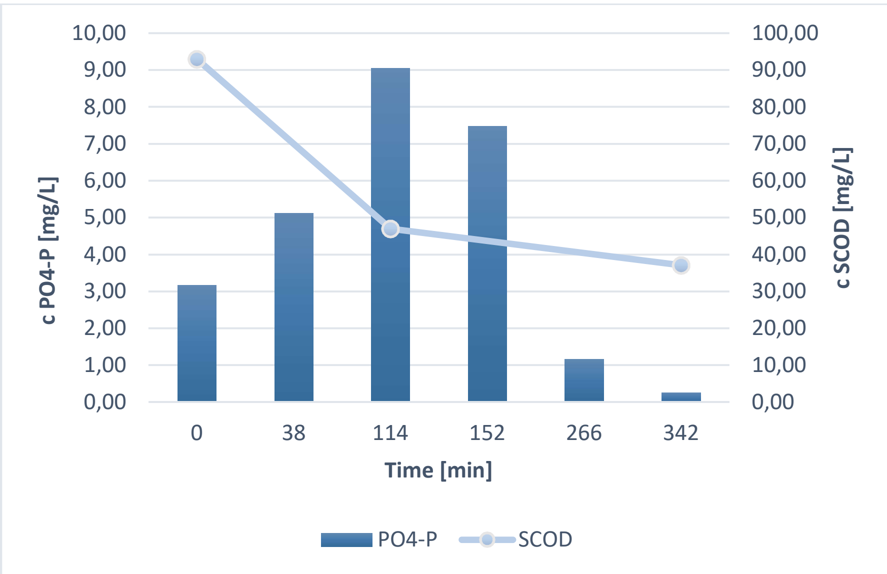

Figure 28 Orthophosphate concentrations and SCOD values as a function of time in Protocol No. 1

Protocol No. 2

The experiment conducted in Protocol No. 2 showed a efficient removal of phosphorus under anoxic conditions. The anaerobic phase in previous experiments was 152 minutes. At t = 0 the PO4-P concentration was 3 mg/L. After 152 min., aeration of wastewater started and the phosphorus concentration at the end of the anaerobic phase at t = 152 was 3.86 mg/L. Increase of phosphorus concentration was caused by activity of PAOs and their release of phosphorus in the anaerobic phase followed by uptook it in the aerobic phase. At t = 228 minutes, the anoxic phase occurred and the PO4-P concentration decreased to 0.32 mg/L. The anoxic growth yield coefficient should be about 70 % of the aerobic growth yield coefficient (Kube et al., 1993).

At the beginning of the process the SCOD was 64.1 mg/L, while at the end of the Protocol No. 2, 90.62 % of SCOD was removed and the final value of SCOD concentration was 6.01 mg/L.

There was no significant difference between the anoxic/anaerobic zone and aerobic zone in terms of COD concentration. This indicated that most of the biodegradable COD was utilized in the anoxic/anaerobic zone for denitrification and phosphorus release (Ahn et al., 2003).

After the anoxic phase, aeration began again and the concentration of PO4-P at t = 304 minutes was 0.26, whereas at t = 342 minutes it was 0.17 mg/L. The 94.3 % of PO4-P was removed.

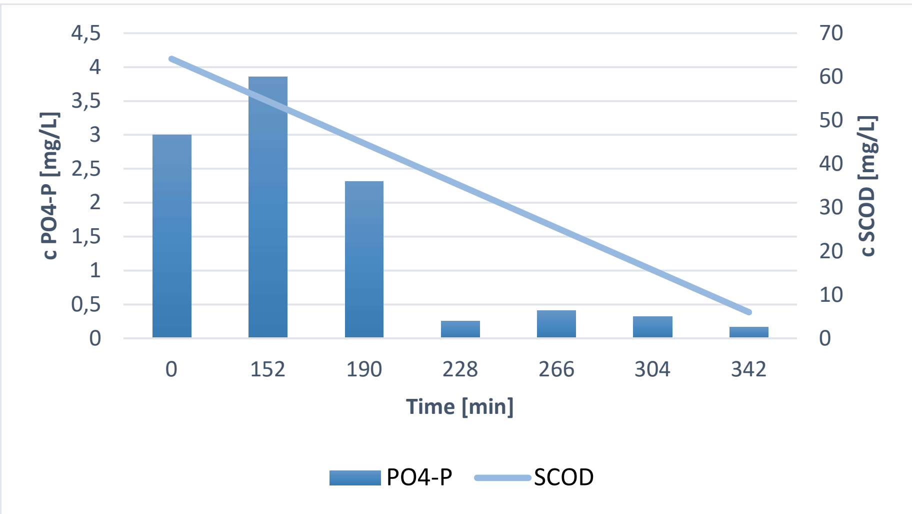

Figure 29 Orthophosphate concentration as a function of time and SCOD in aerobic/anoxic conditions in Protocol No. 2

Protocol No. 3

The Protocol No. 3 was conducted in three bioreactors. Each of three bioreactors was filled with 794 mL of wastewater sample and 600 mL of carriers. The aim of Protocol No. 3 was determination of optimal oxygen concentration for most efficient phosphorus removal. According to obtained results presented on the **Fig. 31** the most successful removal was achieved with a DO level of 6 − 8 mg/L, although the oxygen concentration during the experiment also reached 10 mg/L. The least removal was achieved at low DO concentrations in the range of 2 − 4 mg/L. The initial concentration of PO4-P in the influent was 1.62 mg/L while the SCOD was 61 mg/L as the results of diluted influent by stormwater.

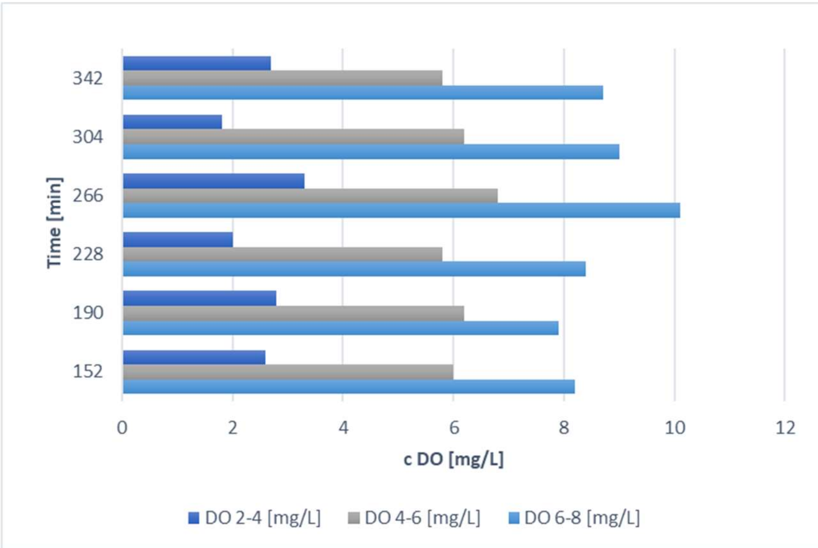

Figure 30 The concentration of dissolved oxygen in all three experiments of Protocol No. 3 over time

DO level 6 – 8 mg/L

The PO4-P concentration after the anaerobic phase was 2.53 mg/L at t = 152 minutes. PAOs consumed 42 mg/L SCOD and 74 % of phosphorus was removed. The concentration of PO4-P at t = 266 minutes was 0.827 mg/L, while at the end of the process at t = 342 minutes it was 0.421 mg/L. By the end of the experiment, 68.85 % of SCOD was removed and the final SCOD concentration was 19 mg/L.

DO level 4 – 6 mg/L

PAOs cosumed 39 mg/L SCOD and by the end of the experiment, 68.66 % of SCOD were removed while the final SCOD concentration was 21 mg/L. After the anaerobic phase, at t = 152 minutes, PAOs released 2.5 mg/L PO4-P, and at t = 266 min. it started to uptake and the concentration was 0.925 mg/L. At the end of the process, the PO4-P concentration was 0.602 mg/L which means that 62.84 % of phosphorus was removed

DO level 2 – 4 mg/L

Among all experiments in Protocol No.3, in the bioreactor with lowest DO level, phosphorus removal was only 47.22 %. At the beginning of the aeration, the concentration of PO4-P was 2.6 mg/L, secondly, at t = 266 minutes was 1.11 mg/L, and, finally, at t = 342 minutes was 0.855 mg/L. PAOs consumed 36 mg/L of SCOD and 59.02 % was removed. In this experiment the phosphorus was removed but based on the previous two experiments the bacteria preferred higher oxygen levels to remove phosphorus and SCOD.

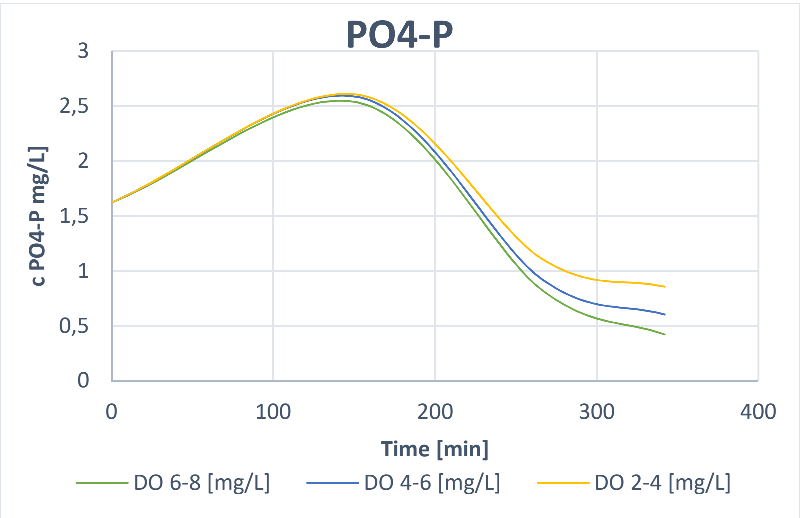

Figure 31 The orthophosphate concentration in three bioreactors over time during the Protocol No. 3

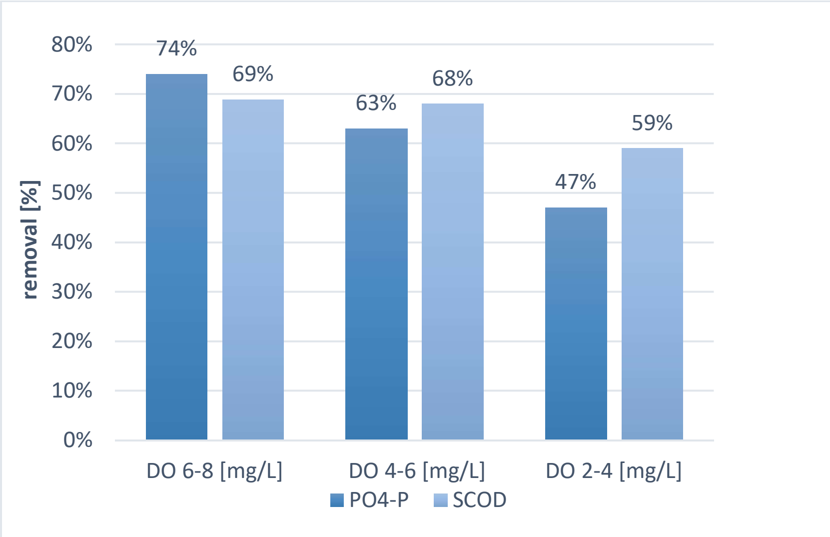

Figure 32 The percentage of phosphorus and SCOD removal in three bioreactors under various DO concentrations in the Protocol No. 3

Protocol No. 4

The Protocol No. 4 examined efficiency of phosphorus removal from wastewater by when PAOs using $NaNO_3$ instead of oxygen. The sample of wastewater was divided two beakers – oxygen was added to one, while $NaNO_3$ to the other in order to compare bacterial activity under different conditions. The initial PO4-P concentration was 1.69 mg/L, and at the end of the anaerobic phase 3.09 mg/L. Phosphorus removal in the oxygen bioreactor was 92.9 % whereas in the $NaNO_3$ supplemented bioreactor 85.8 %. Moreover, the PO4-P concentration in the oxygen bioreactor at t = 266 minutes was 0.17 mg/L, and at the end of the process 0.12 mg/L. On the other hand, the bioreactor to which $NaNO_3$ was added, displayed slightly less successful removal of phosphorus; the concentration of PO4-P at t = 266 minutes was 0.48 mg/L, while at the end of the process 0.24 mg/L.

The concentration of SCOD initially was 44 mg/L. At the end of the process in the oxygen bioreactor the SCOD concentration was 24 mg/L, while in the second bioreactor there was minimal difference, and the concentration was 26 mg/L. The PAOs can use nitrate as electron acceptor, but nitrate is not as efficient as oxygen for phosphorus uptake since more stored carbon was utilized for a given amount of phosphorus taken up (Wentzel et al., 2008).

A fraction of PAOs is able to utilize oxidized nitrogen (nitrate or nitrite) as electron acceptor in the absence of oxygen. Wentzel, Ekama and Marais (1992) claim that PAOs may be divided into two groups:

- Aerobic PAOs (APAOs) which can use only oxygen as electron acceptors.
- Denitrifying PAOs (DPAOs) which can use both oxygen and nitrate as electron acceptors.

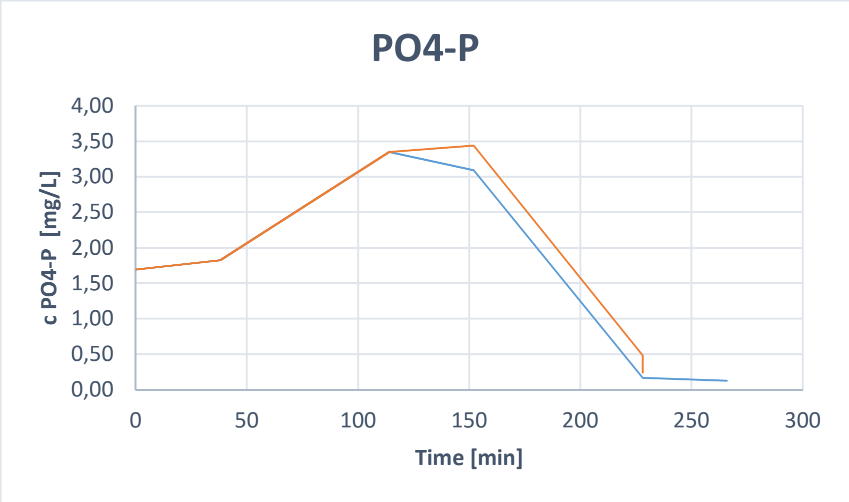

Figure 33 Orthophosphate concentration as a function of time in the Protocol No. 4

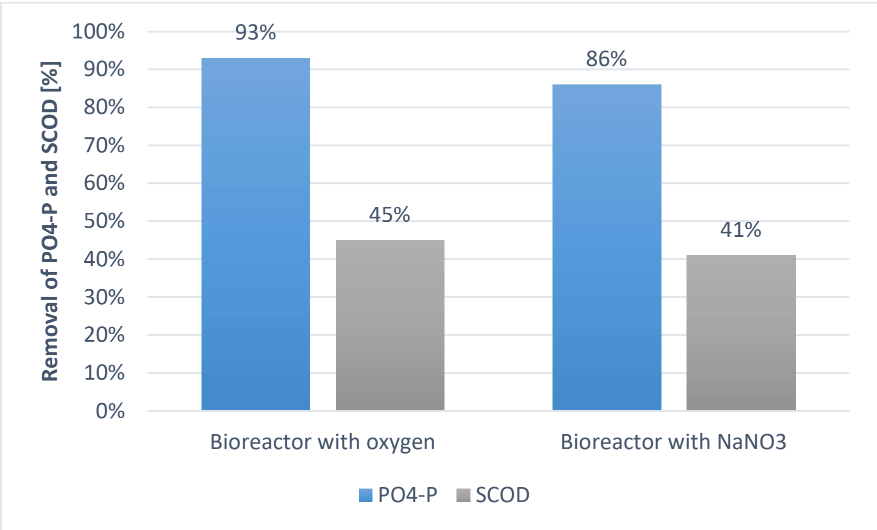

Figure 34 Percentage of phosphorus and SCOD removal in bioreactor with oxygen and with added $NaNO_3$

5. CONCLUSIONS

In this thesis, the efficiency of phosphorus removal from municipal wastewater of city of Trondheim by Moving Bed Biofilm Reactor (MBBR) and the Enhanced Biological Phosphorus Removal (EBPR) were examined under various experimental conditions in a batch bioreactors. The batch experiments with activated sludge attached on carriers and obtained results shown that Enhanced Biological Phosphorus Removal is efficient method for phosphorus removal from municipal wastewater of city of Trondheim and the good activity of bacteria responsible for biological removal of phosphorus in wastewater under different conditions has been demonstrated.

All experimnts were based on the MBBR pilot for nutrient removal from wastewater placed in wastewater laboratory at Norwegian University of Science and Technology in the city of Trondheim, Norway. The concentrations of DO, temperature, pH, SCOD and PO4-P of the municipal wastewater of city of Trondheim were monitored on a daily and hourly basis from January 29 to April 29, 2019. The results of conducted experiments shown that the concentration of dissolved oxygen is most important parameter for successful phosphorus removal as well as the effectiveness of the process. Kinetic experiments have shown the most optimal phosphorus removal was achieved at the DO level of 6 − 8 mg / L.

The activity of bacteria under anoxic conditions and the addition of $NaNO_3$ were tested and both experiments have demonstrated successful phosphorus removal, which indicates that PAOs can use nitrate as an electron acceptor, although bacteria prefer oxygen since lower phosphorus removal efficiency was noted. Moreover, the results presented in this thesis have shown that the PAOs should proliferate and be highly competitive against GAOs in the environment in the pilot. The results have also demonstrated a mixed microbiology which can survive and thrive under differing conditions.

The efficiant phosphorus removal was followed by efficiant SCOD removal in each experiment although the removal of SCOD was not as sensitive to various conditions as P removal. The temperature of wastewater, as well as pH, have been noted also noted as one of the key parameters for successful phosphorus removal from municipal wastewater of city of Trondheim. The samples of tested Lerkendal wastewater, as typical Norwegian wastewater had inlet temperature range from 10 to 14 °C, constant pH of 7.5, and is usually diluted due to often rainfalls or snow melting effect.

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