Biological reject water treatment by using moving bed biofilm reactors (MBBR)

Seyedbehnam Hashemi
Course: FMH606 Master's Thesis, 2018

Title: Biological reject water treatment by using moving bed biofilm reactors (MBBR)

Number of pages: 85

Keywords: reject water, biological treatment, COD removal, nitrification/denitrification, coagulation, jar test, Aqusim, activated sludge model no.1 (ASM1).

Student: Seyedbehnam Hashemi

Supervisor: Rune Bakke
Carlos Dinamarca
Hildegunn H. Haugen

External partner: Knarrdalstrand WWTP
Biowater Technology AS

Availability: Open

The University of South-Eastern Norway takes no responsibility for the results and conclusions in this student report.
Summary:
In this study two moving bed biofilm reactors (MBBRs) (i.e. the reactors called R_1 and R_2 respectively) were subjected to treat reject water from sludge dewatering. Such biological treatment was investigated for possible improvements of discharge water quality to reduce disturbance on, and enhance the main coagulation process. Four mechanisms assumed to be involved in biological reject water treatment evaluated are I) Oxidation of dissolved and colloidal organics in the proposed bioprocess. II) Conversion of these organics in to live biomass through a cell synthesis process and this biomass will leave the reactors and follow reject water into the main inlet. III) The active biomass will capture more dissolved organics and colloidal solids from raw wastewater when introduced to the main inlet. IV) Biological treated reject water causes less disturbance than untreated on the main coagulation process. This study attempted to generate relevant experimental data for testing the hypothesized mechanisms.

The reactors were fed from an equalization tank continuously. During the study period two main condition were observed, unstable and stable conditions. In the stable condition a mesh installed inside the equalization tank in order to reduce the fluctuation of organic loading rate (OLR) to the reactors. The HRT in stable condition was maintained at 24 h and then 12 h. Besides the experimental part, the process further analyzed by modeling and simulation using an activated sludge model (ASM1).

The results show organics (measured as COD) confirming mechanism I. The highest average soluble COD removal in R_1 and R_2 were 50 % and 58 % respectively when the HRT was 12 h. The total COD removal at 12 h HRT were 43 % and 33 % for R_1 and R_2. The ammonium removal in R_1 and R_2 were 28 % and 25 % when HRT was 24 h and it was reduced to 3.5 % and 9.1 % when HRT decreased to 12 h. The simulations show that low alkalinity level in the reject water and low dissolved oxygen (DO) inside the reactors may have limited ammonium removal. In addition, simulations suggest that such bioreactors can obtain efficient ammonium removal and COD removal at much lower HRT than tested experimentally, when optimum condition achieved (i.e. when alkalinity level was 70 mmol HCO_3/L and DO level was 7.5 mg/L). Lower HRTs reduce construction cost and capital investments for the implementation of biological reject water treatment. Optimum conditions can give high biomass production, which may increase coagulation efficiency according to proposed mechanisms II and III. Coagulation experiments in jar tests (carried out by another student) using a relevant mixture of raw wastewater and reject water from the experiments reported here, supports that mechanism IV can be important. The coagulation COD removal efficiency improved by around 10 % when using treated reject water compared to untreated. More COD removal as sludge may also increase biogas production potential in the anaerobic digestion process.

_The University of South-Eastern Norway takes no responsibility for the results and conclusions in this student report._
Preface

This thesis has been written on the topic of “Biological reject water treatment by using moving bed biofilm reactors (MBBR)” to fulfill master degree in Energy and Environment Technology at University of South-Eastern Norway. This study has been performed in the Knarrdalstrand wastewater treatment plant in order to investigate possible effects of biological treatment of reject water on main coagulation process.

I would like to express my greatest appreciates to my main supervisors, Prof. Rune Bakke, for his support and guidance during my work. His advice and encourages strongly contributed to finish this study. The way he thought wastewater engineering course encouraged me to go for a thesis related to wastewater treatment. I would like to thank my supervisor, Associate Prof. Carlos Dinamarca, for his support and technical advice during experimental works. His support and advice played a major role to fulfill this thesis. I also like to appreciate senior engineer Hildegunn H. Haugen for her helps during this study.

I would like to thank researcher Eshetu Janka Wakjera, Ph.D. for helping me to collect samples during the study and his great tips to improve my knowledge. I want to thank Amund Heggholmen for kindly assisting me in the plant and his helpful information about Norwegian culture. I like to extend my thanks to my best friend Sepideh Niazi for her supports and lovely behavior against all challenges during last two years.

I like to thank the representative of Knarrdalstrand WWTP, Rune Hogstad Hansen, and representative of Biowater Technology AS, Shuai Wang, for their helpful information about the plant and biological process and being patient against my infinity questions.

I sincerely appreciate my parents, Ghader and Fakhri, and my brother Ebrahim. Their support, guidance and infinite love made me stronger during my life.

Porsgrunn, May 1 2018

Seyedbehnam Hashemi
Contents

1 Introduction ................................................................................................................. 10
  1.1 Background ........................................................................................................ 10
  1.2 Problem description ......................................................................................... 10
  1.3 Objective of project ......................................................................................... 11

2 Reject water treatment methods ............................................................................. 12
  2.1 Aerobic digestion .............................................................................................. 12
  2.2 Biological treatment of reject water ................................................................. 13
  2.3 Reject water analysis ...................................................................................... 14
    2.3.1 Solids in wastewater .................................................................................. 14
    2.3.2 Organic measurements in the reject water ............................................... 15
    2.3.3 Nitrification and denitrification ................................................................... 16
    2.3.4 Dissolved oxygen and temperature ............................................................ 17
    2.3.5 Alkalinity and pH ..................................................................................... 18
    2.3.6 Hydraulic retention time (HRT) ................................................................. 18
    2.3.7 Organic loading rate (OLR) ....................................................................... 19
  2.4 Coagulation ..................................................................................................... 19
  2.5 Different methods for biomass measurement ..................................................... 19
    2.5.1 Biomass concentration by VSS ................................................................. 19
    2.5.2 Biomass concentration by COD (COD_b) .................................................. 20
  2.6 Anaerobic digestion ......................................................................................... 20

3 Knarrdalstrand WWTP ............................................................................................. 22

4 Methods and materials .......................................................................................... 24
  4.1 Moving bed biofilm reactors ............................................................................ 24
  4.2 Analysis methods ............................................................................................. 25
  4.3 Reject water properties ................................................................................... 26
  4.4 Syringe test ..................................................................................................... 27
  4.5 Simulation Model description ......................................................................... 29

5 Results ..................................................................................................................... 32
  5.1 Performance of MBBR reactors ...................................................................... 32
    5.1.1 Organic loading rate (OLR) and hydraulic retention time (HRT) ............ 32
    5.1.2 Chemical oxygen demand (COD) removal .............................................. 34
    5.1.3 Nitrification and denitrification ................................................................. 38
    5.1.4 Sludge development in the reactors ......................................................... 42
  5.2 Effect of different factors on performance of reactors ...................................... 44
    5.2.1 Effect of alkalinity and pH on ammonium removal ............................... 44
    5.2.2 Variation of pH in denitrification process ................................................. 45
    5.2.3 Dissolved oxygen gradient within the reactors and temperature variation 47
  5.3 Modelling and simulation results in ASM1 ....................................................... 48
    5.3.1 Removal efficiency in current condition of pilot-scale reactors ............ 49
    5.3.2 Using high alkalinity value in order to simulate performance of reactors 51

6 Discussion ............................................................................................................... 55
  6.1 Performance of MBBR reactors ...................................................................... 55
    6.1.1 COD removal and evidences for hypothesis mechanisms ....................... 55
    6.1.2 Nitrification/denitrification and its possible correlations with mechanisms 55
    6.1.3 Biomass concentration and its connections with mechanisms ................ 56
## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>Anaerobic digestion</td>
<td></td>
</tr>
<tr>
<td>AOB</td>
<td>Ammonium oxidation bacteria</td>
<td></td>
</tr>
<tr>
<td>ASM1</td>
<td>Activated sludge model NO.1</td>
<td></td>
</tr>
<tr>
<td>BMP</td>
<td>Bio-methane potential test</td>
<td></td>
</tr>
<tr>
<td>BOD</td>
<td>Biological organic demand</td>
<td>g/L</td>
</tr>
<tr>
<td>Ca(OH)$_2$</td>
<td>Calcium hydroxide</td>
<td></td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>Calcium chloride</td>
<td></td>
</tr>
<tr>
<td>CFIC</td>
<td>Continues flow intermittently cleaning</td>
<td></td>
</tr>
<tr>
<td>CO$_2$</td>
<td>Carbon dioxide</td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>Chemical organic demand</td>
<td>g/L</td>
</tr>
<tr>
<td>COD$_B$</td>
<td>Biomass chemical organic demand</td>
<td>g/L</td>
</tr>
<tr>
<td>COD$_S$</td>
<td>Soluble chemical organic demand</td>
<td>g/L</td>
</tr>
<tr>
<td>COD$_T$</td>
<td>Total chemical organic demand</td>
<td>g/L</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
<td>mg/L</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>Ferric chloride</td>
<td></td>
</tr>
<tr>
<td>HCO$_3$</td>
<td>Bicarbonate (Alkalinity level)</td>
<td>mmol HCO$_3$/L or equivalent g CaCO$_3$/L</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
<td>Day or hour (h)</td>
</tr>
<tr>
<td>LCFA</td>
<td>Low carbon fatty acid</td>
<td></td>
</tr>
<tr>
<td>MBBR</td>
<td>Moving bed or mixed bed biofilm reactor</td>
<td></td>
</tr>
<tr>
<td>NH$_3$</td>
<td>Ammonia</td>
<td></td>
</tr>
<tr>
<td>NH$_4$</td>
<td>Ammonium</td>
<td></td>
</tr>
<tr>
<td>NO$_2$</td>
<td>Nitrite</td>
<td></td>
</tr>
<tr>
<td>NO$_3$</td>
<td>Nitrate</td>
<td></td>
</tr>
<tr>
<td>NOB</td>
<td>Nitrite oxidation bacteria</td>
<td></td>
</tr>
<tr>
<td>OH$^-$</td>
<td>Hydroxide ion</td>
<td></td>
</tr>
</tbody>
</table>
Nomenclature

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLR</td>
<td>Organic loading rate</td>
<td>kg COD/m$^3$.d</td>
</tr>
<tr>
<td>Q</td>
<td>Volumetric flow</td>
<td>m$^3$/d</td>
</tr>
<tr>
<td>sOLR</td>
<td>Soluble organic loading rate</td>
<td>kg COD/m$^3$.d</td>
</tr>
<tr>
<td>SRT</td>
<td>Sludge retention time</td>
<td>day</td>
</tr>
<tr>
<td>R$_1$</td>
<td>Reactor one</td>
<td></td>
</tr>
<tr>
<td>R$_2$</td>
<td>Reactor two</td>
<td></td>
</tr>
<tr>
<td>TOC</td>
<td>Total organic carbon</td>
<td>g/L</td>
</tr>
<tr>
<td>TR</td>
<td>Treated reject water</td>
<td>mL</td>
</tr>
<tr>
<td>TS</td>
<td>Total solid</td>
<td>g/L</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solid</td>
<td>g/L</td>
</tr>
<tr>
<td>UR</td>
<td>Untreated reject water</td>
<td>mL</td>
</tr>
<tr>
<td>V</td>
<td>Volume</td>
<td>m$^3$</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acids</td>
<td></td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solid</td>
<td>g/L</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile suspended solid</td>
<td>g/L</td>
</tr>
<tr>
<td>WW</td>
<td>Row wastewater</td>
<td>mL</td>
</tr>
<tr>
<td>WWTP</td>
<td>Wastewater treatment plant</td>
<td></td>
</tr>
<tr>
<td>X$_{aut}$</td>
<td>Autotroph bacteria concentration</td>
<td>g/L</td>
</tr>
<tr>
<td>X$_{het}$</td>
<td>Heterotroph bacteria concentration</td>
<td>g/L</td>
</tr>
</tbody>
</table>
1 Introduction

Challenges associated with wastewater treatment, such as high energy requirements, disposal of bio-solids and strict environmental regulations are pushing municipalities to use more efficient and innovative technologies [1]. The disposal of bio-solids are expensive and causes environmental difficulties such as Increase ammonia levels in the soil. Therefore, sludge thickening and dewatering process are the most suitable tools in order to achieve sludge volume diminution [2].

Since the excess sludge content generally up to 99% water, the dewatering process has been applied in almost all wastewater treatment plants to reduce water content down to around 80% [3].

1.1 Background

Unlike the physical-chemical processes that typically are considered a costly and lower effectiveness processes, biological processes based on the suspended growth of biomass are proved to be efficient in organic carbon and nutrients removal. Nevertheless, the problem of insufficient sludge settle ability had been faced, as well as higher aeration requirements for larger volume and total biomass recycling. In order to improve the settling ability of the suspended particles, the use of chemicals as coagulant used to be prevalent worldwide [4, 5].

Kibiakova (2013) at Lillevik (Larvik, Norway) wastewater treatment plants (WWTP) conducted a study addressing the effect of reject water treatment on main coagulation performance. This work has proposed the following four mechanisms [5, 6]:

1. Dissolved and colloidal organics in the reject water will be degraded (oxidized) in the introduced bio-process.
2. Dissolved and colloidal organics in the reject water will be converted into biomass through cell synthesis in the introduced bio-process and these cells will be removed by coagulation in the main treatment train.
3. The active biomass synthesized in the introduced bio-process will capture more dissolved organics and colloidal solids from the fresh wastewater when introduced into the treatment plant inlet; all of which will be removed by coagulation in the main treatment train.
4. The biologically treated reject water will cause less disturbance on the main coagulation process than the untreated reject water does today, implying that the coagulation process can become more efficient.

1.2 Problem description

The reject water from the secondary treatment stage is one of the key problems for conventional wastewater treatment plants. The amount of reject water is typically less than 3% of the main inlet, but it contents high concentration of nutrients like orthophosphates and ammonium-nitrogen. The concentration can be within the range of 500 mg/L and 130 mg/L orthophosphates and ammonium respectively [7, 8].
Hence, this can result in a higher load on the system that causes disturbances on the coagulation process, which is associated with higher operational cost and lower discharge water quality. Since the sludge composition changes significantly, the reject water treatment process becomes expensive and complicated, therefore a dedicated method for reject water treatment can be more efficient [9].

1.3 Objective of project

A biological reject water treatment could achieve hypothesized mechanisms to obtain overall better wastewater treatment plant performance. This thesis attempt to investigate possible improvement in discharge water quality due these mechanisms, especially to establish efficient removal of organic matter at wastewater treatment plants (WWTPs) with Knarrdalstrand WWTP (Porsgrunn, Norway) as case. The following study topics were considered as the main objectives of the thesis:

- Investigate the performance of pilot scale moving bed biofilm reactors, as a biological reject water treatment technology, at Knarrdalstrand WWTP in order to generate relevant experimental data for testing the hypothesized mechanisms (section 1.1).
- Investigate effect of different factors such as hydraulic retention time (HRT) organic loading rate (OLR), dissolved oxygen (DO), pH and alkalinity on MBBR reactors in order to identify appropriate conditions.
- Use theoretical evaluations, modeling and simulations to study the effect of biological reject water treatment on the overall plant performance.
2 Reject water treatment methods

Both aerobic and anaerobic processes are widely used in biological sludge treatment. However, each of these biological processes has its own benefits and drawbacks. In order to choose an appropriate treatment method in full-scale reactor design, different factors should be considered. For instance, water characteristics, precipitation rate, sludge handling equipment and the plant capacity can influence the choice of treatment method. In fact, anaerobic digestion is typically applied as a primary sludge treatment process with an additional secondary aerobic treatment [1, 4].

2.1 Aerobic digestion

The aerobic digestion is operated on the same principle as the activated sludge process. The organic matters aerobically oxidized by the microbes to CO$_2$, H$_2$O, NH$_4$, NO$_2$, NO$_3$ through an endogenous phase and cell tissue. In real condition, aerobic digestion oxidizes all kind of biodegradable organic matters and microbial cellular materials by organisms through the following reaction [10]:

$$\text{Organic matter} + O_2 \rightarrow \text{Cellular material} + CO_2 + H_2O \quad (2-1)$$

$$\text{Cellular material} + O_2 \rightarrow \text{Digested sludge} + CO_2 + H_2O \quad (2-2)$$

The second reaction presents the endogenous respiration, which is a predominant reaction in the aerobic oxidation process. According to, Tchobanoglous et al. (2014), under limiting operation condition the minimum required the amount of oxygen level is 1 mg/L. Table 2-1 presents the main benefits and drawbacks of using anaerobic digestion [11].

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatile solid reduction as much as anaerobic digestion</td>
<td>High power requirements</td>
</tr>
<tr>
<td>Lower BOD concentration in outlet</td>
<td>Is not energy source</td>
</tr>
<tr>
<td>Odorless and biologically stable products</td>
<td>Alkalinity consumption</td>
</tr>
<tr>
<td>Simple technology</td>
<td>Affect significantly by location, feed, mixing material, temperature, tank geometry and tank material.</td>
</tr>
<tr>
<td>Low capital cost and small facilities</td>
<td>Poorer dewatering characteristics in mechanical dewatering equipment compare to anaerobic digesters</td>
</tr>
<tr>
<td>Easy to construct of available parts</td>
<td></td>
</tr>
<tr>
<td>Appropriate for nutrient-rich wastewater</td>
<td></td>
</tr>
<tr>
<td>No explosion</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-1: Advantages and disadvantages of aerobic wastewater treatment processes [11].
2.2 Biological treatment of reject water

In the conventional wastewater treatment processes, the water from sludge thickening and dewatering have been directly recycled into the main inlet without any pre-treatment [4]. However, as the wastewater both sources consist of high amount of ammonium and phosphorus, this stream may cause overload in the main treatment train. Hence to avoid overload and process disturbance in the main treatment process the treatment of reject water is vital [12].

To modify different attributes of reject water like pH, temperature, organics quantity, ammonia and ammonium concentration, various treatment methods such as physical, chemical and also biological treatment methods can be applied in order to achieve the environmental goals. The biological treatments methods are more popular as they have low operation cost compared to chemical and physical methods. In fact, the physical methods are highly energy consuming and the chemical treatment methods incur chemical cost [11].

The main application of bio-processes is the removal of phosphorus, BOD, COD and total organic carbon (TOC), nitrification, denitrification and water stabilization. Particularly, removal of dissolved and suspended matters from wastewater is the main target of using biological treatment whereby organic matters become stabilize throughout the bio-processes. Moreover, municipal wastewater contains excess nitrogen and phosphorous which should be removed before discharge to surface water (i.e. it would be another application of biological treatment) [13, 14]. In biological treatment processes, the culture control is crucial to reach ideal growth of microorganisms. Under those circumstances, wastes decomposition can occur faster by controlling the culture. Biological treatment performance can vary in different environmental conditions. However, it is possible to control fluctuations by controlling pH, temperature, feed and convenient mixing [13, 14].

One of the recent version of aerobic bioreactors in wastewater treatment is moving bed or mixed bed biofilm reactor (MBBR). The moving bed or mixed bed biofilm process counts as a novel technology of attached-growth biological process that draws attentions during recent years. MBBR is based on suspended bio carriers with density a little lighter than that of water kept in continuous movement by using aeration. The media fill volume in most MBBR is around 63% of projected volume. The active biomasses will attach to carriers in order to move freely inside the reactor [15].

MBBR process can be applied for BOD removal and nitrification as well as biological nitrogen removal. For BOD removal and nitrification process design, as a primary stage it may be essential to remove most of the soluble BOD, and hence, the heterotrophic bacteria growth will be minimized and the nitrifying bacteria to dominates on the surface area [16, 17].

Overall, the following items can be the main superiority and weakness of MBBRs compare to other biological treatment system such as suspended-growth activated sludge.

**Advantages:** The MBBR processes advantages are 1) Equivalent BOD removal as compared to activated sludge processes, 2) application for biological nitrogen removal, 3) less regular operational attention or interruption for bio-carriers thickness or flash out the solids, 4) less
Reject water treatment methods

wastage of biomass, 5) possibility of existence of aerobic and anoxic region within one system and 6) low up-grading cost and more stable in overloading and toxicity effects [11].

Disadvantages: MBBR process has some disadvantages as follow: 1) energy consumption due to the oxygen supply, 2) specific biofilms requirements, 3) difficult in media removal for air supplier maintenance, 4) media screening and 5) limitation of phosphorous removal [11, 18].

Biofilms are complex structures that microorganisms stuck on, in order to grow and form a diverse array of the microbial population. The balance between biomass formation and detachment rates influence the biofilm thickness [19]. The microbial growth could be influenced by nutrients concentration on the biofilm surface, which depends on fluid flow, as the nutrients are transported by diffusion and convection. The biomass thickness on the biofilm surface highly affected by shear force (i.e. force pushing opposite direction). Thus, the biomass thickness differs in different types of reactors (i.e. due to higher shear forces in the aerobic reactors the biomass thickness will be non-optimal) [20, 21].

Moreover, the performance of MBBR systems depends not only on organic loading rate but also by the bio-carrier type. For the reason that, the biomass thickness changes by the carrier type and organic loading rate. Bio-carriers in MBBR systems plays the major role in bacterial domination within the biofilm, as well as bioreactor performance in a given operational condition. Different types of bio-carriers have been applied in MBBR systems such as polyethylene plastic, polyurethane sponge, biodegradable polymer, granular activated carbon, etc [21].

2.3 Reject water analysis

Various parameters are considered in order to investigate and characterize the reject water properties. Among others, the following Characteristics are the most important factors that should be considered in order to investigate reject water in general.

2.3.1 Solids in wastewater

Suspended and/or dissolved matter in wastewater count as solids. Solids have negative effects on wastewater effluent quality in many ways. Therefore, strong restrictions have been made by environmental authorities. Provided that solid analysis is important to control any biological and physical wastewater treatment processes. The amount and the type of solids in wastewater is an indication of the strength of the wastewater. For instance, if the major part of the solids in the wastewater are organic, the impact on the process of a treatment plant could be more than if the case had been inorganic solids [22, 23]. The solids can be measured as total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS).

TS measures all the suspended, colloidal and dissolved solids in the water sample. This includes dissolved salts such as sodium chloride and solid particles such as silt [11]. These are organic fractions of solids that can be ignited and disappear at high temperature (i.e. 550°C). VS generally represents the number of organic solids in the water and it is helpful when investigating the number of organic matters that converted biologically. Studies have indicated that municipal wastewater solids include around 50 % organic which normally comes from synthetic organic compounds, dead animal matter and plants [11, 24].
Reject water treatment methods

TSS measures the amount of suspended (non-soluble) solids in aeration tank or the effluent of the reactors. In general, the amount of TSS demonstrates the effectiveness of wastewater treatment plant. The amount of solids must be kept at the minimum level to achieve reasonable discharge [11, 22].

Analytically, TSS is weight of particles remained on filters (i.e. smaller pore size) after drying in the oven at 105 °C at least for 24 hours. Hence, the increase in the weight of filter represents the total suspended solids of samples. High TSS is the sign of stress with bacteria like lack of nutrients or increase in BOD loading that typically cause excessive solids generation. High TSS may also demonstrate scant settling time which causes solids to float all around the settling tank. In aeration stabilization tank, high TSS detects inappropriate aeration within the basin [11, 25].

The filtered and dried sample from TSS will be ignited at 550°C (i.e. in laboratory furnace) in order to specify the amount of the volatile suspended solids. VSS represents the organic matter together with biomass concentration in the system. In other words, VSS is a method to measure biomass concentration in the system. In spite of the method is easy to measure samples, it may introduce some errors. For instance, filtration of activated sludge needs longer time and the bacteria smaller than 1µm (i.e. depending on the filter pore size) will pass through the filter and it is hard to measure them [26].

The calculation of volatile per total solids is useful in the control of wastewater plant performance, as this ratio present amount of organics in the solid fraction of the wastewater. VS/TS ratio ranging from 0.75 to 0.79 indicate a high amount of organic matter. Feng also confirmed that sludge with the VS/TS ratio ≥ 0.5 is also considered as high organic content [27, 28].

2.3.2 Organic measurements in the reject water

Both chemical oxygen demand (COD) and biological oxygen demand (BOD) are widely used in wastewater characterization. BOD test determines amount consumed oxygen in the biochemical oxidation of organic matter and ammonia remaining in the effluent. The BOD test is a slow process which takes ≥ 5 days to get the results. Whereas in COD test organic compounds oxidized in more extensive oxidation condition. In the oxidation process, the dichromate in the COD test kits absorbs electrons from organic matters hot sulfuric acid solution with silver cations as a catalyzer. Since carbonaceous (i.e. carbon-containing organic matter) is the only completely oxidized compounds, therefore ammonia is not included in COD value. Reaction 2-3 shows the COD process [11, 14].

\[
C_nH_{2a}O_{b}N_{c} + dCr2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2} H_2O + cNH_4^+ + 2dCr^{3+} \quad (2-3)
\]

One of the COD values determines all oxidizable materials in the sample while it does not provide specific biodegradability. According to International Association on Water Quality (IAWQ) the COD fractions that widely used are as follow [29, 30]:
Reject water treatment methods

- Readily biodegradable COD (rbCOD) which is equivalent to soluble COD. This COD fraction present low molecular weight soluble substances that would be consumed in short time.
- Slowly biodegradable COD (sbCOD) is typically the largest fraction of biodegradable organic matters that largely consist of insoluble biodegradable or particulate. These fractions consist of colloidal substances and/or solid particles with high molecular weight. Hydrolyses Process (i.e. this hydrolysis step include slow reactions) with a contribution of secreted enzymes by the microorganisms is required to convert the sbCODs into soluble molecules.
- Soluble non-biodegradable COD (snbCOD) fraction that will not change during the treatment process. These compounds will discharge to the river or surface water with outlet flow.
- The last fraction is particulate non-biodegradable COD (pnbCOD). The pnbCOD is not consumed by biomass; and hence a large amount of them will settle together with sludge, therefore the concentration of pnbCOD in the effluent is significantly lower than that in the inlet.

Mostly, the COD in reject water is associated with the low fraction of biodegradable substances.

2.3.3 Nitrification and denitrification

The autotrophic microorganisms such as Nitrosomonas (AOB, ammonium oxidation bacteria) and Nitrobacteria (NOB, nitrite oxidation bacteria) are responsible for nitrification process. With sufficient supply of dissolved oxygen and enough amount of Nitrosomonas the ammonium will oxidized to nitrite and then with a contribution of Nitrobacteria the nitrite will be converted to the nitrate. Reactions 2-4 and 2-5 show two steps of nitrification process [31].

\[
NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (2-4)
\]

\[
NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2-5)
\]

The produced nitrate molecules in nitrification process may convert to the nitrogen gas (i.e. in most of the time, it happens with the assistance of carbon sources) through denitrification process as shown in reactions 2-6 and 2-7 [31].

\[
2NO_3^- + 10H^+ + 10e^- \rightarrow 2OH^- + 4H_2O + N_2 \quad (2-6)
\]

\[
2NO_2^- + 6H^+ + 6e^- \rightarrow 2OH^- + 2H_2O + N_2 \quad (2-7)
\]

In aerobic digestion processes, amino acids (i.e. amino acids can be the product of protein degradation which typically exists in the municipal wastewater) release soluble organic matters that may mineralize to ammonium (reaction 2-8). In the further steps, chemolithrophic bacteria (i.e. bacteria that obtain energy from inorganic compounds) consume ammonium as an energy source through synthesis process (i.e. nitrification process) [9].
Reject water treatment methods

\[
C_3H_7NO_2 + 5O_2 \xrightarrow{\text{Enzymes}} 5CO_2 + NH_3 + 2H_2O + \text{Energy} \tag{2-8}
\]

Where \( C_3H_7NO_2 \) is known as biomass compound in activated sludge.

In activated sludge systems, high concentration of organic matters causes the heterotroph bacteria dominates over the autotrophic nitrifies. Hence, a lower organic load is needed in order to have appropriate growth of nitrifying bacteria (i.e. at least two days are required for proper growth of \textit{Nitrosomonas} and \textit{Nitrobacteria}). Provided that temperature plays a vital role for the growth of the microorganism. Optimum nitrification can be achieved a temperature of 30°C [9].

For both steps of nitrification process, the optimum pH value ranges from 6 to 8. Ammonium may accumulate in the non-optimal nitrification condition result in an increase in pH value. Furthermore, high pH condition cause ammonium leaves the system as ammonia gas (Figure 2-1) [32]. Ammonia counts as main energy and nitrogen source for AOB moreover, the equilibrium in reaction 2-9 is highly dependent on pH. In alkaline conditions (i.e. pH higher than 9) concentration of ammonia will start increasing [11].

\[
\text{NH}_3 + \text{H}_2\text{O} \xrightarrow{\text{pH}} \text{NH}_4^+ + \text{OH}^- \tag{2-9}
\]

Figure 2-1: Accumulation of ammonium and/or in different pH [33].

It should be noted that the Nitrobacteria grow slower than Nitrosomonas therefore, nitrate formation is typically less than the nitrite formation rate and nitrate formation starts within 124 hs after nitrite establishment.

2.3.4 Dissolved oxygen and temperature

Dissolved oxygen (DO) is the relative measurement of oxygen dissolved in water to provide sustain life for all aquatic, as well as bacteria. An aerobic activated sludge treats industrial and municipal waste through a biological process in the aeration tank [21], therefore the presence
Reject water treatment methods

of dissolved oxygen is crucial for biological floc bacteria. Living bacteria consume oxygen to oxidize waste to gain energy for growth [21].

In some biological treatments systems, the aeration is not only to supply the dissolved oxygen but also in order to have appropriate mixing. Generally, in such systems, the oxygen requirements can be nearly adequate when the amount of dissolved oxygen is ≥ 2mg/L. In fact, ideal DO range may change depending on treatment methods. For example, the main symptom of low DO can be thick effluent or dark mixed liquor. Moreover, in low DO conditions the quantity of low DO filamentous microorganisms will increase and consequently, settle-ability of the activated sludge will be negatively affected [34].

Oxygen solubility highly depends on temperature. The solubility of oxygen affects the rate of biological activity. Even if the solubility of oxygen at low-temperature increases, the biological treatment in the cold climates can be tough due to the low reaction rate. Rusten et al (2011) indicated that the nitrogen removal has been dependent on temperature. Typically, MBBRs are designed for low-temperature environments in order to meet treatment goals even under worst conditions, without additional energy/heat to maintain the standard temperature [35].

2.3.5 Alkalinity and pH

During the nitrification process the pH drops as 7.14 g of alkalinity is consumed per 1g of removed ammonium (i.e. 2eq alkalinity consumed/mole NH₄-N oxidized)* 50 g CaCO₃/14 g N/mole = 7.14 g CaCO₃ consumed/g NH₄-N oxidized) [36]. Anderson and Mavinic evaluated nitrogen and phosphorus removal in intermittent flow pilot-scale reactor. The results have shown that the pH could decrease to 3.5 due to nitrification (i.e. low alkalinity) and cause a disturbance in the performance of nitrifiers. Therefore, controlling pH between 6 and 8 is an optimal range and it can enhance the metabolic activity [37].

Generally, alkalinity is one of the crucial factors in nitrification process. Moreover, alkalinity plays a vital role as inorganic carbon source for heterotrophic nitrifying bacteria as well as it also balances the acid-base level of the mixture [32].

2.3.6 Hydraulic retention time (HRT)

Hydraulic retention time, which also known as hydraulic residence time, refers to the average time that a compound remains in the system. Conventionally, HRT for the startup step is usually set long for the growth and development of diverse microbial cultures. Later, HRT can decrease depending on the treatment methods and other operational factors. Mathematically, HRT is the ratio of the volume of digester per influent flow [11, 21]:

$$HRT = \frac{V}{Q}$$

Where HRT is hydraulic retention time (d), V is volume of the digester (m³) and Q is inlet flow (m³/d)
2.3.7 Organic loading rate (OLR)

OLR is as the amount of organic matter in a unit of volume of the reactor during a certain unit of time. The ORL in a given reactor volume depends on liquid flow rate and inlet COD concentration. Several studies have shown that increase in OLR has positive effects on the treatment efficiency up to certain levels. However, a further increase over a certain level introduces some operational instability such as sludge bed floatation. Hence, it is crucial to monitor OLR in order to avoid overloading of the system which could impair the system performance. OLR can be regulated by changing influent flow rate, which leads to variation in the inlet COD concentration as follow[11, 21]:

$$ OLR = \frac{Q \cdot COD}{V} $$

Where, OLR is organic loading rate (kg COD/m$^3$.d), Q is flow rate (m$^3$/d), COD is chemical oxygen demand per volume (kg COD/m$^3$), and V is reactor volume (m$^3$).

The above equation can be simplified as [11]:

$$ OLR = \frac{COD}{HRT} $$

2.4 Coagulation

In the conventional wastewater treatment, diverse treatment methods exist such as oxidation, ion exchange, membrane technology, adsorption, biological treatment etc. Each this methods has its own advantages and disadvantages [38]. Among others, the most used method almost in most treatment plants in order to remove organic and inorganic solids is coagulation process. During the coagulation process chemicals (i.e. coagulants, e.g. FeCl$_3$, Ca(OH)$_2$, CaCl$_2$ etc.) will be added to the water to shape settleable flocs from the colloidal solids. The positively charged ion of the metal salt is used as a chemical result in particle neutralization and destabilization. In general coagulation process is an efficient hands-on process as well as cost-effective treatment approach [11].

2.5 Different methods for biomass measurement

The biomass growth and concentration in wastewater treatment processes are measured through various approaches such as mass, volume, cell or organelle count and light scattering. Moreover, as mentioned earlier (section: 2.3.1), the volatile suspended solid also used as a measure of biomass in the water samples. Although this method has certain weaknesses such as the measurement dependent on filter pore size as well as long time required for measurement [39].

2.5.1 Biomass concentration by VSS

Normally the standard methods apply VSS for measuring the biomass. However, this method cannot determine the bacteria smaller than 1μm [22, 39].
2.5.2 Biomass concentration by COD (CODₐ)

In order to measure the CODₐ, it requires determining two different COD values, i.e. soluble and total COD, and then the biomass COD is calculated as follow [39]:

\[ COD_\text{B} = COD_T - COD_S \]

The CODₐ value consists of:

- Viable biomass
- Slowly biodegradable and non-biodegradable particles (i.e. from residual water to be treated)
- Particulate organic matters that is generated by microorganisms in endogenous phase

2.6 Anaerobic digestion

Anaerobic digestion (AD) is a complex process in the absence of oxygen that involves a diverse assemblage of bacteria and methanogenic archaea (Ren et al., 2018). Chemical oxygen demand (COD) and biological oxygen demand (BOD) removal, from food, wastewater sludge and agriculture waste are the basic application of anaerobic digestion. AD counts as a renewable energy source due to its ability to produce biogas. Several factors are involved in order to design and operate AD processes such as reactor shape and design, operational temperature, feeding pattern (i.e. continues feeding or intermittent feeding) and amount of solid. Complex substrates such as carbohydrates, proteins and lipids are hydrolyzed to acetate, hydrogen, carbon dioxide through fermentation, which will end up in methane and carbon dioxide by methanogens organisms. The anaerobic digestion consists of three main stages as follows (Figure 2-2) [11]:

**Hydrolysis:** In this step, the complex particulate materials are converted to soluble materials in order to be hydrolyzed in further steps to monomers. Extracellular enzymes produced by different kind of facultative and obligate anaerobes are responsible for hydrolysis. In most AD processes, the hydrolysis is considered to be the rate-limiting step [11].

**Acidogenesis:** This step is carried out with a specific type of bacteria groups (i.e. acidogenic bacteria). Volatile fatty acids (VFAs), CO₂ and hydrogen are the results of this process; where the substrates act as both electron donors and electron acceptors. The fermentation products of the sugars and amino acids are acetate, propionate, butyrate, CO₂ and hydrogen. However, the fermentation of the LCFAs end to acetate, CO₂ and hydrogen. Hydrogen produced mostly from LCFA COD than from sugar and amino acids. The further fermentation process is also called *acetogenesis* where the intermediate products of acidogenesis convert to also produce acetate, CO₂ and hydrogen. Hence, the final products of fermentation are acetate, hydrogen, CO₂. These products are the precursors of methane formation. In order to proceed the reaction the hydrogen concentration (i.e. most of the hydrogen comes from the oxidation of LCFAs and VFAs) should be at low level [11].

**Methanogenesis:** The final AD process is completed by a group of methanogenic archaea which are collectively named as methanogens. Typically two type of methanogens are involved in methane production. The first group called aceticlastic methanogens convert the acetate into the methane and CO₂ while the second group called hydrogenotrophic methanogens use hydrogen as electron donor and CO₂ as an electron acceptor in order to produce methane [11].
Among others, the following four points are the advantages of anaerobic digestion over aerobic digestion [27, 40]:

1. Treatment of concentrated organic carbons
2. Less sludge production
3. Biogas production that may be used for heat or electricity generation
4. Low energy requirements

Mostly in any AD process, mesophilic and/or thermophilic temperature regime is used widely in anaerobic digestion. Operating temperature from 20°C to 40°C refers to mesophilic condition while the temperature regime between 45°C to 65°C is thermophilic. A change in temperature by 10°C may cause reaction rate either rise or decline by a factor of two. In fact, in some cases it could inhibit the process completely [27].
Knarrdalstrand WWTP

Knarrdalstrand WWTP is a mechanical-chemical treatment plant, which was built in 1990 in order to treat sewage from Porsgrunn and Skien municipalities. The current capacity of Knarrdalstrand WWTP is 50000 m$^3$/d. In the treatment process first, influent water flows into a grit chamber to remove large particles and sand (Figure 3-1). After the grit chamber, the water undergoes in coagulation process by addition of FeCl$_3$ solution as a coagulant. The dosage of coagulant depends on the inlet water concentration (i.e. in the rainy seasons the consumption of coagulant is low due to dilution unlike the dry seasons). On average, the coagulant usage in the treatment plant varies from 0.1 to 0.25 mL FeCl$_3$/L. In the sedimentation, tank particles form easily settle-able flocs while the surface water follows to the river after the fat removal process.

A thickener concentrates sludge by further settling, the thickened sludge hygienised by heating at 60°C for one hour to eliminate harmful microorganisms. The sludge is further treated in anaerobic digesters at 35°C to 37°C in order to produce biogas (i.e. energy) and sludge stabilization (i.e. in anaerobic digester the microorganisms consume organics to growth). The produced biogas is mostly used to provide heat for hygienisation. This energy source may also be used to supply hot water for the city.

The reject water from the sludge dewatering and the thickener returns with the main inlet before grit chamber without any extra treatment process, as per today. Overall, the average hydraulic retention time in primary treatment stage is approximately 24 h.

As described above Knarrdalstrand WWTP does not have any supplement biological process to remove organic matters from reject water. This project has brought the issue that additional biological process before the reject water is returned to the main inlet may improve the process efficiency to achieve better discharge water quality [3]. Figure 3-1 illustrates the current design of the treatment plant together with the proposed approach in order to improve the plant performance.
Figure 3-1: Flow diagram of Knarrdalstrand WWTP. The flow diagram introduced biological process in system in the proposed approach.
4 Methods and materials

4.1 Moving bed biofilm reactors

Two polycarbonate cylinder each has a total working volume of 18.8 L were used for this thesis study. These two reactors were constructed from three polycarbonate cylinders with small, medium and large diameter where the small and medium cylinders were inside the larger cylinder with small separation between each other (Figure 4-1). The small cylinder in the center had 6.7 L volume and 63% of the volume was filled with bio-carriers (i.e. in other words, 22.5% of the total volume was filled by bio mediads) [15]. The carriers were BWTX type, which were supplied by Biowater Technology. The carrier, dimensions were 14.5*14.5*8.2 mm with a protected surface area of 650 m²/m³ [41].

The small cylinder was suspended into the medium cylinder with few centimeters above the bottom of the larger cylinder. Since the medium cylinder is filled with the bio-carrier the aeration pipe was also attached at the bottom. The medium and larger cylinders were mainly to provide an appropriate residence time and circulation of wastewater in the system [6]. In addition to air mixing, the reactors beds were manually mixed occasionally to avoid sludge accumulation and circulate the fast settled particles.

Figure 4-1: Sketch of the MBBR reactors a), different part of reactors (i.e. larger, medium and small cylinder) as well as air pipe, inlet, outlet and expected flow from manual mixing, b) photo of the installed pilot scale reactors at WWTP.
4.2 Analysis methods

Both reactors were continuously operated at a temperature between 13°C to 17°C for 60 days. The hydraulic retention time for both reactors was 24 h. Initially, the reactor one (from now onwards called as R₁) was fed with 4.7 L of reject water four times per day while reactor two (from now onwards called as R₂) was 2.4 L of reject water eight times per day. Later, when the reactors were at the stable condition, the feeding intervals were set equal for both reactors at 20 times per day, which was around ± 0.9 L per feeding pulse. The reactors also operated at HRT of 12 h with the same feeding intervals but only doubling the feeding volume per pulse.

During this study period, a total of 11 parameters (Table 4-1) were measured twice a week. Sampling for the effluent was done either pumping the inlet with high flow rate (i.e. the inlet flow ≥ 1.2 L/min) or the samples were collected by using the 100 ml medical syringes from the point next to the effluent outlet when the pumps were in off mode. Since each of the reactors has three compartments, the DO was measured at four sampling points as shown in Figure 4-2.

While the DO is measured, the temperature in the reactor is also measured at the same time. The DO was measured using portable oxygen meter WTW Oxi 3310 (Weilheim, Germany). The pH is measured using Beckman 390 pH-meter. The alkalinity was determined based on standard titration method (2320 B, APHA 1995) using 0.1 sulfuric acid normal solution [22].

Total COD was determined by using COD test cell () in the range of 300mg/L to 3500mg/L. The samples were homogenized using a homogenizer (Heidolph Diax 900, 8000-26000 rpm, Apeldoorn, the Netherlands) and diluted by distilled water with a ratio of 1 to 10 depending on the concentration of samples. The samples in the COD test cell were digested in a thermoreactor (spectroquant® TR 620) at 148 °C for 2h. The total COD was measured by spectroquant® Pharo 300 UV/VIS spectrophotometer (Darmstadt, Germany). The soluble COD, ammonium (NH₄-N⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) were measured after the samples were centrifuged at 20 000 rpm for 15 minutes (Beckman coulter Avanti J-20i centrifuge, CA, USA) and then filtered.
Methods and materials

at 0.45 µm pore size glass filter (GxF multi-layered, Acrodisc PSF filters). The method corresponds to on the standard protocol (APHA, 1995) [22].

TS, VS, TSS and VSS were determined according to the standard methods (APHA, 1995). For TSS, 5 mL of the samples were filtered with 1.2µm pore size filter where the filters were oven dried at 105°C for 24 h and cooled in the desiccator. The TSS was determined after the oven dried and weighed filters were ignited in a furnace at 550°C for 15 minutes. Table 4-2 provides different method codes in the standard protocols [22, 42].

Table 4-1: The sample types and the different biochemical characteristics analyzed during the experimental period using the standard methods.

<table>
<thead>
<tr>
<th>Samples</th>
<th>COD&lt;sub&gt;T&lt;/sub&gt;</th>
<th>COD&lt;sub&gt;S&lt;/sub&gt;</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;</th>
<th>NO&lt;sub&gt;2&lt;/sub&gt;</th>
<th>TS</th>
<th>VS</th>
<th>TSS</th>
<th>VSS</th>
<th>Alkalinity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>(in&lt;sub&gt;1&lt;/sub&gt;&amp;in&lt;sub&gt;2&lt;/sub&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactors effluent</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>(R&lt;sub&gt;1&lt;/sub&gt;&amp;R&lt;sub&gt;2&lt;/sub&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thickener</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Table 4-2: Different standards and method codes in each standard [22, 42].

<table>
<thead>
<tr>
<th>method</th>
<th>parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHA</td>
<td>4500-F</td>
</tr>
<tr>
<td>APHA</td>
<td>4500-B</td>
</tr>
<tr>
<td>DIN</td>
<td>38405-D9</td>
</tr>
<tr>
<td>APHA</td>
<td>5220-D</td>
</tr>
<tr>
<td>APHA</td>
<td>2540-D</td>
</tr>
<tr>
<td>APHA</td>
<td>2540-E</td>
</tr>
</tbody>
</table>

4.3 Reject water properties

A reservoir tank (1000 Liter IBC (intermediate bulk container) contains a mixture of equal proportion (i.e. 1:1) of the thickener and the centrifuge, where the reactors feed pumped from the reservoir tank continuously. The water in the tank was minimally mixed by aeration, to
ensure that the DO level inside the reservoir was maintained $\leq 0.5$ mg/L. Moreover, the tank was emptied and refilled three times per week to have fresh feed for the reactors. Since the thickener and the centrifuge flow contained high solid particles (i.e. high particulate matter), a filter mesh was installed inside the reservoir to avoid high solid particles as well as frequent blockage of feed pipes (Figure 4-3). The physical, inorganic and organic characteristics of inlet water to the reactors (i.e. thickener and the centrifuge) are given in Table 4-3.

Table 4-3: The physical, inorganic and organic chemical characteristic of reactor’s inlet i.e. the thickener and the centrifuge wastewater of Knarrdalstrand WWTP. All analysis is done based on the standard method (table 4.2).

<table>
<thead>
<tr>
<th>Reject water</th>
<th>Average COD$_T$ (g/L)</th>
<th>Average COD$_S$ (g/L)</th>
<th>Average TS (g/L)</th>
<th>Average VSS (g/L)</th>
<th>Average NH$_4$ (g/L)</th>
<th>Average pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickener</td>
<td>4.76</td>
<td>0.55</td>
<td>5.07</td>
<td>3.13</td>
<td>0.21</td>
<td>6.9</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>2.84</td>
<td>0.95</td>
<td>4.25</td>
<td>2.64</td>
<td>0.45</td>
<td>7.28</td>
</tr>
<tr>
<td>Reactor’s inlet</td>
<td>1.8 (g/L)</td>
<td>0.85 (g/L)</td>
<td>2.62 (g/L)</td>
<td>1.8 (g/L)</td>
<td>0.35 (g/L)</td>
<td>7.37</td>
</tr>
</tbody>
</table>

Figure 4-3: Installed solid filter mesh inside the feeding tank. The filter mesh was approximately 1.5 m long and the feed pipes were inside the mesh

4.4 Syringe test

The method using syringes as batch reactor (Østgaard et.al, 2016) was employed for the biogas potential test (BMP) [43]. A total of ten syringes with the capacity of 100 ml (i.e. medical syringes) (Figure 4-4) were employed as batch anaerobic digester in order to test the potential of biogas production. The granular sludge (GS) provided by E-convert (Appendix 3) originally came from the supplier Opure in the Netherlands, who collects sludge all over Europe, from all sorts of factories and installations. Different substrate (i.e. the samples from thickener, centrifuge, reactors inlet and outlet) were tested for the BMP experiment. The experimental layout and substrate to inoculate ratio for each experimental unit are provided in
**Methods and materials**

Table 4-4. Two parallel for each treatment were performed for statistical reason including blank (i.e. only granular sludge) to control activity of the granular sludge. The BMP test was conducted at incubation temperature of 35°C in a heating cabin (Termaks Lab drying oven, Bergen, Norway). The biogas production was recorded every day during the whole experimental period. Since the syringes had rubber stopper, the produced biogas accumulated in the syringes and the amount of accumulated gas within syringes was equal to syringe expansion.

Table 4-4: The BMP test treatments and the inoculum to substrates ration. Treatments had two parallels including the control (i.e. only granular sludge).

<table>
<thead>
<tr>
<th>Syringes naming</th>
<th>Syringe contain</th>
<th>Amount of GS</th>
<th>Amount of substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1/B-2</td>
<td>GS</td>
<td>20mL</td>
<td>-</td>
</tr>
<tr>
<td>In-1/In-2</td>
<td>GS+ inlet of reactors</td>
<td>20mL</td>
<td>10mL</td>
</tr>
<tr>
<td>R1-1/R1-2</td>
<td>GS+ outlet of R1</td>
<td>20mL</td>
<td>10mL</td>
</tr>
<tr>
<td>R2-1/R2-2</td>
<td>GS+ outlet of R2</td>
<td>20mL</td>
<td>10mL</td>
</tr>
<tr>
<td>T-1/T-2</td>
<td>GS+ thickener</td>
<td>20mL</td>
<td>10mL</td>
</tr>
<tr>
<td>C-1/C-2</td>
<td>GS+ centrifuge</td>
<td>20mL</td>
<td>10mL</td>
</tr>
</tbody>
</table>

Figure 4-4: The 100 mL volume medical syringes used as batch AD reactors
4.5 Simulation Model description

A general simulation model with long sludge retention time (SRT) was applied in order to simulate the organics and ammonium removal in an aerobic/anoxic condition [36]. As bio carriers keep the biomass inside the reactor, a sludge recycle ratio of 0.99 is used. Since the outer layers of the reactors contain low dissolved oxygen (approximate. ≤ 0.5 mg/L) it acts as an anoxic zone, hence it is obvious that simultaneous nitrification/denitrification could occur in the reactors. For the simulation, the reaction equations and growth rates were extracted from activated sludge model no.1 (ASM1) [36].

In the first step of the nitrification process ammonium is converted to nitrite by ammonium oxidation bacteria (AOB) and then in the second step nitrite oxidation bacteria (NOB) consume nitrite as substrate to produce nitrate. However, since nitrite is an intermediate product, the ASM1 consider nitrification as a single-step process. The kinetic model of ASM1 includes a stoichiometric matrix with 8 processes and 13 compounds [44, 45]. ASM1 considers all the organics in the system as easily biodegradable compounds while some of the soluble and particulate organics in the current MBBR reactors were presented in the outlet. Therefore, two hydrolysis processes were developed in the model in order to get closer to real performance of the reactors. The current model consists of 8 biochemical processes provided in Table 4-5 [45]. The 2nd process (i.e. process rate equation 2) indicates the nitrification process which is adapted from Monod kinetics. The heterotrophic bacteria consume dissolved oxygen for growth and in the anoxic zone, where the oxygen level is low heterotrophs use nitrate and/or nitrite as oxygen source. Moreover, the 3rd process (i.e. process equation 3) shows the anoxic growth of heterotrophic bacteria (i.e. denitrification). In our model, as mentioned before in order to provide biodegradable compounds for heterotrophic bacteria growth, two hydrolysis process is introduced for soluble and particulates organics (process rate equation 7th and 8th) [45] (i.e. for further information about parameters and units referred to Rieger, et al. [46]).

In fact, the nitrification process and aerobic growth of heterotrophic bacteria process requiring oxygen. The total oxidation reaction of ammonium is shown in equation 4-1. From the stoichiometry, the overall oxidation reaction of ammonium requires, 4.57 g O$_2$/g N oxidized (i.e. 3.43 g O$_2$/N used for nitrite and 1.14 g O$_2$/g NO$_2$ oxidized to nitrate) [45].

\[ \text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O} \]  
(4-1)

In denitrification process nitrite and nitrate count as electron acceptors and equivalent oxygen can be calculated by oxidation-reduction half reaction equations as follow:

For oxygen:  
\[ 0.25\text{O}_2 + \text{H}^+ + e^- \rightarrow 0.5\text{H}_2\text{O} \]  
(4-2)

For nitrite:  
\[ 0.33\text{NO}_2^- + 1.33\text{H}^+ + e^- \rightarrow 0.17\text{N}_2 + 0.67\text{H}_2\text{O} \]  
(4-3)

For nitrate:  
\[ 0.2\text{NO}_3^- + 1.2\text{H}^+ + e^- \rightarrow 0.1\text{N}_2 + 0.6\text{H}_2\text{O} \]  
(4-4)

According to half reaction equations the oxygen equivalent for conversion of nitrate is 2.86g O$_2$/g NO$_3$. This value theoretically indicates amount of organic requirement for reduction of 1g nitrate. Over all, the stoichiometric matrix of proposed ASM1 is provided in Table 4-6 [44, 45].
### Table 4-5: Proposed processes in activated sludge model no.1 (ASM1) [44, 45].

<table>
<thead>
<tr>
<th>Process</th>
<th>Process rate equation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> Aeration</td>
<td>( K_{la}^a (O_{2,sat} - C_{O_2} ) )</td>
</tr>
</tbody>
</table>
| **2** Conversion of NH4 into NO3 (nitrification) | \[
\frac{\mu_{m,aut}}{K_{NH_4} + C_{NH_4}} \cdot \frac{C_{O_2}}{C_{O_2} + K_{O_2}} \cdot \frac{C_{HCO_3}}{C_{HCO_3} + K_{HCO_3}} \cdot X_{aut}
\] |
| **3** Conversion of NO3 into N2 (denitrification) | \[
\frac{\mu_{m,het}}{C_{NO_3} + K_{NO_3}} \cdot \frac{K_{inhib}}{C_{O_2} + K_{inhib}} \cdot \frac{C_{s,org}}{C_{s,org} + K_{s,org}} \cdot X_{het} \cdot \eta
\] |
| **4** Aerobic Growth of heterotrophic | \[
\frac{\mu_{m,het}}{C_{s,org} + K_{s,org}} \cdot \frac{C_{O_2}}{C_{O_2} + K_{O_2,dhet}} \cdot X_{het}
\] |
| **5** Rate of death nitrifying bacteria | \( X_{aut} \cdot k_{d} \) |
| **6** Rate of death heterotrophic bacteria | \( X_{het} \cdot k_{d} \) |
| **7** Hydrolysis process for particulate organics | \[
\frac{X_{slo}}{K_{hyd} \cdot \left( X_{slo} / X_{het} \right)} \cdot X_{het}
\] |
| **8** Hydrolysis process for soluble organics | \[
\frac{X_{slo,sol}}{K_{hyd} \cdot \left( X_{slo,sol} / X_{het} \right)} \cdot X_{het}
\] |
### Methods and materials

#### Table 4-6: Stoichiometric matrix of proposed model in activated sludge model no.1 (ASM1)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{O_2})</td>
<td>1</td>
<td>-</td>
<td>(\frac{4.57 - Y_{sat}}{Y_{sat}})</td>
<td>-</td>
<td>(\frac{1-Y_{het}}{Y_{het}})</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(X_{het})</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(C_{s,org})</td>
<td>-</td>
<td>-</td>
<td>(\frac{-1}{Y_{het}})</td>
<td>(\frac{-1}{Y_{het}})</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(X_{aut})</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(C_{NO_3})</td>
<td>-</td>
<td>(\frac{1}{Y_{aut}})</td>
<td>(\frac{-1-Y_{het}}{Y_{het}}) (2.86)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(C_{N_2})</td>
<td>-</td>
<td>-</td>
<td>(\frac{1-Y_{het}}{Y_{het}}) (2.86)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(C_{NH_4})</td>
<td>-</td>
<td>(\frac{-1}{Y_{aut}}) (\frac{-I_{mem}}{y_{aut}})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(C_{HCO_3})</td>
<td>-</td>
<td>(\frac{-0.2}{Y_{sat}})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(X_{slo})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1</td>
<td>-</td>
</tr>
<tr>
<td>(X_{slo, sol})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-1</td>
</tr>
</tbody>
</table>


31
5 Results

In this chapter, the experimental results of biological process and operation of two parallel pilot-scale MBBRs are presented. The apparatus details and experimental procedure are described in chapter 4. The results belong to different measurements in two operational conditions (i.e. unstable condition and stable condition). Each measurement was performed twice per week. The average values of important parameters are provided in the tables along with the standard deviations.

The results in this chapter are divided into three subchapters according to the project objectives (1.3). 1) The overall performance of the reactors in terms of operational conditions, COD removal, ammonium removal and sludge development are provided. 2) The effect of different factors such as alkalinity, pH, temperature and DO on reactor performance. 3) Modeling and simulation results in different operational conditions.

5.1 Performance of MBBR reactors

The variation of OLR and HRT in the reactors are provided in this subchapter. According to the OLR and HRT variation different factors as reactor performance parameters (such as COD reduction, nitrification and denitrification and sludge development) are measured and the results are shown here.

5.1.1 Organic loading rate (OLR) and hydraulic retention time (HRT)

The variation of OLR and inlet COD concentration with respect to HRT changes are illustrated in Figure 5-1 and Figure 5-2 for R1 and R2, respectively. The vertical dash line shown in the figures indicates the day that the mesh was installed inside the feed tank. Hence, then after the reactors started being relatively stable after 39th day of experimental run. In both figures, it is seen that the reactors had unstable performance (i.e. not in a steady state) prior to mesh installation, which is observed by high fluctuations in the inlet COD as well as the OLR.

The OLR was equal to inlet COD concentration for both reactors as long as HRT was kept at 24 h (Figure 5-1 and Figure 5-2). Undesirable changes in HRT in R1 on days 28th, 32nd and 37th were due to blockage of feed pipes (i.e. HRT of the R1 was re-calculated based on the amount of feed volume decreased in the feed tank) which caused the OLR became half of the inlet COD concentration. Meanwhile, the OLR had similar values as inlet COD in R2 at the same days. From day 56, the OLR increased more than twice, from 1.24 Kg COD/m$^3$·d to 3.4 Kg COD/m$^3$·d, by reducing the HRT from 24 h to 12 h. During those days, the inlet COD was 1.24 g/L on day 56 and around 1.7±0.015 g/L between days 57 and 60.

Some fluctuations are visible in Figure 5-1 and Figure 5-2 for OLR with a time interval of around 2 weeks that is mainly caused by inlet COD concentration variations. Initially, OLR increased at day 4 reached to 2.65 Kg COD/m$^3$·d, however at the end of second week OLR reduced to 1 Kg COD/m$^3$·d in both reactors. In the 3rd and 5th week a new upward trend observed. These upward trends ended in high OLR values equal to 3 Kg COD/m$^3$·d and 2.8 Kg COD/m$^3$·d on days 23 and 39, respectively. After around one week these values decreased in both reactors on days 28 and 42. For both R1 and R2, these values on day 28 dropped to 0.36 Kg COD/m$^3$·d and 0.73 Kg COD/m$^3$·d respectively whereas on day 42nd the OLR decreased.
slightly to 1.32 Kg COD/m$^3$·d in both reactors. Since flow from the thickener and centrifuge consist of solid particles the fluctuation of inlet COD in the tank is high although, these variations significantly reduced after installation of mesh (i.e. solid particles filter). Therefore, the increase and decrease in OLR (Figure 5-1 and Figure 5-2) were mainly attributed to the high fluctuation in the inlet COD concentration.

The process before installing mesh is named unstable condition due to high OLR variation, sludge accumulation, technical problems such as clogging of pumps and low aeration that caused disturbance in the performance of reactors. However, after the installation of mesh the fluctuation in OLR reduce by approximately 45 % while the HRT was kept at 24 h. Hence, after the mesh installation the highest OLR was 1.4 Kg COD/m$^3$·d on day 49 and later this value reduced to 1.24 Kg COD/m$^3$·d on day 56 (Figure 5-1 and Figure 5-2). Moreover, reducing HRT to 12 h (i.e. increased flow) led to increase in the OLR while the inlet COD did not change a lot. For example, the OLR on day 59 and 60 were 3.4 Kg COD/m$^3$·d and 3.45 Kg
Results

COD/m³·d, respectively while the inlet COD was 1.7 g/L and 1.715 g/L, respectively on the same days in both reactors.

5.1.2 Chemical oxygen demand (COD) removal

Total and soluble COD (COD$_T$ and COD$_S$) changes at inlet and outlet streams of R$_1$ and R$_2$ are depicted in Figure 5-3 and Figure 5-4, respectively. The dash lines with symbols indicate COD concentrations in the inlet flows and the solid lines with symbols represent COD concentrations at the outlet. The vertical dash line divides the graphs into unstable and stable conditions of the reactor process since the date of mesh installation. The inlet COD$_T$ and COD$_S$ values of both reactors are similar however, the COD$_T$ and COD$_S$ at outlet differ for each reactor.

During the unstable period, COD$_T$ at inlet streams fluctuates significantly while the COD$_S$ values at inlet flows are relatively at the same level for both reactors. However, the changes for total and soluble COD values at inlet and outlet streams for both reactors became steady in the stable period (Figure 5-3 and Figure 5-4). It is also worth to mention that the total COD at outlet for both reactors in the unstable period in the graph fluctuates and this observation is more clearly seen for R$_1$.

During the first week of the experiment, the total COD of the inlet reached a peak of 2.65 g/L on the 4th day in R$_1$ and R$_2$ while the amount of inlet soluble COD was 1.28 g/L. On day 7, the difference between inlet and outlet COD$_S$ in R$_1$ reached to highest level experienced in both reactors (Figure 5-3). In second week, the COD$_T$ and the COD$_S$ concentration decreased to 1.1 g/L and 0.88 g/L, respectively. There was slight increase in the outlet CODs in some of the days e.g. on day 14, 17 and 23 both for R1 and R2. Occasionally, there was large fluctuation in the inlet COD, for instance the inlet total COD concentration increased sharply from 1 g/L to 3 g/L during the 3rd week and from 0.73 g/L to 2.8 g/L in week 5th in both reactors (Figure 5-3 and Figure 5-4). In R$_1$, the outlet COD$_T$ on 28th day was 1.95 g/L while the inlet COD$_T$ was 1.2 g/L, similarly on day 32 the inlet COD$_T$ was 0.73 g/L and the outlet COD$_T$ was 1.5 g/L. Meanwhile the outlet COD$_T$ in R$_2$ on 28th day increased by 0.13 g/L compared to the inlet COD$_T$.

However, in the stable conditions, these large fluctuations observed in COD became significantly low, for instance soluble and total COD concentration in the outlet of R$_1$ varied in the range of 0.5±0.01 g/L and 1±0.05 g/L, respectively (Figure 5-3). Meanwhile, in R2 the outlet COD$_S$ varied in the range of 0.45±0.1 g/L, but the total COD fluctuated further between 0.79 g/L and 1.2 g/L.
Results

The average values of the COD$_T$ and COD$_S$ removal at the inlet and outlet streams are summarized in Table 5-1 for unstable and stable conditions. It is worthy to note that a standard deviation for the values are calculated based on average values of each index and by avoiding the negative removals in the calculations. Because these values were caused by technical problems and inappropriate design of the reactors.

The average removal of total COD was 47 % and 42 % in unstable condition for R$_1$ and R$_2$ respectively however, this amount reduced to 32 % and 35 % in stable condition (HRT= 24 h). In R$_1$, efficiency of soluble COD removal decreased form 30 % in unstable condition to 28 % in stable condition while in R$_2$, the efficiency dropped form 29 % to 25 % (Table 5-1). Inlet COD$_T$ concentration in R$_1$ reduced from 2.09±0.65 g/L to 1.63±0.5 g/L for unstable and stable conditions, respectively. Total COD concentration in the inlet of R$_2$ also dropped from 2±0.75 g/L in unstable condition to 1.56±0.5 g/L at the other condition. The soluble COD in inlet of R$_1$ became more diluted from 0.94±0.27 g/L to 0.74±0.26 g/L through approaching stable condition though, these values in R$_2$ changed less compare to R$_1$ (Table 5-1).
The outlet COD_T in R_1 and R_2 in unstable condition were 1.1±0.58 g/L and 0.86±0.3 g/L respectively. The outlet COD_T for R_1 fluctuated around 1.1 g/L and lower deviation in stable condition was observed. The COD_S in the outlet of R_1 and R_2 in unstable condition were 0.6±0.3 g/L and 0.64±0.37 g/L respectively while these values dropped to 0.53±0.1 g/L and 0.5±0.1 g/L in stable condition with HRT equal to 24 h (Table 5-1).

The standard deviation for inlet total COD is higher in the unstable condition although, these values reduced after installation of mesh. The soluble COD changed less compared with total COD in both unstable and stable region of figures. After installation of mesh, less fluctuation was observed in all parameters as shown in Table 5-1. COD removal efficiency in terms of total and soluble COD for R_1 and increased to 43 % and 50 % respectively when HRT decreased. These values for R_2 were 33 % and 58 % in HRT equal to 12 h. The soluble COD removal was rapidly effected by HRT and it doubled in both reactors by reducing HRT from 24 h to 12 h. Difference between average COD_T concentration and average COD_S concentration in the outlet of the reactors for whole experiment period were 0.57±0.21 g/L and 0.25±0.09 g/L respectively.
Results

0.5±0.19 g/L in R₁ and R₂ this values represent concentration of biomass organics in the outlet of the reactors.

Figure 5-5 and Figure 5-6 show the amount of total and soluble COD concentration reduction (i.e. difference between inlet and outlet values for CODₗ and CODₛ concentration) with respect to different total organic loading rate (OLR) and soluble organic loading rate (sOLR) in R₁ and R₂, respectively. The bar charts represent the amount of CODₗ and CODₛ concentration reduction in the reactors while the dashed lines show the variation of OLR and sOLR in reactors. The graphs are divided into unstable and stable part due mesh installation (i.e the vertical dash line shows on day 39 the mesh was installed in the inlet tank).

Figure 5-5: Amount of total and soluble COD removed with standard deviation together with total and soluble organic loading in the R₁

Figure 5-6: Amount of total and soluble COD removed with standard deviation together with total and soluble organic loading in the R₂
Results

The OLR increased in the first week and reached to 2.65 Kg COD/m$^3\cdot$d at day 4 where it resulted in an increase in COD$_T$ removal to around 0.8 g/L in both reactors. R$_1$ removed 1.85 g/L COD$_T$ in 7$^{th}$ day when the OLR declined to 2.2 Kg COD/m$^3\cdot$d (Figure 5-5). Removal of COD$_T$ in R$_2$ at the same day dropped to 0.74 g/L by OLR reduction. Amount of COD$_T$ and COD$_S$ removal decreased in second week when the OLR and sOLR reduced in both reactors. It should be noted that the sOLR had nearly the same trend as OLR in the reactors. (Figure 5-5 and Figure 5-6). In 7$^{th}$ day, R$_1$ removed 1.3 g/L COD$_S$ when the sOLR was 1.35 g/L that is highest amount of COD$_S$ removed during the study period in both reactors. Since the sOLR on day 17$^{th}$ and 23$^{rd}$ was low, the concentration of COD$_S$ in the outlet of R$_1$ increased, in other words, less COD$_S$ was removed (Figure 5-5). Likewise, negative COD$_S$ removal took place in R$_2$ on 14$^{th}$ day (Figure 5-5). OLR increased sharply in the 4$^{th}$ week and reached to roughly 3 Kg COD/m$^3\cdot$d. This increase in OLR in R$_1$ led to higher total COD reduction equal to 1.22 g/L and 2.25 g/L on 23$^{rd}$ and 25$^{th}$ day of the measurement, respectively. Although these values reached to 2.45 g/L and 2.39 g/L with a similar OLR for R$_2$ at those mentioned days (Figure 5-5 and Figure 5-6). COD$_T$ removal in both reactors were negative on 28$^{th}$ day, which is due to insufficient aeration in both reactors. On the 37$^{th}$ day, the OLR and sOLR increased to 1 Kg COD/m$^3\cdot$d and 0.45 Kg COD/m$^3\cdot$d in both reactors and 0.97 g/L of inlet COD$_T$ and 0.35 g/L of inlet COD$_S$ were removed in R$_1$. Afterwards, OLR and sOLR increased more and reached to 2.8 Kg COD/m$^3\cdot$d and 1.28 Kg COD/m$^3\cdot$d. However, due to some technical problems in R$_1$ total COD reduction dropped to 0.7 g/L on 39$^{th}$ day while COD$_S$ reduction increased to 0.55 g/L. With similar OLR and sOLR in R$_2$ the COD concentration reduction reached to 1.72 g/L in day 39. After installation of mesh in the feed tank on day 39$^{th}$, change in the trends for OLR and sOLR became nearly identical. The OLR and sOLR raised to 1.57 Kg COD/m$^3\cdot$d and 0.74 Kg COD/m$^3\cdot$d on day 49, respectively. Also, the soluble COD removal for R$_1$ and R$_2$ on 49$^{th}$ day were 0.7 g/L of COD$_T$ and 0.15 g/L of COD$_S$, respectively.

During stable condition period with HRT of 24h (i.e. between day 39 and 56, the removal of organics influenced highly with OLR and sOLR changes (Figure 5-5 and Figure 5-6). The OLR and sOLR doubled by reducing HRT from 24h to 12h and it resulted in an increase in COD removal in R$_1$ and R$_2$. It is observed from the graphs that the total COD reduction increases by increasing the OLR however, this increment is less in stable condition. Additionally, the negative COD removal was disappeared in both reactors after mesh installation.

5.1.3 Nitrification and denitrification

Unlike COD that was inflicted more by the thickener in the feed, the larger portion of NH$_4$ in the feed is mostly from centrifuge flow due to production of ammonium during degradation of organics in anaerobic digestion process (i.e. more information about thickener and centrifuge characteristics is provided in Appendix 4) [12].

The amount of ammonium in the inlet and outlet flows in R$_1$ and R$_2$ are demonstrated in Figure 5-7 and Figure 5-8, respectively. In addition to the amount of ammonium concentration reduction through each reactor. The bars illustrate amount of NH$_4$ concentration reduction and the lines are showing amount of ammonium nitrogen in the inlet and outlet of the reactors in stable and unstable condition.
Results

In unstable condition, the highest inlet NH$_4$ were 0.65 g NH$_4$/L and 0.6 g NH$_4$/L on the 1$^{st}$ and 7$^{th}$ day in both reactors while, the highest amount of ammonium in stable condition was 0.56 g NH$_4$/L on the 60$^{th}$ day for both reactors. On the 4$^{th}$ day when the OLR reached a peak in the first two weeks, the inlet ammonium reduced by half compared to the first day’s result where negative ammonium removal was observed in both reactors. However, increasing the inlet ammonium to 0.6 g/L increased the NH$_4$ removal. The highest ammonium removal efficiency occurred on the 7$^{th}$ day for both reactors (i.e. 96.6 % and 67 % for R$_1$ and R$_2$ respectively). On the day 28$^{th}$ nitrification rate dropped due to lake of dissolved oxygen. Reduction in HRT influenced ammonium removal and hence, removal efficiency in R$_1$ and R$_2$ dropped.

The NH$_4$ concentration reduction in R$_1$ became negative on 23$^{rd}$ day while this negative value was also observed on the 37$^{th}$ day for R$_2$. In both cases, the OLR was doubled compared to previous days (i.e. referred to Figure 5-5 and Figure 5-6). However, R$_1$ faced another negative ammonium removal on the 17$^{th}$ day when the OLR was low. This is clear from Figure 5-7 and Figure 5-8 that the removal of the ammonium after installation of the mesh became positive in all the remaining experimental days. In stable condition, ammonium removal decreased by reducing the HRT in the last two analyses.
The average values of ammonium removal in both reactors for both unstable and stable part of figures are presented in Table 5-2. The average efficiency of reactors in terms of ammonium removal in unstable condition decreased from 42.5 % and 33 % for R₁ and R₂, respectively, to 28% in stable condition for both reactors when HRT was 24 h.

Concentration of inlet ammonium in unstable condition reduced from 0.4 ±0.15 g/L and 0.36 ±0.15 g/L for R₁ and R₂ to around 0.32 g/L in stable condition with HRT of 24 h for both reactors. Moreover, the average outlet concentrations ammonium in the reactors was around 0.23 ±0.1 g/L in stable condition (HRT=24 h). These values in unstable condition were 0.23 ±0.17 g/L and 0.24 ±0.14 g/L for R₁ and R₂, respectively. In unstable condition, 0.17 g/L and 0.12 g/L of inlet ammonium was removed in R₁ and R₂. Though, these values dropped to 0.09 g/L in stable condition with HRT equal to 24 h for both reactors. It is worthy to mention that negative removal values were not considered for calculation of average values in Table 5-2.

By reducing HRT to 12 h removal efficiency in both reactors dropped and reached to 3.5 % and 9.1 % for R₁ and R₂, respectively. It should be noted that, the inlet ammonium concentration for shorter HRT increased and reached to 0.43 ±0.12 g/L and 0.44 ±0.1 g/L for R₁ and R₂, respectively.

Fluctuation of inlet and outlet ammonium concentration decreased after installation of mesh in both reactors. In unstable condition, R₁ is more efficient in terms of NH₄ removal but nearly equal amount of ammonium was removed in both reactors in stable condition.

Table 5-2: Average NH₄-N in inlet and outlet of the reactors at stable and unstable condition.

<table>
<thead>
<tr>
<th>Index</th>
<th>R₁ in unstable condition, HRT ≈ 24 h</th>
<th>R₂ in unstable condition, HRT ≈ 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inlet (g/L)</td>
<td>Outlet (g/L)</td>
</tr>
<tr>
<td>NH₄</td>
<td>0.4 ±0.15</td>
<td>0.23 ±0.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Index</th>
<th>R₁ in stable condition, HRT = 24 h</th>
<th>R₂ in stable condition, HRT = 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inlet (g/L)</td>
<td>Outlet (g/L)</td>
</tr>
<tr>
<td>NH₄</td>
<td>0.32 ±0.04</td>
<td>0.23 ±0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Index</th>
<th>R₁ in stable condition, HRT = 12 h</th>
<th>R₂ in stable condition, HRT = 12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inlet (g/L)</td>
<td>Outlet (g/L)</td>
</tr>
<tr>
<td>NH₄</td>
<td>0.43 ±0.12</td>
<td>0.41 ±0.11</td>
</tr>
</tbody>
</table>
The amount of nitrite and nitrate production through nitrification process within R₁ and R₂ are demonstrated in Figure 5-9 and Figure 5-10, respectively (i.e. average concentration of NOₓ and average ammonium reduction in unstable and stable condition provided in Appendix 5).

Before installation of mesh, the highest amount of nitrite was approximately 0.23 g NO₂/L on 7th day in R₂, while the highest value for R₁ was to 0.16 g NO₂/L on the 32nd day. Nitrate concentration in R₂ reached a highest amount 0.19 g NO₃/L on 14th day of the experiments period, while the highest nitrate concentration in R₁ observed on 7th day (Figure 5-9 and Figure 5-10). Between 23rd and 32nd day, nitrite and nitrate concentration in the R₂ dropped to around zero though the NH₄ removal was positive during these days (referred to Figure 5-8). Total concentration of nitrite and nitrate in most of the days in unstable condition is less than ammonium removal i.e. positive removal in both reactors in Figure 5-7 and Figure 5-8.

In stable condition, the highest concentration of nitrite and nitrate in R₁ reached to 0.22 g NO₂/L to 0.05 g NO₃/L on the day 42 (Figure 5-9). However, form Figure 5-7, it is observed that this value is less than ammonium removal in the R₁. After installing mesh in the feed tank,
Results

The production of nitrate and nitrite in R₂ dropped to around zero on the 39th day though, on day 46th, nitrite concentration increased to 0.1 g NO₂/L and nitrate concentration was around 0.02 g NO₃/L (Figure 5-10). In the last two measurements where the HRT was reduced 12 h, the NO₃ production dropped to around zero in R₂ while this value increased in R₁ in last analysis.

5.1.4 Sludge development in the reactors

The TSS and VSS removal in the reactors are shown in Figure 5-11 and Figure 5-12, respectively. Measurements in some of the days are removed in the data processing due to negative removal in the reactors. In fact these negative values occurred in the same days that COD removal were also negative.

Initially, TSS removal in R₁ increased from 0.3 g/L in the first day to approximately 1 g/L on day 4th, but it reduced after an increase in OLR and then become zero on day 14th. Afterward, in the 3rd week, the TSS removal increased to 4 g/L on 23rd day and remained unchanged until day 25 in R₁. In R₂, the same trend was observed however, the highest TSS removal occurred
on 23rd day when 10.2 g/L removed but the TSS removal dropped to around 4 g/L on day 25 (the TSS and VSS removal on day 23rd and 25th are unusual and it can be neglected). (Figure 5-11) In general, all measurements in unstable condition for both reactors showed that the VSS removal was nearly half of TSS removal (Figure 5-11).

On day 39 the VSS and TSS concentration reduction in R1 was 0.68 g/L. These values for R2 were 1.6 g/L and 2.12 g/L, respectively. In stable condition, the TSS removal and VSS removal reduced in both reactors as concentration of inlet reduced due to the mesh. The average TSS and VSS removal in R1 measured as low as 0.18 ±0.08 g/L and 0.1 ±0.03 g/L. R2 removed more suspended solids in stable condition compared to R1 where average TSS and VSS removal in R2 were 0.26 ±0.1 g/L and 0.16 ±0.07 g/L. While all values were still lower than 5% of total inlet suspended solids concentration.

Table 5-3 present the average concentration of solids in terms of TS, VS, TSS and VSS. These values were from the influent and effluent of the reactors at stable condition when the manual mixing employed.

Table 5-3: Amount of solids and removal efficiency in the reactors after manual mixing (42nd) in stable condition

<table>
<thead>
<tr>
<th></th>
<th>TS (g/L)</th>
<th>VS (g/L)</th>
<th>TSS (g/L)</th>
<th>VSS (g/L)</th>
<th>VS/TS (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>1.7 [±0.2]</td>
<td>1.3 [±0.5]</td>
<td>0.6 [±0.1]</td>
<td>0.4 [±0.1]</td>
<td>0.65</td>
</tr>
<tr>
<td>R1</td>
<td>1.4 [±0.2]</td>
<td>0.9 [±0.2]</td>
<td>0.5 [±0.05]</td>
<td>0.3 [±0.1]</td>
<td>0.64</td>
</tr>
<tr>
<td>Removal R1</td>
<td>18%</td>
<td>18%</td>
<td>17%</td>
<td>25%</td>
<td>-</td>
</tr>
<tr>
<td>R2</td>
<td>1.3 [±0.3]</td>
<td>0.8 [±0.2]</td>
<td>0.4 [±0.1]</td>
<td>0.3 [±0.1]</td>
<td>0.62</td>
</tr>
<tr>
<td>Removal R2</td>
<td>24%</td>
<td>27%</td>
<td>33%</td>
<td>25%</td>
<td>-</td>
</tr>
</tbody>
</table>

In general, measurement showed that concentration solids reduced in the outlet of the reactors however particularly the reduction for VSS is negligible. The average removal efficiency for R2 in terms of TS, VS and TSS was higher than that in R1. The VS/TS ratio in the inlet and outlet of the reactors stayed unchanged around 0.64 ±0.03.

Table 5-4 illustrates the concentration of sludge in terms of total COD, soluble COD and ammonium inside the reactors measured in three different days. Before installing mesh (day 28), concentration of COD\(_T\) in R1 and R2 were 40.15 g/L and 44.55 g/L. Meanwhile on day 42, when the mesh was installed, the total COD concentration dropped to 29.35 g/L and 22.75 g/L within R1 and R2, respectively. After mixing manually (i.e. measured in day 56), the COD\(_T\) concentration in the sludge became more diluted to 6.98 g/L for R1 and 6.65 g/L for R2. The Soluble COD in the sludge of R1 after installing mesh increased from 2.225 g/L to 2.86 g/L while in R2, COD\(_S\) concentration reduced from 2.2 g/L to 1.37 g/L on day 42. On day 56, soluble COD concentration in R1 and R2 decreased to 0.52 g/L and 0.47 g/L, respectively. In R1, the amount of NH\(_4\) increased from 0.615 g/L on day 28 of the measurement to 0.685 g/L on day 42, whereas these values for R2 reduced from 0.64 g/L to 0.36 g/L. After applying manual mixing, ammonium concentration decreased to 0.23 g/L and 0.14 g/L for R1 and R2, respectively.
Results

Table 5-4: Concentration of sludge in terms of COD and ammonium inside the reactors before installing mesh (day 28th), after installing mesh (day 42nd) and after manual mixing (56th).

<table>
<thead>
<tr>
<th>Sampling days</th>
<th>COD T R1,s (g/L)</th>
<th>COD S R1,s (g/L)</th>
<th>NH4-N R1,s (g/L)</th>
<th>COD T R2,s (g/L)</th>
<th>COD S R2,s (g/L)</th>
<th>NH4-N R2,s (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28th</td>
<td>40.15</td>
<td>2.225</td>
<td>0.615</td>
<td>44.55</td>
<td>3.585</td>
<td>0.635</td>
</tr>
<tr>
<td>42nd</td>
<td>29.35</td>
<td>2.86</td>
<td>0.685</td>
<td>22.75</td>
<td>1.37</td>
<td>0.36</td>
</tr>
<tr>
<td>56th</td>
<td>6.98</td>
<td>0.52</td>
<td>0.235</td>
<td>6.65</td>
<td>0.47</td>
<td>0.14</td>
</tr>
</tbody>
</table>

5.2 Effect of different factors on performance of reactors

Alkalinity, pH, DO and temperature have been considered as factors influencing the performance of the reactors. These parameters measured and the results are presented in this subsection.

5.2.1 Effect of alkalinity and pH on ammonium removal

Alkalinity consumption, ammonium reduction and pH variations in stable condition are shown in Figure 5-13 and Figure 5-14 for R1 and R2, respectively. The line graphs present the inlet and outlet pH, while the bar charts provide alkalinity consumption (dark bar chart) and ammonium concentration reduction (light bar chart). The inlet pH in both reactors was fluctuating from 7.1 to 7.5 with an exception of day 42 when the inlet pH reached to 8.3. In stable condition, the outlet pH was higher than inlet pH in both reactors except on day 42, which could be due to high oxygen level in the feed tank.

On day 39, the alkalinity consumption was almost equal to ammonium removal though, the outlet pH reached to 8.1, which was the highest outlet pH recorded during stable condition in R1 (Figure 5-13). On the day 42, 0.28 g/L of inlet ammonium was removed in R1 as 0.45 g CaCO3/L was consumed and the outlet pH dropped to 6.95. The highest amount of alkalinity consumption in R1 were 0.53 g CaCO3/L and 0.5 g CaCO3/L on 46th and 49th days of the measurement. The ammonium removal on day 49 was 0.06 g/L which was lower than 0.1 g/L on day 46 in R1. The inlet and outlet pH for 46th day was 7.16 and 7.46, respectively, while these values for day 49th increased to 7.53 and 7.55 in R1 as shown in Figure 5-13. On day 53 and 56 of the measurement, the alkalinity consumption and ammonium removal remained almost constant nearly to 0.28 g CaCO3/L and 0.05 g/L, respectively in R1. In the last two experimental measurements, when the HRT reduced to 12 h, the difference between inlet and outlet pH increased, whereas alkalinity consumption and ammonium removal in R1 reduced (Figure 5-13).
5.2.2 Variation of pH in denitrification process

The effect of pH change in on the total amount of nitrite and nitrate production in R_1 and R_2 are demonstrated in Figure 5-15 and Figure 5-16, respectively. The outlet pH value was nearly to 9.0 in the beginning of experiments and it reached to 8.0 at the end of experiments. While the inlet pH within the reactors had been constant around 7.2 except on day 42 where the inlet pH increased significantly. Moreover on day 32 and 42, roughly 0.19 g NO_x/L and 0.27 g
Results

NO\textsubscript{x}/L produced in R\textsubscript{1} with an inlet pH value of 7.2 and 6.9. Meanwhile, in the remaining days, when the concentration of nitrate and nitrite reduced through denitrification process (i.e. the ammonium reduction was higher than NO\textsubscript{x} concentration) where the outlet pH increased in the R\textsubscript{1} as shown in Figure 5-15.

![Figure 5-15: Variation of pH together with total amount of nitrite and nitrate produced in R\textsubscript{1}](image)

On day 7, the highest amount of NO\textsubscript{x} was accumulated in R\textsubscript{2} however, the amount was around half of ammonium removed in the R\textsubscript{2} (i.e. referred to Figure 5-8). Therefore, the outlet pH increased by 0.72 compared to inlet pH and reached to 8.13. The inlet and the outlet pH on days 14 and 42 were equal in R\textsubscript{2} while on day 14, only 0.2 g/L ammonium removed in R\textsubscript{2} (Figure 5-8) and was accumulated in the R\textsubscript{2} as nitrate and nitrite as shown in Figure 5-16. Since the ammonium removal on day 28 was negligible in R\textsubscript{2} and the amount of NO\textsubscript{x} was around zero, the pH stayed the same in the inlet and outlet (Figure 5-16). Between days 23 and 39, most of the nitrite and nitrate produced in R\textsubscript{2} disappeared (probably as nitrogen gas), which resulted in higher pH in the outlet of the reactor.

![Figure 5-16: Variation of pH together with total amount of nitrate and nitrate produced in R\textsubscript{2}](image)
5.2.3 Dissolved oxygen gradient within the reactors and temperature variation

Temperature measurements and concentration of dissolved oxygen (DO) at different measurement points as discussed in Figure 4-2 in chapter 4 are shown in Figure 5-17 and Figure 5-18 for R₁ and R₂, respectively. Initially, temperature increased in both reactors and reached to 16 °C in R₁ on day 4 and 18.2 °C in R₂ on the 7th day. Afterwards, temperature in both reactors were 14 ±0.5 °C until day 28 when the temperature increased to 16 °C and 15.2 °C in R₁ and R₂, respectively. In stable condition (after 39th day), temperature within both reactors varied around 14 ±0.8 °C. The oxygen level on the surface of central layer in all measurements were higher than oxygen level in the bottom of central layer in both reactors except for the first day. Average difference between DO level on the surface and bottom of central layer were 0.35 mg/L and 0.48 mg/L within R₁ and R₂, respectively.

![Figure 5-17: Temperature and DO measurement in different points of R₁. Surface of central layer (A₁), bottom of central layer (A₂), surface outer layer next to discharge point (B₁) and bottom of the reactor (B₂)](chart1)

![Figure 5-18: Temperature and DO measurement in different points of R₂. Surface of central layer (A₁), bottom of central layer (A₂), surface outer layer next to discharge point (B₁) and bottom of the reactor (B₂)](chart2)
Results

After discharging the sludge on day 9, DO level in all points of the reactors increased. These values for $R_1$ and $R_2$ were 8.45 ±0.2 mg/L and 8.5 ±0.1 mg/L, respectively. However, few days later, the accumulated sludge in the outer layer resulted in low oxygen level in the bottom of reactor (i.e. line B2 in Figure 5-17 and Figure 5-18). The DO level in both reactors significantly dropped on day 28 due to insufficient aeration within the reactors. In fact the DO level in $R_2$ reduced between on day 49 and 56 that was mainly due to lower aeration flow.

After installing the mesh inside the feed tank and applying the manual mixing, DO level in the outlet of the reactors (B1) increased. The outlet flow in both reactors contains dissolved oxygen (B1) (Figure 5-17 and Figure 5-18). The outlet streams from $R_1$ and $R_2$ contained low values of DO with only 1.26 mg/L and 0.8 mg/L, except on day 28 where both reactors contained less than 0.06 mg/L DO in the outflow. The accumulated sludge at the bottom of the reactor has caused a reduction in DO level up to 0.05±0.014 mg/L. In the last two measurement when the HRT reduced to 12 h, higher amount of particles flowed into the reactors and the DO level in the outlet of the reactor reduced to 3 mg/L and 3.25 mg/L within $R_1$ and $R_2$ respectively on day 60.

5.3 Modelling and simulation results in ASM1

The Syringe test results were considered in the simulations for two main reasons. First, to demonstrate what fraction of the substrates are biodegradable, second, to estimate the maximum hydrolysis rate in proposed hydrolysis processes.

Figure 1 shows the accumulated biogas production in syringes from different substrates after one week (i.e. the syringe tests run for 60 days and the results are provided in the Appendix 2). The substrates from inlet and outlet of $R_1$ and $R_2$ had similar trend in terms of biogas production with low amounts of biogas. Thickener water produced the highest amount of biogas and it started producing biogas from the first day. The centrifuge water also had a significant biogas potential but it took longer for the production to get start (i.e. lag period).

![Figure 5-19: Accumulative amount of biogas production in syringe test](image)

Table 5-5 presents the average inlet values that were used in simulation of nitrification and organic removal within the reactors. Average values are based on the inlet characteristics of inlet flow in stable condition. The values in the Table 5-5 are presenting the experimental
condition of pilot-scale reactors that is called current condition in the further discussion. It is assumed that the initial concentration of both type of bacteria in the bulk volume of the reactors were 10 mg/L. The volume of the reactors assumed to be equal 18.8 L.

Table 5-5: Inlet characteristics based on average values of experimental measurements

<table>
<thead>
<tr>
<th>NH₄-N (mg/L)</th>
<th>CODₛ (mg/L)</th>
<th>CODₚ (mg/L)</th>
<th>CODₜ (mg/L)</th>
<th>NO₃ (mg/L)</th>
<th>HCO₃ (mmol HCO₃/L)</th>
<th>Xₐₙₜ (g/L)</th>
<th>Xₜₙₜ (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>353</td>
<td>815</td>
<td>785</td>
<td>1600</td>
<td>3</td>
<td>16</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

5.3.1 Removal efficiency in current condition of pilot-scale reactors

In this sub section overall performance of the reactors in the real condition is simulated and the results are presented in Figure 5-20. The bars present the removal efficiency in terms of ammonium, soluble COD and total COD while the lines illustrating the nitrite concentration in the system. Inside the reactors the average dissolved oxygen (include aerobic and anoxic zones) did not reach to more than 4.5 mg/L. Even in some days this value was lower than 4.5 mg/L. Therefore, for simulating the current performance of reactors this value selected as oxygen level. The other parameters are presented in Table 5-5.

Figure 5-20: Simulation of experiment condition in different HRTs when the alkalinity level is 16 mmol HCO₃/L and DO concentration was 4.5 mg/L.

In general, by decreasing HRT from 30 h to 2 h the ammonium removal decreased from 0.23 to zero respectively. Similar trend observed for soluble COD and total COD. So that, these values dropped from 0.4 and 0.39 respectively, to 0.04 for both parameter when the HRT was 2 h. When the HRT equal to 30 h the nitrite concentration was 0.038 g/L and by decreasing HRT to 24 h this value increased to 0.047 g/L. However, ammonium removal at HRT equal to 30 h and 24 h was 0.23. Ammonium removal decreased to 0.14 when HRT was 12 h. The simulation showed that when HRT decreases, the ammonium removal and nitrite concentration also decreased.

The highest soluble and total COD removal was 0.4 and 0.39 respectively, which occurred in the condition with HRT equal to 30 h. These values dropped to 0.35 and 0.34 when HRT was
24. For shorter HRTs the COD$_S$ and COD$_T$ removal continue to decrease and reached to around 0.04 at HRT of 2 h.

Figure 5-21: Biomass concentration in different HRTs when the alkalinity level was 16 mmol HCO$_3$/L and DO concentration was adjusted on 4.5 mg/L.

Figure 5-21 provides effect of HRT on biomass growth and development inside the reactors when the oxygen level was 4.5 mg/L. By decreasing HRT from 30 h to 12 h concentration of both type of bacteria in the reactors increased. Concentration of heterotroph bacteria increased from 6.6 g/L at HRT of 30 h to 12 g/L when HRT was 12. These values for autotroph bacteria was 0.1 g/L and 0.2 g/L when HRT was set on 30h and 12 h, respectively.

The autotroph bacteria concentration reduced to 0.04 g/L in HRT equal to 8 h and for shorter HRT the concentration of autotroph bacteria declined to zero. The concentration of heterotroph bacteria increased to 15.4 g/L by decreasing HRT to 8 h while for further reduction of HRT heterotroph concentration decreased and reach to 9.7 g/L when HRT was 2 h.

Figure 5-22 provides the COD and ammonium reduction per day in different HRTs. By reducing HRT from 30 h to 12 h the ammonium removal increased from 1.2 Kg NH$_4$/d to 1.9 Kg NH$_4$/d as the concentration of autotroph bacteria increased (Figure 5-21 b). However, when the HRT decreases further the ammonium removal continuous to decrease and reached to zero when the autotroph bacteria washed out from the reactors in HRT < 4h. The amount of daily reduction was calculated based on daily load to the reactors and the removal efficiency. Therefore, even in low removal efficiency at short HRT the amount of daily removal is high.

Since heterotroph bacteria population increased by reducing HRT from 30 h to 8 h, the COD$_T$ reduction per day increased from 9.3 Kg COD/d to 17.9 Kg COD/d. These values for COD$_S$ was 4.9 kg COD/d and 9.1 kg COD/d, respectively. By reducing HRT to 4 h the COD$_T$ reduction and COD$_S$ reduction dropped to 15.1 Kg COD/d and 7.7 Kg COD/d and the these values were similar at HRT of 2 h.
5.3.2 Using high alkalinity value in order to simulate performance of reactors

In this part, the set point for oxygen level in the model was 7.5 mg/L before each run. Hence, dissolved oxygen was not a limiting factor in simulation. Figure 5-23 shows the ammonium and organic removal efficiency in different alkalinity conditions when HRT is 24 h.

In the first run, that present current operational condition of pilot-scale reactors, the alkalinity was 16 mmol HCO$_3$/L. Ammonium removal efficiency was 0.23 and the efficiency of organic removal in terms of soluble and total COD were 0.33. These results are quite close to removal efficiency in reactors (Table 5-1). By increasing the alkalinity in the reactors the COD removal did not change and stayed equal to 0.33 for COD$_s$ and COD$_T$. By increasing alkalinity to 20 mmol HCO$_3$/L the ammonium removal efficiency increased to 0.29. The nitrite concentration also increased from 0.058 g/L to 0.078 g/L when alkalinity level increased from 20 mmol HCO$_3$/L to 50 mmol HCO$_3$/L. When alkalinity increased to 70 mmol HCO$_3$/L the ammonium removal efficiency and nitrate concentration increased to 1 and 0.322 g/L, respectively. After
Results

the optimum level of alkalinity (i.e. 70 mmol HCO$_3$/L) the increase in alkalinity level did not affect ammonium removal efficiency and nitrate concentration in the system.

Effect of alkalinity concentration on autotroph and heterotroph bacteria growth is shown in Figure 5-24 when HRT is 24 h and DO level set to 7.5 mg/L. Concentration of heterotroph bacteria were 7.7 when alkalinity were 16 mmol HCO$_3$/L. By increasing alkalinity level from 16mmol HCO$_3$/L to 100 mmol HCO$_3$/L concentration of heterotroph bacteria did not change and stayed equal 7.7 g/L. The lowest concentration of autotroph bacteria was 0.17 g/L when the alkalinity was 16mmol HCO$_3$/L. When alkalinity increases, the concentration of autotroph bacteria increased until reached a pick of 0.75 g/L at alkalinity level of 70 mmol HCO$_3$/L. However, further increase in the alkalinity level, concentration of autotroph bacteria remained at 0.75 g/L.

The effect different HRTs on the performance of the reactors on COD, ammonium removal efficiency and the variation of nitrate production are shown in Figure 5-25. The result shows that, the alkalinity level of 70 mmol HCO$_3$/L was chosen as an optimum alkalinity level. In other words, alkalinity was not a limiting factor for high ammonium removal efficiency in results that are presented in Figure 5-25. Increasing HRT from 2 h to 30 h, the ammonium removal efficiency and nitrate concentration did not change, which was 0.32 g/L in each HRT. Lowest COD$_S$ and COD$_T$ removal efficiency of 0.05 recorded when HRT set to 2 h. These values increased to 0.4 and 0.39 when HRT increased to 30 h.
Figure 5-25: Ammonium (NH₄), soluble and total COD removal efficiency together with nitrate production in different HRT when the alkalinity level is 70mmol HCO₃/L.

Effect of HRT variation on different bacteria growth is shown in Figure 5-26. It is clear from Figure 5-26 that, concentration of heterotroph bacteria in the system increased from 7.8 g/L to 32 g/L as HRT decreased from 30h to 2h. On the other hand, the autotroph bacteria concentration increased from 0.6 g/L when HRT set to 30 h to 4.5 g/L at HRT of 2 h. According to the simulation results the autotroph concentration reached to the 0.7 g/L when the alkalinity level was 70 mmol HCO₃/L and HRT set to 24 h while at the real condition with lower alkalinity level (i.e. 16 mmol HCO₃/L) the autotroph concentration was 0.2 g/L. Reducing the HRT to 2 h resulted in the autotroph concentration to 4.5 g/L (Figure 5-21 and Figure 5-26).

Figure 5-26: Variation of biomass concentration in different HRT when the alkalinity level is 70mmol HCO₃/L. a) Heterotroph bacteria concentration, b) Autotroph bacteria concentration

Effect of HRT on daily reduction of COD and ammonium in optimum condition is provided in Figure 5-27. The ammonium reduction increased from 5 Kg NH₄/d to 79 Kg NH₄/d by reducing HRT from 30 h to 2 h. Since, at similar condition the autotroph concentration increased from 0.603 g/L to around 4.5 g/L.

COD reduction in the reactor has followed similar trend to ammonium reduction, however this trend for both total and soluble COD is smoother when compared to ammonium. Daily CODₜ and CODₛ reduction were 9 Kg COD/d and 5 Kg COD/d, respectively when the HRT was 30
h whereas at the same condition the heterotroph bacteria concentration was 6.6 g/L. When HRT reduced to 2 h the heterotroph bacteria growth was 30 g/L and hence, the SOD\textsubscript{T} reduction and COD\textsubscript{S} reduction increased and reached to 17 Kg COD/d and 9 Kg COD/d, respectively.

![Figure 5-27: Daily reduction of ammonium and COD in different HRTs when the alkalinity set to 70 mmol HCO\textsubscript{3}/L and the DO level was 7.5 mg/L.](image-url)
6 Discussion

In this chapter, the results are discussed to demonstrate to what extent the results are supporting the proposed mechanisms. This chapter has five subchapters. In the first three sub-sections, the possible relation between the results and proposed mechanisms are discussed. In the fourth sub-section, the simulation result are discussed, and the last sub-section generalizes the discussion with the overall performance of the reactors and possible weakness in the performance of the reactors.

6.1 Performance of MBBR reactors

This sub-section considered different factors as reactor performance (i.e. such as COD removal, nitrification and denitrification process, and biomass production inside the reactors) in order to investigate possible correlation between these factors and hypothesis mechanisms (section 1.1).

6.1.1 COD removal and evidences for hypothesis mechanisms

In both unstable and stable conditions, average COD removal in both reactors was higher than average COD removal when HRT was 24 h. This shows that the conversion of organics and solid particles into biomass [11, 47]. Such biomass growth (i.e. cell synthesis) from COD in the reject water is in accordance with the second mechanism proposed for the system (Table 5-1) [6]. Moreover, the relatively high effluent COD (i.e. sometimes even leading to negative COD removal) shows that much of the produced biomass leaves the reactors which in fact contributes for the transfer of active biomass to the plant inlet (i.e. the third mechanism)[6, 11]. In fact, the COD removal in the reactors shows the first mechanism. Organics consumed as food aid to biomass growth and presence of this biomass in the outlet of the reactors may contribute to the third mechanism.

6.1.2 Nitrification/denitrification and its possible correlations with mechanisms

The ammonium removal in R1 and R2 supports the presence of nitrifier bacteria in the reactors [9]. It can be considered that nitrifiers could also leave the reactors to the main inlet of the plant and may contribute in the third mechanism [6, 11]. However, according to the simulations the concentration of autotroph organics are very low (i.e. because of low nitrification rate) compared to concentration of heterotroph and hence the results verified that autotrophs may not have a significant contribution to the third mechanism.

Lower concentration of nitrite and nitrate in the outlet of reactors compared to ammonium reduction show presence of denitrification process (Appendix 5) [9, 48]. Denitrification in anoxic part aids to oxidation of organics results in biomass growth in the reactors [49]. Therefore, denitrification process that has occurred in the reactors contributes for the first and second mechanisms. However, our result can not verify this as its effect is not significant until high nitrification/denitrification range is achieved.

Moreover, in some experimental measurements the observed high oxygen level in the reactors has leads to the high amount of nitrite and nitrate left the reactors (i.e. this represents a condition
without denitrification). Under conditions without denitrification process, it is possible to have positive effects of bringing some electron acceptor (i.e. such as nitrate as product of nitrification process) back to the inlet. Several studies have reported that the presence of nitrate makes heterotroph more efficient to capture dissolved organics [11, 34]. All in all, the concentration of nitrifiers are not significant in this study due to low nitrification rate. In a case with higher nitrification rate and the presence of enough oxygen, more ammonium will remove through nitrification process and concentration of nitrifiers and nitrate will increase inside the reactor as well as outlet of the reactor that cause higher contribution in the third mechanism.

6.1.3 Biomass concentration and its connections with mechanisms

Low reduction of total and volatile solids in Table 5-3 show that in optimum operational condition (i.e. presence of enough oxygen, appropriate circulation of water and free movement of carriers), the outlet of the reactors contains high amount of biomass. The high COD\textsubscript{B} (i.e. this value includes active biomass concentration, slowly biodegradable organics and particulate organics) and the average VSS of 0.3±0.1 g/L (i.e. VSS expresses the biomass concentration in the samples) in the outlet of the reactors have confirmed that some heterotroph and autotroph bacteria left the reactors [39]. The study suggests that high concentration of solids in the outlet does not conflict with the objectives rather the presence of biomass can contribute to the third mechanism.

The results from Table 5-4 express that installation of mesh in the feed tank and using manual mixing method reduced the sludge concentration in the reactors compared to unstable condition. The manual mixing was to enable sludge movement into the central layer in order to activate degradation of particulate organics inside the central layer of the reactor. It suggests that in the optimum operating condition some of the dissolved and particulate organics in the sludge were degraded inside the reactor and it enhanced the growth of biomass on the surface of biocarriers [6, 11]. Such biomass growth may supports first and second mechanisms.

It can be concluded that, high concentration of the active biomass (measured as COD\textsubscript{B} and VSS) in the outlet of the reactor can contribute in the third mechanism. Moreover, a good mixing process in the reactors (mainly led by aeration) is essential to prevent sludge sedimentation, and it leads to degradation of particulates, furthermore, it improves the biomass development (first and second mechanisms).

6.2 Effect of alkalinity and DO on hypothesis mechanisms

Alkalinity plays an important role as a growth substrate for nitrifiers and balancing the pH level in the reactors [32]. Simulations indicate that alkalinity level affects nitrification rate as well as autotroph bacteria concentration. The results suggest that presence of enough alkalinity not only increased the nitrification rate and the nitrate concentration in the outlet of the reactors but also increased the autotroph concentration in the reactor outlet. So that, the higher concentration of autotrophs and nitrate may leave the reactors and contribute significantly to the third mechanism.

The difference between DO level in the surface and bottom of central layer clearly showed the oxygen consumption in order to oxidize the organics and growth of biomass on the carriers’
High aeration level increases the carrier’s movement in the reactors which ultimately result in a continuous collision of the surface of carriers and enhanced the detachment process [34, 50]. Hence, detached biomass may flow with the reject water through the main inlet and contribute to the third mechanism. Moreover, the results in Figure 5-17 and Figure 5-18 have shown that dissolved oxygen is high in the outlet of the reactors (B1). The presence of oxygen in the outlet of the reactors can improve performance of the heterotroph organics in grabbing of dissolved organics when they introduced into the main inlet [6].

Altogether, high alkalinity level may enhance the ammonium removal; nitrate concentration and growth of nitrifiers in the reactors. These nitrate and nitrifiers can contribute to the third mechanism when introduced into the main inlet. DO consumption in the reactors supports first mechanism moreover, high DO level in the outlet can improve the active biomass performance in the main treatment train.

### 6.3 Improvement in plant performance based on proposed mechanisms

The case study on the Knarrdalstrand WWTP showed that the highest flow from reject water to the main inlet is less than 0.2 % of plant inlet flow (i.e. based on mass balance in Appendix 2). Nevertheless, the reject water can cause a disturbance on the main coagulation process by overloading organics [14]. According to the jar test results, the coagulation COD removal efficiency improved by adding treated reject water to the main inlet compared to the untreated reject water (i.e. jar tests carried out by another master student using a relevant mixture of wastewater and reject water from the experiments that are reported in this thesis, and the results are provided in Figure 5 in Appendix 7). This phenomenon also confirmed the presence of active biomass in the outlet of the reactor which readily capture more dissolved organics in the coagulation process and improves the quality of discharge water (3rd and 4th mechanisms). When more dissolved organics are captured in the coagulation process, it may also lead to higher biogas production potential in the sludge anaerobic digestion process. This is not experimentally confirmed.

Overall, the proposed approach has been demonstrated to improve the COD removal efficiency of the main coagulation process explained by the presence of active biomass from the reject water treatment bioreactors. Obtaining better quality outlet water with less organics, a major concern, can thereby be obtained by biological reject water treatment.

### 6.4 Simulation and modeling

The performance of reactors is simulated in various operational conditions that did not test experimentally to get an overview of the reactors function in full-scale and possibly to choose the most appropriate operational condition.
6.4.1 Simulation reliability

Based on the results in Figure 5-20 and Figure 5-23 when alkalinity level in the inlet is 16 mmol HCO$_3$-L at HRT of 24 h, ammonium, COD$_T$ and COD$_S$ removal efficiency were 0.23, 0.35 and 0.36 respectively. These values were not significantly different from the measured removal efficiency of both reactors in stable condition shown in Table 5-1 and Table 5-2. It indicates that inlet values and kinetics of biochemical reactions proposed in ASM1 [45] are well-fitted with reject water treatment and the experimental conditions tested here.

The experimental data in Figure 5-5 and Figure 5-6 show that by increasing HRT in the last two tests the COD removal increased while the ammonium removal reduced. In R$_1$ the average total COD removal efficiency for the two analysis was 0.43 and this value in the R$_2$ was 0.33 (Table 5-1). In fact, these values were not significantly different from total COD removal efficiency in the simulation. The soluble COD removal in the simulation was 0.33 at HRT of 12 h while in experimental data the average COD$_S$ removal reached more than 0.5. This could be due to two reasons, first, these average values are only from two measurements (i.e. not statistically enough data); second, the initial values of the model are the average of inlet characteristics in whole stable condition, while the inlet characteristics changed continuously.

The results suggest that the proposed hydrolysis processes [45, 46] with significant fractions of slowly degradable organics for particulate and soluble are reliable to simulate the real condition of the reactors. Since the thickener is located before anaerobic digestion process, its flow contains more biodegradable organics compare to the stream from the centrifuge. Syringe tests results confirm that the organics are slowly biodegradable and oxidation and/or solubilization of organics requires time since it took at least two days to produce biogas (Figure 5-19).

All in all, the model, the initial values and kinetic coefficients produce simulations of the biological process that fit quite close to the experiment data. It is therefore reasonable to use the model to simulate conditions not tested experimentally, to extrapolate on experimental data and to plan future tests.

6.4.2 Simulation achievements

The ammonium removal was limited by alkalinity and the amount of oxygen concentration (Figure 5-23 and Figure 5-25). When the alkalinity level was 16mmol HCO$_3$-L ammonium removal does not change even by changing HRT (Figure 5-20) whereas increasing alkalinity level to 70 mmol HCO$_3$/L the removal efficiency reached to 100% even in shortest HRTs (i.e. 2 h and 4 h).

Currently, FeCl$_3$ is used as coagulate in the plant. In fact, alkalinity is consumed in the coagulation process due to low pH of FeCl$_3$. However, if the plant switch to a calcium-based coagulant (i.e. for instance calcium hydroxide), not only the alkalinity level in the reject water will be high but also it will increase the alkalinity level in the MBBR reactors (i.e. thickener flow) and improves ammonium removal. Positive effects of using calcium hydroxide as coagulant such as improving the performance of coagulation process, providing rich sludge and increasing the pH level of the wastewater are reported by Niazi (2018) [52].

COD removal mostly depends on HRT than alkalinity level as in all alkalinity levels the COD removal behaved similarly at a fixed HRT (Figure 5-20 and Figure 5-23). On the other hand as
shown in Figure 5-26 biomass development in the reactors is dependent on HRT at an optimum alkalinity level. If the oxygen and alkalinity level in the reactors are at high set point these two factors do not limit the nitrification process. Otherwise, it advisable to reduce HRT based on the treatment requirements as short HRT produces more biomass (i.e. better contribution to the third mechanism).

In Figure 5-26 it is shown that HRT of 2 h and 4 h have produced high amount of autotroph and heterotroph bacteria. In fact, based on the hypothesized mechanisms in this study, presence of biomass (especially heterotroph bacteria) and dissolved oxygen in the outlet of the reactors will improve the performance of coagulation process. In the condition with high concentration of DO and alkalinity, if HRT reduced to less than 4 h it reduces the construction cost and capital investment for the implementation of biological reject water treatment. Tchobanoglous et al. (2014) reported that, in general, the appropriate HRT for MBBR reactors is around 4 h in order to remove organics in different types of wastewaters (it is not specifically tested on reject water) [18].

The removal efficiency in terms of COD and ammonium dropped when HRT was reduced from 30 h to 2 h. However, the COD and ammonium removed per day were high at short HRT which is expected in biological treatment processes (i.e. when the HRT was 2 h the OLR in the system was 19.2 Kg COD/m$^3$·d and in HRT equal to 30 h the OLR was 1.28 Kg COD/m$^3$·d.) (Figure 5-22 and Figure 5-27) [51]. It means that in short HRT when the high alkalinity level and DO level are fulfilled in the reactors, heterotrophic bacteria get access to a large amount of feed, therefore, their population grows rapidly even by removing a low amount of COD (Figure 5-25 and Figure 5-26). A similar phenomenon is also expected for autotroph bacteria.

It can be concluded that, the ammonium removal is highly dependent on alkalinity level in the reject water and DO level inside the reactors so that if surplus of these are provided, the maximum removal efficiency will be achieved. Shorter HRTs increased OLR and enhanced biomass growth and COD removal in the reactors. Short HRT (4 h or 2 h) seems sufficient for biological reject water treatment suggesting an optimum at HRT < 4 h.

6.5 Overall performance of the reactors and possible errors

This sub-section investigates the overall performance of the reactors regarding COD removal, ammonium removal, and sludge development. During the study period, some technical errors that described here affected the performance of the reactors.

6.5.1 COD removal in different HRTs and OLRs

It is worthy to note that R2 was also used for the similar study in Lillevik project and the average operating temperature was 20 ºC [6]) while in this study the average temperature was around 14 ºC in stable condition [5, 6]. Young et al (2015) claimed that cold culture can slow down the reaction rate and reduce the removal efficiency nevertheless, average COD$_T$ removal of 42 % in unstable condition and 35% in stable condition (HRT= 24 h) is a good improvement compare to COD removal efficiency at Lillevik with average 16 % and 22 % when the HRT was 30 h and 12 h respectively [53]. COD$_S$ removal efficiency in stable condition increased and crossed 50 % in both reactors when HRT reduced from 24 h to 12 h. 12 h HRT improved COD$_T$ removal efficiency in R1 and this value reached to 43% (Figure 5-5 and Figure 5-6).
Negative values for COD removal is also reported in the Lillevik project with similar reactor [5, 6]. Low oxygen level, low OLR, sampling method and/or the sludge accumulation in the system could be some of the reasons for increasing COD₇ concentration in the outlet of the reactors [15]. Whenever the OLR increased, the total COD removal started to increase in the respective reactors moreover, the shorter HRT increased the COD removal efficiency (especially COD₅) in reactors. The temperature did not affect the reactors performance significantly.

6.5.2 Nitrification /denitrification in different HRTs and OLRs

Average NH₄ removal in unstable condition for R₁ and R₂ were 42.5 % and 33 % respectively while these values dropped to around 28% in both reactors in stable condition when the HRT was 24 h that was similar to the results from Lillevik (i.e. average ammonium removal in Lillevik was 27%) [5, 6]. The pH in the whole system fluctuated between 6.9 and 8.5 and it indicates the concentration of ammonium nitrogen is dominated in both reactors and ammonium did not disappear as ammonia gas (Equation 2-9 and Figure 2-1) [11, 33].

Negative removal of ammonium in the reactors may have had three main reasons. First, the settled sludge contains high amount of ammonium (as shown in Table 5-4) and it served as substrate for nitrifiers. Therefore, excess ammonium deposited in the reactors resulted in higher ammonium concentration in outlet samples [9]. Second, inlet and outlet samples were collected at the same time (i.e. while the HRT of the reactors were 24 h) so that some biological variations during the time interval may cause negative removals. Third, as municipal sewerage contains protein and the degradation of protein provides ammonium which may increase the ammonium concentration inside the reactors [11]. As shown in Table 5-2 ammonium removal efficiency dropped in both reactors by reducing HRT to 12 h. Since shorter HRT increase the OLR and the biomass grows faster, the oxygen consumption in the reactors will increase. In pilot-scale reactors, by reducing HRT to 12 h the oxygen level did not increase (especially in R1) compared to the condition with HRT equal to 24 h and it led to lower removal efficiency in the reactors [36, 54].

The amount of ammonium reduction in both reactors were higher than the concentration of nitrite and nitrate in the outlet of the reactors due to low concentration of DO in the outer layers (Figure 5-7, Figure 5-8, Figure 5-9, Figure 5-10 and Appendix 5). This phenomenon suggests that denitrification is simultaneously occurred [50]. When the oxygen level in the outer layers increased the concentration of nitrite and nitrate in the outlet samples increased (Figure 5-9 and Figure 5-10 ) [34]. It indicates that heterotrophs tend to consume oxygen when it is available instead of consuming NOₓ as an oxygen source. The simulation results indicate a difference between nitrate concentration and amount of removed ammonium. This difference has confirmed existence of denitrification process in the reactors when the oxygen level was low. Altogether, Low nitrification rate in the reactors led mainly by several factors such as low alkalinity, high pH, high organic loading rate and low DO level [11, 55].

6.5.3 Sludge development

At some point in the experimental period it was observed that the bio carries were blocked (Figure 6-1). Thick biofilm on the carriers may cause substrate and oxygen cannot diffuse into the inner layers of biofilm result in heavier carriers and these may lead to detachment of biofilm
Discussion

(e.g. some weaknesses of reactor design is provided in Appendix 6) [11, 18]. High air flow provides uniform and thin biofilm on the carriers’ surface. Therefore, an appropriate aeration flow for mixing is essential in MBBRs (i.e. Due to the mentioned problem the reactors replaced with CFIC reactors as described in section 6.5.5)[11].

Figure 6-1: Biomass density on the bio carriers. a) Carriers from R1 b) Carriers from R2

6.5.4 pH variations and DO level in the reactors

Denitrification process release hydroxide ion and it cause an increase in the pH. However, its effect is negligible under high aeration flow. The result from Figure 5-15 and Figure 5-16 have shown that NO₃ accumulation under sufficient alkalinity created similar pH in the inlet and outlet of the reactors even if ammonium has been removed in the reactors (i.e. the average concentration ammonium, nitrite and nitrate in the outlet of the reactors is provided in Appendix 5) [18, 56]. It indicates that alkalinity plays a major role to stabilize pH variation in the reactors due to nitrification whereas in opposite denitrification increased reactors’ pH. Therefore, pH measurement can be vital in order to monitor the performance of reactors [47]. Moreover, several studies have documented that the CO₂ stripping from wastewater by high aeration flow has the most significant effect on pH rise from 7 to 8 while this phenomenon did not tested in this study [57-59].

During this thesis study period it has been attempted to keep the oxygen level close to the regular oxygen level of Standard MBBRs (i.e. between 4mg/L and 6mg/L) while results and observations suggest that this aeration level was not sufficient for the aims of this study [34]. Low aeration level leads to inappropriate mixing in the reactors and increased settle ability of the particles. Moreover, it causes rapid development of biomass on the carrier surface due to poor detachment process [34]. In general, for complete dominated nitrification process a minimum of 1 mg/L oxygen is essential (Appendix 8) [34]. This DO level was not sufficient for mixing the carriers in the center of reactors because of the compact design of central layer. Therefore, the high DO and alkalinity level will improve the performance of the reactors in both ammonium removal efficiency and uniform biofilm development (section 6.5.5).
6.5.5 CFIC reactors

The reactors replaced by two new continues flow intermittently cleaning (CFIC®) moving bed reactor (i.e. for more information about CFIC reactors referred to Rusten, et al. [35], Ghimire [21] and Ødegaard [16]) by Biowater Technology due to the technical problems (i.e. such as sludge accumulation, dead zones inside the reactors, poor detachment process, blockage of the carriers, inappropriate mixing and poor water circulation). However, the results from the new reactors in terms of removal efficiency and overall performance are not provided in this report. The volume of the reactors was 68 L and carrier filling ratio was 70 %. The reactors were fed from the bottom next to the aeration pipe. It was observed that bio carriers moving freely in the reactors and oxygen level had almost uniform value in different points within the reactors. The reactors are currently operating under HRT of 16 h, which will be reduced over time. The COD removal efficiency was 35±4 % and CODS removal was 41±1 %. The biofilm was developed uniformly on the surface of carriers and sludge sedimentation in the reactors was negligible. It observed that blockage of carriers have been minimized in CFIC reactors when the DO level was between 7 mg/L and 8 mg/L therefore an average value of 7.5 mg/L selected for simulation. It is also mentioned that excess oxygen in the outlet of the reactors will not waste and it returns back together with biomass to the main treatment.
7 Conclusion and further studies

7.1 Conclusion

I. Performance of the reactors have verified the proposed hypothesis/mechanisms (section 1.1) as follow:
   - The results show the degradation of particulate organics and dissolved organics (measured as COD) according to the proposed first mechanism.
   - The growth of biomass on the carriers’ surface show biomass developed through cell synthesis.
   - High concentration of active biomass in the outlet samples together with excess oxygen in the reactors outlet may support the proposed third mechanism of soluble organics uptake from the main plant in let when the treated reject water is introduced there.
   - The coagulation COD removal efficiency was improved by around 10 % when using treated reject water compared to untreated, supporting the idea that reject water treatment improves the coagulation.

II. High oxygen level in the reactors is essential for mixing process and to supply sufficient oxygen for the biological reactions. There is insufficient alkalinity level in the reject water to obtain complete ammonium removal. The pH variation is an indicator of reactor performance in terms of nitrification/denitrification process.

III. Higher OLR leads to higher organic removal and biomass production rates in the reactors. Lower HRTs to increase OLR will also reduce construction cost and capital investments for the implementation of biological reject water treatment. The optimum HRT appear to be < 4 h. Proposed approach may improve the discharge water quality due to less disturbance of coagulation process and may increase the biogas production potential.

7.2 Further works

- The simulations indicate shorter HRT is more efficient and reduce capital cost, therefore, it is recommended to run the new reactors in lower HRTs to prove the simulations results.

- The syringe test results show that the thickener and the centrifuge have high biogas potential and it is possible to use this capacity in order to produce biogas. It is recommended to evaluate biogas production of each stream in anaerobic digesters.

- Testing hybrid vertical anaerobic biofilm reactor (HyVAB) can be the way forward alternative to exploit the biogas potential of the reject waster as well as for nutrient removal such as nitrogen and phosphorus.
References


References


A. Zafarzadeh, B. Bina, M. Nikaeen, and H. M. Atta, Effect of dissolved oxygen and chemical oxygen demand to nitrogen ratios on the partial nitrification/denitrification process in moving bed biofilm reactors vol. 9, 2011.


E. Contreras, N. Bertola, L. Giannuzzi, and N. Zaritzky, A modified method to determine biomass concentration as COD in pure cultures and in activated sludge systems vol. 28, 2002.


References


Appendices

Appendix 1: Master thesis description
Appendix 2: WWTP Mass balance
Appendix 3: The granular sludge properties and syringe tests results
Appendix 4: Thickener and centrifuge characteristics
Appendix 5: NO\textsubscript{x} production and average ammonium removal
Appendix 6: Reactors weaknesses
Appendix 7: Jar test results
Appendix 8: The minimum required amount of oxygen for dominate nitrification in simulation and analysis of biomass recycling rate
Appendix 9: Correlations between different factors
Appendices

Appendix 1: Master thesis Description

FMH606 Master's Thesis

Title: Biological sludge reject water treatment by using moving bed biofilm reactor (MBBR)

USN supervisor: Rune Bakke, Carlos Dinamarco and Hildegunn H. Haugen

External partner: Knarrdalstrand WWTP, Biowater Technology AS

Task background: The purpose is to establish efficient removal of organic matter at wastewater treatment plants (WWTP) such as Knarrdalstrand WWTP, through biological treatment of reject water from the sludge dewatering processes before returning the reject water to the treatment plant inlet. This is a continuation of previous projects to gain deeper understanding and to obtain more data to estimate the overall potential of this concept. It is hypothesized that mainly four mechanisms will be involved:

1. Dissolved and colloidal organics in the reject water will be degraded (oxidized) in the introduced bio-process.

2. Dissolved and colloidal organics in the reject water will be converted into biomass through cell synthesis in the introduced bio-process and these cells will be removed by coagulation in the main treatment train.

3. The active biomass synthesized in the introduced bio-process will capture more dissolved organics and colloidal solids from the fresh wastewater, when introduced into the treatment plant inlet, all of which will be removed by coagulation in the main treatment train.

4. The biologically treated reject water will cause less disturbance on the main coagulation process than the untreated reject water does today, implying that the coagulation process can become more efficient.

Task description:

- Investigate the performance of pilot scale reactors, moving bed biofilm, as a biological reject water treatment at Knarrdalstrand WWTP.
- Generate relevant experimental data testing the hypothesized mechanisms.
- Investigate effect of different factors such as HRT, DO, pH, alkalinity and temperature in order to achieve optimum performance.
- Discuss effects on the plant overall performance.

Student category: EET student

Practical arrangements: Work will be carried out both at USN and at Knarrdalstrand WWTP.

Signatures:

Student (date and signature): | 2.5.2018 Sedehbemn Hashemi

Supervisor (date and signature): 2.5.2018 Carlos Dinamarco
Appendix 2: WWTP Mass balance

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_s$</td>
<td>27500 m³/day</td>
</tr>
<tr>
<td>$j_s$</td>
<td>27 kg/m³</td>
</tr>
<tr>
<td>TN$_s$</td>
<td>25 g/m³</td>
</tr>
<tr>
<td>AP$_s$</td>
<td>15 g/m³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{O_2}$</td>
<td>8740.976 mol/day</td>
</tr>
<tr>
<td>$O_2$</td>
<td>21571.2 g/day</td>
</tr>
<tr>
<td>$O_2$$_{removed}$</td>
<td>8628.45 g/day</td>
</tr>
<tr>
<td>$j_{O_2}$</td>
<td>96 g/m³</td>
</tr>
<tr>
<td>$j_{N_2}$</td>
<td>505.997 g/m³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{COD}$</td>
<td>430 g/m³</td>
</tr>
<tr>
<td>$j_{COD}$</td>
<td>1000 kg/m³</td>
</tr>
<tr>
<td>TN$_{COD}$</td>
<td>56.17 g/m³</td>
</tr>
<tr>
<td>AP$_{COD}$</td>
<td>24 g/m³</td>
</tr>
<tr>
<td>$j_{N_2}$</td>
<td>1000 kg/m³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{CO_2}$</td>
<td>145 m³/day</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>249.0 g/m³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{CO_2}$</td>
<td>126 m³/day</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>375.35 g/m³</td>
</tr>
<tr>
<td>$j_{PO_4}$</td>
<td>2860.6 g/m³</td>
</tr>
<tr>
<td>TN$_{PO_4}$</td>
<td>376.964 g/m³</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{Sludge}$</td>
<td>0.005 m³/day</td>
</tr>
<tr>
<td>$TN_{Sludge}$</td>
<td>273.354713 g/day</td>
</tr>
<tr>
<td>AP$_{Sludge}$</td>
<td>273.354713 g/day</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{Centrifuge}$</td>
<td>118.54 m³/day</td>
</tr>
<tr>
<td>$j_{COD}$</td>
<td>125 g/m³</td>
</tr>
<tr>
<td>$PO_4$</td>
<td>1000 kg/m³</td>
</tr>
<tr>
<td>$j_{N_2}$</td>
<td>16.11 g/m³</td>
</tr>
<tr>
<td>AP$_{N_2}$</td>
<td>3.4282 g/m³</td>
</tr>
</tbody>
</table>
Appendix 3: The granular sludge properties and Syringe test results

The reactor from which the sludge was taken, was a 4 m diameter 24 m high IR reactor (upflow: 8 m/h), at a (recycled) cardboard factory. OLR of about 0.5 – 0.6 kg COD per kg VSS per day. HRT between 6 – 10 hours COD in: 5.000 – 9.000 mg/l

After it came to our Lab. We measured some parameters as follow:

<table>
<thead>
<tr>
<th>Granular sludge</th>
<th>Density (g/cm3)</th>
<th>Diameter range</th>
<th>Settling velocity (m/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-convert</td>
<td>1.0 – 1.09</td>
<td>0.6 – 2.7</td>
<td>68 – 71</td>
</tr>
</tbody>
</table>

The characteristics of substrates used in syringe tests and the amount of biogas production for each substrate are presented in Table 1. The syringes that contained thickener and centrifuge produced 78 mL and 82.5 mL biogas, respectively. Syringes that were filled with outlet of the reactors had higher biogas production when compared to one was fed by the inlet of the reactors. For instance, the biogas production from the outlet of R₁ and R₂ were 27.5 mL and 48 mL, respectively, while the syringe contained the inlet samples produced only 24.5 mL. The thickener had very high total COD, but it also had significantly low soluble COD which was to 0.2 g/L. Hence, the thickener produced low amount of methane per COD i.e. 0.42 L CH₄/g COD. The amount of COD in R₂ was 0.61 g/L, which was low COD compared to the tested substrates nevertheless, methane production per COD in R₂ was the highest (i.e. 5.11 L CH₄/g COD). Methane production per COD for centrifuge and R₁ were 2.85 L CH₄/g COD and 2.38 L CH₄/g COD as shown in Table 1. Theoretically, the highest value for methane production per COD is 0.38 L CH₄/g COD while these values in this study are much higher than the theoretical value. These values may be led by some errors such as underestimation of COD of substrate.

Table 1: Amount of COD for different substrate as well as average biogas production in syringe test for each substrate. It is assumed that 65% percent of the biogas is methane and 5% of it is CO₂.

<table>
<thead>
<tr>
<th>Sample</th>
<th>COD₇ (g/L)</th>
<th>COD₅ (g/L)</th>
<th>Total biogas (mL)</th>
<th>L CH₄/g COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>3</td>
<td>0.71</td>
<td>24.5</td>
<td>0.53</td>
</tr>
<tr>
<td>Reactor 1</td>
<td>0.75</td>
<td>0.35</td>
<td>27.5</td>
<td>2.38</td>
</tr>
<tr>
<td>Reactor 2</td>
<td>0.61</td>
<td>0.29</td>
<td>48</td>
<td>5.11</td>
</tr>
<tr>
<td>Thickener</td>
<td>12</td>
<td>0.2</td>
<td>78</td>
<td>0.42</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>1.88</td>
<td>1.1</td>
<td>82.5</td>
<td>2.85</td>
</tr>
</tbody>
</table>
Figure 1 shows the accumulated biogas production in syringes from different substrates. The substrates from inlet and outlet of R₁ showed similar trend in terms of biogas production since both substrates produced the lowest amount of biogas at the end of the incubation period. However, thickener and centrifuge produced the highest amount of biogas at the end of experiment period whereas the biogas produced from outlet of R₂ was on average between these treatments.

In the first week of the incubation period, the thickener started by producing high amount of biogas and reached to 44 mL on day 7, while the other substrates were in lag phase. However, these treatments began to produce biogas in the second and third weeks. The syringes that contained centrifuge and outlet of R₂ produced more biogas up to 30.5 mL and 12 mL on day 7, respectively, whereas the accumulated biogas for R₁ and inlet in the same period were only 8 mL. However, in second week biogas production speeded up in all the syringes, especially in syringes that contained centrifuge. On day 13, accumulated biogas in the syringes that contained centrifuge and thickener reached to 59.5 mL and 53.5 mL, respectively. However, the accumulated amount of biogas in the syringes that had R₁ and inlet was less at the end of second week. Meanwhile, at the end of second week, biogas production in the syringes that contained R₂ increased to 25.5 mL. After 50 days of incubation period from the start of experiment, the biogas production from the centrifuge and thickener became stable at 82 mL and 78 mL, respectively. When the biogas production became stable, R₁ and R₂ produced 27.5 mL and 48 mL, respectively that were higher than biogas production in the syringes that contained inlet (24.5 mL).
Appendix 4: Thickener and centrifuge characteristics

Table 1: Thickener and Centrifuge tests

<table>
<thead>
<tr>
<th>project day</th>
<th>Thickener</th>
<th></th>
<th>Centrifuge</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tCOD (g/L)</td>
<td>sCOD (g/L)</td>
<td>NH4 (g/L)</td>
<td>tCOD (g/L)</td>
</tr>
<tr>
<td>8</td>
<td>2.93</td>
<td>1.39</td>
<td>0.38</td>
<td>2.40</td>
</tr>
<tr>
<td>15</td>
<td>1.24</td>
<td>0.77</td>
<td>0.12</td>
<td>3.02</td>
</tr>
<tr>
<td>25</td>
<td>0.94</td>
<td>0.59</td>
<td>0.15</td>
<td>2.64</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td>2.49</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>7.06</td>
<td>0.40</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>2.03</td>
<td>0.92</td>
<td>0.53</td>
<td>2.43</td>
</tr>
<tr>
<td>112</td>
<td></td>
<td></td>
<td></td>
<td>2.19</td>
</tr>
<tr>
<td>116</td>
<td>10.90</td>
<td>0.20</td>
<td>0.10</td>
<td>2.48</td>
</tr>
<tr>
<td>119</td>
<td>4.18</td>
<td>0.89</td>
<td>0.38</td>
<td>1.77</td>
</tr>
<tr>
<td>122</td>
<td>0.36</td>
<td>0.14</td>
<td>0.10</td>
<td>2.15</td>
</tr>
<tr>
<td>128</td>
<td>6.68</td>
<td>0.37</td>
<td>0.15</td>
<td>3.81</td>
</tr>
<tr>
<td>130</td>
<td>12.00</td>
<td>0.20</td>
<td>0.11</td>
<td>1.88</td>
</tr>
<tr>
<td>133</td>
<td>2.80</td>
<td>0.65</td>
<td>0.11</td>
<td>5.65</td>
</tr>
<tr>
<td>137</td>
<td>2.04</td>
<td>0.70</td>
<td>0.28</td>
<td>3.49</td>
</tr>
<tr>
<td>142</td>
<td>2.89</td>
<td>0.74</td>
<td>0.19</td>
<td>2.62</td>
</tr>
<tr>
<td>144</td>
<td>1.92</td>
<td>0.77</td>
<td>0.39</td>
<td>1.86</td>
</tr>
<tr>
<td>147</td>
<td>1.74</td>
<td>0.69</td>
<td>0.22</td>
<td>2.19</td>
</tr>
<tr>
<td>151</td>
<td>1.61</td>
<td>0.63</td>
<td>0.27</td>
<td>4.13</td>
</tr>
<tr>
<td>154</td>
<td>2.44</td>
<td>0.95</td>
<td>0.47</td>
<td>2.49</td>
</tr>
<tr>
<td>158</td>
<td>4.56</td>
<td>0.43</td>
<td>0.12</td>
<td>3.62</td>
</tr>
<tr>
<td>161</td>
<td>12.67</td>
<td>0.39</td>
<td>0.16</td>
<td>2.75</td>
</tr>
<tr>
<td>165</td>
<td>3.43</td>
<td>0.55</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2: COD concentration in the centrifuge flow

Figure 3: COD concentration in the thickener flow
Figure 4: Ammonium concentration in the thickener and the centrifuge flow
### Appendix 5: NO\textsubscript{x} production and average ammonium reduction

Table 2: Average ammonium reduction and NO\textsubscript{x} production in the reactors in stable and unstable condition

<table>
<thead>
<tr>
<th></th>
<th>Unstable condition</th>
<th>Stable condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{NH}_4 ) reduction R\textsubscript{1} (g/L)</td>
<td>( \text{NH}_4 ) reduction R\textsubscript{2} (g/L)</td>
</tr>
<tr>
<td>Average values</td>
<td>162</td>
<td>121</td>
</tr>
<tr>
<td>Standard deviations</td>
<td>( \pm 22 )</td>
<td>( \pm 67 )</td>
</tr>
<tr>
<td>Average values</td>
<td>84</td>
<td>87.5</td>
</tr>
<tr>
<td>Standard deviations</td>
<td>( \pm 78 )</td>
<td>( \pm 38.5 )</td>
</tr>
</tbody>
</table>
Appendix 6: Reactors weaknesses

Some weaknesses in the shape of the reactors were observed by author as follow:

- Lake of full use of entire volume of the reactors. By placing carrier in the central layer, a compact compartment is provided that led to low detachment rate and avoid freely movement of carrier. This design problem caused thick layer of biofilm covered the carriers' surface and affected ammonium removal efficiency.

- The gap between central layer and reactor bottom and also installing the aeration pipe in the bottom of central layer (instead of bottom of the reactor) provided dead zones in the bottom of reactors with high dense sludge and low oxygen level.

- Inappropriate circulation of water inside the reactors provided anoxic zones in the outer layer of reactors.

- Manual mixing provides better circulation of the oxygen inside the reactors, however based on the definition of MBBR the aeration flow is responsible for mixing in the reactor and typically there are no additional mixing devices in the MBBRs.

Nevertheless, reactors had acceptable performance after installation of mesh and manual mixing that compensated design weaknesses. Having anoxic zones in the reactor can be also one of the positive aspects of reactors because in these zones produced nitrite and nitrate converted to nitrogen gas.
Appendices

Appendix 7: Jar test results

In order to evaluate the effect of MBBRs on coagulation process, four jar tests were performed by using Kemira flockulator 2000. Two jars were filled with different samples in order to simulate and compare the current condition of the plant and the effect of the reactors. The samples were mixed with a proportion based on plant mass balance from Niazi and Hashemi (2017, unpublished student project) which shows in the Appendix 2 [14]. The Ferric III chloride used as coagulant was equal proportion that has been using by the treatment plant. Table 3 shows amount of raw wastewater and amount of inlet and outlet of the reactors in each jar test.

Table 3: Proportion of sample mixture in each jar.

<table>
<thead>
<tr>
<th>Jar number</th>
<th>Row wastewater (WW) (mL)</th>
<th>Untreated reject water (UR) (mL)</th>
<th>Treated reject water (TR) (mL)</th>
<th>Chemical FeCl₃ (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>989</td>
<td>11</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>989</td>
<td>-</td>
<td>11</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The jar test was conducted in three main steps. The first step was fast mixing where the stirrers were rotated at 200 RPM for 60 seconds just after adding the coagulant. The second step, which was called slow mixing, the jars were mixed at 50 RPM for 20 minutes and the final third steps were settling for one hour. The turbidity of the samples was measured using laboratory turbidity meter model 2100 N (Colorado, USA) calibrated with Gelex Secondary Standards.

Results from the jar tests are demonstrated in Figure 5 in terms of COD and turbidity removal in different COD concentrations of row wastewater (WW). The jars are filled based on the proportions described in In order to evaluate the effect of MBBRs on coagulation process, four jar tests were performed by using Kemira flockulator 2000. Two jars were filled with different samples in order to simulate and compare the current condition of the plant and the effect of the reactors. The samples were mixed with a proportion based on plant mass balance from Niazi and Hashemi (2017, unpublished student project) which shows in the Appendix 2 [14]. The Ferric III chloride used as coagulant was equal proportion that has been using by the treatment plant. Table 3 shows amount of raw wastewater and amount of inlet and outlet of the reactors in each jar test.

Results clearly show that by increasing COD concentration in wastewater, the COD removal efficiency improved in the jars including treated reject water (TR) or untreated reject water (UR). However, the turbidity removal was almost constant with an exception of first day. In the first day, the jar included untreated reject water removed 59% while the jar contained treated reject water was able to remove 70 % of COD in the wastewater. By increasing wastewater COD concentration to 195 g/L in 39th day, the COD concentration improved to 68.7 % and 78.5 % for jars including untreated and treated reject water, respectively. At the same day, more than 90 % of turbidity was removed in both jars. In 56th day, since wastewater COD concentration reduced to 150 g/L, the COD removal decreased to 57.6 % and 69 % for untreated and treated reject water, even though more than 95 % of turbidity removed in both jars.
In the last jar test, the highest COD removal took place when concentration of wastewater reached to 276 g/L, these values for untreated and treated reject water were 80 % and 82 %, respectively. Turbidity removal remained almost unchanged with a value of 95 % in both jars. It is clear from the results when the inlet COD concentration was between 10 g/L and 200 g/L the treated reject water performed better than untreated reject water in terms of COD removal by removing more COD.

Figure 5: COD removal and turbidity removal in jar tests by using a mixture of raw wastewater with treated reject water (TR) and untreated reject water (UR).
Appendix 8: The minimum required amount of oxygen for dominate nitrification in simulation and analysis of biomass recycling rate

Effect of DO on ammonium removal (HCO_3= 70mmol/L)
Figure 6 provide effects of different biomass recycle rate on removal efficiency in terms of total COD and ammonium removal together with concentration of biomass in the reactor. In this condition the HRT was set to 24 h and the DO level adjusted on 7.5 for each run and alkalinity level was 70 mmol HCO$_3$-/L. As shown in Figure 6 the concentration of heterotroph and autotroph bacteria reduced by decreasing recycle rate. Concentration of autotroph and heterotroph organics were 0.8 g/L and 7.7g/L, respectively, when recycle rate was 0.99. These values dropped to zero and 0.7g/L for autotroph and heterotroph bacteria, respectively in recycle rate of 0.1. Concentration of autotroph bacteria was 0.05 g/L when recycle rate was 0.2. The COD removal efficiency was 0.31 ±0.03 in the conditions when recycle rate was higher than 0.9 while in the similar condition ammonium removal efficiency were 1. The COD removal dropped to 0.25 by decreasing recycle rate to 0.7 but the ammonium removal was
remained the same. The COD removal for recycling rate of 0.2 and 0.1 declined to around 0.14 while the ammonium removal for these conditions were 0.9 and zero.

Figure 6: Effect of different biomass recycle rate on performance of the reactors in terms of COD removal and ammonium removal when the HRT is 24 h and alkalinity level set to 70mmol HCO$_3$/L. The bars presenting the biomass concentration (left axis) and the lines show removal efficiency in terms of ammonium and total COD removal (right axis).

Biomass recycling rate in ASM1 is an interpretation of sludge retention time (SRT) [44]. In an ideal MBBR biomass recycle rate more than 0.95 is expected. As shown in Figure 6 in lower biomass recycling rate (i.e. between 0.9 and 0.7) the removal efficiency of the reactors will not change a lot. It may indicate that lower filling ratio of carriers can be sufficient if HRT is long enough, but high amount of biofilm mass is required at low HRT.
Appendix 9: Correlations between different factors

COD/N ratio: The effect of inlet COD/N ratio on nitrite and nitrate concentration (NO$_x$ production) in stable condition in R$_1$ and R$_2$ is shown in Figure 7. In 39$^{th}$ day, NO$_x$ concentration for R$_1$ and R$_2$ were 0.02 g/L and zero, respectively, while the COD/N ratio was 8 gCOD/gNH$_4$-N that was highest ratio in stable condition. NO$_x$ concentration in R$_1$ reached a peak of 0.28 g/L at day 42 when COD/N ratio dropped to 3.5 gCOD/gNH$_4$-N and this value rise to 0.07 g/L for R$_2$. Afterwards, variation in COD/N became smoother so that, the average COD/N ratio was 4.3±0.06 gCOD/gNH$_4$-N in the following days. Between 46$^{th}$ and 60$^{th}$ day of experiment, the average NO$_x$ production for R$_1$ and R$_2$ was 0.06±0.02 g/L and 0.06±0.03 g/L, respectively. It is clear from Figure 7 that the NO$_x$ concentration declined by increasing COD/N ratio. However, between 46$^{th}$ and 60$^{th}$ day, NO$_x$ concentration fluctuated less in both reactors due to lower variations in COD/N ratio.

![Figure 7: Amount of COD/NH$_4$-N and NO$_x$ production in R$_1$ and R$_2$](image)

![Figure 8: Relation between biomass COD and VSS in the outlet of R$_1$](image)
Figure 9: Relation between biomass COD and VSS in the outlet of $R_2$