Biološko uklanjanje dušika iz komunalne otpadne vode grada Trondheima

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

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Biološko uklanjanje dušika iz komunalne otpadne vode grada Trondheima
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Sažetak:
Pojava povećanih koncentracija hranjivih tvari u površinskim vodama, prije svega dušikovih i fosfatnih spojeva, jedan je od najznačajnijih ekoloških problema današnjice, jer prisutnost navedenih nutrijenata u površinskim vodama šteti vodnim ekosustavima, uzrokujući smanjenje koncentracije otopljenog kisika i eutrofikaciju. Eutrofikacija je proces smanjenja kakvoće vodnog tijela koje se očituje prekomjernom rastu algi, povećanju koncentracije fitoplanktona te bujanjem biomase što za posljedicu može imati isušivanje vodnog tijela. Nekoliko tehnologija može učinkovito uklanjati hranjive sastojka iz otpadnih voda. U ovom radu ispitana je mogućnost uklanjanja dušika iz otpadne vode norveškog grada Trondheima pomoću tzv. Moving Bed Biofilm Reactor (MBBR) tehnologije. MBBR je nova, ekološki prihvatljiva i učinkovita tehnologija biološke obrade otpadnih voda usmjerena k uklanjanju hranjivih tvari iz otpadnih voda. Učinkovitost procesa i kinetika uklanjanja dušika određena je svakodnevnim praćenjem koncentracije otopljenog kisika, temperature, pH, kemijske potrošnje kisika te koncentracije dušika u različitim uvjetima.

Ključne riječi: obrada otpadne vode, MBBR, dušik, nitrifikacija, denitrifikacija

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

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Summary:
The occurrence of elevated nutrient concentrations in surface waters, mainly nitrogen and phosphorous, has become one of the main global concerns since their presence in surface waters harm to the aquatic habitats and ecosystems causing the depletion of dissolved oxygen and eutrophication. Eutrophication is the process of impairing water quality manifested by excessive growth of algae, an increase of phytoplankton, and large production of biomass in the aquatic system. Therefore, nutrients removal is one of the main issues in wastewater treatment technologies nowadays. Several technologies can be used for nutrients removal from wastewater. In this thesis, nitrogen removal from wastewater of the city of Trondheim, Norway, was examined by the Moving Bed Biofilm Reactor (MBBR). The MBBR is a new biological wastewater treatment technology focused on nutrients removal from wastewaters. It is an ecologically acceptable and efficient process with reduced needs for space. The efficiency of the process and kinetics of nitrogen removal was determined by daily monitoring of dissolved oxygen, temperature, pH, chemical oxygen demand, and nitrogen concentrations under various conditions.

Keywords:	wastewater treatment, MBBR, nitrogen, nitrification, denitrification
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The experimental part of this thesis was done at the Department of Civil and Environmental Engineering at Norwegian University of Science and Technology (NTNU) in Trondheim, Norway, during February, March, and April of 2019.

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List of Abbreviations

AS	Activated Sludge
BNR	Biological Nutrient Removal
BOD_5	Biological Oxygen Demand
C	Carbon
Ca	Calcium
CO_2	Carbon Dioxide
SCOD	Soluble Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
DNPAOs	Denitrifying PAOs
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
EPS	Extracellular Polymeric Substance
GAOs	Glycogen Accumulating Organisms
H_2O	Water
HAB	Harmful Algal Blooms
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor
MCL	Maximum Contaminant Level
Mg	Magnesium
N	Nitrogen
N_2	Nitrogen Gas
N_2O	Nitrous Oxide

NH_4-N	Ammonium Nitrogen
NO_3-N	Nitrate Nitrogen
NO_2-N	Nitrite Nitrogen
NTNU	Norwegian University of Science and Technology
OHOs	Ordinary Heterotrophic Organisms
P	Phosphorus
PAOs	Polyphosphate Accumulating Organisms
rpm	revolutions per minute
SND	Simultaneous Nitrification and Denitrification
TN	Total Nitrogen
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acids
WHO	World Health Organization
WWTP	Wastewater Treatment Plant

Contents

1. INTRODUCTION

Urbanization and population growth have increased the need for wastewater treatment. Recent decades, scientific researches shown that wastewater with elevated pollutants and nutrient concentrations destroys the ecological balance in water to which it is discharged. To avoid negative impact on the environment, nitrogen (N) and phosphorus (P) must be removed from the wastewater at a concentrations that are not above aquatic ecosystem bearing capacity. More stringent wastewater discharge standards have been adopted recently. Removal of nitrogen from wastewater is required due to its contribution to climate change, contamination of the receiving water bodies, the occurrence of eutrophication, and toxicity of the environment. Total nitrogen in municipal wastewater consists of 60 – 70% of ammonium nitrogen (NH_4-N) and 30 – 40% of organic nitrogen (Tebbutt, 1990; Slagstad and Brattebø, 2013; Al-Rekabi, 2015; Lagesen Richardsen, 2017; Kyrkjeeide Finstad, 2018; Guo et al., 2019).

Biological methods are increasingly used in wastewater treatment because of their low cost, environmental friendliness, and sustainability. Keys for sustainability are high treatment efficiency and low energy consumption. Biological removal of nutrients from wastewater implies the simultaneous removal of the organic substrate, nitrate and phosphorus. Microbial community performing biological nutrient removal (BNR) is various and complex. Biological removal of nitrogen occurs in two steps: aerobic nitrification and anoxic denitrification (Al-Rekabi, 2015; Trikoilidou et al., 2016; Lagesen Richardsen, 2017).

BNR in biofilm processes has potential advantages compared to conventional processes. New and innovative technologies have been proposed in the last years and Moving Bed Biofilm Reactor (MBBR) technology imposes itself as a solution. MBBRs are compact units with small footprints, high effluent quality, and specialized biomass. It has been established worldwide as high efficiency, simple maintenance, low cost, flexible, and compact wastewater technology (Helness and Ødegaard, 2001; Leiknes and Ødegaard, 2007; Wang et al., 2019). The aims of this master thesis were: (i) determination of characteristics of typical Norwegian municipal wastewater by conducting daily measurements of organic materials, nutrients, DO, pH, and temperature and (ii) to investigate and monitor the kinetics of biological nitrogen removal in batch experiments using influent wastewater and biomass from a continuous MBBR pilot dedicated to enhanced biological phosphorus removal (EBPR) placed in

Wastewater Laboratory. Batch experiments were carried out under different conditions in order to define the key parameters for efficient nitrogen removal.

In total, 19 kinetic experiments were performed during February, March, and April of 2019. All experiments were conducted at the Department of Civil and Environmental Engineering, at Norwegian University of Science and Technology (NTNU) in Trondheim, Norway.

2. THEORETICAL PART

2.1. WASTEWATER AND WASTEWATER TREATMENT

Wastewater is potentially polluted water formed by the use of water from numerous water supply systems for specific purposes, with changes in its original physical, chemical, and microbiological characteristics. The wastewaters can be classified as:

- Municipal wastewater – domestic wastewater from rural and urban settlements, a combination of human and animal excreta and water used for washing, bathing, and cooking;
- Industrial wastewater – wastewater generated by the implementation of various technological procedures in industrial and other production plants including industrial cooling water with elevated temperature;
- Precipitation wastewater – wastewater generated from precipitation, polluted by the contact with lower atmospheric layers, soil, roofs, etc.;
- Leachate wastewater – wastewater formed by contamination of precipitation or groundwater discharged through landfills of solid waste, and
- Wastewater from livestock farms – wastewater originating from stock and poultry farms (Bitton, 2005; Perušina, 2010).

World Health Organization (WHO) states that 80% of diseases in developing countries are caused by water. The importance of appropriate sanitary measures was recognized in the era of early civilizations. Since then, wastewater treatment plants (WWTP), along with pollution control, have played a crucial role in improving community health standards. The pollutants mostly found in wastewater are suspended solids, organic compounds (biodegradable and volatile), nutrients (N and P), xenobiotics, heavy metals, pathogenic microorganisms, and parasites. Initially, the wastewater treatment plants (WWTPs) were designed to remove suspended solids and organic matter from wastewater, but nowadays WWTPs are, besides suspended solids and organic matter removal, mostly designed for nitrogen and phosphorus removal in order to reduce the pollutions and eutrophication of receiving water bodies. WWTPs seek to emulate natural processes and consist of preliminary, primary, secondary, and sometimes tertiary treatment (**Figure 1**). The degree of required treatment depends on the location of the wastewater discharge (Tebbutt, 1990; Bitton, 2005; Salgot and Folch, 2018; Lopez et al., 2019).

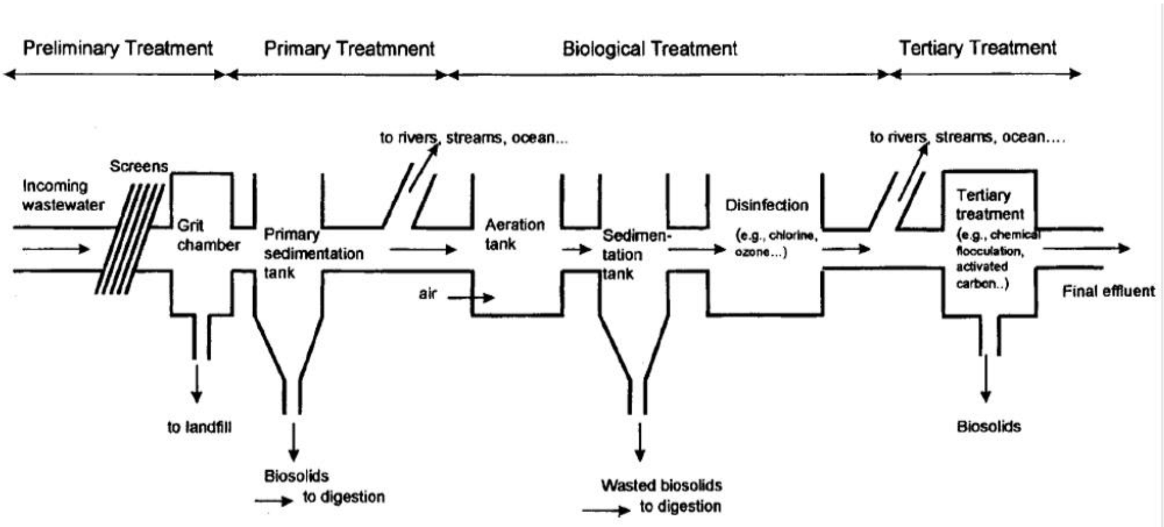

Figure 1 Typical design of wastewater treatment plant (Bitton, 2005)

Wastewater treatment methods may be physical, chemical, and biological. Preliminary treatment of wastewater includes the removal of suspended solids using screens or conditioning of wastewater. The purpose of preliminary treatment is to remove material that may clog equipment in the plant. Primary wastewater treatment involves the removal of sand, gravel, grease and oil. These pollutants are removed from wastewater by methods such as screening, sedimentation, flotation, and neutralization (Ramalho, 1977; Bitton, 2005). By primary wastewater treatment, the initial biological oxygen demand (BOD_5) value is reduced by at least 20% and total suspended solids (TSS) by at least 50%, according to the Regulation of Wastewater Emission Limit Values (Ministry of Agriculture, 2013). Methods used in the secondary wastewater treatment can be chemical and biological; individual or in combination. Chemical wastewater treatment implies the application of chemicals that assist in separating, destruction or neutralization of pollutants from wastewater or wastewater disinfection. Chemical methods are applied individually or in combination with physical methods. The biological treatment uses and exploits various bacteria species to remove pollutants and is optimal because of its efficiency, reliability, and low-cost. Commonly used biological processes are activated sludge, trickling filter, oxidation ponds, etc. (Cheremisinoff, 2002; Bitton, 2005; Al-Rekabi, 2015; Trikoilidou et al., 2016; Salgot and Folch, 2018).

Indeed, biological wastewater treatment is most widely used and it is elaborated in this master thesis. Tertiary or advanced treatment includes further reduction of nutrients, suspended solids, pathogens, parasites, or other pollutants still present after secondary treatment.

Tertiary treatment includes filtration, oxidation, ozonation, adsorption, activated carbon, membrane treatment, etc. The biological aerated filter is used for the removal of residual NH_4-N and COD. If microbial removal is necessary, the disinfection step is included. The by-product of wastewater treatment is a solid waste, sludge. The composition of sludge reflects the composition of wastewater. Sludge contains active (live) and inactive (dead) microorganisms (Bitton, 2005; Trikoilidou et al., 2016; Zhao et al., 2017; Salgot and Folch, 2018; Lopez et al., 2019).

The efficiency of wastewater treatment is determined by comparing the quality of influent and effluent of wastewater. If wastewater is not treated properly, it can cause serious environmental deterioration and ultimately impact human health. Once a WWTP is built, special attention has to be paid to the start-up phase since the treatment processes are strongly influenced by seasonal conditions. Sometimes it takes more than a year to reach a state where all processes occur to the desired extent. The cost of wastewater treatment depends on the required treatment standard. Costs are increasing with additional process requirements (Boller, 1997; Trikoilidou et al., 2016; Salgot and Folch, 2018).

2.1.1. Environmental Impact of Wastewater Treatment Plants

Trondheim's wastewater effluent is discharged into the nearby fjord. While the nitrogen entering the sea has eutrophication potential, nitrous oxide (N_2O), as a greenhouse gas, contributes to global warming almost 300 times more than carbon dioxide (CO_2). Because of the energy and chemical use, as well as N_2O emission, WWTPs have a large impact on the environment. Only a small part of the nitrogen content is removed from wastewater before it is discharged into the fjord. Therefore, improved nitrogen removal from wastewater is required although the contribution to climate change from the wastewater system in Trondheim is of minor concern (Slagstad and Brattebø, 2013). When treated wastewater is reused or returned to the environment, the main concern is safety (Salgot and Folch, 2018).

2.1.2. Nitrogen in Wastewater

Nitrogen is a major element of proteins and cells. It is essential for plants, animals and humans. Almost 80% of the atmosphere consists of nitrogen. Nitrogen present in wastewater originates mainly from excreta, detergents, and fertilizers. People excrete 1 – 1.3 L of urine and 100 – 500 g of faeces per capita per day (Bitton, 2005; Al-Rekabi, 2015; Kyrkjeeide Finstad, 2018). Forms of nitrogen present in wastewater are divided into four groups:

- organic nitrogen,
- ammonium nitrogen (NH_4-N),
- nitrate (NO_3-N), and
- nitrite (NO_2-N).

Total nitrogen (TN) in municipal wastewater consists of 60 – 70% of NH_4-N and 30 – 40% of organic N. NH_4-N is derived from urea and is rapidly transformed into ammonia in wastewater. Occurrence of heightened ammonia concentrations in wastewater discharged in the environment can cause acute and chronic toxicities in receiving water bodies (Al-Rekabi, 2015; Ashkanani et al., 2019).

2.2. ISSUES OF NUTRIENTS OCCURENCE IN AQUATIC SYSTEMS

Nitrogen and phosphorus, if excessively discharged, cause significant contamination of the receiving water bodies. As a result, eutrophication appears. Eutrophication is a natural process of rapid algae and bacteria growth, which consume oxygen in large quantities. Cyanobacteria are the largest and the most important group of green algae that develop in water and form harmful algal blooms (HAB) caused by nitrogen and phosphorus. Besides nitrogen and phosphorus, eutrophication is also triggered by the lack of light and high temperature. As a consequence of eutrophication, the proliferation of opportunistic plants occurs, replacing the existing species. Some of them may be toxic. Furthermore, biodiversity is lost and anoxia appears. It leads to the death of most forms of aquatic life. Eutrophication has become a serious global problem. Also, high levels of nitrogen and phosphorus cause toxicity in the environment. Even excessive amounts of discharged organic matter cause exhaustion of oxygen since microorganisms use organic pollutants as a source of food and consume dissolved oxygen (DO). Therefore, many pollution issues are related to the DO in water

(Tebbutt, 1990; Al-Rekabi, 2015; Álvarez et al., 2017; Lagesen Richardsen, 2017; Le Moal et al., 2019).

To avoid negative impact on the environment, nitrogen and phosphorus must be removed from the wastewater at a concentration that is not above aquatic ecosystem bearing capacity and that is in accordance with the regulations. Treated wastewater must be of sufficient quality to be reused safely and legally. Maximum Contaminant Level (MCL) is determined by permissible pollutant concentrations and loads in wastewater according to the Regulation. It is the highest level of an allowed contaminant (Cheremisinoff, 2002; Ministry of Agriculture, 2013; Kyrkjeeide Finstad, 2018; Salgot and Folch, 2018). **Table 1** shows the MCL of municipal wastewater depending on the discharge site.

Table 1 MCL of municipal wastewater depending on the discharge site according to Regulation of wastewater emission limit values (Ministry of Agriculture, 2013)

	DISCHARGE SITE	
PARAMETER	**SURFACE WATER**	**SEWAGE SYSTEM**
pH	6.5 – 9	6.5 – 9.5
T [°C]	30	40
BOD_5 [mg/L]	25	250
COD [mg/L]	125	700
TP [mg/L]	2	10
TN [mg/L]	15	50
NH_4-N [mg/L]	10	-
NO_2-N [mg/L]	1	10
NO_3-N[mg/L]	2	-

2.3. BIOLOGICAL NUTRIENT REMOVAL (BNR) FROM WASTEWATER

Biological removal of nutrients from wastewater implies the simultaneous removal of the organic substrate, nitrogen and phosphorus. BNR process relies on a combination of different environmental conditions; hence it consists of anaerobic/anoxic and aerobic reactors. In the aerobic reactor, aerobic bacteria perform nitrification, oxidation of the organic substrate, and remove phosphorus. Moreover, in anaerobic/anoxic reactor, denitrification occurs. Biomass is cycling through alternating anaerobic and aerobic reactors where they are subjected to anaerobic feast and aerobic famine. Microorganisms affect nutrient removal by assimilation and other biological processes. Ordinary heterotrophic organisms (OHOs) remove nitrogen through assimilation. Denitrifying Polyphosphate Accumulating Organisms (DNPAOs) are strings of different bacteria that perform nitrification and denitrification (Al-Rekabi, 2015; Kyrkjeeide Finstad, 2018).

The conventional BNR system is expensive in terms of investment and space, requiring large reactor volumes (Mannina et al., 2017).

2.3.1. Nitrogen Removal from Wastewater

Biological removal of nitrogen occurs in two steps: nitrification and denitrification. In conventional nitrogen removal processes, NH_4-N is oxidized to NO_3-N by nitrification and then NO_3-N is reduced to nitrogen gas (N_2) by pre- or post-denitrification. Nitrification and denitrification take place in two different reactors (Lagesen Richardsen, 2017; Wang et al., 2019).

Nitrification

Nitrification is the first step in the biological removal of nitrogen from wastewater which occurs in the presence of oxygen. Nitrification implies the conversion of NH_4-N into NO_2-N by autotrophic bacteria such as *Nitrosomonas* and further oxidising into NO_3-N by microorganisms such as *Nitrobacter*. Nitrifying bacteria inside the biofilm are developed with the presence of oxygen and ammonia. Microorganisms responsible for these processes use oxygen as the electron acceptor and organic carbon (C) as the source of food. No carbon addition is required (Garzón-Zúñiga and González-Martínez, 1996; Al-Rekabi, 2015; di Baise et al., 2019). Stoichiometry of described processes and the total reaction are given below in Equations 1, 2 and 3:

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad (2)$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

Based on stoichiometric reactions, NH_4-N oxidation to NO_2-N requires 75% of the total oxygen while the remaining 25% of the oxygen is used to oxidize NO_2-N to NO_3-N. pH between 8 and 9 is optimal for nitrification. Also, the nitrification rate strongly depends on the level of DO. In the MBBR process, the efficiency of nitrification is enhanced by increasing DO. The diffusion of oxygen from outer to inner layers of biofilm also contributes to the nitrification rate. Indeed, DO concentration, as well as organic loading rate, is the main factor triggering nitrification. Complete nitrification requires longer or stronger aeration (Garzón-Zúñiga and González-Martínez, 1996; Al-Rekabi, 2015; di Baise et al., 2019). Bryant et al. (1997) point out that moderate wastewater temperature (22°-35°C) and the pH around 7.3 favour nitrification.

Denitrification

Denitrification is an anoxic process which completes the total process of biological nitrogen removal. Denitrification is performed by heterotrophic bacteria, facultative anaerobic organisms, with the ability to use NO_3-N or NO_2-N as well as oxygen. While the organic matter is oxidised, NO_3-N and NO_2-N are used as electron acceptors. DO level must be under 0.5 mg/L (Garzón-Zúñiga and González-Martínez, 1996; Al-Rekabi, 2015). Denitrification involves several reduction steps shown in Equation (4):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (4)$$

Each reduction step is catalysed by the different sequentially operating enzyme: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase, respectively. Denitrifying heterotrophs consume biodegradable carbon and reduce soluble oxidized form of nitrogen to N_2. In this final step of biological nitrogen removal, 60% of the carbon source is consumed. The optimal pH for denitrification is between 7 and 9. Since most of the denitrifying bacteria are facultative anaerobic organisms, the presence of oxygen is detrimental for the denitrification process because heterotrophic bacteria will use oxygen as an electron acceptor and consume organic matter needed for denitrification. Denitrification can be improved by adding an external source of carbon. To avoid additional costs, conventional treatment of wastewater is being modified. Post-denitrification and pre-denitrification are often used as well as simultaneous phosphorus removal, nitrification, and denitrification (Al-Rekabi, 2015; Zhang et al., 2018; di Baise et al., 2019).

Most of the nitrogen removal technologies are based on nitrification and denitrification in two separate reactors. However, both of the processes can occur simultaneously in the same reactor (Zinatizadeh and Ghaytooli, 2015). More on simultaneous nitrification and denitrification (SND) see below.

2.3.2. Activated Sludge Nutrient Removal from Wastewater

Activated sludge (AS) has become well established and one of the most common technologies for BNR. The nutrients are removed through phosphorus removal, nitrification, and denitrification. Each process takes place in a separate reactor. Activated sludge recirculates through different reactors with different conditions, thus makes this process continuous (Kyrkjeeide Finstad, 2018). See **Figure 2** for the scheme.

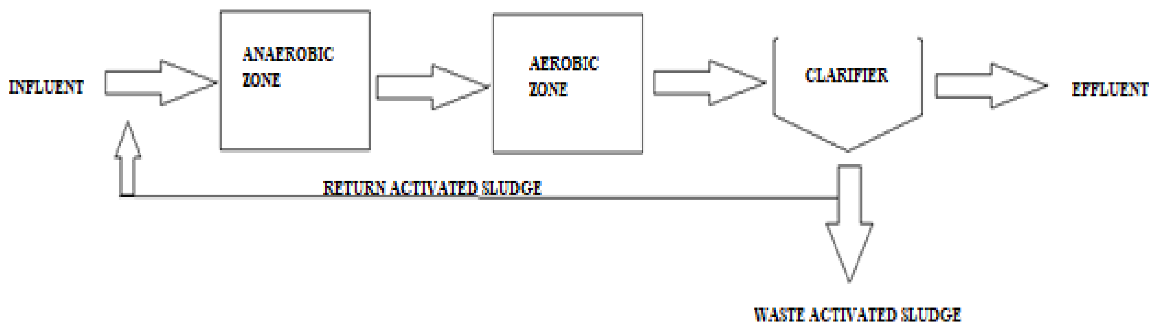

Figure 2 Activated sludge technology (Kyrkjeeide Finstad, 2018) for biological nutrient removal (BNR).

Figure 2 shows activated sludge technology. Influent enters an anaerobic zone where Polyphosphate Accumulating Organisms (PAOs) store Volatile Fatty Acids (VFA) and release phosphorus. In aerobic zone, PAOs use carbon for growth and uptake phosphorus. In the clarifier, activated sludge is separated from treated wastewater. Part of the activated sludge is returned at the beginning of the process while waste activated sludge is disposed of. Although the activated sludge process is considered conventional and well-established in wastewater treatment, it has some disadvantages. Investment costs, when establishing this process, are quite high and process demands huge space areas. Furthermore, solids and sludge load are high and the process may suffer from separation problems due to sludge settle ability. Consequently, large quantities of activated sludge are generated as a product and have to be frequently disposed of. Another problem that arises is sludge disposal. Landfill disposal of sludge can cause significant environmental, economic, and social problems. Sludge treatment doubles wastewater treatment costs (Trikoilidou et al., 2016; Kyrkjeeide Finstad, 2018).

2.4. DEVELOPMENT OF MOVING BED BIOFILM REACTOR (MBBR)

Moving Bed Biofilm Reactor (MBBR) technology was developed in Norway in the late 1980s and early 1990s. MBBR is a biological wastewater treatment process based on the conventional activated sludge process and biofilm system. It has evolved due to the necessity of higher quality effluent while having minimum space used. Biofilm processes have proved to be very successful in terms of carbon and nitrogen removal avoiding limitations of activated sludge processes. MBBR is widely applied because of its high efficiency, simple maintenance, and low cost (Pastorelli et al., 1997; Al-Rekabi, 2015; Wang et al., 2019).

MBBR process is based on the movement of carriers through the reactor. The carriers are small plastic cylinders (**Figure 3**) made of high-density polyethylene (HDPE), less dense than the water with a cross on the inside and ribs on the outside, which makes big surface area where microorganisms grow. Microorganisms tend to form colonies to grow faster and facilitate access to food by forming a biofilm (Leiknes and Ødegaard, 2007; Al-Rekabi, 2015; Lagesen Richardsen, 2017; Wang et al., 2019). di Baise et al. (2019) have defined biofilm as a complex heterogeneous micro-ecosystem of microbial community interactions sharing the same environment. Biofilm can attach on both the inner and the outer surface of the carrier by adhesion, forming a tight connection by producing extracellular polymeric substances (EPS), a mixture of polysaccharides, proteins, and extracellular deoxyribonucleic acid (DNA). EPS spontaneously develops into dense aggregates that adhere to a surface. Biofilm systems largely depend upon biofilm formation. Biofilm formation occurs through several phases beginning with adsorption of nutrients and macromolecules to the surface, initial cellular transport, adhesion, and detachment. On the other hand, detachment of biomass occurs through abrasion by carrier collision, erosion by bulk liquid shear forces and loss of biofilm segments from the carriers. An even and thin biofilm is desirable and achieved by turbulence and action of shear forces through homogenous mixing. When it comes to biofilm formation, several factors play a decisive role, including (i) physical and chemical properties of the carrier surface; (ii) surface roughness; (iii) pore structure; (iv) specific area; (v) material of carriers (Al-Rekabi, 2015; di Baise et al., 2019; Wang et al., 2019; Zhao et al., 2019).

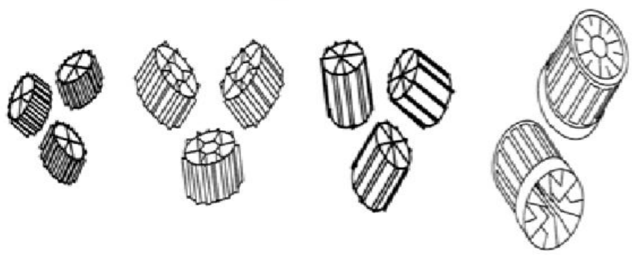

Figure 3 Different types and sizes of carriers (Kyrkjeeide Finstad, 2018)

The biomass attaches to certain areas on a carrier where microbial turnover is happening by using substances from liquid bulk. Effective surface area is a protected area of the carrier that has no contact with other carriers during mixing and is where biofilm attaches, amounting 70% of the total surface. That high biofilm concentration in small reactor volume controls the performance of the process. Biofilm includes different layers with different microbial communities. SND occurs inside the biofilm. The biofilm needs to be specialized to work well under different conditions. Oxygen will penetrate to a certain depth in the biofilm-forming outer aerobic layer where nitrification occurs, while deeper layers of the biofilm where denitrification occurs are anoxic. Except for the attached biomass, in MBBR are present suspended flocs of biomass, phenotypically and genotypically different from biofilm (Helness and Ødegaard, 2001; Al-Rekabi, 2015; Mannina et al., 2017; Kyrkjeeide Finstad, 2018; di Baise et al., 2019).

Continuous EBPR-MBBR pilot

Therefore, carriers with attached biomass in the continuous EBPR-MBBR pilot flow with the wastewater through the treatment process. Carriers are exposed to anaerobic and aerobic conditions. By dividing the reactor into separate entities, each chamber is designed and adjusted to optimal conditions. For each anaerobic chamber, the mixer speed is adjusted separately as is the airflow rate in aerobic chambers. The anaerobic phase should be long enough to minimise the competition of anaerobic bacteria and aerobic heterotrophs. On the other side, the aerobic phase should be long enough to achieve complete nitrification. Size and number of chambers depend on wastewater characteristics and effluent limit requirements. In anaerobic reactors, the movement is caused by mechanical stirrer and by aeration in aerobic reactors.

In MBBRs there is no dead or unused space in the reactor due to good mixing (Helness and Ødegaard, 2001; Leiknes and Ødegaard, 2007; Al-Rekabi, 2015; di Baise et al., 2019). di Baise et al. (2019) reported that filling fraction must not exceed more than 70% of total reactor volume to achieve proper mixing. Proper mixing is very important since high turbulence can cause detaching of biomass from carriers. Also, faster bulk flow means thinner biofilm. The thickness of the boundary layer influences the utilization of oxygen. The thinner boundary layer means a more efficient utilization of oxygen. Growth and detachment processes should be balanced to maintain constant biofilm thickness at a steady state due to shear forces from mixing or aeration. Mixing is especially challenging in the early stage of biofilm development. When the biofilm is not yet established, carriers float due to their lower density compared to water. As microorganisms start to attach and develop on carriers, carriers become heavier and mixing capabilities improve. In MBBR, the process efficiency depends on system setup, active biomass concentration, mass transfer effectiveness, feed distribution, and mixing (Kyrkjeeide Finstad, 2018; di Baise et al., 2019; Wang et al., 2019). Active biomass concentration is relatively constant in a stable process.

10 years after the development of MBBR, 400 large-scale wastewater treatment plants based on this process took place in 22 different countries all over the world (Al-Rekabi, 2015).

2.4.1. Types of Carriers Used in Moving Bed Biofilm Reactor

Carriers differ according to their surface area, size, price, shape, material, etc. Carriers can be made of stones, gravels, sand, soil, wood, rubber, agglomerates of the biomass, plastic, or any other synthetic material. Material selection is important to maintain a high quantity of active biomass (Wang et al., 2019) since biofilm architecture and microbial composition are influenced by carrier material. HDPE is the most preferred material because of its plasticity, density, and durability (di Baise et al., 2019). The most important property of carriers is the surface area. The large surface area provides the development of biofilm on the media and efficient adsorption of substrates from wastewater (Wang et al., 2019). If the surface area is difficult to reach, diffusion issues occur. To repeat, the total surface area of the carrier consists of the inner and the outer surface of which the effective surface is where the biomass attaches (Kyrkjeeide Finstad, 2018). Ashkanani et al. (2019) point out that the carrier surface area and carrier-specific surface should be considered when designing MBBR. Carriers have to obey following conditions for their geometry to be regular: (i) the carrier geometry should protect biofilm produced on it and provide enough area for the proper biofilm development; (ii) the carrier should not have dead places where oxygen is limited; (iii) the carrier should provide a suitable film thickness. Lengths of carriers vary from 7 to 15 mm and diameters from 10 to 15 mm (Leiknes and Ødegaard, 2007; Kruszelnicka et al., 2018). Kruszelnicka et al. (2018) conclude that by increasing the length of the carrier, maximal velocity in the internal region is reduced. Moreover, they investigated that maximal velocity is increased by increasing the diameter of the carrier. To sum up, it is possible to optimize the maximal values of the velocity of the fluid interacting with biofilm by changing the values of the radius and the length of the carrier. Carriers can be made of different shapes like sphere, round, or square. The shape is important because it affects the carrier's strength, shearing, and colliding conditions. The density of carriers is normally lower than wastewater so they can be suspended in wastewater. Well-designed carriers enable stable biofilm (Wang et al., 2019).

2.4.2. Microbial Diversity in Moving Bed Biofilm Reactor

Microbial communities on MBBR carriers are various and complex. A large number of bacteria colonies are cultivated in activated sludge or immobilised on carriers. Biomass utilizes carbon, nitrogen and phosphorus for growth and energy production. Different environment means more differences in the growth and abundance of bacteria. Microbial communities vary from inactive to rapidly growing and autotrophic to heterotrophic organisms. Heterotrophic bacteria grow fast and reduce oxygen available for autotrophic nitrifiers. Indeed, the coexistence of different microorganisms means competition for oxygen. MBBR can provide different kinds of bacteria such as: PAOs for phosphorus removal, bacteria for nitrification-denitrification, heterotrophic bacteria, etc. The diversity and composition of the bacterial community is a crucial factor for MBBR performance. A more diverse bacterial community means higher efficiency in pollutant removal. The biomass should be stringently active to accomplish optimal biological removal rates. Biomass died due to the lack of nutrients do not contribute to the efficient removal of pollutants. Active biomass is all microorganisms that are sufficiently supplied with nitrogen, phosphorus, and carbon to be metabolized and with oxygen if required. The establishment of a steady microbial community usually requires 4-8 months for all systems (Garzón-Zúñiga and González-Martínez, 1996; Al-Rekabi, 2015; Trikoilidou et al., 2016; Geiger and Rauch, 2017; Lagesen Richardsen, 2017; Kyrkjeeide Finstad, 2018; Guo et al., 2019; Layer et al., 2019; Wang et al., 2019).

2.4.2.1. Ordinary Heterotrophic Organisms (OHOs)

Ordinary Heterotrophic Organisms, known as OHOs, are bacteria with high affinity for aerobic carbon degradation and release of organic nitrogen as ammonia. OHOs also perform denitrification in anoxic conditions and fermentation of fermentable organics to VFAs in anaerobic conditions. OHOs use either oxygen or nitrate as electron acceptor and receive their carbon (anabolism) as well as energy (catabolism) requirements for biomass production from the same organic compounds. That results in OHOs having much higher biomass growth coefficient than autotrophic nitrifiers (5:1). Active OHOs are involved in total organic matter in influent and in some wastewaters active OHOs could account for 20% of total COD concentration. OHOs are usually outcompeted by slower growing carbon-storing organisms (PAOs and GAOs). Otherwise, lower nutrient removal performances are observed (Ekama, 2011; Layer et al., 2019).

2.4.2.2. Polyphosphate Accumulating Organisms (PAOs)

Polyphosphate Accumulating Organisms (PAOs) are a community of different strings of bacteria responsible for enhanced biological phosphorus removal (EBPR). PAOs release phosphorus in stress anaerobic conditions and then uptake phosphorus in the aerobic phase using oxygen as electron acceptor. Combination of aerobic and anaerobic conditions promote PAOs growth as well as presence of SCOD. On the other hand, denitrification intermediates such as nitrite or nitric oxide could have an inhibitory effect on PAOs. The presence of nitrate induce the activity of denitrifiers which use COD as electron donor and result in less COD available for PAOs. PAOs can be divided into two groups according to their denitrifying capabilities: (i) group of PAOs which is able to denitrify nitrate as well as nitrite and uptake phosphorus simultaneously and (ii) group of PAOs which can only use nitrite in presence of oxygen (Ekama, 2011; Guerrero et al., 2011; Tayà et al., 2013; Kyrkjeeide Finstad, 2018).

2.4.2.3. Denitrifying Polyphosphate Accumulating Organisms (DNPAOs)

Polyphosphate accumulating organisms, known as PAOs, are capable of performing denitrification and are then referred to as Denitrifying PAOs. DNPAOs can use either oxygen or NO_3-N to consume carbon source and carry out internal biochemical processes. Since they remove phosphorus, while denitrifying NO_3-N, DNPAOs are of an advantage when simultaneous nitrogen and phosphorus removal is desired. An important advantage of DNPAOs is the high utilization of the substrate. Simultaneous removal of nitrogen and phosphorus reduces the need for a substrate that is present in small concentrations in diluted Norwegian wastewater (Lagesen Richardsen, 2017; Kyrkjeeide Finstad, 2018).

2.4.2.4. Glycogen Accumulating Organisms (GAOs)

Glycogen Accumulating Organisms (GAOs) are slow growing organisms which uptake carbon under anaerobic and aerobic conditions. Presence of GAOs in MBBR could be a drawback because GAOs outcompeting PAOs results in lower phosphorus removal. Moreover, GAOs increase carbon and chemical requirements and total cost of EBPR process. Researchers have been struggling finding optimal conditions that favour PAOs over GAOs. Several studies have demonstrated that the key factors that promote the growth of PAOs over GAOs are pH, temperature, concentration of DO and C/P ration in influent (Tayà et al., 2013; Acevedo et al., 2017; Kyrkjeeide Finstad, 2018;).

2.4.2.5. Ammonia Oxidizing Bacteria (AOB)

Ammonia Oxidizing Bacteria (AOB) are autotrophic bacteria responsible for the oxidation of NH_4-N to NO_3-N. AOB are obligated aerobes, i.e. grow under aerobic conditions only. AOB require a long start-up period due to a low growth rate and long doubling time (10-14 days). Biofilm attached to carriers is protected from the environment and slow-growing organisms are preserved from washing out of the system. AOB better retain and perform in MBBR at lower temperatures (Ekama, 2011; Kowalski et al., 2019; Wang et al., 2019).

Anaerobic oxidation of ammonia is considered a specific type of denitrification. Ammonia oxidation is associated with NO_2-N reduction. Anammox bacteria initiate the catabolic reaction of merging nitrogen atoms from ammonia and NO_2-N to form N_2. Anammox bacteria are autotrophic bacteria that use inorganic carbon as source of food for biomass production (van Loosdrecht et al., 2016).

2.4.2.6. Nitrite Oxidizing Bacteria (NOB)

Nitrite Oxidizing Bacteria (NOB) convert NO_2-N to NO_3-N. At temperatures < 20°C, NOB has an advantage over AOB and its specific growth rates are higher than AOB's. Consequently, NOB must be constantly washed out. Free ammonia, free nitrous acid, and low DO level (1-2 mg/L) might inhibit NOB (Ekama, 2011; di Baise et al., 2019; Kowalski et al., 2019).

Since AOB and NOB are slow-growing autotrophic organisms, the time required for the development of nitrifying biofilm is long. AOB and NOB have limited abilities to produce EPS, the main factor of biofilm formation. On the other hand, heterotrophs have a doubling time of only a few hours (Abtahi et al., 2018).

The ideal carrier for fast-growing aerobic heterotrophs would have wider openings to avoid loss of effective surface area caused by clogging. On the contrary, slow-growing autotrophs grow better in smaller openings and larger surface areas (di Baise et al., 2019). Gu et al. (2018)

reported that the duration of the aeration significantly influences the autotrophic activity and longer aeration time means higher autotrophic activity. Also, AOB usually shows a better affinity for oxygen than NOB.

2.4.3. Advantages of Moving Bed Biofilm Reactor

- One of the advantages of MBBR is a possibility for SND. The outer layer of the biomass attached to carriers consists of nitrifying bacteria that consume available oxygen, while denitrifying bacteria perform in the inner layer where oxygen is limited (Lagesen Richardsen, 2017).
- One of the most important advantages of the MBBR process is specialized and active biomass. Inactive biomass erodes off carriers. Such specialized biomass is a result of having carriers fitted into a single environment full-time so microorganisms adapt to the given conditions. Self-regulating biofilm requires less monitoring and ensures a stable treatment process (Kyrkjeeide Finstad, 2018; Wang et al., 2019).
- Also, MBBRs are especially suitable for slow-growing microorganisms like nitrifying bacteria that have to be kept in the process (Al-Rekabi, 2015).
- Moreover, there is no need for recycling biomass since the biomass is retained as a biofilm on carriers which results in increasing efficiency for a given reactor volume. Therefore, the removal rate in the MBBR is several times higher than in the activated sludge process (Al-Rekabi, 2015; di Baise et al., 2019).
- Furthermore, low sludge production results in lower costs as there is no need for the removal and disposal of the sludge (Wang et al., 2019).
- MBBR technology does not need large reactors, precipitators, and biomass recycling. Therefore, MBBR is particularly convenient if there are space limitations (Al-Rekabi, 2015).
- Reactor volume is fully active as a result of completely mixed chambers. MBBR must be properly designed. If not, hydrodynamic properties may lead to the appearance of stagnant zones within the reactor (Kyrkjeeide Finstad, 2018; di Baise et al., 2019).
- Since the treatment performance is proportional to the biofilm surface, the improvement of the process can easily be achieved by adding more carriers to the reactor. Thus, better performances are achieved and the volumetric capacity of the

reactor is increased. Indeed, one can upgrade performance and volumetric treatment capability with minimal additional costs (Al-Rekabi, 2015; di Baise et al., 2019).

2.4.4. Disadvantages of Moving Bed Biofilm Reactor

- The most significant drawback of MBBR is the slow biofilm formation rate during the start-up phase (Gu et al., 2018).
- The transport of compounds in and out of the biofilm happens through molecular diffusion which happens to be the main limitation in the MBBR process. Diffusion limitations can occur both in the anaerobic and the aerobic chambers. Generally, it is desirable to have a thin biofilm so the substrate can diffuse into the biofilm as the products can diffuse out of the biofilm. It is also preferable to have both aerobic and anoxic layers on the biofilm. The diffusion problem increases if the biofilm is thicker. Although, if the biofilm is as thin as it is desirable, there is a possibility that there is a lack of active biomass which reduces removal efficiency. On the other side, if the biofilm is too thick, detachment of active biomass can be very damaging for the process. There must be a balance on the surface of the biofilm, therefore the same quantity that diffuses in the biofilm is either removed, converted in the biofilm or diffuses from the biofilm (Kyrkjeeide Finstad, 2018).
- Furthermore, scaling on biofilm carriers also causes negative effects on the reactor's performance since scaling reduces effective surface area and disrupts mass transfer demanding more energy to keep carriers in suspension. Such carriers become heavier and settle down at the bottom of the reactor (Wang et al., 2019).
- One of the MBBR technology drawbacks is high energy costs due to aeration (di Baise et al., 2019).

2.5. COMPOSITION OF MUNICIPAL WASTEWATER

Municipal wastewater consists of 40 – 60% proteins, 25 – 50% carbohydrates, 10% fats and oils, urea and trace organic compounds such as phenols, surfactants, and pesticides. Most of the organic matter is biodegradable and contain amino acids, peptides, proteins, carbohydrates, volatile acids, fatty acids and their esters. The test used for the determination of the organic matter in wastewater is soluble chemical oxygen demand (SCOD). It represents the amount of oxygen required to completely oxidize the organic carbon into ammonia, H_2O, and CO_2 (Bitton, 2005).

The concentration of nutrients in influent depends on urban consumption; respectively city wastewater will naturally have higher nutrient levels than apartment complex wastewater (Lagesen Richardsen, 2017).

Influent carbon-to-nitrogen (C/N) ratio has a strong influence on the system performance in terms of organic carbon and nitrogen removal. For C/N ratio 5 – 10, the removal efficiency is satisfactory. If C/N ratio is reduced to < 5, bacterial activity significantly decreases causing stress for bacteria (Mannina et al., 2017). Mannina et al. (2017) have found that the C/N ratio equals to 5 presents a limit value at which denitrification cannot increase due to insufficient C source. A sudden decrease in the C/N ratio causes a decrease in denitrification and nitrogen removal efficiency. On the contrary, the C/N ratio of 10 improves nitrogen removal. This ratio can affect the growth of AOB, NOB, and DNPAOs and affect both suspended and attached biomass. Due to high hydraulic retention time (HRT) of biofilm on the carriers, attached biomass has greater nitrification capability while the suspended biomass shows a greater organic removal capability. Moreover, the attached biomass is more resistant to external disturbances and has higher activity if C/N is low.

2.6. TYPICAL NORWEGIAN WASTEWATER

Norwegian wastewater, in general, is cold and diluted because of the high amount of precipitation. Also, it contains a low concentration of nutrients and organic material when there is a limited amount of discharge from the industry. The concentration of DO in the wastewater is often high as the transport systems in Norway are affected by the varying topography. Also, heavy rainfall and groundwater infiltration contribute to a high level of DO. Wastewater contains a high concentration of calcium (Ca) and magnesium (Mg) since Norwegian drinking water is declared as hard water and wastewater consists of drinking water. The characteristics of wastewater depend on the hour, day, and season. During winter, low wastewater temperatures slow down processes. The pH of Norwegian wastewater is around 7 (Lagesen Richardsen, 2017). **Table 2** shows a comparison of the characteristics of typical municipal wastewater and characteristics of Norwegian wastewater.

Table 2 Comparison of typical municipal wastewater and Norwegian wastewater (Bitton, 2005; Lagesen Richardsen, 2017)

Parameter	**TYPICAL MUNICIPAL WASTEWATER**			**NORWEGIAN WASTEWATER**	
[mg/L]	strong	medium	weak	concentrated	diluted
COD	1000	500	250	1200	500
NH_4-N	50	25	12	75	20
TN	85	40	20	100	30
TP	15	8	4	25	6
TSS	350	220	100	600	250

3. EXPERIMENTAL PART

3.1. MATERIALS AND METHODS

Experiments were conducted in order to define and control the process of biological nitrogen removal. In this study, a single MBBR was applied to simultaneously remove carbon and nutrients from real samples of Trondheim municipal wastewater. This master thesis aimed to characterize local Norwegian wastewater by conducting daily measurements of organic materials, nutrients, DO, pH, and temperature; and to investigate the efficiency of nitrogen removal in batch experiments using influent wastewater and biomass from MBBR. Batch experiments were carried out under different conditions to detect the most suitable ones for efficient nitrogen removal. In total, 19 kinetic experiments were performed. Some experiment was repeated and only those with the most efficient results are analysed and presented in this thesis. The experiments were performed during February, March, and April 2019.

Materials and methods used, and the tests performed during experiments, are described in the following chapter. All analyses of influent were performed due to national or international standards.

3.1.1. Trondheim's Wastewater Influent

Influent wastewater comes from a local pump station, which collects wastewater from a nearby housing apartment complex area of Lerkendal (Gnr/Bnr/64/17), Trondheim (Norway), which is close to the Wastewater Laboratory. The wastewater is pumped into a storage tank inside the laboratory. The tank volume is 3.5 m^3. Locations of the apartment complexes and wastewater laboratory are shown in **Figure 4**.

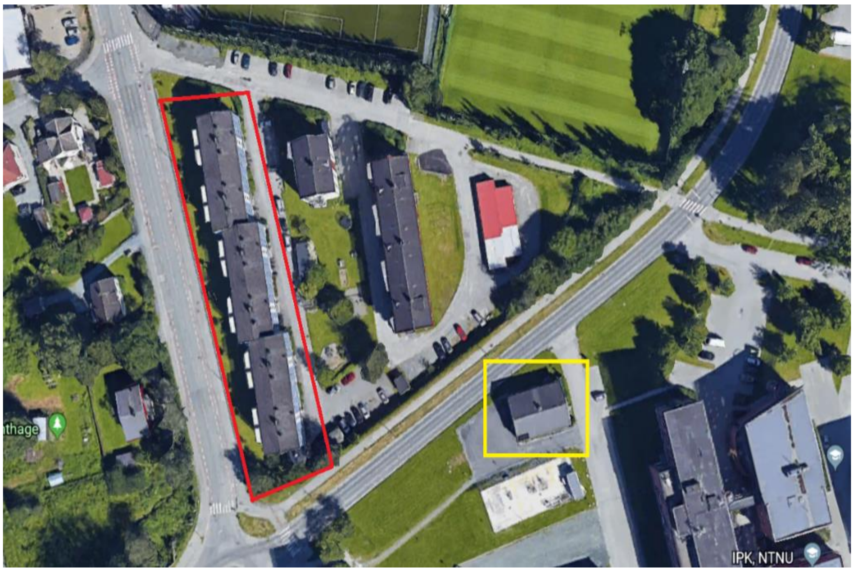

Figure 4 Position of apartment complex area (red) and wastewater laboratory (yellow). Source: Google Earth

3.1.2. Wastewater Sampling

Samples were taken from the tank by opening the valve on the influent pipe. Before taking samples, it was necessary to let the wastewater drain for a while to avoid the collection of high fraction of settled solids since the tank has no mechanical mixer. The sample is usually collected into plastic containers.

3.1.3. Analytical Methods and Wastewater Analysis

The samples were filtrated through 0.45 µm cellulose and nitrate filter (Sartorius). Before using, filters needed to be immersed in the distilled water and filtrated with distilled water at least three times.

Cuvette tests (Hach Dr. Lange) were used for determination of following parameters: NH_4-N, NO_3-N, NO_2-N, and SCOD (**Figure 5**). Some of the samples were diluted, depending on the measuring range of the used cuvette test.

Figure 5 Hach Dr. Lange cuvette test LCK 342 Nitrite

Once the cuvette test had been completed, the cuvette was placed in a spectrophotometer Hach DR 1900^{TM} (**Figure 6**) for measuring the concentration of a certain parameter.

Figure 6 Spectrophotometer Hach DR 1900 used for wastewater analysis

3.1.3.1. Soluble Chemical Oxygen Demand (SCOD)

The determination of SCOD was performed by adding 2 mL of filtrated sample in the appropriate cuvette (LCK 314, LCK 614, LCK 514, LCI 400 or LCK 1414) and then putting it in a heating block instrument. Tempering of SCOD samples was performed in LT 200 (Hach)

Thermostat shown in **Figure 7** at 148°C for 2 hours. After cooling, the concentration of SCOD was measured by inserting the cuvette into the spectrophotometer (**Figure 6**).

Figure 7 Thermostat Hach LT 200

3.1.3.2. Ammonium Nitrogen (NH_4-N)

The determination of NH_4-N was performed by adding 0.2 mL of filtrated sample in the appropriate cuvette (LCK 302, LCK 303 or LCK 304). After 15 minutes, the concentration of NH_4-N was measured by inserting the cuvette into the spectrophotometer (**Figure 5**).

3.1.3.3. Nitrate Nitrogen (NO_3-N)

The determination of NO_3-N was performed by adding 1 mL of filtrated sample in the appropriate cuvette (LCK 339 or LCK 340). After 15 minutes, the concentration of NO_3-N was measured by inserting the cuvette into the spectrophotometer (**Figure 5**).

3.1.3.4. Nitrite Nitrogen (NO_2-N)

The determination of NO_2-N was performed by adding 2 mL of filtrated sample in the appropriate cuvette (LCK 341 or LCK 342). After 10 minutes, the concentration of NO_2-N was measured by inserting the cuvette into the spectrophotometer (**Figure 5**).

3.1.3.5. Dissolved Oxygen (DO)

DO was measured by portable Dissolved Oxygen meter (VWR DO 220) shown in **Figure 8**. The electrode of the device was inserted in the continuous MBBR pilot and the value was obtained after the stabilization. The electrode was transferred from one chamber to another. DO in the batch experiment was measured by inserting the electrode of the DO meter in the reactor and reading the values when necessary.

Figure 8 Dissolved Oxygen Meter VWR DO 220

3.1.3.6. Temperature and pH value

The temperature was measured together with pH using WTW Oxi 3315 shown in **Figure 9**.

The electrodes of the device were inserted in MBBR and the values were obtained after the stabilization. The electrodes were transferred from one chamber to another. Temperature and pH in the batch experiment were measured by inserting the electrodes of the WTW Oxi 3315 meter in the reactor and reading the values when necessary.

Figure 9 WTW Oxi 3315 for measuring temperature and pH

3.2. DETERMINATION OF CHARACTERISTICS OF TRONDHEIM'S WASTEWATER

Samples were taken every Tuesday and Friday at 8 am during March, April and May 2019. Samples were filtrated through 0.45 µm cellulose and nitrate filter, and parameters (SCOD, NH_4-N, NO_3-N, NO_2-N) were determined by using cuvette tests. To measure pH, DO, and temperature of wastewater, pH meter and DO meter were used. Electrodes were inserted in wastewater. Both devices simultaneously measured the temperature and electrodes were transferred from one chamber to another.

The composition of wastewater changed due to the precipitation of a certain day. High precipitation is typical for Norwegian winter and spring when characterization was performed. Precipitation dilutes wastewater and lowers the temperature of wastewater. Characterization was carried out twice a week for 3 months and values of analysed parameters are reasonably similar.

3.3. BATCH EXPERIMENTS

Batch experiments were conducted to monitor the kinetics of nitrogen removal from Trondheim's wastewater and to simulating the processes and conditions presented in real MBBR. Batch experiments were carried out in laboratory bioreactors of volume 1 L, completely mixed by magnetic stirrer with rotational speed of 150 rpm to achieve homogenous and representative samples. Dissolved oxygen and pH were continuously controlled and measured with the DO meter and pH meter during each experiment. Samples were taken from the batch reactor, generally, every 38 minutes and analyses were done by previously described methods.

3.3.1. Batch experiment procedure

The samples were prepared by collecting 794 mL of influent wastewater from the receiving tank, 600 mL of carriers from a conveyor belt of MBBR and transferred to the bioreactor. All experiments were conducted at the temperature and pH equal to temperature and pH in MBBR of a specific day. The pH was constant so there was no need to adapt it and low temperature was maintained by placing bioreactor in a plastic bucket with snow. Hydraulic retention time in continuous EBPR-MBBR pilot was 380 minutes and consequently, every batch experiment lasted 380 minutes to mimic the same conditions. The anaerobic phase lasted for 152 minutes after which aeration started by supplying the bioreactor with compressed air. A small electric pump shown in **Figure 10** was used for aeration. Aeration lasted for 228 minutes. DO level below 0.05 mg/L was the limit set for anaerobic conditions. The described process is shown in **Figure 11**.

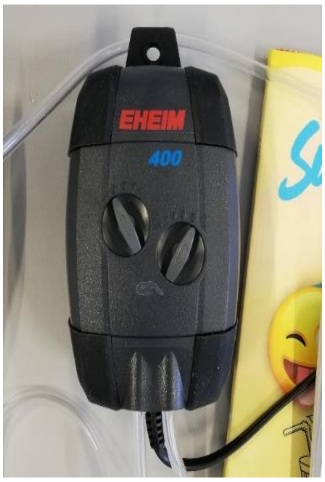

Figure 10 An electric pump EHEIM 400 for supplying the bioreactor with air

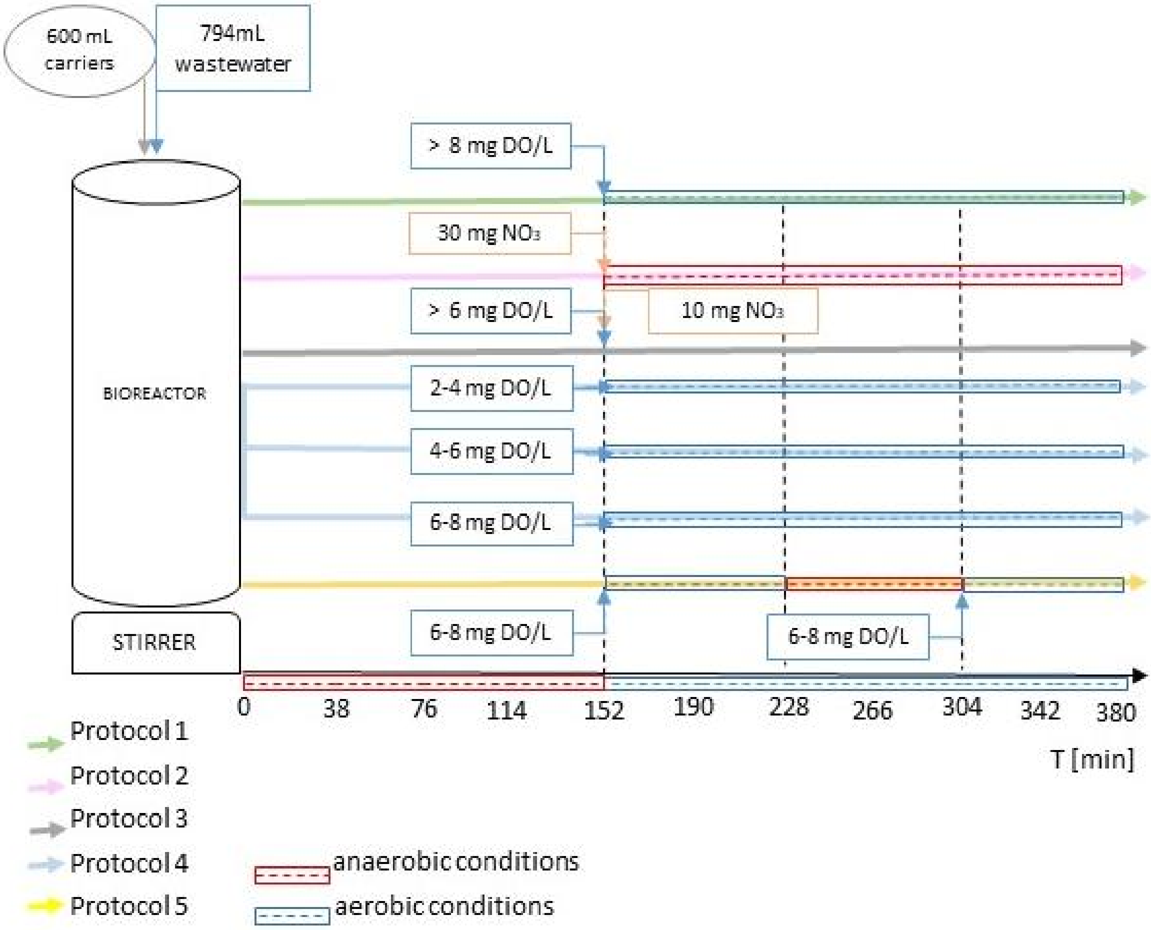

Figure 11 Scheme of batch experiments

The activity in the experiments was tracked by using Hach Dr. Lange cuvette tests. The results of batch experiments can be used for indicating what can be done to optimize the process.

3.3.2. Protocol 1

794 mL of wastewater was placed in the bioreactor together with 600 mL of carriers collected at the end of the aerobic zone of the EBPR-MBBR pilot with a conveyor belt. The content of the bioreactor was mixed as described in the previous chapter. Measuring of time began after the mixer had been switched on. Aeration started after 152 minutes. After 5 minutes of oxygen exposure, the DO level increased to 8.17 mg/L. Aeration lasted for 228 minutes and DO concentration was kept above 8 mg/L. Samples were collected at t = 0, 38, 114, 152, 266, and 342 minutes to determine concentrations of SCOD, NH_4-N, NO_2-N, and NO_3-N. Samples

were filtrated and used in cuvette tests and then placed in a spectrophotometer to read concentrations. **Figure 12** is showing Protocol 1. This experiment was carried out to gain insight into the nitrogen removal process at higher oxygen concentrations which proved to be appropriate. An experiment was done on 1st March when the oxygen level in MBBR was high. The temperature was kept 10-13°C and pH was 7.5.

Figure 12 Conducting Protocol 1

3.3.3. Protocol 2

Protocol 2 began with the same procedure as Protocol 1, except adding 0.14375 g of $NaNO_3$ corresponding to 30 mg/L of NO_3 as electron acceptor instead of oxygen in 152 minutes when the aeration should have started. Equation (5) shows the calculation of required amount of salt ($NaNO_3$) that correspond to the NO_3 concentration of 30 mg/L. This experiment was carried out to investigate the activity of bacteria when using $NaNO_3$ instead of oxygen. Samples for determination of SCOD, NH_4-N, NO_3-N, and NO_2-N were collected at t = 0, 114, 152, 228, 266, and 342 min. The pH was around 8 and the temperature was 13.5-14°C. The samples were prepared and parameters were determined as previously described.

$84.49\ mg\ NaNO_3 \rightarrow 14\ mg\ NO_3 - N$

$x \rightarrow 30\ mg\ NO_3 - N$

$$x = \frac{84.49 \cdot 30}{14} = 181.05\, mg\, NaNO_3$$

$$181.05\, mg\, NaNO_3 \rightarrow 1000\, mL$$

$$x \rightarrow 794\, mL$$

$$x = \frac{181.05 \cdot 794}{1000} = 143{,}75\, mg\, NaNO_3 = 0.14375\, g\, NaNO_3 \tag{5}$$

3.3.4. Protocol 3

Protocol 3 began with the same procedure as Protocol 1. In t = 152 min 0.047 g of $NaNO_3$ corresponding to concentration of 10 mgNO_3/L was added in bioreactor together with oxygen. Equation (6) shows the calculation of required amount of $NaNO_3$ that correspond to the NO_3 concentration of 10 mg/L. $NaNO_3$ was added at t = 152 min and re-added at 228 minutes when measurements showed that NO_3-N concentration is low. Aeration started at t = 152 min and the DO level was above 6 mg/L. The temperature was around 13°C and a pH was around 7.5. Samples for determination of SCOD, NH_4-N, NO_3-N, and NO_2-N were collected and prepared as described. The analyses of the parameters were conducted as in Protocol 1.

$$84.49\, mg\, NaNO_3 \rightarrow 14\, mg\, NO_3 - N$$

$$x \rightarrow 10\, mg\, NO_3 - N$$

$$x = \frac{84.49 \cdot 10}{14} = 60.35\, mg\, NaNO_3$$

$$60.35\, mg\, NaNO_3 \rightarrow 1000\, mL$$

$$x \rightarrow 794\, mL$$

$$x = \frac{60.35 \cdot 794}{1000} = 47\, mg\, NaNO_3 = 0.047\, g\, NaNO_3 \tag{6}$$

3.3.5. Protocol 4

Three bioreactors were used as shown in **Figure 13**. Each beaker was filled with wastewater and carriers as previously described. Once the anaerobic phase had been completed, different oxygen concentrations were blown into each reactor. In the first bioreactor, the DO level was between 2 and 4 mg/L; in the second bioreactor, the DO levels were between 4 and 6 mg/L;

and in the third bioreactor, the DO levels were between 6 and 8 mg/L. This experiment was conducted to detect differences in nitrogen removal from wastewater depending on different DO levels. The results were used to determine the most appropriate oxygen concentration for efficient removal of nitrogen from wastewater. Samples for determination of SCOD, NH_4-N, NO_3-N, and NO_2-N were collected at t = 0, 152, 190, 266, and 342 min from each bioreactor were prepared and analyses were carried out as previously described. In all three reactors, the temperature was between 13.40 and 14.70°C and pH was around 7.5.

Figure 13 Mixed bioreactors of the same content into which different oxygen concentrations are blown

3.3.6. Protocol 5

Protocol 5 began with the same procedure as Protocol 1, anaerobic phase was the same. Aeration started at t = 152 min. DO level was between 6 and 8 mg/L. At t = 228 min, aeration was stopped and the anoxic phase started. The anoxic phase lasted for 76 minutes. At t = 304 min, aeration started again. DO level was between 6 and 8 mg/L. The temperature was 12-13°C and the pH was 8. Samples for determination of SCOD and NO_2-N were collected at t = 0 and 342 min. Samples for determination of NH_4-N and NO_3-N were collected at t = 0, 152, 190, 228, 266, 304, and 342 min. Samples were prepared and determinations were carried out as previously described. All results of parameter analyses were expressed as mg per litre.

4. RESULTS AND DISCUSSION

4.1. WASTEWATER CHARACTERISTIC IN THE CITY OF TRONDHEIM

The experiments were performed in order to establish optimal conditions for efficient nitrogen removal from real wastewater samples of the city of Trondheim. The results vary depending on the day when the samples were taken since samples were not equally homogenous.

The characteristics of wastewater were determined to establish the composition of wastewater and behaviour of bacteria under certain conditions. Composition of wastewater varies with day and time. Temperature and precipitation significantly affect the composition of wastewater as well as snow melting. More precipitation and stronger snow melting dilute wastewater and make it easier to treat.

4.1.1. Temperature and pH value of Trondheim's wastewater

The temperatures of wastewater entering the laboratory were between 10°C and 15°C due to the season and frequent rainfall. The lowest temperature of 10.06 °C was noted on 1 March 2019, while the highest temperature of 14.6°C was noted on 8 February 2019. The average value of temperature in monitored period was 13.55°C. Since heightened temperature accelerates chemical and biochemical processes, the efficient nitrogen removal were expected (Kyrkjeeide Finstad, 2018). A sharp drop in temperatures was observed on days when snow was melting. Daily change in temperature and pH value in Trondheim's wastewater in the monitored period is presented in **Figure 14**.

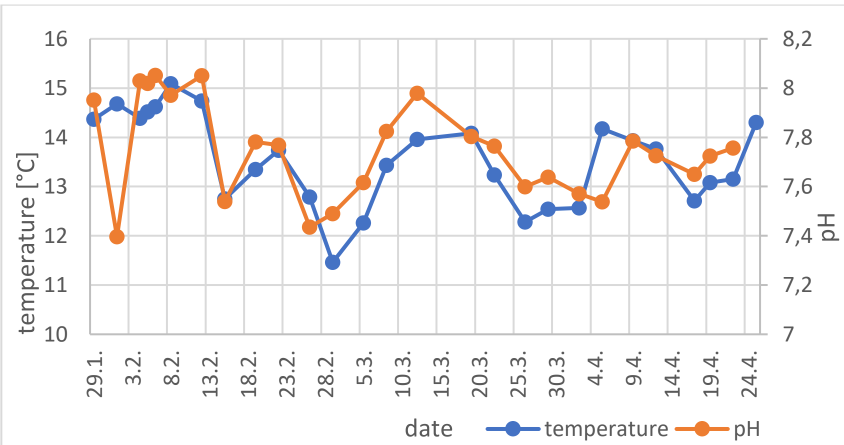

Figure 14 Temperatures and pH values of Trondheim's wastewater in MBBR during the monitored period

Previous researches indicated the pH sensibility of the microorganisms in MBBR process by pH since, pH can have a large influence on the morphology and metabolism of biofilms (Kyrkjeeide Finstad, 2018; Kowalski et al., 2019). If pH is high, microorganisms require more energy for substrate uptake due to the energy gradient. Energy gradient occurs as the pH of the internal cell stays constant and the pH of external environment changes the pH of Trondheim's wastewater is constant, and between 7 and 8. The pH decreased during snowmelt, and the lowest pH value of 7.3 was recorded on 26 February 2019 while the highest pH of 8.3 was recorded on 12 March 2019. The average pH value for the measured period was 7.75. It is desirable to keep the pH high to control the competition between bacteria. Maintaining pH value over 7 is crucial for favouring PAOs over GAOs (Al-Rekabi, 2015; Acevedo et al., 2017; Kyrkjeeide Finstad, 2018).

4.1.3. Dissolved Oxygen in Aerated Chambers of MBBR with Trondheim's Wastewater

The concentration of dissolved oxygen in aerobic chambers of MBBR was changing during monitored period from day to day and noted values are presented on **Figure 15**. The lowest DO concentration of 0.08 mg/L in wastewater was recorded in the first aerated chamber, while the highest DO concentration of 10.24 mg/L was observed in the fourth aerated chamber.
For the process to be efficient, DO level in the anaerobic zone must be under 0.2 mg/L, while DO concentration needs to be around 4 mg/L in the aerobic phase if nitrification is desired. DO level affects the competition between GAOs and PAOs. Thus, if DO level is low, PAOs have a higher affinity for DO than GAOs (Kyrkjeeide Finstad, 2018).

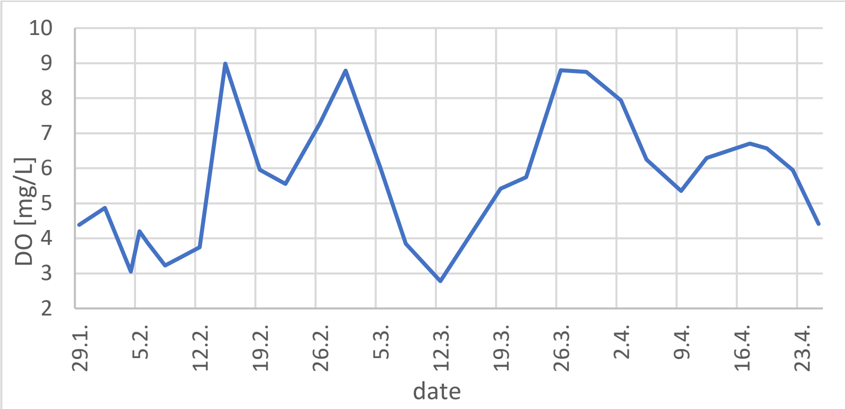

Figure 15 Average concentration of dissolved oxygen (DO) in aerobic chambers of MBBR in the monitored period

4.1.4. Soluble Chemical Oxygen Demand and Nitrogen in Trondheim's Wastewater

Trondheim's wastewater is typical Norwegian wastewater, normally diluted and has low influent 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 (Kyrkjeeide Finstad, 2018).

Due to obtain results of monitoring conducted in this study, soluble chemical oxygen demand, NH_4-N, NO_3-N, and NO_2-N concentrations were varied with day and time, and measured values are presented in **Figure 16.**

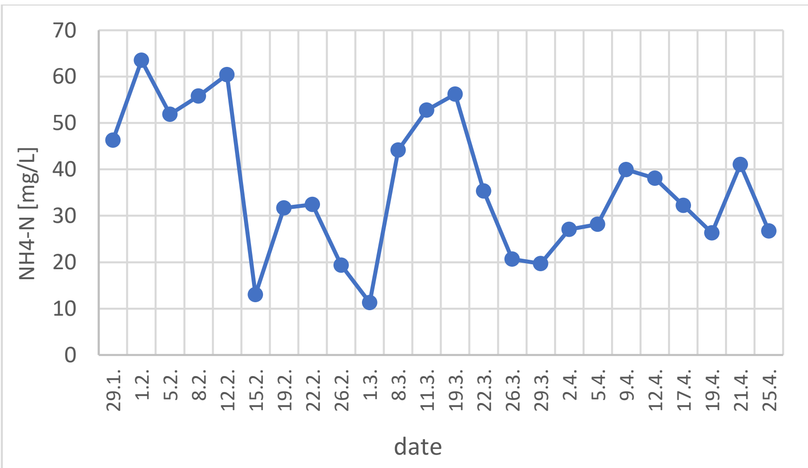

Figure 16 The concentrations of NH_4-N in wastewater influent during the measuring period

The lowest concentration of NH_4-N of 11.3 mg/L in wastewater was noted on 1 March 2019, while the highest NH_4-N concentration of 63.5 mg/L was observed on 1 February 2019. The **Figure 17** presents the concentrations of NO_3-N in wastewater influent during measuring period.

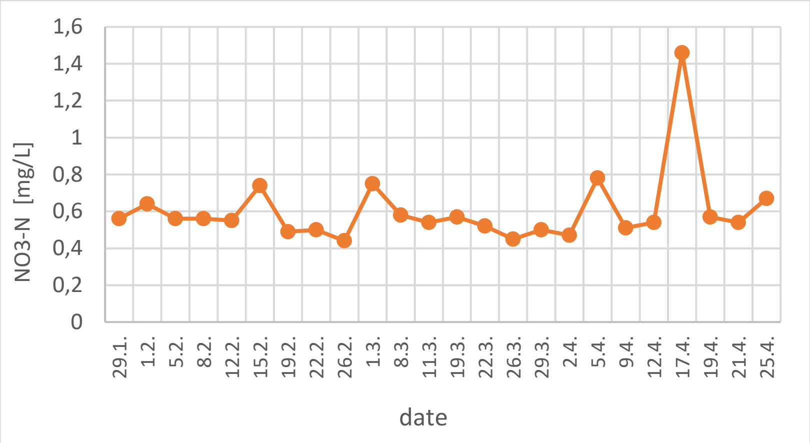

Figure 17 The concentrations of NO_3-N in wastewater influent during the measuring period

The lowest NO_3-N concentration of 0.44 mg/L was recorded on 26 February 2019, while the highest nitrate concentration of 1.46 mg/L was noted on 17 April 2019**.**

The **Figure 18** presents the concentrations of NO_2-N in wastewater influent in the measuring period. The lowest concentration of NO_2-N in wastewater was obtained on 19 and 21 April 2019 and amounted 0.005 mg/L. The highest concentration of NO_2-N in wastewater was observed on 26 February 2019 and amounted 0.271 mg/L.

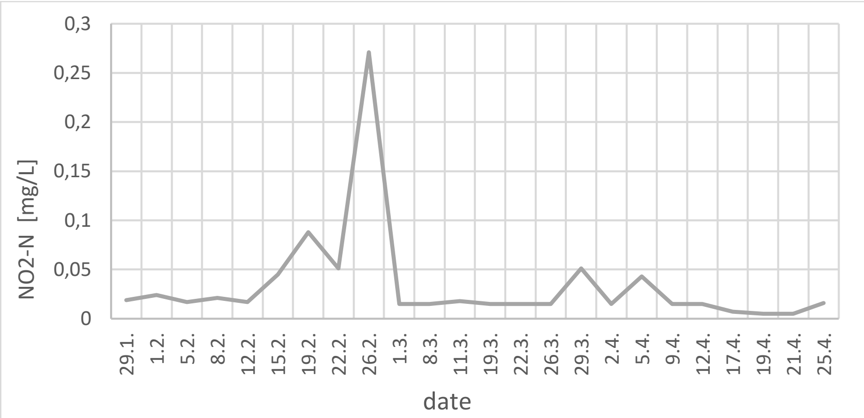

Figure 18 The concentrations of NO_2-N in wastewater influent during the measuring period

The **Figure 19** presents the concentrations of SCOD in wastewater influent measured during the measuring period. From presented data, it is evident that the lowest SCOD concentration of 196 mg/L in influent wastewater was measured on 25 April 2019. The highest concentration of SCOD in wastewater was recorded on 15 February 2019 and amounted 31 mg/L.

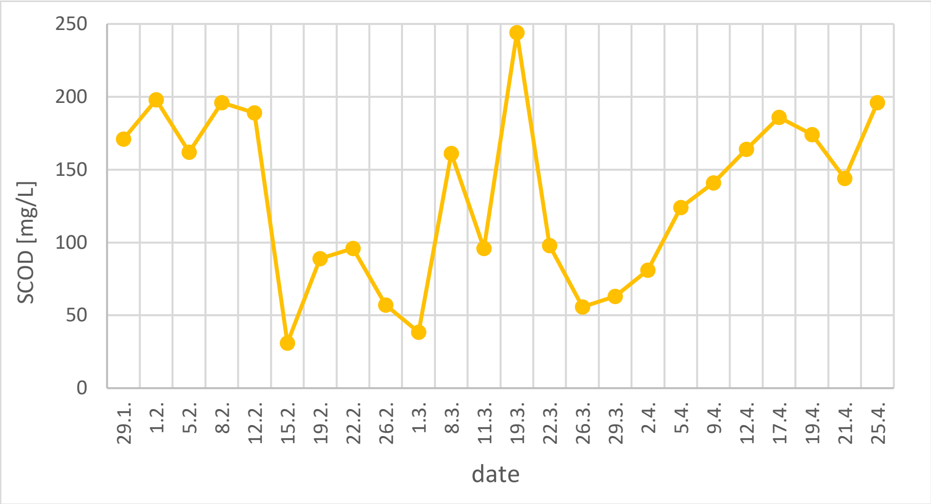

Figure 19 The concentrations of SCOD in wastewater influent during the measuring period

The average values of measured parameters, SCOD, NH_4-N, NO_3-N, NO_2-N, TN, temperature and pH, in Trondheim's wastewater during monitored period are summarized in **Table 3**.

Table 3 Average influent values of measured parameters of Trondheim's wastewater during the monitored period

PARAMETER	AVERAGE VALUE
SCOD [mg/L]	131.47
NH_4-N [mg/L]	36.42
NO_3-N [mg/L]	0.60
NO_3-N [mg/L]	0.03
TN [mg/L]	37.06
T [°C]	12.82
pH	7.62

Figure 20 shows concentrations of total nitrogen in influent in the measuring period. Methods and materials that were previously presented were used to characterize wastewater.

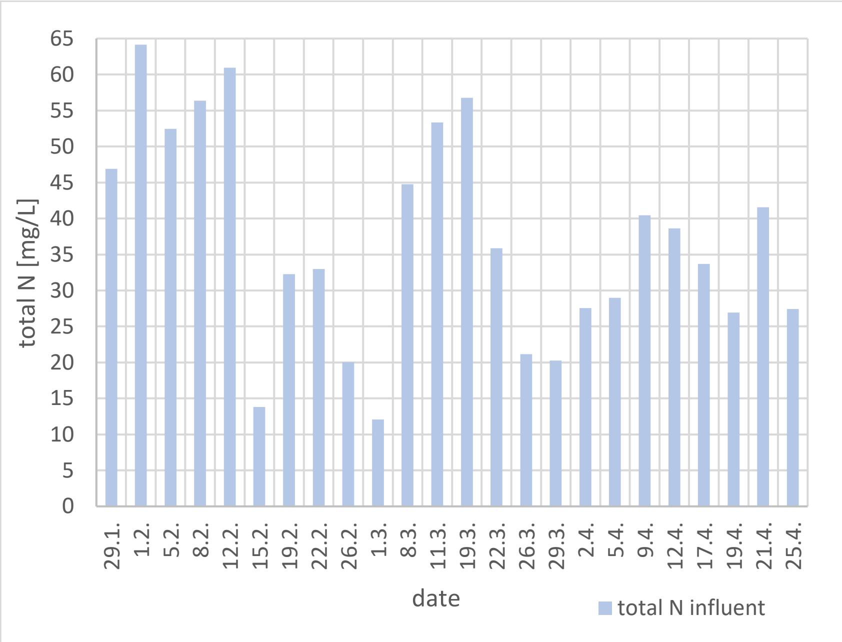

Figure 20 Influent concentrations of total nitrogen in the measuring period

The concentration of total nitrogen in influent varies from a minimum of 12 mg/L to a maximum of 64 mg/L. The major part of total nitrogen belongs to NH_4-N which makes most of the total nitrogen. Shares of NH_4-N, NO_3-N, and NO_2-N of total nitrogen are shown in **Figure 2**.

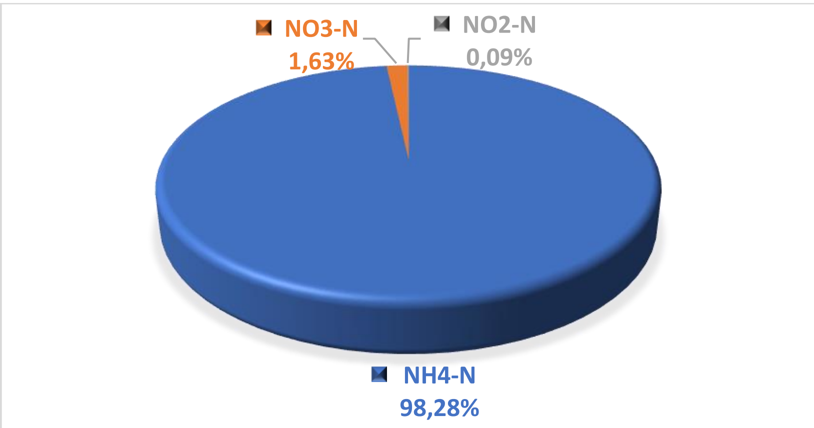

Figure 21 Total nitrogen present in Trondheim's wastewater

Most of total nitrogen occupies NH_4-N with its 98.28%. NO_3-N is following with content of 1.63%. The smallest part of total nitrogen occupies NO_2-N with a share of 0.09%.

Figure 22 shows concentrations of SCOD in influent in the measuring period.

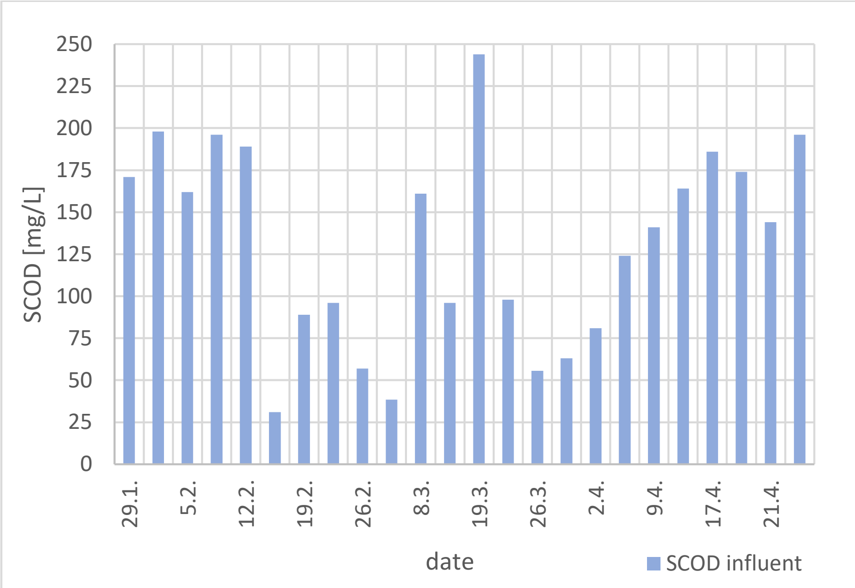

Figure 22 Concentrations of SCOD in influent in the measuring period

SCOD concentrations were affected by dilution. The concentration of SCOD in influent varied from a minimum of 38 mg/L to a maximum of 244 mg/L. It is apparent that low SCOD concentration does affect the efficiency of nitrogen removal. The efficient removal of SCOD is due to OHOs in the aerobic phase (Pastorelli, 1997; Kyrkjeeide Finstad, 2018).

4.2. BATCH EXPERIMENTS

The batch experiments were performed to in order to define the kinetic and efficiency of nitrogen removal under different conditions. In most cases, the work of the actual MBBR and the conditions in the MBBR on a particular day were imitated. On the other hand, conditions changed to eventually optimize the biological wastewater treatment process in MBBR.

4.2.1. Protocol 1

Batch experiments for nitrogen removal were investigated. The concentration of NH_4-N in t = 0 min was 10.01 mg/L while at the end of the process it decreased to 0.22 mg/L, which means that approximately 98% of NH_4-N was removed. The concentration of NO_3-N increased from 0.75 mg/L at the beginning to 11.05 mg/L at the end of the process and concentration of NO_2-N increased from 0.05 mg/L to 0.08 mg/L due to nitrification. Bacteria used 18 mg/L SCOD. An experiment was conducted on 1st March 2019 and the wastewater was diluted due to high precipitation week. Besides the low concentration of nutrients, carbon concentration was also low. Bacteria successfully carried out the process of nitrification, the lack of carbon source is favourable for nitrification since it is autotrophic process (**Figure 23**). Conditions in the bioreactor, as well as in MBBR, were suitable for bacterial activity and NH_4-N removal. Experiments shown that high oxygen concentration (above 8 mg/L) in the aerobic phase is crucial for efficient process implementation. Full nitrification was achieved since all NH_4-N was converted to NO_3-N. di Baise et al. (2019) referred that nitrification rate should be constant at saturating oxygen concentrations. Oxygen concentration can be used in controlling the nitrification process (Pastorelli et al., 1997).

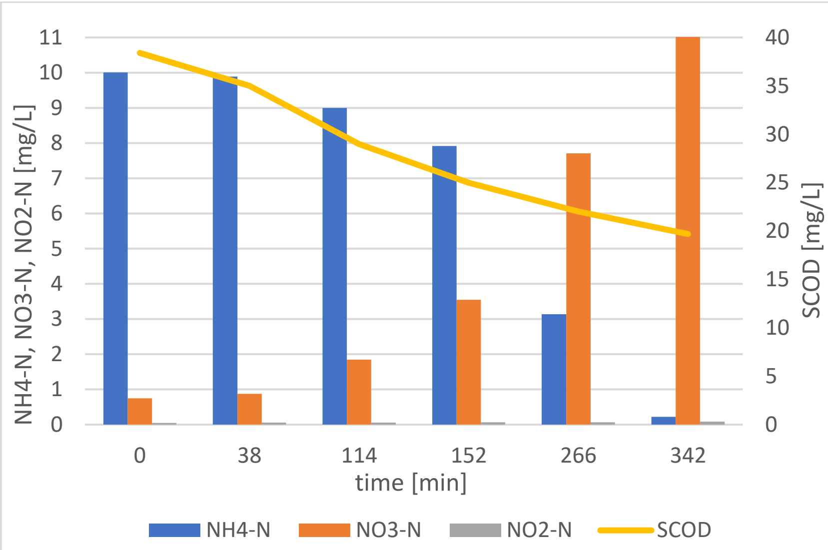

Figure 23 Change in the concentration of NH_4-N, NO_3-N, and NO_2-N with time in the batch experiment carried out on 1 March 2019

4.2.2. Protocol 2

Protocol 2 investigated whether nitrifiers and denitrifiers could successfully remove nitrogen from wastewater in anoxic conditions using NO_3 instead of oxygen and NO_3-N as the electron acceptor. The concentration of NH_4-N at the beginning of the process of 44.55 mg/L, and at the end of the process, 43.01 mg/L, indicating that nitrification did not occur which was expected due to the lack of oxygen. Namely, nitrification is an aerobic process and there was no oxygen in the reactor. Assuming there were errors in measuring the concentration of NH_4-N, it can be concluded that the concentration of NH_4-N in this experiment did not change.

Approximately 30 mg/L of NO_3 at t = 152 min was added to the reactor. Interestingly, the bacteria consumed almost all of NO_3. More specifically, bacteria consumed 22.513 mg/L of NO_3-N and 78 mg/L of SCOD. The concentration of NO_2-N decreased from 0.01 to 0.09 mg/L. The changes in the concentrations of SCOD, NO_3-N, NO2-N, and NH_4-N during this experiment are shown in **Figure 24**. Replacing oxygen with NO_3 did not accelerate the nitrogen removal process. On the contrary, the process took place only partially. It is assumed that the nitrification did not occur because of saturation with NO_3, while removal of NO_3-N was very effective. Since denitrification is an anoxic process, the anoxic conditions in this experiment were suitable and the results were expected. However, the conventional anoxic-aerobic nitrogen removal process cannot be replaced by anoxic due to constant NH_4-N concentration. Lee et al. (2009) assumed that SCOD was consumed by heterotrophic bacteria rather than denitrifiers so incomplete denitrification occurred in the anoxic process.

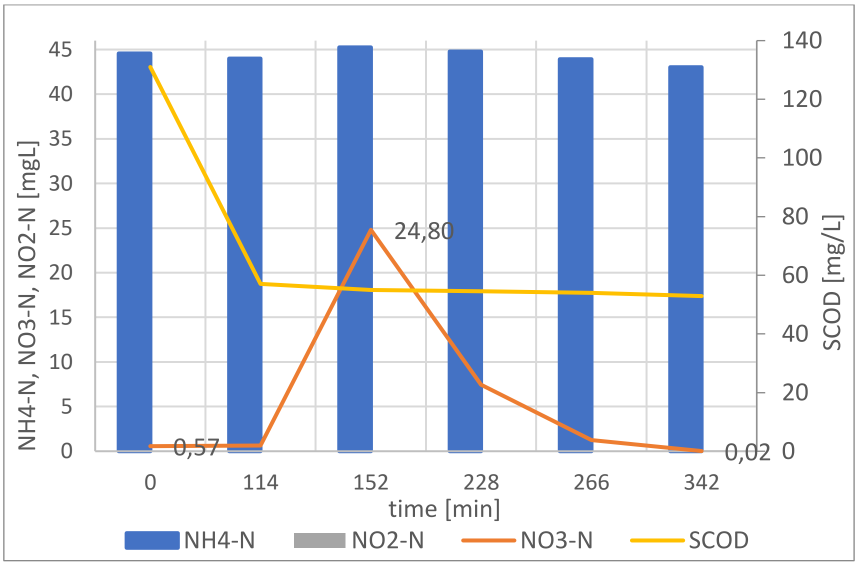

Figure 24 Changes in the concentrations of NH_4-N, NO2-N, NO_3-N and SCOD during addition of NO_3-N in the aerobic zone

4.2.3. Protocol 3

The efficiency of nitrification and denitrification under various oxygen and NO_3 concentrations were investigated. During the experiments bacteria were supplied with carbon source, oxygen and NO_3 at the same time. Results shown that bacteria used 19 mg/L SCOD. The concentration of NO_3-N at the end of the process was high because of added NO_3 and conversion of NH_4-N into NO_3-N. 99.77% of NH_4-N was converted into NO_3-N, while the concentration of NO_2-N increased from 0.05 to 1.41 mg/L. Simultaneous addition of oxygen and NO_3 to the reactor resulted in partial removal of nitrogen. Obviously, bacteria using oxygen were more active and carried out the nitrification in bioreactor. Denitrification did not occur or it occurred at a small rate, which means that denitrifiers lost the competition with aerobic nitrifiers. **Figure 25** shows a change in the concentrations of nitrogen compounds in wastewater over time.

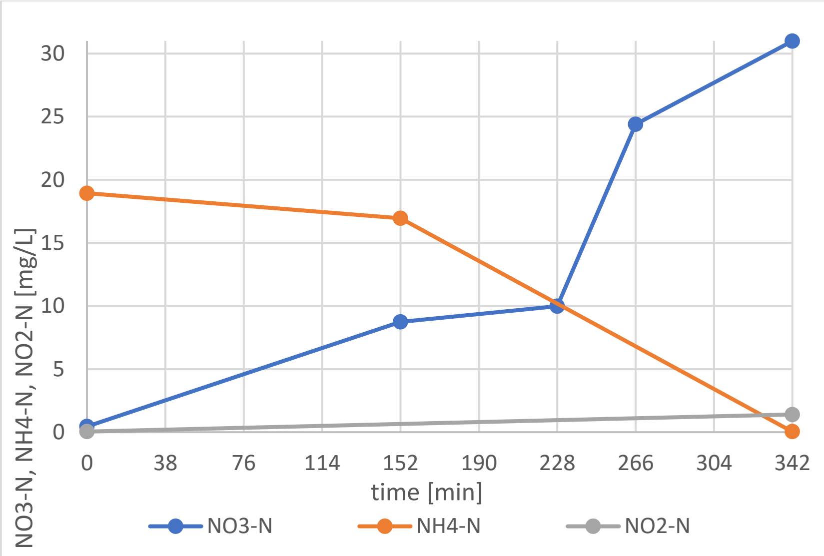

Figure 25 Concentrations of nitrogen compounds in bioreactor during simultaneously addition of oxygen and NO_3-N

4.2.4. Protocol 4

The Protocol 4 was carried out to determine the most appropriate oxygen concentration for efficient removal of nitrogen from Trondheim's wastewater. As expected, the most efficient nitrogen removal was achieved when DO concentration was above 6 mg/L, while the smallest efficiency in nitrogen removal was observed under reduced oxygen concentration. The **Figures 26-29** shows changes in the concentrations of NH_4-N, NO_3-N, NO_2-N, and SCOD during reaction time under various oxygen concentrations. Obviously, the conversion of NH_4-N to NO_3-N and NO_2-N is the most favourable at DO level of 6-8 mg/L and the lowest at DO level of 2-4 mg/L. Also, bacteria consumed most of the carbon at the highest concentration of oxygen and vice versa.

Dissolved Oxygen Level 2-4 mg/L

Bacteria used 19 mg/L of SCOD and removed 43% of NH_4-N. NO_3-N concentration increased from 1.02 to 1.09 mg/L and NO_2-N concentration increased from 0.341 to 0.377 mg/L. The lowest oxygen concentration was 2.06 mg/L and the highest was 3.70 mg/L. 40% of total nitrogen was removed. The nitrogen removal process takes place under conditions of low DO concentrations, but efficiency is low. It is assumed that nitrification was carried out at a small rate due to low oxygen level, suggesting heterotrophic bacteria have won the competition for oxygen, and is active in the outer layer of the biofilm (Pastorelli et al., 1997).

Dissolved Oxygen Level 4-6 mg/L

DO concentration was kept between 4.12 mg/L and 4.55 mg/L. 23 mg/L of SCOD was used by bacteria and 43.3% of NH_4-N was removed, similar to the previous experiment. NO_3-N concentration increased from 1.02 to only 2.81 mg/L. Under this experimental condition, the 89% of NO_2-N and 36% of total nitrogen were removed.

Dissolved Oxygen Level 6-8 mg/L

27 mg/L of SCOD was used and 84% of NH_4-N was removed which is the best result from all three experiments. Nitrification occurred in total since NO_3-N concentration increased from 1.02 to 13.30 mg/L and NO_2-N concentration increased from 0.341 to 1.659 mg/L. A higher DO level means better performance. A higher DO concentration benefits the rate of nitrogen removal. Nitrification is favoured by a high oxygen level while denitrification occurs simultaneously in the deeper anoxic layers of the biofilm, only at a smaller rate. DO concentration was kept between 6.38 mg/L and 6.74 mg/L.

The effect of time and DO concentrations on NH_4-N removal in the bioreactor of Protocol 4 is presented on **Figure 26.**

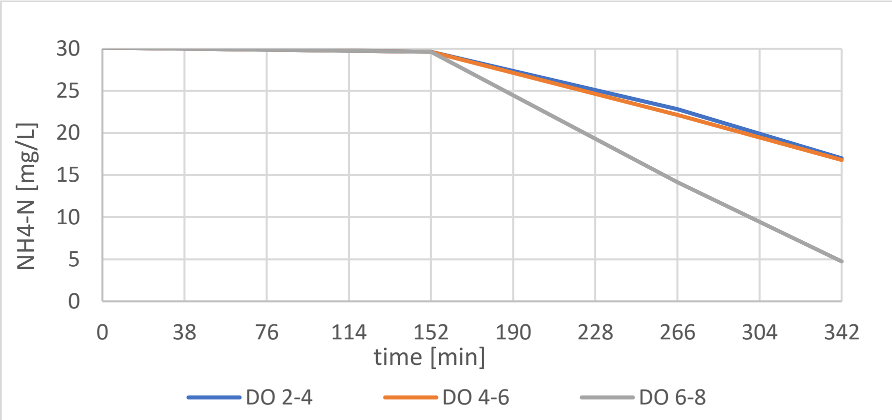

Figure 26 The effect of time and DO concentrations on NH_4-N concentrations in the bioreactor of Protocol 4

Figure 27 present the effect of DO concentration on NO_3-N concentrations in the bioreactor of Protocol 4 with time.

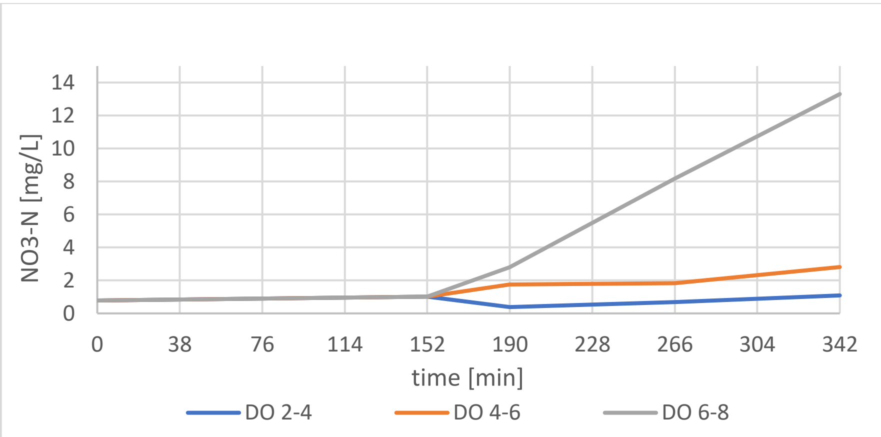

Figure 27 The effect of time and DO concentrations on NO_3-N concentrations in the bioreactor of Protocol 4

Figure 28 shows the relationship among NO_2-N and DO concentrations in the bioreactor of Protocol 4 with time.

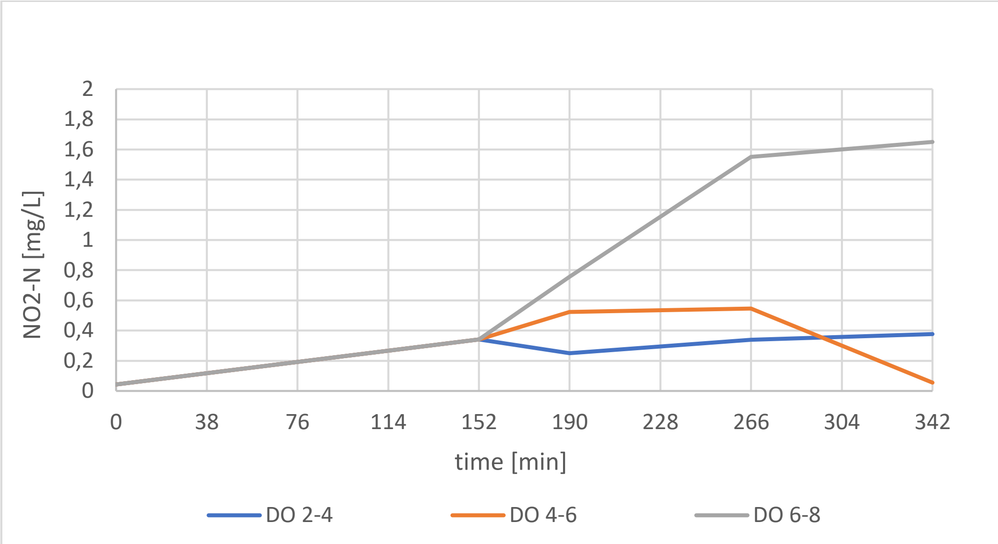

Figure 28 The effect of time and DO concentrations on NO_2-N concentrations in the bioreactor of Protocol 4

In **Figure 29** relationship among SCOD and DO concentrations in the bioreactor of Protocol 4 with time is shown.

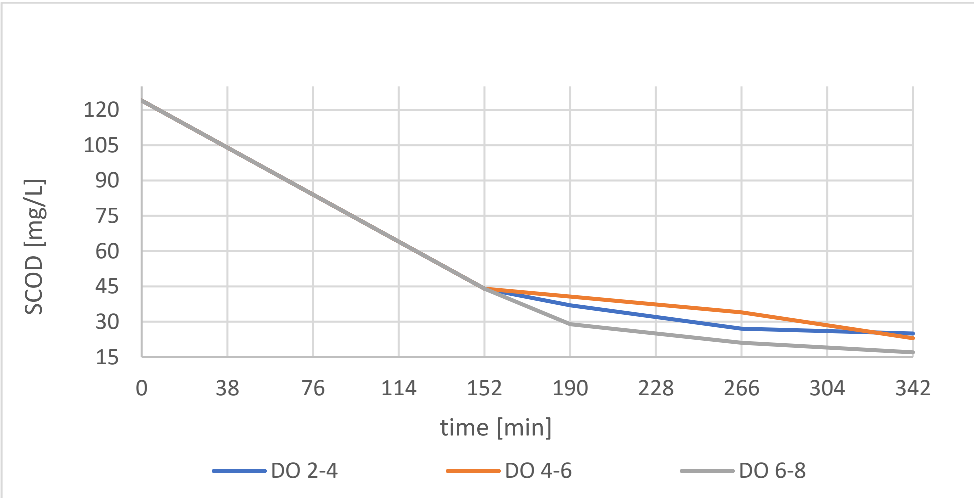

Figure 29 The effect of time and DO concentrations on SCOD concentrations in the bioreactor of Protocol 4

4.2.5. Protocol 5

The experiment in Protocol 5 was conducted in order to examine nitrogen removal under additional anoxic phase. After the aeration started as in the previous protocols at t = 152 min, the reactor was subjected under anoxic conditions from t = 228 min to t = 305 min. After the anoxic phase, the aeration started again. The aeration lasted from t = 305 min to t = 342 min. The efficiency of NH_4-N removal was higher than 85%. From **Figure 30,** it is apparent that the concentrations of NO_3-N increased until the anoxic phase occurred. Once the anoxic phase started, the NO_3-N concentrations decreased from 7.5 mg/L to 5.46 mg/L, while the concentrations of NO_2-N increased. Obtained results impels that the initiation of an anoxic phase between two aerobic phases favours the process of nitrogen removal. Apart from the conversion of NH_4-N to NO_3-N, the conversion of NO_2-N to NO_3-N was unusually high. 58 mg/L of SCOD was used.

Since aerobic processes, i.e. aeration, require additional energy consumption, high efficiency obtained in this Protocol 5 indicate that additional anoxic phase between aerobic phases would save energy and decrease cost of wastewater treatment. This fact is additional fortified with claim of Wang et al. (2019) that aeration energy consumption takes more than 70% of the complete treatment energy demand. Moreover, Trikoilidou et al. (2016) assert that different conditions enhance the growth of specific bacteria. This means that imposing specific operating conditions in the bioreactor results in the growth of desired microbial species.

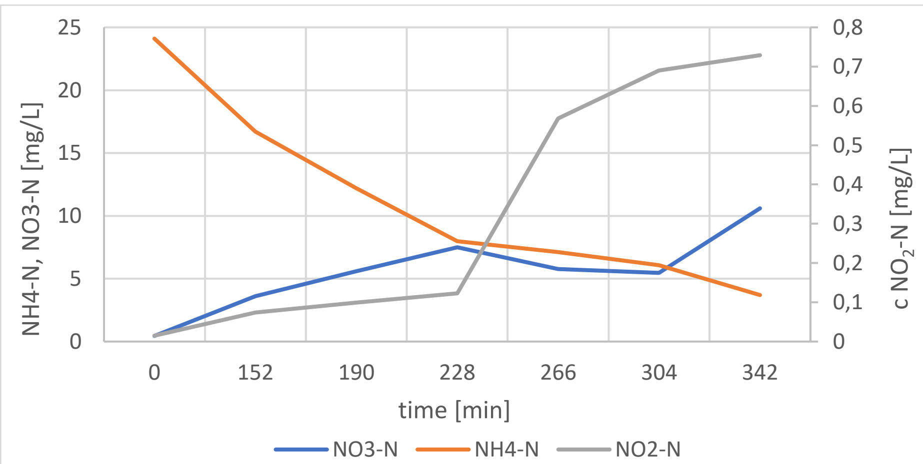

Figure 30 The effect of additional anoxic phase on NH_4-N, NO_3-N and NO_2-N concentrations with time in the bioreactor of Protocol 5

5. CONCLUSION

The aims of this master thesis were (i) determination of Trondheim's wastewater characteristics and (ii) nitrogen removal from Trondheim's wastewater using MBBR in order to define key parameters for efficient nitrogen removal. For this purpose, daily monitoring of carbon and nutrients concentrations, pH, temperature, and DO were used and following conclusions can be drawn:

- Biological nitrogen removal can be achieved in an MBBR under different conditions.
- Nitrogen removal from municipal wastewater of Trondheim through SND was successful in the single MBBR process.
- MBBR proved to be a compact, efficient, and robust solution.
- Microbial community present in MBBR is various and can survive under different conditions. More diverse microbial community means higher efficiency in pollution removal. Experiments conducted in this research showed domination of heterotrophic bacteria (OHO) and PAOs under slow growing autotrophic bacteria (AOB, NOB) in aerobic conditions. It is proved that longer aeration means higher autotrophic activity. Generally, bacteria using oxygen were more active. In anaerobic conditions, PAOs, DNPAOs, and GAOs were active competing for substrate.
- Changes in effluent composition implies that different conditions enhance the growth of specific bacteria. This means that imposing specific operating conditions in the bioreactor the growth of desired microbial species will occur.
- Trondheim's wastewater examined in this study has the characteristics of typical Norwegian wastewater. Norwegian wastewater is generally cold, diluted, and low in nutrients due to high precipitation.
- A several process parameters were determined as important for effective nitrogen removal, but DO level seems to be essential. Strong correlation between DO level and nitrogen removal efficiency was observed. When the wastewater in reactor was saturated with oxygen, aerobic bacteria were active and successfully carry out the nitrification process which implies that nitrification can be controlled by DO level.
- The average temperature of wastewater in the period from 29th January 2019 to 29th April 2019 was 13.55°C and average pH value was 7.75.
- Based on results from kinetic experiments, efficient nitrogen removal was followed by efficient SCOD removal.

- Analysis of wastewater influent and effluent indicates that the MBBR process achieved around 20% nitrogen removal and 70% of SCOD removal. Since the initial concentration of total nitrogen in influent is not high, the average nitrogen removal of 20% can be estimated as effective since the final concentrations in effluent were in accordance with national and international regulations. Furthermore, when the influent contained higher amounts of nitrogen, more nitrogen was removed.
- Replacement of oxygen with $NaNO_3$, resulted with decrease in efficiency of nitrogen removal. However, the conventional anoxic-aerobic nitrogen removal process cannot be replaced by anoxic due to 4.44% of total nitrogen removal. However, approximately 60% of SCOD was removed.
- If bacteria were supplied with carbon source, oxygen, and NO_3-N at the same time, partial removal of nitrogen occurs. Bacteria using oxygen were dominant and carried out nitrification. Consequently, denitrifiers lost the competition with aerobic nitrifiers.
- When determining the most appropriate oxygen concentration, the removal of total nitrogen was alike in all of three cases. At lower oxygen concentration denitrifying bacteria (such as DNPAOs) prevailed conducting denitrification. On the other side, high oxygen concentrations can favour nitrification.
- Introduction of the anoxic phases between aerobics fortified nitrogen removal.
- The forward research on microorganisms behaviour during the wastewater treatment and under tested conditions is needed for complete understanding of the mechanism of MBBR process.

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