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Enhanced Digestate Nitrification in Sequencing Batch Reactors (SBR)

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Summary:

The liquid fraction of digestates from anaerobic digestion contains a high concentration of Ammonium, which can be transformed into liquid "organic fertilizer". Direct application of these digestates as fertilizer would lead to a significant nitrogen loss as ammonia. Therefore, it is necessary to convert it to a stable form of nitrogen for nitrate capture and slow release in biochar (i.e., nitrate impregnated biochar) that could be accepted commercially. Hence, nitrification of reject water was proposed for making the product stable. Two lab-scale parallel nitrification reactors were set up as sequential batch reactors (SBR). The main aim of this thesis is to achieve complete nitrification by tuning the operating parameters of SBR and finding optimum conditions. The study was performed using reject water (having 500 mg/L NH_4-N) as feed obtained after dewatering of digested sludge from the Knarrdalstrand Wastewater Treatment Plant in Norway. Along with the reject water, synthetic feed was also used.

A series of laboratory analyses were performed for determining the concentration of Ammonium, Nitrite, Nitrate, organic matter (as TCOD, SCOD), and alkalinity. Moreover, analysis for measuring solids as TS, VS, TSS, VSS was carried out. In addition, the measurement of pH and dissolved oxygen were also carried out daily during the study period. Different operating conditions were tested by tuning one parameter at a time for achieving stable nitrification. The study is complemented with a continuous study of literature review.

During the early stage of the project, 47% of Ammonium was converted into Nitrite and only 11% of Ammonium was converted to Nitrate. However, after the addition of sufficient alkalinity, 98% of Ammonium was removed of which 80% was converted to Nitrite and only 18% was converted to Nitrate. Hence, alkalinity was one of the limiting factors for nitrification in this study. After alkalinity change, increasing the HRT from 1.67 days to 3.34 days by lowering the nitrogen loading rate from 0.3 kg/m^3day to 0.14 kg/m^3day helped to achieve complete nitrification. Moreover, changing the feeding sequence from two to one time a day helped to give enough contact time for bacterial biofilms and wastewater. Hence, HRT, nitrogen loading rate, and feeding sequence played a significant role and can be considered as important operating parameters for nitrification.

It is concluded that stable nitrification can be achieved using sequential batch reactors. Moreover, through nitrification, the liquid part of effluents from anaerobic digestors that treat municipal organic wet wastes can be successfully transformed into a high-quality liquid organic fertilizer that can be impregnated in biochar – as slow releasing and commercially acceptable fertilizer.

Preface

This master thesis study has been performed as part of a master's degree program in Energy and Environmental Technology at the University of South-Eastern Norway. This is an ongoing project initiated by the Environmental Biotechnology Research Group in collaboration with Standard Bio AS to investigate the suitability and performance of sequential batch reactors for the complete and stable nitrification of reject water from anaerobically digested sludge. SBRs were tuned for the purpose of nitrification by changing the operating parameters based on the analysis of experimental data through which optimum condition was accomplished.

I would like to show my deepest gratitude to researcher Dr. Eshetu Janka Wakjera for providing me continuous support during the operation of reactors, helping me to tackle the process challenges, giving beneficial suggestions, guidance and feedback throughout the project.

I would also like to express my sincere appreciation to Associate Professor Carlos Dinamarca for his important guidance, technical support, dedication, commitment, and feedback which helped me to finalize the project successfully. I am so thankful to senior laboratory engineer Hildegunn H. Haugen for providing valuable information about safety job analysis, laboratory activities, and important feedback. I would also like to pay my special regards to external partner Standard Bio As for their support and funding to run the project.

I wish to acknowledge the great support and love of my family, and friends during this difficult period of the COVID-19 situation which helped me to keep going and this project work would not have been accomplished without their input.

Porsgrunn, 14-05-2021

Sandeep Gyawali

Contents

Nomenclature

AD	Anaerobic Digestion
AGS	Aerobic Granular Sludge
Anammox	Anaerobic Ammonium Oxidation
AOB	Ammonia Oxidizing Bacteria
BOD	Biochemical Oxygen Demand
C/N	Carbon to Nitrogen Ratio
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substances
FA	Free Ammonia
F/M	Food to Microorganisms Ratio
FNA	Free Nitrous Acid
GAOs	Glycogen Accumulating Organisms
HRT	Hydraulic Retention Time
KRA	Knarrdalstrand Renseanlegg/ Wastewater Treatment Plant
NLR	Nitrogen Loading rate
NOB	Nitrite Oxidizing Bacteria
OCR	Oxygen Consumption Rate
OTR	Oxygen Transfer Rate
PAOs	Polyphosphate Accumulating Organisms
RAS	Return Activated Sludge
SBR	Sequential Batch Reactor
SCOD	Soluble Chemical Oxygen Demand
TCOD	Total Chemical Oxygen Demand
TS	Total solids
TSS	Total Suspended Solids
UAE	United Arab Emirates
VS	Volatile Solids
VSS	Volatile Suspended Solids

1 Introduction

Nutrient mishandling is becoming a significant threat to environmental changes due to the redundant accumulation of nitrogen in water and soil. Various nitrogenous compounds that usually come from untreated nutrient-rich organic waste harm the environment. Eutrophication of surface water bodies and high level of ammonia toxicity for aquatic life are the main encountered problems when these untreated nutrients release open habitats. This thesis discusses the complete nitrification of the nutrient-rich liquid fraction of anaerobic digestion called reject water obtained from dewatering of anaerobically digested sludge [1].

The digestate from the anaerobic digestion (AD) running on municipal organic wastes comparatively contains a large amount of Ammonium. Moreover, other organic nitrogenous compounds such as urea and amino acids are also converted to ammonia in AD. Due to the high nitrogen content of the digestate, it is also applied directly as a fertilizer but it is controlled in many countries because of strict rules and regulations on toxic contaminants. Besides, it is not economically feasible as the Ammonium present in the digestate is unstable above neutral pH values, leading to ammonia gas release, reducing the overall fertilizer quality and creating air pollution [1]. The use of untreated digestate, coming from AD, can result in 70% of nitrogen release as NH_3 in the environment [2]. Hence, Nitrification of digestate can be a reasonable measure for stabilizing the nitrogen and making digestate suitable for effective commercial fertilizer [1].

Nitrification is a biological method for wastewater treatment that contains a two-step biochemical aerobic process. In the first step, ammonium is converted into nitrite by a group of bacteria called "*Ammonia Oxidizing Bacteria*" (AOB) and in the second step, nitrite is further converted to nitrate by a species of bacteria known as "*Nitrite Oxidizing Bacteria*" (NOB). The overall nitrification reaction can be represented by equation 1.1. Nitrate is the most stable form in the soil and is an extremely flexible nutrient source for plants [1]. The reject water used for this thesis is from the Knarrdalstrand Wastewater Treatment Plant (KRA) which contains around 520 (±50) mg/L of ammonium nitrogen. Therefore, two lab-scale parallel nitrification reactors were set up as sequential batch reactors (SBR) to investigate the operating parameters of SBR for complete nitrification of reject water.

$$NH_4^+ + 1.863O_2 + 0.098CO_2 \rightarrow 0.0196C_5H_7NO_2 + 0.98NO_3^- + 0.0941H_2O + 1.98H^+ \quad (1.1)$$

1.1 Problem description

Digestate used directly as a fertilizer harms the environment and the quality of the fertilizer will not be good enough. The nitrogen present in the digestate is unstable, resulting in the loss of nitrogen as NH_3 in the environment. Therefore, it is necessary to treat the reject water from anaerobic digestion containing a high amount of Ammonium. The main challenge is to convert Ammonium to Nitrate by tuning the SBR with suitable operating conditions.

1.2 Aim and objectives

This thesis main objective is to study sequencing batch reactor (SBR) conditions that can promote desired nitrogen transformation mainly from Ammonium, based on changes in process parameters such as loading rates, sequences, and aeration strength. Specific objectives are outlined as follows:

- Operate existing SBRs to monitor the effects of changes in physical conditions.
- Tuning the reactors to establish process limitations and safe operating ranges.
- Analyzing and collecting relevant experimental data for this research and finding out the operational variables required for full-scale industrial applications.
- Literature review about aerobic granular sludge as well as studying it through microscopic investigation.

However, the focus will be on enhancing nitrification i.e., converting Ammonium into Nitrate as well as investigating the effect of process parameters such as pH, alkalinity, dissolved oxygen (DO), hydraulic retention time (HRT), and loading rates.

1.3 Structure of the report

There are seven main chapters in this study. The first chapter gives an overview of the introduction, objective, and problem description to have background information and a proper understanding of the topic. The second chapter includes the literature review and theory related to biological processes, reactor operating principle, nitrification, and parameters affecting nitrification. The third chapter is about the material and methods used in this study. Reactor setup, operating conditions, and analysis procedures are included in the third chapter. The fourth chapter presents the results obtained during this study with proper graphs. The fifth chapter is the discussion which explains and discusses the result obtained with proper arguments. The sixth chapter gives a short overall conclusion and findings during this study. The seventh chapter presents the recommendation for future on making the reactor more efficient and economical.

2 Literature review

This topic includes detailed knowledge about biological processes for wastewater treatment, use of sequential batch reactor (SBR), nitrification, denitrification, and oxygen transfer theory. Moreover, this chapter covers information about aerobic granular sludge, and impregnating nitrate into biochar.

2.1 Biological processes for wastewater treatment

Biological treatment is a natural process to break down the organic waste as well as to remove nutrients like nitrogen, phosphorous, etc. using different types of microorganisms (bacteria, nematodes, algae, fungi, etc.). The two main predominant biological processes used for wastewater treatment are: *attached growth (or biofilm)* process and *suspended growth* process [3].

2.1.1 Attached growth process

In this process, microorganisms are stick to an inert packing material such as rock, slag, gravel, sand, redwood, and a wide range of plastic and other synthetic materials [3]. Material selection is essential for ensuring the high amount of active biomass and a diverse microbial population because packing material provides a broad surface area per unit volume for the growth of biofilm. Polymers are widely used as packaging material for attached growth process because of their low cost, lightweight, adaptability to various shapes and sizes, and relatively large surface area [4]. In this process, organic material and nutrients are removed by passing the wastewater flow through the attached growth also known as a biofilm [3]. A biofilm is a highly moisturized biological structure attached to a packing material, which consists of microorganisms, extracellular polymeric substances generated by them, as well as abiotic particles stored from the liquid medium and integrated into the film [5].

Attached growth process have many advantages like higher biomass concentration in the aeration tank resulting in less biomass waste and high removal rates at relatively small hydraulic retention times. This process also reduces the lengthy sludge settling periods. Moreover, there is metabolic coexistence between aerobic and anoxic activity within the same biomass ecosystem [6]. The attached growth process can also be used as an aerobic or anaerobic process. The most common example of the attached growth process is a trickling filter [3].

A trickling filter is an aerobic (mostly) attached growth process that generally consists of a fixed bed (sand, gravel, rock, wide range of plastic, etc.). Wastewater (to be treated) flows downward over the packed medium where microorganisms attached to the medium, as a biofilm, removes the organic material and nutrients present in the wastewater. As the biofilm layer thickens, oxygen cannot pass through the medium, and anaerobic organisms grow due to the absence of oxygen. Further growth of the biofilm layer reduces the microorganism's ability to hold on to the medium; hence, a portion of the biofilm layer falls off the filter. This process is known as sloughing. The sloughed solids are then transferred to the clarifier through the under-drained system to retain the biomass [7].

2.1.2 Suspended growth process

Suspended growth process are designed to promote the growth of specific microorganisms that are capable of carrying out the reactions required to achieve the desired transformation of influent wastewater [8]. In the suspended growth process, microorganisms and the bacteria treating wastes are suspended within the liquid (being treated) with the help of pneumatic aeration or mechanical agitation. Microorganisms in this type of process form floc particles between 50 and 200 µm in diameter. During mixing, these flocs move through the liquid removing organic material and nutrients present in the wastewater. So, in this process, both the microorganisms and wastewater are in motion [9].

Suspended growth process have many benefits like increased active microbial mass per unit volume, minimized suspended solids loading to the clarifier, enhanced capacity for nitrification, better sludge settling characteristics, flexible to various influent conditions (shock load) [8]. It can be used as aerobic or anaerobic process .The activated sludge process and SBR are common examples of an aerobic suspended growth process [9].

The activated sludge process is mostly used for the treatment of municipal wastewater. A conventional activated sludge process generally consists of three parts: an aeration tank, a secondary clarifier, and a recycling system. In an aeration tank, influent wastewater is kept in an aerated and well-mixed environment, where microorganisms responsible for organic matter decomposition are maintained in a suspension. Microorganisms balance the organic matter in the aeration tank during aeration. The effluent from the aeration tank is directed to the secondary clarifier where the resulting biomass can settle and separate from the liquid, which is the primary mechanism for removing biochemical oxygen demand (BOD) in the activated sludge process. A part of the sludge settled in the secondary clarifier is returned back to the aeration tank and is referred as return activated sludge (RAS). The excess biomass at the bottom of the secondary clarifier is removed for further treatment and successive disposal. A typical activated sludge process diagram is shown in Figure 2.1 [10].

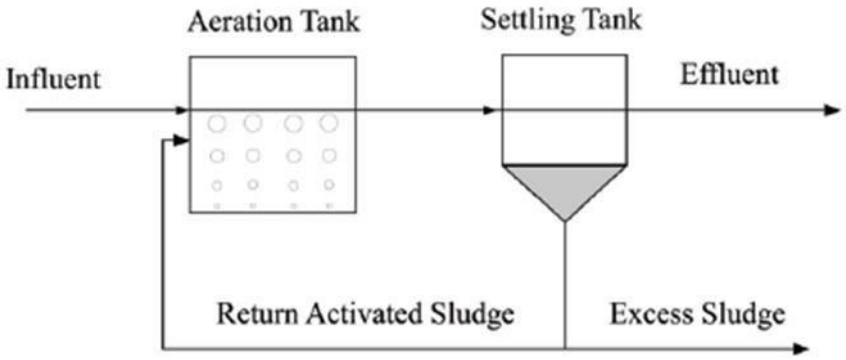

Figure 2.1: Layout of a conventional activated sludge system [11].

2.2 Sequential batch reactor (SBR)

The sequential batch reactor technology is the modification of the traditional activated sludge process which operates in time rather than space. SBR is a fill and draw type reactor. The term SBR was originally created by R.L. Irvine [12]. It was famous during 1914-1920 but the concern in SBR in its present form took place during the late 1950s and early 1960s due to the advancement in aeration and process control technology. It is generally used for the municipal sewage treatment but due to the better control in its process as well as flexibility in design, SBR has also established a broad range of acceptance in the biological treatment of industrial wastewater containing complex organic chemicals [12].

SBR performs equalization, neutralization, biological treatments, primary clarification, secondary clarification in a single tank following a timed control sequence. SBR can be established in a small space as compared to any other aeration plants of same capacity. Moreover, nutrient removal can be achieved by operational changes with high effluent quality. It can also handle the shock loads. There are generally five basic operating modes in SBR-1) Fill, 2) React, 3) Settle, 4) Decant, and 5) Idle [13].

1. ***Fill***: In this mode, the feed liquid generally reject water is given to the reactor either through the pump or by gravity or can be done manually. The volume of influent depends on the reactor volume. There are three variations in the fill step: i) static fill, ii) mixed fill, and iii) aerated fill. Static fill is governed by no mixing or no aeration which means there will be high substrate concentration when aeration starts or mixing begins. A high food to microorganisms (F/M) ratio is favorable for floc forming organisms as compared to filamentous organisms, providing better settling characteristics for biosolids. Mixed fill is characterized by mixing influent organics with biomass. Mixed fill is used to create an anoxic zone suitable for denitrification. In mixed fill, bacteria utilize alternate electron acceptors (such as nitrate-nitrogen) as residual oxygen to degrade the organics biologically. Aerated fill is defined by aerating the contents of the reactor to start the aerobic reactions subsequently thus reducing the react phase time [13].

2. ***React***: In this phase, aeration is provided by aerators/blowers using a flow meter to supply sufficient dissolved oxygen (DO) and mixing to the filled reject water. The time allocated for this phase can be as high as 50% or more of the total time cycle. There are two modes namely mixed react and aerated react. In aerated react, aerobic reaction takes place achieving complete Nitrification whereas, in mixed react, anoxic conditions can be maintained to achieve Denitrification [13].

3. ***Settle***: In this step, the separation of solids takes place under no inflow/outflow conditions. Sludge starts to settle as a flocculent mass, creating a distinctive interface leaving a clear supernatant. It generally covers 20-25% of the total cycle time [13].

4. ***Decant***: This step is used to draw the supernatant effluent from the reactor which is also use as a sample for the chemical analysis. The supernatant can be drawn by using decanters as well as manually by the skilled man-power known as operators. The time given to this step is generally 15% of the total cycle time [13].

5. ***Idle***: It is the period between draw and fill. It is mainly allocated for sludge wasting but in SBR, sludge wasting is done once every 2 to 3 months. It generally covers 5% of the total cycle time [13].

The schematic diagram showing the basic operating principle in SBR is represented by Figure 2.2.

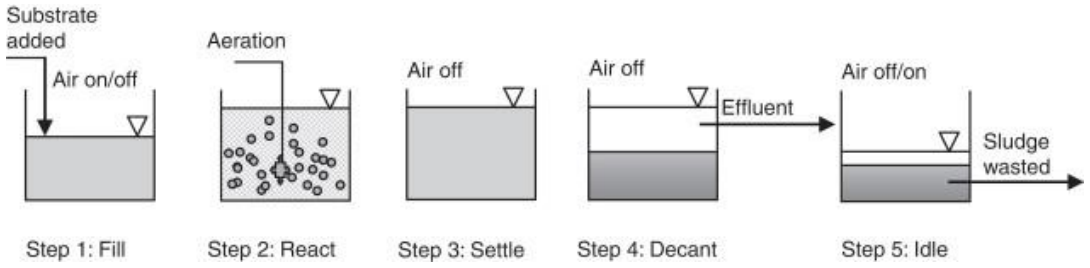

Figure 2.2: SBR operation for a single tank for one cycle for the five different time periods of Fill, React, Settle, Draw, and Idle [10].

However, it is very difficult to operate in SBR system as it includes automatic valves, automatic switches, and instrumentation. These types of controls in larger system are very sophisticated. Higher level of sophistication increases the risk to fail or may require frequent maintenance. Therefore, in U.S, most of the SBR installations used for wastewater treatment are smaller and can treat below two million gallons per day (MGD). But larger SBR systems are also exist, the largest SBR system which can treat ten million gallons per day (MGD) is in United Arab Emirates (UAE) [14].

2.3 Nitrification

Nitrification is the biological oxidation of Ammonia (NH_3) or Ammonium (NH_4^+) into Nitrite and then into nitrate through two steps using ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in the presence of oxygen [15]. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria are chemoautotrophs as they find their carbon source from CO_2 and oxidize inorganic compounds using dissolved oxygen to obtain cell energy [3]. Five AOB genera have been recognized and classified into proteobacteria class in which four of them lies in the *β-Proteobacteria* subclass including *Nitrosomonas* (including *Nitrosococcus mobilis*), *Nitrosospira*, *Nitrosovibrio,* and *Nitrosolobus*, whereas one cluster of *Nitrosococcus* belongs within the *γ-Proteobacteria* subclass [16]. The NOB phylogeny has more variety with four genera in three Proteobacteria groups. *Nitrobacters* are within *α-Proteobacteria*, *Nitrococcus* within *γ-Proteobacteria*, and *Nitrospina* and *Nitrospira* within the *δ-Proteobacteria*. The two-step nitrification reaction is represented by equation 2.1 and equation 2.2, whereas the total ammonium oxidation reaction is represented by equation 2.3 [3].

$$2NH_4^+ + 3O_2 \xrightarrow{AOB} 2NO_2^- + 4H^+ + 2H_2O \tag{2.1}$$

$$2NO_2^- + O_2 \xrightarrow{NOB} 2NO_3^- \tag{2.2}$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (2.3)$$

The growth balance between AOB and NOB plays a key role in optimizing a nitrifying community because of their sequential oxidation property. If ammonia-oxidizing rate is higher than nitrite-oxidizing rate due to rapid growth of AOB as compared to NOB then nitrite as an intermediate will be easily accumulated in the reactor. Moreover, the accumulated nitrite will be converted to nitrous oxide under anoxic condition by *Nitrosomonas*, which is a lethal greenhouse gas causing ozone layer depletion. Therefore, to optimize and improve the nitrification process, it is very important to know the population and interlinkage of AOB and NOB in the nitrifying group in biological nutrient removal treatment plants [17].

2.4 Factors affecting Nitrification

The rate of nitrification process depends on the activity of nitrifying bacteria, environmental factors, and operating parameters like pH, dissolved oxygen (DO), hydraulic retention time (HRT), alkalinity, temperature, carbon to nitrogen (C/N) ratio.

2.4.1 pH

pH is the most sensitive parameter for nitrification because both *Nitrosomonas* (i.e. AOB) and *Nitrobacters* (i.e. NOB) are susceptible to their own unionized ammonia and nitrite substrates, and the unionized-ionized equilibria depend on pH [18]. The optimal pH range for *Nitrosomonas* is approximately between 7.0 and 8.0 whereas, *Nitrobacter* has an optimal pH range of approximately 7.5 to 8.0 [19]. As the nitrification starts, ammonium ion is oxidized to nitrite, which releases hydrogen ion causing decrease in pH to an extent related to the buffering capacity of the system. This nitrite formed will be in equilibrium with unionized nitrous acid (FNA) which is represented by the equation 2.4. Further decrease in pH increases the concentration of free nitrous acid which will directly impact the performance of both *Nitrosomonas* and *Nitrobacters.* Free nitrous acid (FNA) concentration ranging from 0.22 to 2.8 mg/l strongly inhibits the nitrifying organisms. FNA can be calculated using the equation 2.5 and equation 2.6 [20].

$$H^+ + NO_2^- \rightleftharpoons HNO_2 \quad (2.4)$$

$$FNA\ (mg/l) = \frac{46}{14} \times \frac{NO_2^-\text{-}N\ (mg/l)}{k_a \times 10^{pH}} \quad (2.5)$$

$$k_a = e^{(-2300/273+℃)} \quad (2.6)$$

K_a represents the ionization constant of the nitrous acid equilibrium equation 2.4 [20].

Furthermore, higher pH above the certain optimum range also affects the nitrification rate. The concentration of the un-ionized ammonia will increase with increase in pH which can also be represented by the equation 2.7. The un-ionized ammonia also known as free ammonia (FA) can inhibit both *Nitrosomonas* and *Nitrobacter*. However, *Nitrobacter* is more vulnerable to free ammonia than *Nitrosomonas*. Therefore, at lower concentration of free ammonia, only *Nitrobacters* may be inhibited resulting in accumulation of nitrite. The free ammonia (FA)

concentration that inhibits *Nitrosomonas* ranges approximately between 10 to 150 mg/l whereas, for *Nitrobacters* the inhibitory value of free ammonia concentration ranges approximately between 0.1 to 1 mg/l. FA can be calculated using equation 2.8 and 2.9 [20].

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O \tag{2.7}$$

$$FA\ (mg/l) = \frac{17}{14} \times \frac{NH_4^+\text{-}N\ (mg/l) \times 10^{pH}}{\frac{k_b}{k_w} + 10^{pH}} \tag{2.8}$$

$$\frac{k_b}{k_w} = e^{(6344/273+℃)} \tag{2.9}$$

K_b represents the ionization constant of the ammonium equilibrium equation 2.7 and k_w represents the ionization constant of water [20].

2.4.2 Dissolved Oxygen (DO)

In terms of wastewater, DO is the measure of amount of oxygen available to microorganisms responsible for the inorganic nitrogen conversion present in the wastewater. For the biological oxidation of ammonia to nitrite and then to nitrate, nitrifying microorganisms consumes dissolved oxygen. Based on the equation 2.1 and equation 2.2, it is supposed that 3.43 mg of oxygen is required for the oxidation of 1 mg of NH_4-N to NO_2-N, and only 1.14 mg of oxygen is utilize to oxidize 1 mg of NO_2-N to NO_3-N [21]. The optimum dissolved oxygen concentration for complete nitrification is 4 mg/l and the nitrification process ceases when dissolved oxygen concentration is below 0.2 mg/l. The concentration of dissolved oxygen above 1 mg/l does not influence the growth rate of *Nitrosomonas* whereas, the growth rate of *Nitrobacters* is independent when dissolved oxygen is more than 2 mg/l [22]. Therefore, when the dissolved oxygen is low, ammonia oxidation rate is higher than the nitrite oxidation rate resulting in accumulation of nitrite [23]. However, NOB bacteria like *Nitrospira* can survive even in low DO (~0.5 mg/l) conditions. Under long-term low DO conditions, *Nitrospira* (NOB) has higher oxygen affinity which makes them better competitor for oxygen than other AOBs [24].

Furthermore, the dissolved oxygen profile in SBR can be controlled based on the balance between the oxygen consumption rate (OCR) and the oxygen transfer rate (OTR) from air to liquid. The volumetric mass-transfer coefficient depends on the airflow to the SBR, so controlling the airflow rate can maintain the balance between OCR and OTR. The OCR is the change in dissolved oxygen concentration with change in time. It is normally expressed as mg O_2/L/hr whereas the OTR can be described by the equation 2.10 [23].

$$OTR = k_l a(c_s - c) \tag{2.10}$$

Where, $k_l a$= overall oxygen transfer coefficient, c_s= saturation concentration of dissolved oxygen at the specified temperature and salinity, and c=dissolved oxygen concentration in the bulk liquid [25].

2.4.3 Alkalinity

Alkalinity in wastewater is defined as the ability of water to neutralize the hydrogen ions produced during the oxidation of ammonium ion to nitrate ion. Alkalinity is also called as buffering capacity of water. It plays a significant role to maintain a toxic-free environment suitable for the nitrification process because nitrifying bacteria are sensitive to toxic environments. 7.14 g of alkalinity (as $CaCO_3$ equivalent i.e., $\frac{2}{14}$ x (50 g $CaCO_3$/eq)) is consumed for the oxidation of 1 g of ammonium nitrogen into nitrate nitrogen in a closed nitrification system. As the ammonium oxidation begins, there will be a release of hydrogen ions resulting in a decrease pH not suitable for nitrification (as given in equation 2.3). Therefore, there should be enough alkalinity in the wastewater to counteract the hydrogen ion and maintain the pH (7.5-8) favorable for complete nitrification. Equation 2.11 shows the stoichiometry of alkalinity requirement in nitrification process [26] [27].

$$NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O \qquad (2.11)$$

However, the larger amount of alkalinity also affects the nitrification process. pH and alkalinity are different but closely related parameters. Water with high alkalinity will always have high pH but water with high pH may not always have high alkalinity. Therefore, if alkalinity is added more than enough it will raise the pH, as a result, the ammonium nitrogen will be converted to free ammonia. Free ammonia inhibits the performance of both AOB and NOB bacteria. AOB can survive for a wide range of free ammonia whereas NOB are very sensitive to free ammonia. Due to this reason, accumulation of nitrite occurs in many nitrification reactors [27].

2.4.4 Temperature

The effect of temperature on oxygen transfer rate is very important for determining the overall efficiency of a biological treatment process [3]. The solubility of oxygen is lower at a high temperature which means that hot water surface needs less dissolved oxygen to reach saturation point than cooler water. Moreover, oxygen transfer rate (OTR) becomes low at high temperature due to smaller driving force (C_s-C) ,which can also be understood by equation 2.10 [25].

However, higher temperature increases the diffusion rate of oxygen and at the same time decreases the liquid viscosity and surface tension. These effects tend to increase the $k_l a$ value which might compensate the smaller driving force, as a result, increasing the overall OTR slightly. $k_l a$ value at any temperature can be determined by using the equation 2.12 [25].

$$k_l a(T) = k_l a_{20} \theta^{T-20} \qquad (2.12)$$

Where, $k_l a(T)$= overall oxygen transfer coefficient at temperature (T), $k_l a_{20}$= overall oxygen transfer coefficient at 20℃, T is the temperature and θ= theta factor. Under well-defined experimental conditions, very different values for this factor were found [25]. Reported values of θ are in the range of 1.015 to 1.040 [3].

On the other hand, temperature also affects microbial nitrification activity. The nitrification activity increases with an increase in temperature but under a certain limit. Because high

temperature increases the level of free ammonia as free ammonia is related to the temperature which can be seen through equations 2.8 and 2.9. At the lower temperature of wastewater like below 10℃, nitrification activity is limited [28]. The optimum temperature for nitrification is in the range 28℃ and 36℃ [18].

2.4.5 Hydraulic retention time (HRT)

Hydraulic retention time (HRT) is the average amount of time holding the wastewater inside the biological reactor. Mathematically, it is defined as the ratio of total working volume (V) of the reactor to the feeding/discharge rate (Q) as shown in the equation 2.13 [3].

$$HRT = \frac{V}{Q} \tag{2.13}$$

HRT affects the population dynamics of nitrifying organisms and their performance. The contact time between microorganisms and wastewater becomes less in shorter HRT, which results in lower nitrification efficiency. The growth rate of nitrifying organisms is slow hence, in shorter HRT the proportion of nitrifying organisms will be low in the reactor. Therefore, at the startup of process, longer HRT is preferred to grow the nitrifying bacteria good enough for nitrogen transformation [29]. Moreover, shorter HRT leads to the high nitrogen loading rate, which causes overload to the reactor. High nitrogen loading rate also causes partial nitrification resulting in nitrite accumulation. As AOB oxidizes large amount of ammonium ion to nitrite, the pH of wastewater decreases, as a result, free nitrous acid (FNA) will produce. FNA inhibits the *Nitrobacters*. However, after sufficient growth of nitrifying microorganisms, HRT can be optimized by monitoring the effluent concentration of nitrification reactor [20].

2.4.6 Carbon to nitrogen (C/N) ratio

Carbon to nitrogen (C/N) ratio plays a vital role in controlling the population of heterotrophs bacteria and autotrophs bacteria. It determines which bacteria plays a dominant role on inhibiting the other one. Heterotrophs bacteria survives on organic carbon which is generally measured in terms of COD in wastewater. Autotrophs bacteria used bicarbonate as an inorganic carbon source. If the amount of organic carbon in wastewater is much higher than inorganic nitrogen concentration, then heterotrophs bacteria will easily grow and can suppressed the nitrifying bacteria resulting in poor nitrification rate [30]. Moreover, under aerobic conditions, heterotrophic bacteria utilize large amount of organic carbon and dissolved oxygen for their metabolism. Hence, heterotrophs and autotrophs bacteria compete for the dissolved oxygen and space. Therefore, high C/N ratio favors the heterotroph bacteria [31]. For the complete and efficient nitrification, it is suggested that the C/N ratio should be less than 0.25 [30]. In some research paper, it is found that the ammonium oxidation time increased with increase in C/N ratio [32].Feeding strategy also helps in the sludge control. Low C/N feed provides limited amount of nutrients for the growth of microorganisms which can result in less sludge production. An autotrophic community produced growth energy by oxidizing ammonium/nitrate, as a result, fine micro-colony structure will develop in bioreactors [31].

2.5 Denitrification

Denitrification is a biological process of removing nitrogen from wastewater. It reduces the nitrate produced during nitrification to nitrogen gas. A broad range of heterotrophic microorganisms can do denitrification. Some of their genera are *Acinetobacter*, *Agrobacterium*, *Achromobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, *Corynebacterium*, *chromobacterium*, *Flavobacterium*, *Halobacterium*, *Hypomicrobium*, *Moraxella*, *Methanomonas*, *Neisseria*, *Paracoccus*, *Pseudomonas*, *Propionibacterium*, *Rhodopseudomonas*, *Rhizobium*, *Spirillum*, and *Vibrio* [3]. Many of them are facultative aerobic microorganisms with the capacity to consume oxygen as well as nitrite or nitrate. In this process, soluble organic substrates present in wastewater are biologically oxidized using nitrite/nitrate as the electron acceptor rather than oxygen. In an oxidation-reduction reaction, the organic substrate act as an electron donor and nitrite/nitrate as an electron acceptor. The oxidation-reduction stoichiometry for wastewater is represented by equation 2.14 [3].

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \qquad (2.14)$$

In the above denitrification reaction, it is seen that one equivalent of NO_3-N reduction produces one equivalent of alkalinity, which means 3.57 g of alkalinity (as $CaCO_3$ equivalent) is produced with the reduction of per gram of nitrate nitrogen. So, one-half amount of alkalinity used for nitrification can be restored by the denitrification process. Nitrate reduction goes via a series of intermediate products, which is represented by equation 2.15. Firstly, NO_3^- (nitrate) is converted to NO_2^- (nitrite), and then NO_2^- to NO (nitric oxide), and further NO to N_2O (nitrous oxide), and finally N_2O to N_2 (nitrogen gas). N_2O is a strong greenhouse gas, so it is of important concern while performing the denitrification process [3].

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (2.15)$$

2.6 Oxygen transfer from gas to suspended microorganisms

During the aeration process, the oxygen transfer from the gas-phase to the suspended microorganisms in the liquid must take place through a definite pathway. Figure 2.3 shows the path along which oxygen is transfer from gas-phase to suspended microorganisms [33].

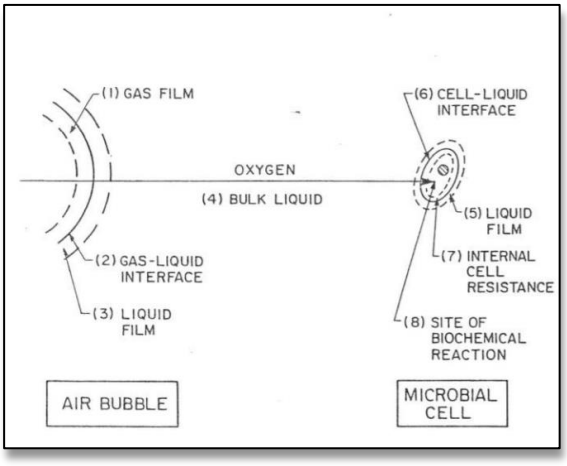

Figure 2.3: The oxygen transport path to the suspended microorganisms [33].

As shown in the Figure 2.3, there are eight resistances in the path of oxygen transfer. However, all other resistances are neglected due to their less impact so, only one resistance associated with the gas-liquid interface is considered [33].

2.6.1 The two-film theory of oxygen transfer

Oxygen transfer at the gas-liquid interface is based on the two-film theory which was firstly given in Whitman and Lewis's paper in 1924 [34]. A graphical representation of oxygen mass transfer in two-film theory is given in Figure 2.4. The two films are gas film and liquid film. These films produce resistance to the movement of gas molecules. In both bulk-liquid and bulk-gaseous phase, it is presumed that the concentration and partial pressure are uniform [3].

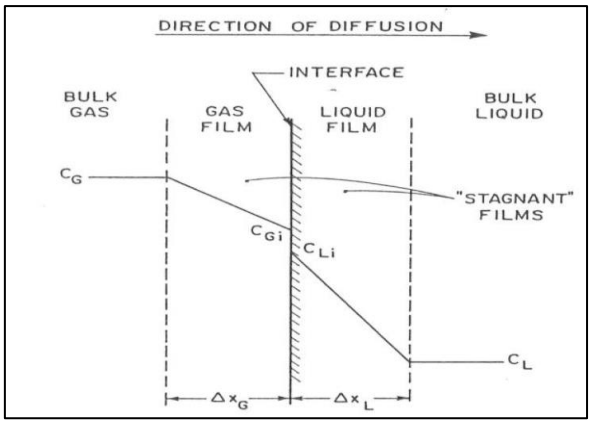

Figure 2.4: Oxygen mass transfer mechanism in gas-liquid interface [33].

In steady-state conditions, the rate of mass transfer along the gas film is equal to the rate of mass transfer along the liquid film. The mass transfer for each phase can be written using Fick's law which is represented by equation 2.16 [33]:

$$r = k_G(C_G - C_{Gi}) = k_L(C_{Li} - C_L) \tag{2.16}$$

Where, r= rate of mass transferred per unit area per unit time (g/m^2/h), k_G= gas film mass transfer coefficient (m/h), C_G= concentration of oxygen in the gas phase (g/m^3), and C_{Gi}, C_{Li} are the interface concentration in gas and liquid phase, respectively.

If it is assumed that only the liquid film causes resistance to the oxygen mass transfer for soluble gases like O_2, then the rate of mass transport can be increased by decreasing the thickness of the liquid film. Moreover, the interfacial concentrations of gas and liquid are in equilibrium. Hence, the rate of mass transfer per unit area per unit time in respect of overall liquid mass transfer coefficient can be understood by equation 2.17 [3].

$$r = K_L(C_s - C_L) \tag{2.17}$$

Where, K_L= overall liquid mass transfer coefficient (m/h), C_s= oxygen concentration in liquid equilibrium with gas phase (g/m^3), and C_L= concentration of oxygen in the liquid (g/m^3).

C_s can be expressed in terms of C_G by using Henry's law which is given by equation 2.18:

$$C_G = HC_s \tag{2.18}$$

Equation 2.17 can also be written as per unit volume per unit time by dividing with the area (A) and Volume (V), which is:

$$r_v = K_L \frac{A}{V}(C_s - C_t) = K_L a(C_s - C_t) \tag{2.19}$$

Where, r_v= rate of mass transfer per unit volume per unit time (g/m^3/h), C_t= concentration in liquid bulk phase at time t (g/m^3), $K_L a$= volumetric mass transfer coefficient (h^{-1}), A= area through which mass is transferred (m^2), V= volume in which constituent concentration is increasing (m^3), and a= interfacial area of mass transfer per unit volume (m^{-1}).

In the biological reactor, oxygen uptake by microorganisms should be considered in the mass balance equation, which is represented by equation 2.20. The value of this respiration rate can be determined by maintaining the oxygen level constant (i.e., $\frac{dC}{dt} = 0$) which is shown in equation 2.21 [3].

$$\frac{dC}{dt} = K_L a(C_s - C) - r_M \tag{2.20}$$

$$r_M = K_L a\,(C_s - C) \tag{2.21}$$

Where, C= concentration of oxygen in solution (g/m^3), and r_M= rate of oxygen used by the microorganisms (g/m^{3}h).

2.7 Aerobic granular sludge (AGS) process

Aerobic granular sludge (AGS) process is the fast-growing technology for the biological treatment of municipal, domestic, and industrial wastewater. It can be a better substitution for activated sludge (AS) process as it addresses the several issues of AS like poor settling characteristics of biomass, nutrient removal, high energy required for recirculation of sludge and wastewater, larger land footprint, and complex design process due to several process units. AGS technology is getting popularity due to decrease in land footprint by 75%, reduction in operational costs and capital costs by 50% as compared to conventional AS system [35]. AGS process was first revealed in the early 1990s. However, detailed investigation regarding the effects of operational parameters is going on to understand the evolution of microbial community formation of aerobic granules [36].

Generally, SBR are used for the cultivation of AGS. The sludge from the AS process is seeded in SBR as an inoculum and the reactor is operated with aeration bubble and shorter settling periods [37]. These operating conditions helps for the selection of slow growing microbes like nitrifying bacteria, anammox bacteria, glycogen accumulating organisms (GAOs), and polyphosphate accumulating organisms (PAOs) as dense aggregates. The settling characteristics of these type of aggregates is much faster than that of floc forming microorganisms, as a result, high biomass retention in SBR can be seen. Further growth of bio-aggregates can lead to the development of millimeter-sized granules [35]. It is possible to keep aerobic, anoxic and anaerobic environmental condition within a single granule due to its big particle size, dense microbial formation, and presence of oxygen in the outer part of the granule.

These types of redox conditions present in a single granule is favorable for the simultaneous removal of nutrients (like nitrogen, phosphorous), and organic carbon from wastewater [35].

Formation of AGS in SBR are triggered by two forces: i) feast-famine feeding pattern, and ii) hydrodynamic shear force [37]. In feast regime, there is availability of substrate in the extracellular medium which is consumed by the microbes whereas in the famine phase, substrate is depleted which creates food scarcity condition for cell in the presence of oxygen through air supply. The feast-famine condition creates physical appearance alterations, biofilm formation and aggregates. It also produces extracellular polymeric substances (EPS), which enhances cell attachment [35]. Shear force also influence the formation of AGS and its characteristics. Shear force can be obtained by dividing the air flow rate by the cross-sectional area of reactor. It is also known as superficial air velocity. Superficial air velocities higher than 1.2 cm/s are found to be suitable for the formation AGS. These higher velocities detach the filamentous outgrowth thereby enhancing the stability and density of AGS. However, other factors like volume exchange ratio, settling time, and discharge time also plays an important role for the formation of AGS as the superior form of biomass. To understand the overall effect of these parameters, a single unified term is introduced known as minimum settling velocity $(V_s)_{min}$. The $(V_s)_{min}$ greater than 1 mh^{-1} is good for the formation of aerobic granules and becomes the dominant biomass in SBR when the value reach above 4 mh^{-1} [37]. However, molecular level research is still necessary to know the function of aerobic starvation on microorganisms aggregates and granulation. In addition, the long start-up time for granulation and instability problem when operated for longer period should be addressed for successful implementation of AGS method [35].The graphical representation of a single aerobic granule is represented by Figure 2.5 whereas formation mechanism of AGS is represented by Figure 2.6.

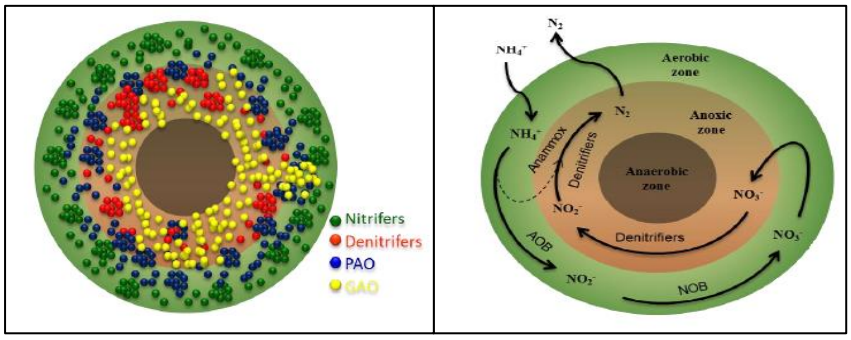

Figure 2.5: Graphical representation on distribution of microorganisms on left side, and nitrogen removal pathways on right side in an individual aerobic granule [37].

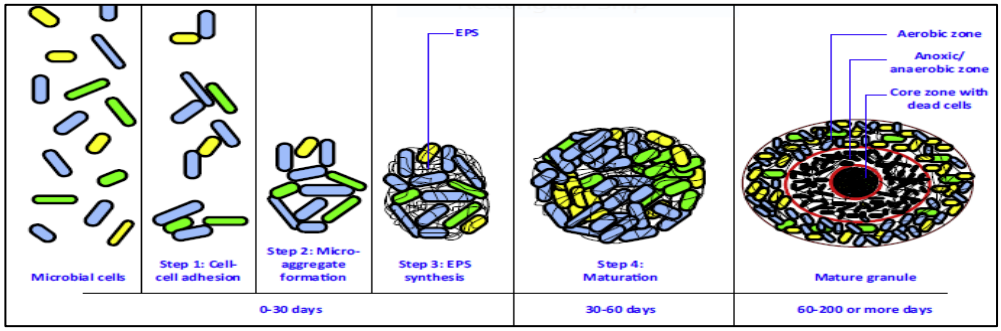

Figure 2.6: Formation mechanism of aerobic granule [38].

2.8 An overview on impregnating nitrate into biochar

The demand and use of fertilizer, required to produce crops, is increasing day by day with increase in the population of the world. The extensive use of these kind of fertilizers causes nitrate leaching, eutrophication, groundwater pollution, and emissions of the greenhouse gas like nitrous oxide. Therefore, it is a great concern to develop better agricultural practices and to promote accumulation of soil organic carbon in soils. Moreover, effective use of N fertilizer should be established to reduce the future hazards, global warming, and loss of nitrogen (N) in the form of nitrate leaching [39].

Biochar is rich in carbon source produced from the thermal decomposition of agricultural residues in a closed system under limited oxygen or absence of oxygen. It can be used as composting additive and soil amendment [40]. It has specific physical and chemical properties like porous structure, presence of large number of functional groups, and rich in mineral elements. These properties help to enhance the water-storing capacity, controls the immobilization of heavy metals like cadmium (Cd), provides good environment for microbial activity, and optimize the release of nutrients in the soil reducing overloading of nutrients [40][41].

However, for the proper and effective use of biochar, it should be combined with nitrogen (N) fertilizer, as biochar does not have enough nutrients for the growth of agricultural crops. It is found that the production of crops decreased when only giving biochar due to inadequate supply of nitrogen (N) [41]. The involvement of biochar with organic nitrogen species and minerals, particularly with nitrate, has been studied and was recently recommended as one essential mechanism of biochar required for plant growth. In both co-composted biochar and soil-aged, nitrate was found to be slowly released. Slow-release rate of nitrate due to biochar helps to prevent the nitrate leaching. Moreover, it supplies nitrate to plants for a long-term use as compared to non-biochar fertilized soils [42]. It also adjusts pH of the soil, improves permeability and soil ventilation by reducing bulk density, and increase the crop production considerably [41].

Although the combined form of biochar with nitrogen fertilizer possesses many advantages, their storage, movement, and implementation to soil remain difficult due to their irritation of human eyes, skin, and respiratory system. During the field expanding, almost 25% of applied biochar was wasted. In addition, heavy rainfall ran off 20-53% of applied biochar. Therefore, it is important to develop biochar-based fertilizer with minimal application loss as well as that can sustain and provide a long-term adequate supply of nutrients [41].

3 Materials and methods

This chapter gives detailed information about reactor setup, design and operating conditions. Moreover, it includes sampling frequency and laboratory analysis procedures.

3.1 Feed source

Reject water is obtained from the full-scale wastewater treatment plant (*Knarrdalstrand, Telemark, Porsgrunn, Norway*). The reject water is generally classified into two groups: i) reject water from thickener and another ii) reject water from the centrifuge. The feed used in this thesis is the reject water from centrifuge which is the effluent from the anaerobic digestion (AD) reactor. Feed is brought to the campus laboratory once in month to ensure enough feed is available for the reactor. The brought reject water was stored in a cold room at temperature 4℃. The characteristics of the feed (reject water) is given in **Table 3.1**.

Table 3.1: **Organic and inorganic chemical characteristics of reject water**

Parameter	**Value**	**Units**
TCOD	3000±500	mg/L
SCOD	2000±500	mg/L
pH	7.7±0.3	
NH_4^+-N	520±50	mg/L
Alkalinity	2100±400	mg/L

Along with the reject water, synthetic feed was also used in this study. Synthetic feed was prepared in a solution with tap water. Ammonium chloride (NH_4Cl) was added for the concentration of ammonium-nitrogen (NH_4-N) and sodium bicarbonate ($NaHCO_3$) was added to maintain enough alkalinity for the nitrification process. Vitamins and minerals were also added to the feed to support bacteria growth. The constituents of these vitamins and minerals are shown in **Table 3.2**. The concentration of ammonia in the synthetic feed was maintained the same as the concentration of ammonium nitrogen in the reject water feed. The calculation of synthetic feed is given in Appendix B.

Table 3.2: Constituents of vitamins and minerals used in synthetic feed [43]**.**

Vitamin solution (g/L)	**Mineral solution (g/L)**
Biotin: 0.02	$MnSO_4 \cdot H_2O$: 0.04
Folic acid: 0.02	$FeSO_4 \cdot 7H_2O$: 2.7
Nicotinic acid: 0.05	$CoCl_2 \cdot 6H_2O$: 0.05
p-aminobenzoic acid: 0.05	$CuSO_4 \cdot 5H_2O$: 0.055
Pantothenic acid: 0.05	$NiCl_2 \cdot 6H_2O$: 0.1
Pyridoxine hydrochloride: 0.1	H_3BO_3: 0.05

Riboflavin: 0.05	$ZnSO_4 \cdot 7H_2O$: 0.088
Thiamine: 0.05	-
Thioctic acid: 0.05	-
Vitamin B_{12}: 0.001	-

3.2 Nitrification reactor setup

Two parallel lab-scale sequential batch reactors (SBR) were set up in September 2020 and have been in operation since that period. The first reactor (R_1) has a height of 151.5 cm and a diameter of 4.2 cm whereas; the second reactor (R_2) has a height of 136.5 cm and a diameter of 3 cm. Three *Tygon*® tubes were inserted into the reactor from the top for feeding, supplying aeration, and taking samples. The feeding tube and the aeration tube were inserted to the bottom of the reactor whereas the effluent tube for taking samples was suspended up to the middle of the reactor to prevent the washing out of the sludge while taking samples. Plastic tubing adjustable clamps were provided for closing the flow of the tube. The top of the reactor was covered by aluminum foil to prevent the overflow of the reactor. Aeration was provided from the compressor regulated by an airflow meter. The continuous up-flow aeration helped proper mixing in the reactor. Figure 3.1 illustrates the experimental setup of two SBR for the nitrification process.

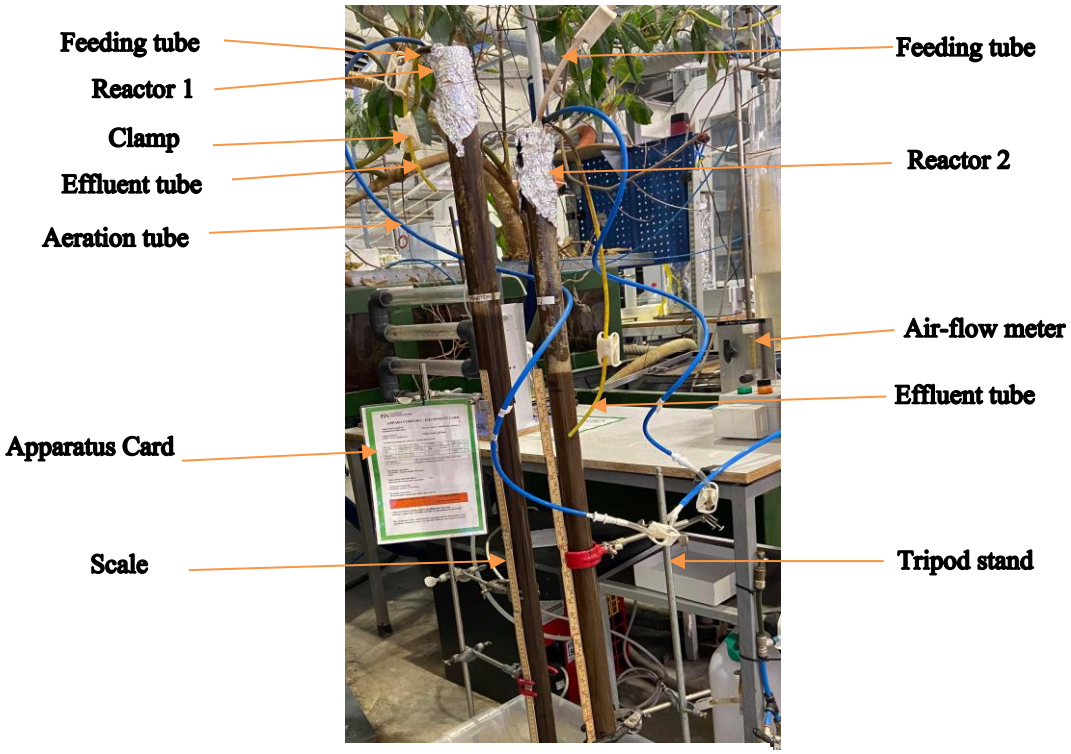

Figure 3.1: Laboratory setup for SBR

The inoculum used in both reactors was the mixture of aerobic and anaerobic sludge from the Risør wastewater treatment plant. The volume of inoculum used was one-third (1/3rd) of the reactor volume and the rest of the space in the reactor was filled by reject water. The total working volume of the reactor was only 70% of the total reactor volume. Feeding to the reactor was increased gradually from 10% to 30% of the total working volume, which percentage generally depends on the operating condition like HRT and the performance of the reactor. Similarly, different feeding sequences (one, two, and three times a day) were tested for tuning and investigating the optimum condition in the reactors. The procedure for feeding was at first the aeration was turned off and the biomass was allowed to settle for 20 minutes. After that, a certain amount of sample was drawn out from the effluent tube and then the same amount of reject water was feed through the feeding tube. After feeding, aeration was turned on and leave the reactor in the reaction phase. The feeding procedure was done manually throughout the experimental period. Different HRTs were tried to see the effect of loading rate on the performance of nitrification. **Table 3.3** gives an overview of the design parameters of SBRs used in this thesis.

Table 3.3: Design parameters of SBR

Parameters	Reactor 1	Reactor 2	Units
Reactor volume	2.1	0.96	L
Working volume	1.47	0.68	L
Internal diameter	4.2	3	cm
Height	151.5	136.5	cm
Tygon® tubes diameter	4.8, 7	4.8, 7	mm

3.3 Operation of reactors

Both reactors (1 and 2) were operated with the reject water feeding until day 154. From day 155, reactor 1 was continued with reject water whereas, reactor 2 was operated with synthetic feed. The airflow rate was 25 L/h remaining constant for the whole period and the reactors were operated at a room temperature of 18±2℃. The change in operating conditions over a different period of time is shown in **Table 3.4** for both reactors.

Table 3.4: Operating conditions applied to both reactors over different period.

Parameters	Operation period in days					Units
	100-124	125-133	134-138	139-176	177-222	
Feeding rate	20	20	30	30	30	%
Feeding sequence	3 times	1 time	2 times	2 times	1 time	Per day
HRT	1.67	5	1.67	1.67	3.34	day
NLR	0.321	0.112	0.27±0.02	0.27±0.02	0.14±0.005	kg/m^{3}day
Alkalinity	2100±4	2100±400	2100±400	3400±200	3400±200	mg/L

3.4 Sample analysis frequency and procedures

Analysis of samples was performed two times per week, normally Tuesday and Friday. Samples were taken in the morning time during feeding. During sampling first aeration was turned off and left the biomass to settle. After complete settlement of biomass, the sample was taken from the effluent pipe of both the reactors by a 100 mL syringe and if needed, the sample was stored at 4℃ in a refrigerator. The standard procedures to measure dissolved oxygen (DO), temperature, pH, ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), alkalinity (as $CaCO_3$), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS) are described in the following subsection.

3.4.1 Dissolved oxygen (DO) and temperature

The dissolved oxygen meter used was calibrated once and does not need to calibrate for every measurement. The dissolved oxygen was measured by inserting the probe into the reactor and waiting for the meter to show constant reading. The DO meter used was WTW Oxi 3310 oxygen meter (Weilheim, Germany). It measures the DO concentration in terms of mg/L and the temperature in ℃.

3.4.2 pH

pH was measured and monitored every time the sample was extracted. A Beckman-390 pH meter was used for measuring the pH of the sample. At first, the pH meter was calibrated by using standard buffer solutions of pH 4.0 and 7.0 before measuring the pH of the sample.

3.4.3 Organic matter measurement in terms of TCOD and SCOD

TCOD gives a quantification of total organics present in the extracted sample. A sufficient sample was stored for crosscheck of the results if needed. The measurement procedure complies with the US standard 5220 D [44]. It was measured by following the procedures given in the Spectroquant prove 300 instruction manual [45]. As per the US standards, the method number for the measurement of COD (500-10000 mg/L) is **114555** [45] [46].

SCOD measures the value of soluble organics present in the extracted sample. In the case of SCOD, the extracted sample was centrifuged at 12500 rpm for 15 min using Heraeus Megafuge 16 centrifuge by ThermoFisher Scientific. Then the centrifuge sample was filtered through 0.45 µm GxF multi-layered, Acrodisc PSF filters. After that, the filtered sample was measured using the same procedure and instructions used for measuring TCOD.

3.4.4 Nitrogen measured as NH_4^+-N, NO_2^--N, NO_3^-N and alkalinity as $CaCO_3$

The extracted sample was centrifuged and filtered using the same procedure as in the SCOD. After filtration, the procedure given in the Spectroquant prove 300 instruction manual was followed for the measurement of ammonium, nitrite, nitrate, and alkalinity [45]. The measurement procedure complies with the US standard 4500-NH_3 [44]. As per the US standards, the method number for measurement of ammonium (4-80 mg/L), nitrite (1-90

mg/L), nitrate (0.5-25 mg/L), and alkalinity (20-400 mg/L) are **114559**, **100609**, **114563**, and **101758** respectively [45].

3.4.5 Measuring solids as total Solids and volatile Solids

All the solids like biodegradable and non-biodegradable, suspended and soluble, organics and inorganics represent the total solids (TS) in wastewater whereas only biodegradable organics (suspended and soluble) represents the volatile solids (VS). The measurement procedure of these solids complies with the American standard method APHA 2540 B [44].

A washed porcelain crucible was dried at 105℃ in an oven and kept in a desiccator for cooling to room temperature and then it was weighed (W_1) using an analytical balance (Sartorius). 10 to 30 mL sample was mixed thoroughly and poured into the crucible and kept in the oven at 105℃ at least for 24 hours for drying the sample. After 24 hours, it was taken out and cooled in a desiccator to room temperature and again weighted (W_2). Then the total solid was calculated using equation 3.1 :

$$TS\ (g/L) = \frac{W_2(g) - W_1(g)}{V(L)} \tag{3.1}$$

For VS, the dried sample weighted as W_2 was kept in a muffle furnace at 550℃ for 20 minutes and then cooled in a desiccator to room temperature. The cooled crucible with the sample was again weighted as W_3 using the same analytical balance. The volatile solid was calculated using equation 3.2.

$$VS\ (g/L) = \frac{W_2(g) - W_3(g)}{V(L)} \tag{3.2}$$

3.4.6 Total suspended solids and volatile suspended solids

The procedure for measuring TSS and VSS also follows the American standard method APHA 2540 D and 2540 E, respectively [44]. At first, 1.5 µm of glass microfibers filters (VWR European Cat No. 516-0875) was put in a porcelain crucible and dried at 105℃ for 20 minutes and cooled in a desiccator. Then it was weighted as W_1. 10 to 60 mL of sample was passed through filter paper with the help of diaphragm vacuum pump and the sample retained on filter paper was put back to the crucible and dried at 105℃ for 24 hours and weighted as W_2. The TSS was then calculated by using the equation 3.3.

$$TSS(g/L) = \frac{W_2(g) - W_1(g)}{V(L)} \tag{3.3}$$

After TSS, the sample was ignited in a muffle furnace at 550℃ for 20 minutes and cooled in a desiccator to room temperature. The cooled sample was again weighted as W_3 using an analytical balance. The VSS was calculated by using the equation 3.4.

$$VSS\ (g/L) = \frac{W_2(g) - W_3(g)}{V(L)} \tag{3.4}$$

3.5 Microscopic analysis of the sludge

The OLYMPUS IX70 microscope (Figure 3.2) was used to perform the analysis of the sludge (i.e., biofilms) from both nitrification reactors 1 and 2. The OLYMPUS IX70 microscope is a high-tech device that has the ability of imaging specimens under different illumination modes. The microscope is using a Moticam 5.0-megapixel (MP) camera which has various magnification lens with live resolution (2592x1944) capability. It is coupled with its own shift capture system called Motic Images Plus 3.0 ML through which the diameter of the sludge can be measured. For our sludge, a magnification of 20X was used to observe the microbial biofilm structure of the sludge.

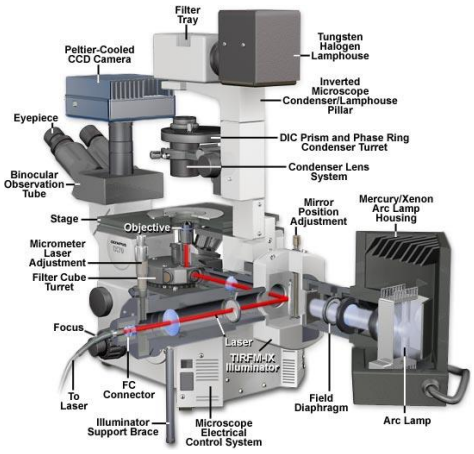

Figure 3.2: Olympus IX70 Microscope labelled cutaway diagram [47].

4 Results

All the results presented here are in graphical form. Graphs were plotted based on the experimental data obtained after analysis in the laboratory. MS-excel was used for data processing, calculation, and plotting of the graphs. Different results obtained during the tunning of reactors are presented in the following subsection and the equations are given in Appendix C. Since it was an ongoing project, the graph was plotted from day 100 to show the previous trend and condition of the reactor. The thesis study started from day 124.

4.1 Tunning Reactor 1 with different operating parameters

Reactor 1 was tuned by changing one operating parameter (at the time). It was in an operating condition of HRT 1.67 days and nitrogen loading rate (NLR) of 0.328 kg/m^3·day up to day 124 (Figure 4.2**A**). The average ammonium removal was 62% of which 47% was converted to nitrite, 11% was converted to nitrate, and only 4% was converted to nitrogen gas (Figure 4.1). In terms of concentration, the average influent NH_4-N, NO_2-N, and NO_3-N concentration was 535±13, 50±30, and 9±1 mg/L, respectively. Whereas the average effluent NH_4-N, NO_2-N, NO_3-N, and N_2 was 206±30, 275±15, 68±5, and 20±90 mg/L respectively up to day 124 (Figure 4.2**A**).

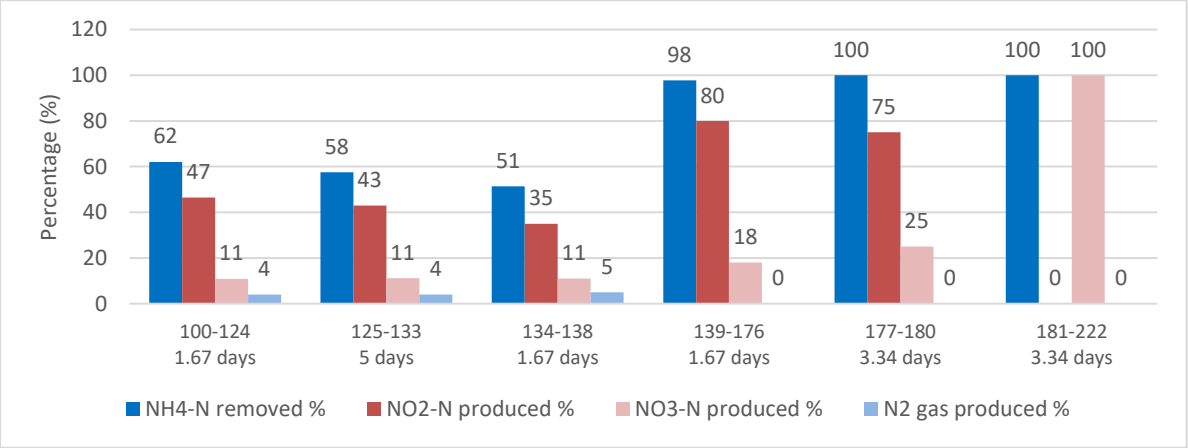

Figure 4.1: Ammonium conversion into nitrite, nitrate and nitrogen gas in reactor 1.

As presented in Figure 4.1, on day 125, HRT was increased to 5 days reducing the nitrogen loading rate to 0.112 kg/m^{3}day as a next step on tunning the reactor. However, such a low nitrogen loading rate led to a decrease in the average ammonium removal from 62 to 58% and nitrite production from 47 to 43%. Nitrate and nitrogen gas production was approximately constant. The average influent concentration of NH_4-N, NO_2-N, and NO_3-N was 561±1, 74±1, and 16±1 mg/L whereas the average effluent concentration of NH_4-N, NO_2-N, NO_3-N, and N_2 was 238±4, 315±20, 79±7, and 20±30 mg/L respectively up to day 133 (Figure 4.2**A**).

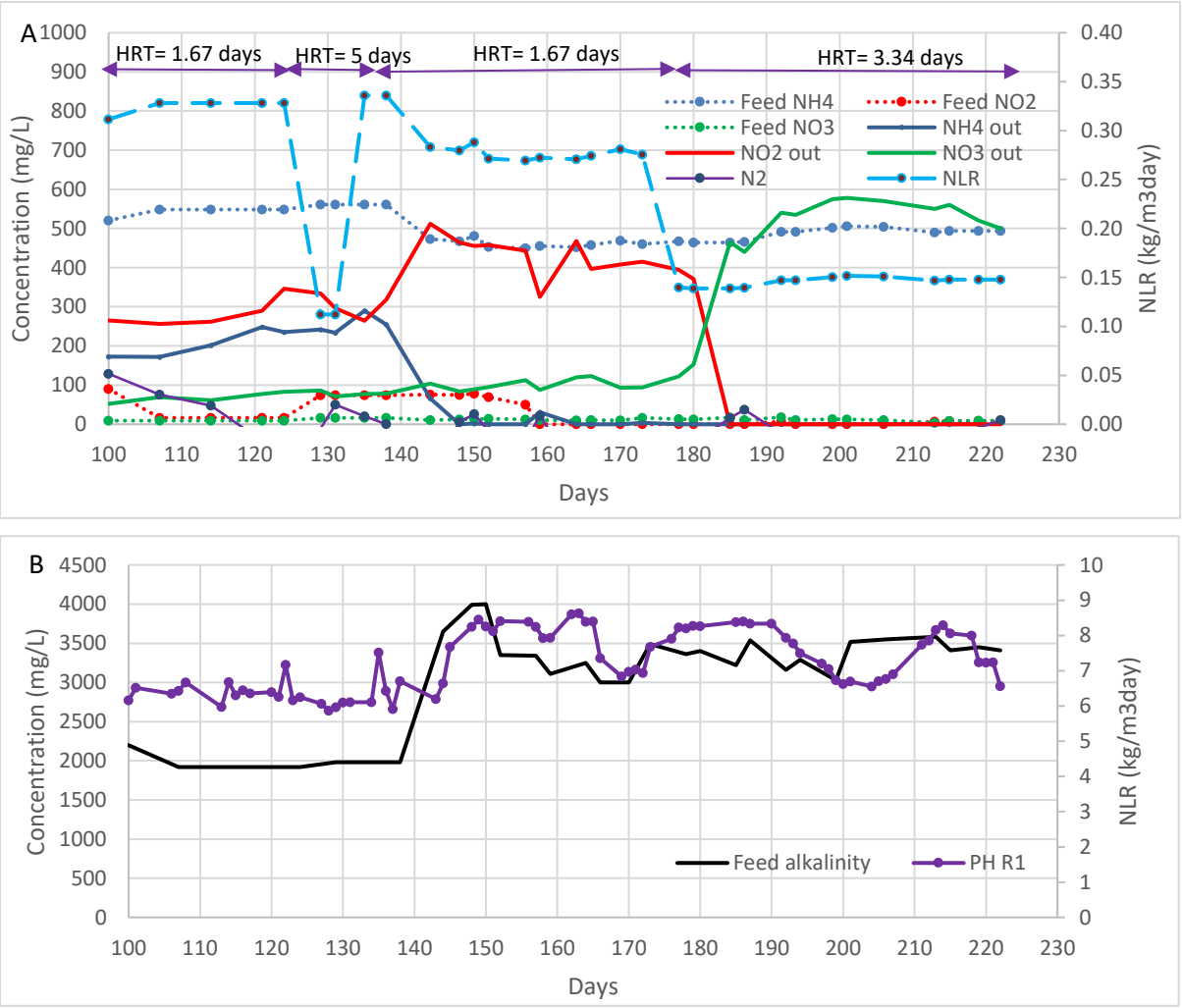

Figure 4.2: A) Concentration of different forms of nitrogen in the influent and effluent of the reactor 1 with NLR, B) Concentration of feed alkalinity and pH in reactor 1.

On day 134, HRT was brought back to 1.67 (NLR: 0.3±0.036 kg/m^{3}day) days just to recover the previous growth and continued up to day 138. However, the ammonium removal efficiency further decreased to 51% and only 35% was converted to nitrite. Nitrate production was constant whereas nitrogen gas production increased to 5% (Figure 4.1). On day 139, sufficient alkalinity was added as per the stoichiometric calculation and this step was considered as the first transition phase. As seen in Figure 4.2**A** and Figure 4.2**B**, with the addition of alkalinity, pH increased to 7-8, effluent NH_4-N concentration started to decrease and at the same time, effluent NO_2-N concentration started to increase. Moreover, the effluent NO_3-N concentration was gradually increasing. On day 150, almost all the NH_4-N was removed and converted to NO_2-N, whereas after a certain increment, NO_3-N concentration became approximately constant (Figure 4.2**A**). This condition was kept for a certain period up to day 176. During this period, the average ammonium removal reached 98% of which 80% was nitrite and 18% was nitrate. Nitrogen gas production was zero which indicates no denitrification in the reactor.

Almost all the ammonium was converted to nitrite resulting in nitrite accumulation in the reactor. Therefore, based on this result, HRT was increased to 3.34 days reducing the loading rate to 0.146±0.005 kg/m^{3}day, as another step, on day 177 (Figure 4.2**A**). This step was the

second transition stage because over time the produced nitrite was started to convert into nitrate (Figure 4.1). Therefore, the reactor was maintained in the same condition to enhance NOB growth. On day 181, all the 100 % ammonium was converted to nitrate leaving no nitrite in the reactor. Hence, complete nitrification was achieved from day 181 (Figure 4.1). In terms of concentration (from day 181 to 222), the average influent concentration of NH_4-N, NO_2-N, and NO_3-N was 485±20, 4±4, and 13±5 mg/L respectively, whereas the average effluent concentration of NH_4-N, NO_2-N, NO_3-N, and N_2 was 0, 0, 500±60, and 0 mg/L, respectively.

4.2 Tunning reactor 2 with different operating parameters

Reactor 2 was also tuned with the same procedure as reactor 1. However, there are some little differences in feeding and the results. Reactor 2 was at NLR of 0.328 kg/m^{3}day and HRT of 1.67 days up to day 124 (Figure 4.4**A)**. At these conditions, the average ammonium removal percentage was 64% of which 43% was converted to nitrite, 11% was converted to nitrate, and 10% was converted to nitrogen gas, respectively (Figure 4.3). The average influent concentration of NH_4-N, NO_2-N, and NO_3-N was 535±13, 50±30, and 9±1 mg/L, respectively whereas the average effluent concentration of NH_4-N, NO_2-N, NO_3-N, and N_2 was 200±50, 260±30, 70±8, and 40±80 mg/L, respectively (Figure 4.4**A)**.

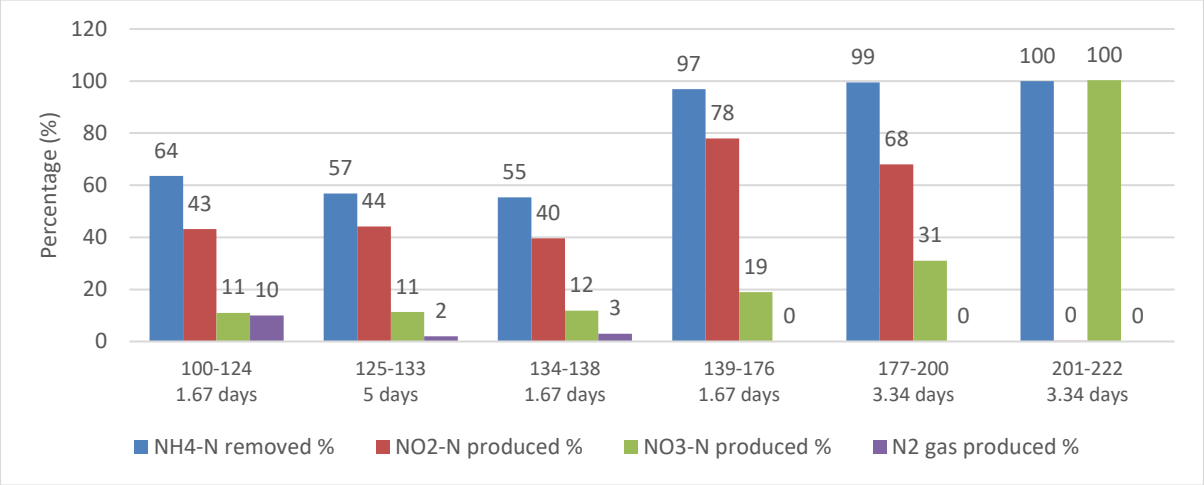

Figure 4.3: Ammonium conversion into nitrite, nitrate and nitrogen gas in reactor 2.

From day 125 to day 133, HRT was increased to 5 days reducing the NLR to 0.112 kg/m^3 day to see the effect of loading rate. However, low NLR did not help to enhance the nitrogen conversion. The average ammonium removal decreased to 57% (Figure 4.3). On day 134, the reactor was brought back to the previous condition, just to recover the bacteria growth, which was HRT of 1.67 days and NLR of 0.3±0.036 kg/m^{3}day. But the average ammonium removal further decreased to 55% up to day 138. Since variation in NLR did not help to enhance ammonium removal, alkalinity addition was selected as a next step to tune the reactor. Sufficient alkalinity was added as per the stoichiometric calculation on day 139 (Figure 4.4**B**). Just after the alkalinity addition, pH increased to 8±0.5, the average effluent NH_4 concentration started to decrease and effluent NO_2 concentration started to increase, whereas the NO_3 concentration was constant, resulting in nitrite accumulation in the reactor (Figure 4.4**A** and Figure 4.4**B)**.

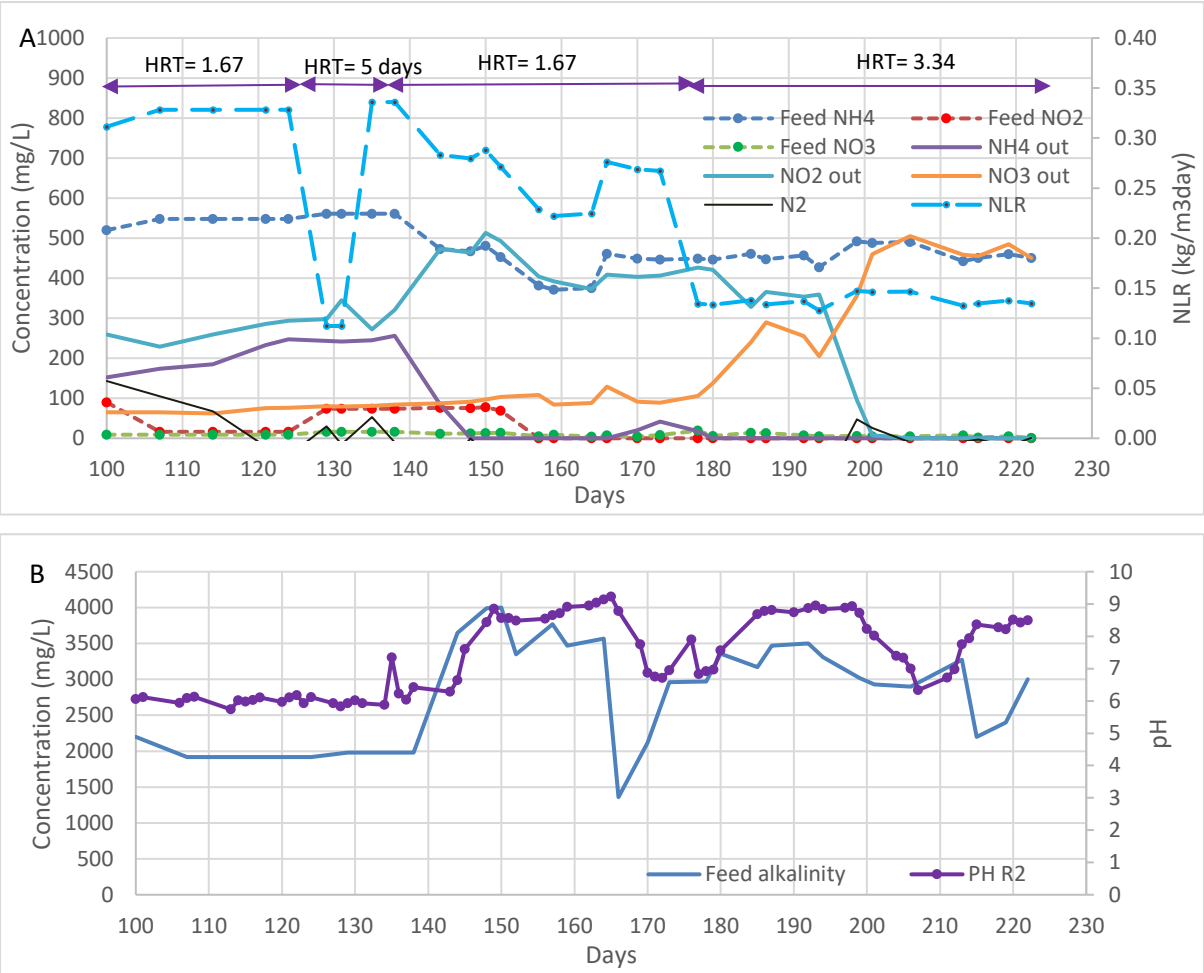

Figure 4.4: A) Concentration of different forms of nitrogen in the influent and effluent of the reactor 2 with NLR, B) Concentration of feed alkalinity and pH in reactor 2.

From day 139 to day 154, the average influent concentration of NH_4, NO_2, and NO_3 was 465±15, 74±5, and 12±2 mg/L, respectively, whereas the average effluent concentration of NH_4-N, NO_2-N, NO_3-N, and N_2 was 44±40, 485±25, 90±10, and 0 mg/L, respectively. From day 155, the reactor was given synthetic feed as a next step to see the effect of the COD/N ratio on AOB and NOB bacteria. However, as seen in Figure 4.4**A**, the effluent concentrations were constant even with the synthetic feed up to day 176. The NH_4 removal reached 97% of which 78% was converted to nitrite and 19% was converted to nitrate. No production of nitrogen gas was seen.

As there was nitrite accumulation in the reactor which means AOB was active whereas NOB bacteria were not able to perform. Hence, NLR was reduced to 0.14±0.006 kg/m^3day by making HRT to 3.34 days on day 177 (Figure 4.4**A**). This change in NLR helped to increase ammonium conversion to nitrate. Up to day 200, the average ammonium removal was 99% of which 68% was converted to nitrite, 31% was converted to nitrate. The conversion from nitrite to nitrate was increasing. Hence, the reactor was maintained on the same condition to get complete nitrification. On day 201, complete conversion of ammonium to nitrate was achieved. Up to day 222, the average ammonium removal was 100% of which all 100% was converted to nitrate achieving complete nitrification (Figure 4.3). In terms of concentration from day 201 to 222 (Figure 4.4**A**), the average influent concentration of NH_4, NO_2, and NO_3 was 470±20, 0, and

4±3 mg/L and the average effluent concentration of NH_4-N, NO_2-N, NO_3-N, and N_2 was 0, 0, 475±25, and 0 mg/L, respectively.

4.3 Effect of sequence on reaction time of nitrification

For both reactors (from day 100 to 124), the feeding sequence was three times a day and on day 125, it was made one time a day up to day 133. However, the change in feeding sequence from 3 times to one time did not show any effect on the nitrification process which can be observed from Figure 4.2**A** and Figure 4.4**A**. Alkalinity was added on day 139 and the sequence was twice a day. From that day, the ammonium removal efficiency was almost 100% but most converted to nitrite resulting in nitrite accumulation. However, when the feeding sequence was changed from two times to one time a day (on day 177), accumulated nitrite started to convert into nitrate and the nitrate percentage started to rise. After certain days, all nitrite was converted to nitrate achieving complete nitrification (Figure 4.1 and Figure 4.3).

4.4 pH, free ammonia (FA) and free nitrous acid (FNA)

There was a fluctuation of pH over the days in both reactors R_1 and R_2 which created some conditions to produce free ammonia and free nitrous acid depending upon the pH value. The variations in pH, FA, and FNA in both reactors are described in the following subsection.

4.4.1 pH, free ammonia, and free nitrous acid variations in reactor 1

As seen in Figure 4.5, from day 100 to 138, pH in reactor 1 was around 6.0±0.2 and the free ammonia (FA) concentration was around 0.5±0.2 mg/L. After the addition of alkalinity from day 139, pH started to increase gradually and was varying between 6.5-8.6 whereas free ammonia was varying between 44.4-0.0 mg/L. The highest free ammonia detected was 44.4 mg/L on day 164.

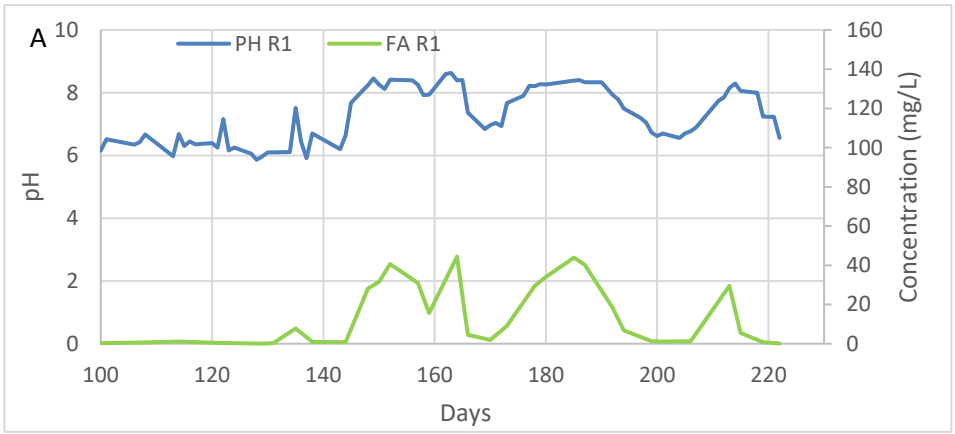

Figure 4.5: Variations in pH and concentration of free ammonia

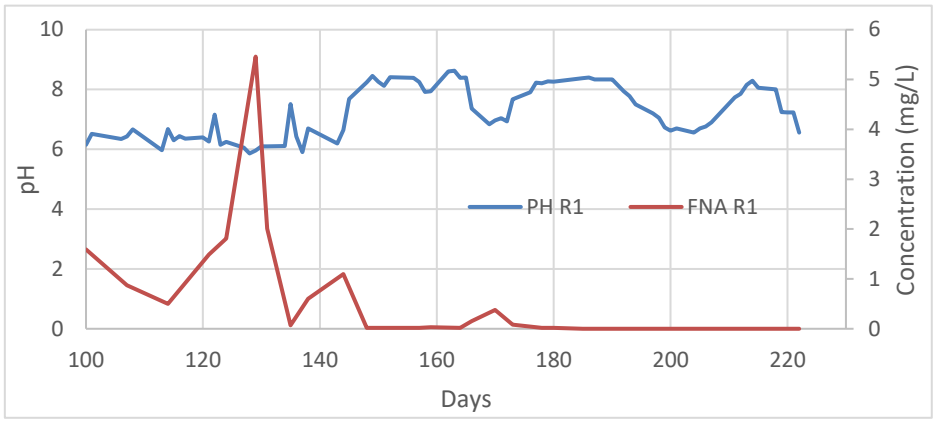

Figure 4.6: Variations in pH and concentration of free nitrous acid

From Figure 4.6, it can be observed that the FNA was below 2.2 mg/L up to day 124. On day 129, it rose to 5.5 mg/L which was the highest concentration throughout the whole period. After that, it started to decrease and was fluctuating between 0-1 mg/L up to day 180. From day 181 to 222, the FNA concentration was none.

4.4.2 pH, free ammonia, and free nitrous acid variations in reactor 2

As seen from Figure 4.7, up to day 138, pH of the reactor 2 was around 6.0±0.1 approximately and the free ammonia concentration was 0.1±0.1 mg/L. After the alkalinity was added on day 139, pH started to increase which helps to increase free ammonia concentration too. On day 164, pH reached 9.14 resulting in a higher concentration of FA to 149.37 mg/L. However, the pH was adjusted by pouring some drops of HCl in the synthetic feed which helped to maintain the pH around 7.0-8.0. But, when the new feed was prepared; the pH was always high at the beginning until it was adjusted by HCl, as can be observed on day 191. On day 191, pH again reached around 8.9 leading to the FA concentration to 118.1 mg/L. After the pH was maintained, the FA concentration was 15±5 mg/L only.

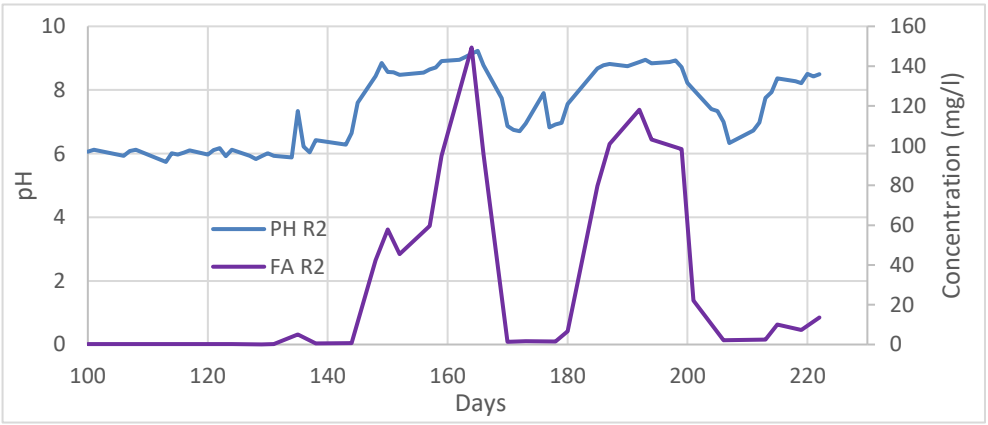

Figure 4.7: Variations in pH and concentration of free ammonia

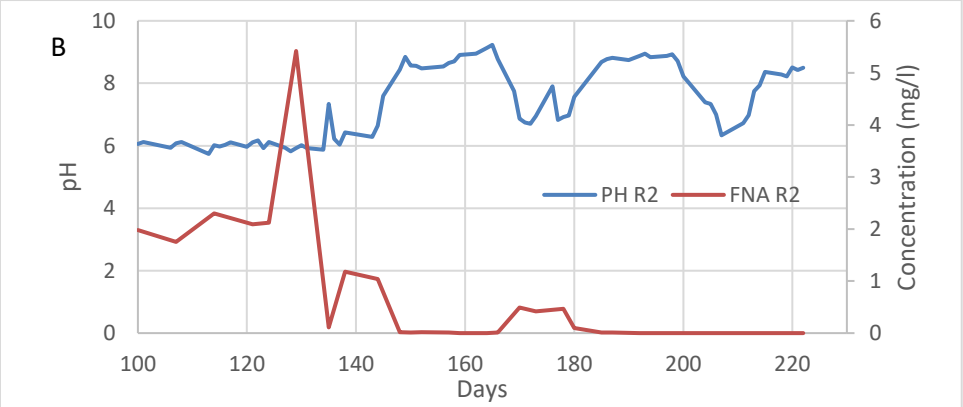

Figure 4.8: Variations in pH and concentration of free nitrous acid

From Figure 4.8, it can be noticed that the pH was around 6±0.1 up to day 138. FNA was 3.5±0.5 mg/L up to day 124 but after that, it started to increase and reached 5.42 mg/L (on day 129) which was the highest concentration throughout the period. On day 139, alkalinity was added which increased the pH and decreased the FNA concentration to approximately zero. However, when the pH was dropped below 7 (from day 170 to 178); the FNA concentration increased to 0.5±0.005 mg/l. In our case for both reactors, the optimum pH was between 7-8.5.

4.5 Dissolved oxygen (DO) profile for one cycle

Oxygen was provided continuously at the aeration rate of 25 L/h to both reactors from the compressor regulated by an air flow meter. The DO profile of both reactors is presented and described in the following subsection.

4.5.1 DO profile of reactor 1

DO concentration level was recorded for 10 hours. In Figure 4.9, aeration was ON for the first 10 minutes recording the level of dissolved oxygen concentration in the reactor which was 9.6±0.03 mg/L. After 10 minutes, aeration was turned OFF for settling of sludge, decanting of effluent, and feeding to the reactor. It took 20 minutes time. During this period, the DO concentration started to decrease gradually. However, when the aeration was turned ON just after the feeding (from 30 min on the graph), DO concentration decreased instantly from around 8 mg/L to around 2 mg/L just in 10 minutes time which can be clearly observed in Figure 4.9 as well. It can be predicted that the dissolved oxygen was consumed by the bacteria for the nitrification process. DO concentration remained constant around 1.8 mg/L for another 70 minutes. Hence, there was high consumption of dissolved oxygen in the reactor after the feeding for 80 minutes. Up to here, we reached 110 minutes time on the x-axis of Figure 4.9. After that, the consumption of DO was less so, the DO concentration in the reactor was gradually increasing and reached 8.93 mg/L at 260 min (i.e., 150 min after it started to increase). From then on, the DO level was constant. The lowest DO level observed was 1.62 mg/L.

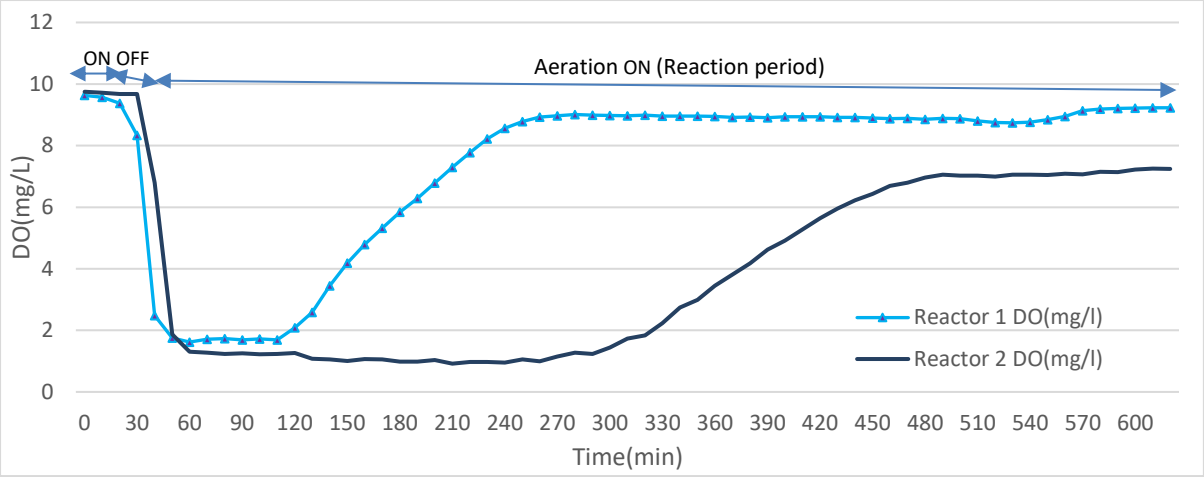

Figure 4.9: DO variations for one cycle in both reactors

4.5.2 DO profile of reactor 2

DO level concentration was recorded for 10 hours. As observed in Figure 4.9, the first 10 minutes is the ON period which helped to know the DO concentration level (i.e., 9.73±2 mg/L). After that the aeration was turned OFF for 20 minutes for the purpose of settling of sludge, decanting of effluent, and feeding the reactor. When the feeding was completed and aeration was turned ON, the DO level started to decrease rapidly from 9.73±2 mg/L to 1.27±0.03 mg/L just in 30 minutes. After that, the DO level remained constant for 210 minutes. This decreased in DO level was due to the consumption of oxygen by the bacteria required for the nitrification process. Hence, there was high consumption of oxygen at the beginning but 240 minutes later (counted just after the feeding), the DO level started to increase gradually. The DO level reached 7±0.04 mg/L after 450 minutes from the feeding (480 minutes on the graph) and from there on it was constant. The lowest DO level observed was 0.92 mg/L.

4.5.3 DO differences between two reactors

In Figure 4.9, initial stage up to the decant and feeding phase (30 minutes), both reactors trend was same. However, in the reaction period, reactor 1 DO decreased below 2 mg/L just for 80 minutes and then it started to increase gradually and reached constant value at 260 min whereas, reactor 2 DO decreased below 2 mg/L for 210 minutes which was longer as compared to reactor 1. Moreover, the increasing rate was also lower in reactor 2 as compared to reactor 1. Hence, the DO was consumed for a longer period in reactor 2 than in reactor 1.

4.6 Sludge settling time and microscopic analysis of the sludge

The sludge settling pattern was observed after the aeration was turned off. The time was recorded using a stopwatch. The experiment was performed and the data was recorded after the reactor was stable with complete nitrification. The height of the sludge was measured using the scale as can be seen in Figure 3.1. Reactor 1 internal diameter is 4.2 cm and reactor 2 internal diameter is 3 cm. In reactor 1 (Figure 4.10**A**), for the first 15 minutes, the sludge was settling rapidly from the initial height of 106 cm to 55 cm whereas for another 15 minutes it just went

A

from 55 cm to 40 cm. Therefore, within the first 15 minutes of time, almost all the sludge was settled while in another 15 minutes it was getting compacted.

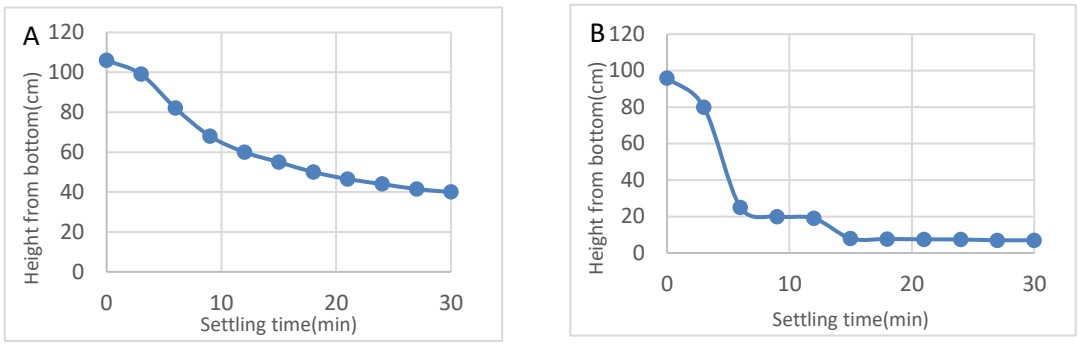

Figure 4.10: Sludge settling characteristic with time

In reactor 2 (Figure 4.10**B**), for the first 9 minutes, the sludge settled quickly from the initial height of 96 cm to 20 cm whereas for the next 21 minutes it just went from 20 cm to 7 cm. Hence, almost all the sludge was settled within 9 minutes and in the next 21 minutes, it was getting dense and compacted.

Figure 4.11 shows the structure of a microbial aggregate observed using a microscope from the sludge sample of reactor 1. It normally consists of biofilms and granules. The length of the microbial aggregate, which in fact varies depending on the sample, was 873.87 µm whereas the breadth was 776.90 µm. Similarly, Figure 4.12 shows the structure of the microbial aggregate observed from the sludge sample of reactor 2. The length of the aggregate was 759.16 µm whereas the breadth of the aggregate was 476.89 µm.

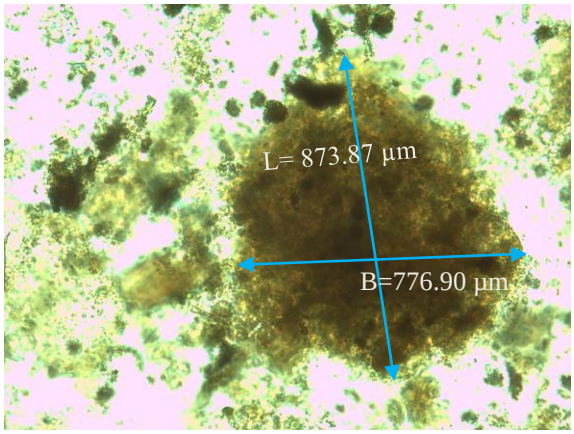

Figure 4.11: Microscopic image (20x magnification) of the sludge from reactor 1.

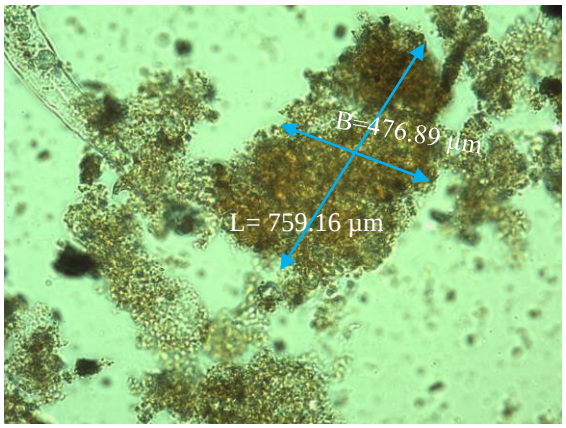

Figure 4.12: Microscopic image (20x magnification) of the sludge from reactor 2.

4.7 Organics removal (TCOD and SCOD) from reactor 1

Figure 4.13 depicts the concentration of TCOD at both influent and effluent of reactor 1. As per the graph, it can be clearly observed that the average TCOD removal efficiency was 8±2% between day 107 to 124 excluding day 114. On day 114, the removal percentage was negative which might be due to contamination of the effluent sample with some of the unsettled suspended biomass. After certain fluctuations between day 125 to 138, the removal percentage

increased to 35±5% with increase in feed TCOD up to day 170. After decrease in feed TCOD, the removal percentage also decreased and remained around 15±5 %.

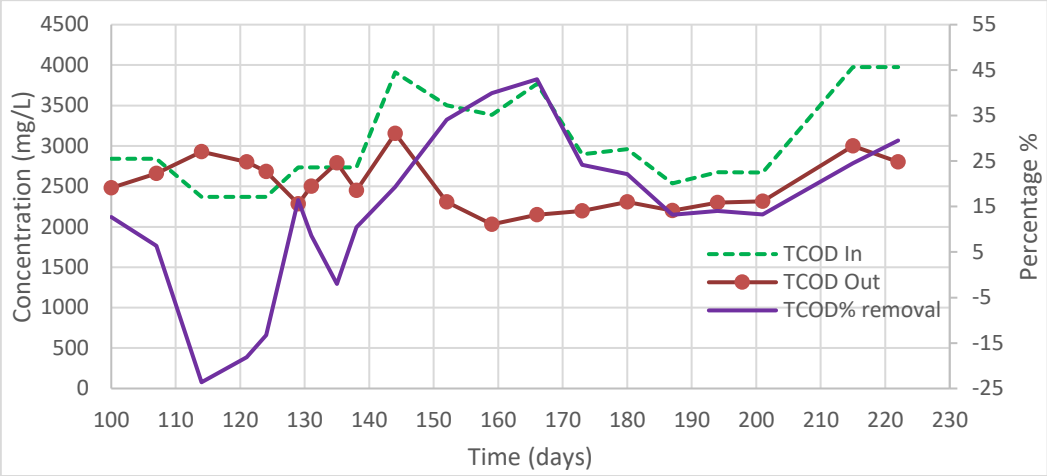

Figure 4.13: Concentration and removal efficiency of TCOD in influent and effluent of the reactor 1.

Figure 4.14 represents the concentration of SCOD at both influent and effluent of the reactor1 along with its removal efficiency. As seen from the graph, SCOD average removal efficiency was 10±2% between day 100 to 124. Afterwards, there were certain fluctuations in removal efficiency but overall SCOD removal efficiency after day 185 was around 2%.

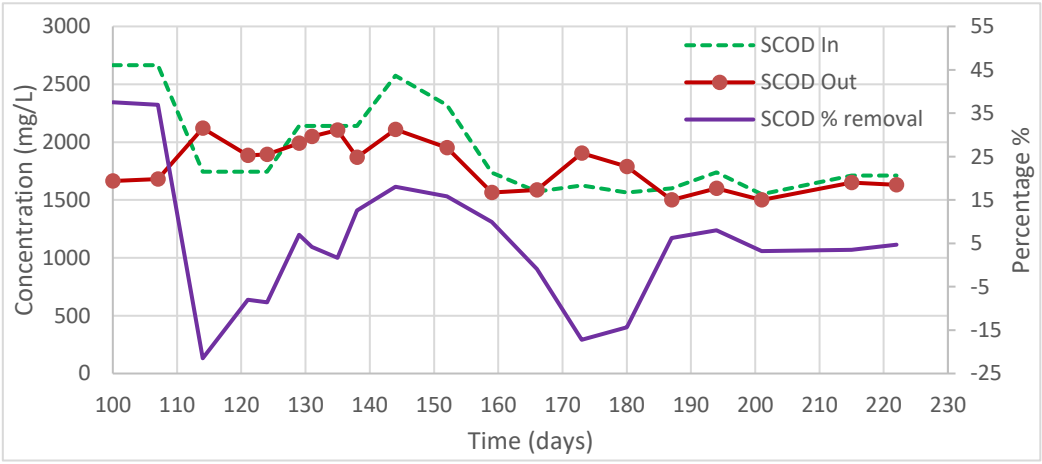

Figure 4.14: Concentration and removal efficiency of SCOD in influent and effluent of the reactor 1.

4.8 Total solid (TS) and Volatile solid (VS) removal

The outlet concentration of TS and VS was greater than the inlet concentration of TS and VS between day 107 to 124 which results in decreasing removal efficiency. During this period, the removal efficiency was negative becoming the lowest. This might be due to the weighing machine error and minute deviation from the human error and standard procedures. But thereafter, the outlet concentration started to decrease, resulting in positive removal efficiency. The maximum removal efficiency was 29% reached on day 173 whereas the average TS and VS removal efficiency was 15±10% and 5±10%, respectively excluding small fluctuations. The data link is provided in Appendix E.

4.9 Volatile suspended solid and total suspended solid ratio

Figure 4.15 shows the variations in the ratio between volatile suspended solid and total suspended solid in both influent and effluent of reactor 1. As can be seen on the graph, the average ratio of influent VSS/TSS was 0.7±0.1 excluding small fluctuations. The effluent ratio of VSS/TSS was below 1 and the average ratio was 0.8±0.2. The proportion of volatile suspended solids in the effluent was higher than in the influent concentration which indicates the growth of biomass in the reactor.

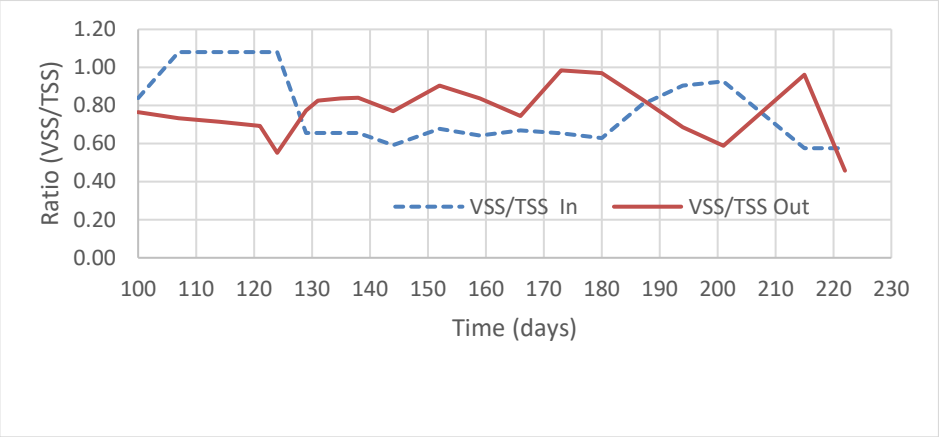

Figure 4.15: VSS/TSS ratio in the influent and effluent of reactor 1

5 Discussion

The results from chapter 4 are discussed in the following subsection under six different headings; effect of alkalinity on nitrification, effect of nitrogen loading rate, HRT, feeding sequence, effect of COD/N ratio, effect of FA and FNA, comparison of DO level between reactors, and organics removal.

5.1 Effect of alkalinity on nitrification

In both reactors from day 100 to day 138, alkalinity was 2100±400 mg/L whereas the ammonium concentration was 535±13 mg/L. Hence, comparing this amount of alkalinity with the theoretical stoichiometric value (as described in chapter 2.4.3), alkalinity in the feed was not enough for full nitrification as well as to counteract with hydrogen ion produced during oxidation of ammonium into nitrate [27]. As a result, there was a drop in pH level around 6.0±0.2 (Figure 4.2**B** and Figure 4.4**B**). The overall performance of AOB and NOB in such an acidic environment was quite low. Hence, only 60±5% ammonium was removed of which 42±2% was converted to nitrite and only 11% was converted to nitrate (Figure 4.1 and Figure 4.3).

However, from day 139, alkalinity as per the stoichiometric calculation was added to the feed. The alkalinity and ammonium concentrations were 3400±200 and 480±20 mg/L, respectively. The addition of alkalinity in the feed helped to maintain the pH value stable around 7.5±0.5 (Figure 4.2**B** and Figure 4.4**B**). This range value was suitable for nitrification [18]. Hence, 100 % of the ammonium was removed of which all 100% was converted to nitrate after tunning other parameters with this amount of alkalinity (Figure 4.1 and Figure 4.3). Hence, alkalinity was one of the limiting factors for the nitrification process in this study.

Along with neutralizing the acid, produced during the nitrification process, carbonate alkalinity also fulfills the necessity of inorganic carbon for the growth and cellular synthesis of nitrifying bacteria. Apart from the stoichiometric criterion, residual alkalinity could be needed to keep the pH level required for the complete nitrification process [27].

5.2 Effect of NLR, HRT, and feeding sequence

Before the addition of alkalinity, from day 100 to 138, the NLR was changed from 0.328 kg/m^{3}day to 0.112 kg/m^{3}day and to 0.3±0.036 kg/m^{3}day. However, the various change in NLR did not help to enhance the nitrification process due to the limited alkalinity. In addition, there was a small decrement in ammonium removal efficiency that might be due to the sudden decreased and increased in NLR making the reactor difficult to recover exactly as previous over a short period of time.

When the alkalinity was added on day 139, the reactor was in the NLR of 0.3±0.036 kg/m^{3}day. Since the ammonium concentration in the feed was constant, the NLR was only related to the HRT and the feeding sequence. This loading rate caused nitrite accumulation in the reactor (Figure 4.2 and Figure 4.4). Looking at the trend in Figure 4.2**A** and Figure 4.4**A** from day 139 to 176, nitrite accumulation in the reactor indicates that the percentage of AOB is greater than NOB in the reactor resulting in a high ammonia-oxidizing rate as compared to nitrite-oxidizing

rate [17]. Also, the growth rate of AOB bacteria like *Nitrosomonas* is higher than the growth rate of NOB bacteria like *Nitrospira* [48]. Therefore, AOB was in a strong dominant position than NOB. Hence, the low proportion of NOB bacteria could not handle such a high nitrogen loading rate and resulted in nitrite accumulation [29]. Another reason for the nitrite accumulation was the short contact time between inorganic nitrogen and the nitrifying sludge. Decrease in HRT to 1.67 days and two times feeding sequence (from day 139 to 176) led to the shorter interaction between microorganisms and wastewater resulting in a nitrite accumulation [29].

From day 177 to 222, HRT was increased to 3.34 days by decreasing the feeding sequence from two to one time per day. As a result, the nitrogen loading rate was reduced to 0.146±0.005 kg/m^3day. This longer HRT and one-time feeding sequence gave enough contact time for the interaction between feed and the nitrifying sludge. Moreover, the low NLR favored the condition for the growth of NOB. Hence, the accumulated nitrite started to convert into nitrate. As a result, on day 181, complete nitrification was achieved in reactor 1 whereas, in reactor 2, it was achieved 20 days later on day 201 (Figure 4.2**A** and Figure 4.4**A**).

5.3 Effect of COD/N ratio

Reactor 2 was given synthetic feed from day 155 to investigate the effect of the COD/N ratio by comparing with the condition when fed with the reject water. A high COD/N ratio provides a sufficient carbon source for the growth of heterotrophic bacteria which can easily outcompete autotrophic bacteria decreasing nitrification efficiency whereas a low COD/N ratio creates carbon limited condition which restricts the growth of heterotrophic bacteria [31]. However, after the synthetic feeding, no change in the result was observed. The nitrite was accumulated and the nitrate production was constant (Figure 4.4**A**) similar as it was with the reject water feed. Moreover, even with the reject water feed in reactor 1, complete nitrification was achieved on day 181. COD/N ratio in the feed was around 4. Hence, it can be concluded that the COD/N ratio of 4 was still a low ratio which did not favor the heterotrophic bacteria to outcompete autotrophic bacteria [49]. There was sufficient growth of nitrifying bacteria resulting in complete nitrification under this COD/N ratio.

5.4 Effect of free ammonia and free nitrous acid

During the early days from 100 to 139 (in both reactors), free nitrous acid concentration in the reactor was high with a maximum value of 5.45 mg/L and the pH was dropped to 5.9 while the free ammonia value was low (i.e., below 1 mg/L). FA and FNA are the unionized nitrogen forms that could inhibit the growth of AOB and NOB. NOB bacteria are more vulnerable to FA and FNA than AOB bacteria [20]. As represented by equation 2.4 and equation 2.7, FA and FNA are in equilibrium with ammonium and nitrite. With certain fluctuations in pH value, ammonium can convert into free ammonia and nitrite can convert into free nitrous acid. The inhibitory range value for both FA and FNA is mentioned in Chapter 2.4.1. Hence, from day 100 to 139, the average FNA value was 2 mg/L which is nearly equal to the upper inhibitory range. During this period, FNA may strongly affected NOB whereas AOB was affected to a lesser extent. Therefore, FNA might be one of the reasons for nitrite accumulation in the reactors during this period.

In the case of FA, reactor 1 was not affected by FA concentration. In reactor 2 (from day 185 to 199), FA concentration was higher around 100±20 mg/L and pH was around 8.6-8.8. There was nitrite accumulation in reactor 2 even after reducing the loading rate and adding enough alkalinity. Hence, higher FA might have inhibited NOB resulting in nitrite accumulation in reactor 2 during this period.

5.5 Comparison of DO level between reactors (1 and 2)

The oxygen consumption period was higher in reactor 2 than in reactor 1(Figure 4.9). The two reactors were geometrically different in terms of height and diameter (**Table 3.3**). Moreover, the amount of sludge in reactor 1 was higher than in reactor 2 (data is presented in Appendix E). Due to the higher proportion of sludge in reactor 1, the ammonium oxidation rate might be higher resulting in a shorter consumption period. Nowobilska-Majewska *et el*. (2020) found that the concentration of sludge had a greater impact on the consumption rate of oxygen [50].

In addition, oxygen transfer rate depends on the volumetric mass transfer coefficient ($k_L a$). $k_L a$ is influenced by the bioreactor's geometrical parameters. The difference in geometrical parameters results in different $k_L a$ values [51]. Hence, the oxygen transfer rate in reactor 1 might be different than in reactor 2 because of variation in $k_L a$ values, which could be the reason for DO level variations between the two reactors.

5.6 Organics removal

Organics present in the wastewater were measured in terms of TCOD and SCOD. During the early period (day 100 to 138), the average COD removal was around 10% (Figure 4.13 and Figure 4.14) and there was nitrogen gas production of 4% (Figure 4.1) which signifies some denitrification in reactor 1. However, on day 114, the COD removal percentage was negative which might be due to the washout of biomass while taking a sample. Due to the short settling time of SBR, some of the poorly settling biomass might be mixed with effluent sample resulting in deterioration of effluent sample quality [37]. These biomass contributes addition COD in the effluent concentration, sometimes, resulting in a negative removal percentage. The reactors were operated in a way to get complete nitrification. Moreover, carbon source was limited due to the low COD/N ratio of 4 [49]. Hence, the condition was much more in a favor of autotrophic bacteria. As a result, over a period, the heterotrophic bacteria were suppressed by autotrophic bacteria resulting in complete nitrification. Heterotrophic bacteria are responsible for denitrification [3]. Since they were suppressed, there was no denitrification in the later stage of operation.

6 Conclusion

The nitrification of reject water obtained from dewatering of anaerobically digested sludge containing high ammonium nitrogen (520±50) can be achieved using sequential batch reactors. Nitrification of reject water helps to avoid nitrogen loss as ammonia in the environment resulting in a concentrated form of nitrogen. In addition, it improves the stability of the reject water and becomes a high-class organic fertilizer. The findings during tuning of reactors to achieve complete nitrification are concluded here:

1. Alkalinity in the reject water was not sufficient for nitrification. Hence, enough alkalinity should be added as per stoichiometric calculation to achieve 100% ammonium conversion.
2. At the initial stage of the nitrification, nitrogen loading rate should be lower to support the growth of NOB bacteria. In this study, NLR of 0.3±0.036 kg/m^3day limited the growth of NOB bacteria resulting in nitrite accumulation whereas reduced NLR close to by half i.e., 0.14±0.006 kg/m^3day enhanced the growth of NOB resulting in complete ammonium conversion.
3. Feeding sequence significantly affected nitrification. The feeding sequence two times a day resulted in nitrite accumulation whereas one time a day resulted in complete nitrification. Because one-time feeding sequence increased the contact time between bacterial biofilms and wastewater.
4. pH around 6 caused free nitrous acid production, and pH above 8.5 caused free ammonia production resulting in NOB inhibition. The optimum pH value was 7.0-8.5.
5. There was high dissolved oxygen consumption just after the feeding which dropped from 9.63 mg/L to 1.62 mg/L in reactor 1 and from 9.75 mg/L to 0.92 mg/L in reactor 2.
6. The reject water COD/N ratio of 4 did not affect the nitrification process.
7. The volumetric exchange ratio (i.e., feeding and decanting amount) was 30% of the working volume of the reactor during the stable nitrification period. Hence, this volumetric exchange ratio was in favor of nitrification.

7 Recommendations

The sequential batch reactor can be further improved and make it more economical through the following points:

- Nitrogen loading rate should be increased by increasing the ammonium concentration in the feed without changing other parameters.
- The reaction cycle time should be further investigated by tracking the ammonium concentration in the effluent such that the feeding sequence can be increased without affecting nitrification.
- If possible, the feeding and drawing out should be made automatic. The automatic procedure will help to continue the feeding cycle in the nighttime which is not possible during manual operation.
- Big size reactor is suggested with a proper mixing mechanism such that the DO level should be monitored and control properly.
- Sludge growth should be monitored and if necessary proper amount of decanting of sludge should be planned through literature review.

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Appendices

Appendix A Master thesis description

University of South-Eastern Norway

Faculty of Technology, Natural Sciences and Maritime Sciences, Campus Porsgrunn

FMH606 Master's Thesis

Title: Enhanced digestate nitrification in sequencing batch reactors (SBR)

USN supervisor: Eshetu Janka Wakjera, Hildegunn H. Haugen and Carlos Dinamarca

External partner: Standard Bio (by research Council funded "Decarbonize project")

Task background:
Nitrification can improve the fertilizer quality and stability of the digestate coming from anaerobic digestion (AD) for biogas production based on organic wastes. The cost of such depends mainly of the efficiency of bioreactor design and operations conditions. SBRs that can select for aerobic granular sludge (GS) is the most promising option for such and therefore tested here using digestate from various sources relevant for the Decarbonize project.

Task description:
Study SBR conditions that promote desired nitrogen transformations from mainly ammonia, based on changes in physical conditions (sequences, loading rates, aeration strength etc.).

Sub tasks:

- Experimentally operate existing SBRs to monitor effects of changes in physical conditions.
- Tune the reactors to establish process limitations and safe operating ranges.
- Generate relevant experimental data for this research and establish which operational variables are required for full-scale industrial applications.
- Study the aerobic granular sludge formation through microscopic investigation, sampling for microbial genomic analysis and literature review

Student category: EET or PT students
Practical arrangements: Experimental work, analysis and theoretical work at USN.
Supervision:- As a general rule, the student is entitled to 15-20 hours of supervision. This includes necessary time for the supervisor to prepare for supervision meetings (reading material to be discussed, etc.
Is the task suitable for online students (not present at the campus)? No

Signatures:

Supervisor (date and signature): 31/1/21

Student (write clearly in all capitalized letters): SANDEEP GYAWALI

Student (date and signature): 01/20

Address: Kjølnes ring 56, NO-3918 Porsgrunn, Norway. Phone: 35 57 50 00 Fax: 35 55 75 47.

Appendix B Synthetic feed calculation

1. Calculation of NH_4^+ amount in NH_4CL

Ammonium chloride (NH_4CL) dissociates into its component ion when dissolved in water, which can be seen in equation G.1.

$$NH_4Cl \rightarrow NH_4^+ + Cl^- \quad \text{(G.1)}$$

Here, 1 mol of NH_4CL → 1 mol of NH_4^+

Molar mass of NH_4CL (MW_{NH4CL})= (14+4+35.5) g/mol = 53.5 g/mol

Molar mass of NH_4^+= 14+4= 18 g/mol

Hence, 18 g of NH_4^+ → 53.5 g of NH_4CL

18*(14/18) g of NH_4^+-N →53.5 g of NH_4CL

1 g of NH_4^+-N → 3.8214 g of NH_4CL

Therefore, 1 g of NH_4^+-N is equivalent to 3.8214 g of NH_4CL.

2. Conversion of $NaHCO_3$ to $CaCO_3$

Sodium bicarbonate ($NaHCO_3$) when dissolved in water dissociates into its following component ion:

$$NaHCO_3 \rightarrow Na^+ + HCO_3^- \quad \text{(G.2)}$$

Here, 1 mole of $NaHCO_3$ → 1 mole of HCO_3^-

Molar mass of $NaHCO_3$ (MW_{NaHCO3}) = 84.01 g/mol

Molar mass of HCO_3^- (MW_{HCO3}) = 61 g/mol

Hence, 84.01 g $NaHCO_3$ → 61 g of HCO_3^-

1 g of $NaHCO_3$ → 0.72 g of HCO_3^-

So, 1 g of $NaHCO_3$ contains 0.72 g of HCO_3^-.

We measure alkalinity in terms of $CaCO_3$ in the lab. For the synthetic feed, we add $NaHCO_3$ which contains alkalinity in the form of HCO_3^-. So, we need to find equivalent of HCO_3^- to $CaCO_3$.

Equivalent weight of HCO_3^- = 61 g/ 1 (charge) = 61 g/Eq.

Equivalent weight of $CaCO_3$ = 100/2 (oxidation state) = 50 g/Eq.

No. of Eq. HCO_3^- per liter = 0.72 (g/L)/ 61 g = 0.00118 Eq/L

Alkalinity as $CaCO_3$ = No. of Eq. HCO_3^- * Eq. wt. of $CaCO_3$

= 0.0118 (Eq/L) * 50 (g/Eq)

= 0.59 g/L

Hence, 1 g of $NaHCO_3$ is equivalent to 0.59 g of $CaCO_3$.

1 g of $CaCO_3$ is equivalent to 1.695 g of $NaHCO_3$.

Appendix C Equations

Nitrogen mass Balance :

$(NH_4^+\text{-}N)_{IN} + (NO_2^-\text{-}N)_{IN} + (NO_3^-N)_{IN} = ((NH_4^+\text{-}N)_{OUT} + (NO_2^-\text{-}N)_{OUT} + (NO_3^-N)_{OUT} + N_2$

Percentage Calculation:

Ammonium removal percentage (%) = $\frac{(NH_4^+)IN-(NH_4^+)Out}{(NH_4^+)In} x\ 100$

Nitrite production percentage (%) = $\frac{(NO_2^-)Out-(NO_2^-)In}{(NH_4^+)In} x\ 100$

Nitrate production percentage (%) = $\frac{(NO_3^-)Out-(NO_3^-)In}{(NH_4^+)In} x\ 100$

Nitrogen gas production (%) = $\frac{(N_2)produced}{(NH_4)In} x\ 100$

Nitrogen Loading rate (NLR)= $\frac{(NH_4)In}{HRT*1000}$, NH_4 in mg/L, HRT in days

Free ammonia (FA) and free nitrous acid (FNA) calculation:

$$FA\ (mg/l) = \frac{17}{14} \times \frac{NH_4^+\text{-}N\ (mg/l) \times 10^{pH}}{\frac{k_b}{k_w} + 10^{pH}}$$

$$\frac{k_b}{k_w} = e^{(6344/273+℃)}$$

$$FNA\ (mg/l) = \frac{46}{14} \times \frac{NO_2^-\text{-}N\ (mg/l)}{k_a \times 10^{pH}}$$

$$k_a = e^{(-2300/273+℃)}$$

TCOD and SCOD removal %:

$$TCOD\ removal\ \% = \frac{(TCOD)In - (TCOD)Out}{(TCOD)In} x\ 100$$

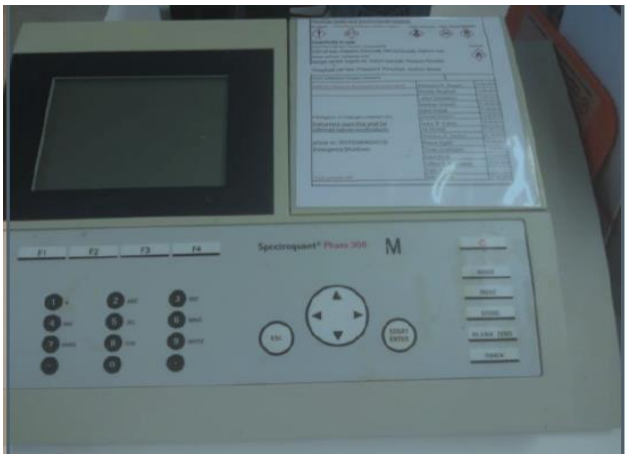
$$SCOD\ removal\ \% = \frac{(SCOD)In - (SCOD)Out}{(SCOD)In} x\ 100$$

TS and VS removal %:

$$TS\ removal\ \% = \frac{(TS)In - (TS)Out}{(TS)In} x\ 100$$

$$VS\ removal\ \% = \frac{(VS)In - (VS)Out}{(VS)In} x\ 100$$

Appendix D Laboratory instrument Pictures



A spectroquant pharo 300 used for measuring the concentrations of COD, NH_4-N, NO_2-N , NO_3-N, and alkalinity.

Thermoreactor TR620 used for heating the COD cells to 148℃.

Porcelain basins used for keeping sample for the measurement of TS, VS, TSS, VSS.

A picture indicating the procedures, and materials used for preparing the sample.

	
Oven at temperature 105℃ for drying TS, and TSS.	Muffle furnace at temperature 550℃ to ignite volatile solids.

Appendix E Measured data

Data was collected and updated in the Microsoft teams. All the safety analysis and procedures are uploaded in separate folder in Microsoft teams which can be read using the same link for the data. The link for data is provided below:

https://teams.microsoft.com/_#/school/files/General?threadId=19%3A07b707f1751041bf899b83b6e0ce383d%40thread.tacv2&ctx=channel&context=General&rootfolder=%252Fsites%252FDecarbonize%252FShared%2520Documents%252FGeneral