Novel Submerged Membrane Electro-Bioreactor-Anaerobic/Anoxic Ammonia Oxidation (SMEBR-Anammox)

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ABSTRACT

Novel submerged membrane electro-bioreactor-anaerobic/anoxic ammonia oxidation

(SMEBR-Anammox)

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Conventional membrane bioreactor (MBR) is unable to remove nutrients to an acceptable level without additional operation units. However, submerged membrane electro-bioreactor (SMEBR) featured by a compact hybrid vessel, where biological processes, membrane filtration and electrokinetic phenomena take place simultaneously, is able to increase efficiency of nutrient removal. In SMEBR, nitrification and denitrification process happen due to creating alternately aerobic and anoxic conditions by supplying adequate direct current density (CD) and dissolved oxygen (DO). In order to upgrade total nitrogen (TN) removal, the research objective was to develop a novel SMEBR-Anammox. This research was the first attempt to create simultaneously aerobic condition for nitrifiers and anoxic for denitrifiers and anammox in a sole reactor. The experiments consisted of four phases where performances of Anammox, MBR, SMEBR and SMEBR-Anammox were tested and compared. All the reactors were fed with the synthetic wastewater while mixed-liquor suspended solids (MLSS) concentrations, current densities (CD) and dissolved oxygen (DO) were the influencing factors and they underwent optimization process. The same UF membrane module, temperature, SRT and HRT were applied in all tests.

In SMEBR-Anammox system, nitrifiers transform ammonia to nitrite and then to nitrate in aerobic phase, then, denitrifying bacteria generate gas nitrogen from nitrate in an anoxic phase.

Furthermore, anammox bacteria use remaining nitrite to transform ammonia directly to nitrogen gas. Anammox (anaerobic/anoxic ammonium oxidation bacteria) was proven to be an effective method to eliminate high quantities of ammonium from wastewater; however their growth requires preserving special anoxic conditions during the entire process. Subsequently, an additional operation unit was constructed. Variation in these parameters affected the concentration of ammonia nitrogen, nitrate nitrogen, phosphorous and chemical oxygen demand (COD) in the effluent. At steady state operation, the removal efficiencies of ammonia, nitrate improvement, phosphorus and COD in the SMEBR-Anammox were 97%, 99.95%, 99.91% and 99.87%, respectively. Accordingly, phosphorous removal was due to electrocoagulation process and phosphorous accumulative organism (PAO) growth while COD removal was the result of biological processes and flocculation.

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DEDICATION

I would like to express a heartfelt thank you to my family who always encouraged and supported my educational and personal dreams and to dedicate this thesis to the special person in my life; my husband Ali. His unconditional moral support and love gave me the strength and encouragement to accomplish my goals.

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LIST OF ABBREVIATIONS

Symbols	Definitions
Anammox	Anaerobic Ammonium Oxidation
AS	Activated Sludge
AR	Aeration Rate
BOD	Biochemical Oxygen Demand
BNR	Biological Nutrient Removal
CD	Current Density
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EC	Electrical Conductivity
F/M	Food/Mass of microorganisms
HRT	Hydraulic Retention Time
MBR	Membrane Bio-Reactor
MSBR	Membrane Sequencing Batch Reactor
MLSS	Mixed Liquor Suspended Solid (mg/L)
MLVSS	Mixed Liquid Volatile Suspended Solid (mg/L)
ORP	Oxygen Reduction Potential
SMEBR	Submerged Membrane Electro-Bioreactor

1 Chapter INTRODUCTION

1.1 Background

Daily, trillions of liter wastewater containing ammonia and phosphorous are produced by hospitals, households, livestock waste, leachate, industries and detergent manufactures in Canada. They should extensively be treated before discharging to environment since ammonia, phosphorous and nitrate as nutrients have negative impacts on the aquatic lives (Eini 2012). The release of ammonia can be toxic to some forms of aquatics and can be chronically toxic for fish and plants and/or reduces reproductive capacity and may cause death (Metcalf and Eddy 1972). Moreover, the release of nitrate is a source of algal blooms and phosphorous as a principal limiting factor for eutrophication allow for unfavorable plant growth and reduces the water quality by decreasing the oxygen level in the water bodies (Eini 2012).

On the other hand, adequate amounts of nutrients are necessary for the water living organisms (Pasquini et al. 2014). The Environmental Protection Agency (US EPA) imposes some standards for all contaminants of surface water in order to control point sources, containing ditch, pipe, ship or factory smokestack, which discharge pollutants into rivers or lakes (Kang et al. 2008). Therefore, the central objectives for wastewater treatment plants are to remove nutrients such as ammonia, nitrate and phosphorous to a satisfactory level.

Designing of a contemporary wastewater treatment plant requires a high nutrient removal, low energy use, low costs and small footprint. The offered methods to remove nutrients from wastewater include: activated sludge, filtration (reverse osmosis), ultraviolent radiation, aerated lagoon, rotating biological contactor, membrane bioreactors, sequencing batch reactor, biotrickling filter and etc. (Metcalf and Eddy 1972). Pretreatment and primary treatments remove only large objects and coarse materials. However, biological treatment as a secondary treatment is applied to remove organic pollutants (Morera et al. 2016).

Nowadays, the aerobic activated sludge reactor is applied to eliminate biodegradable organics from industrial and municipal wastewater (Bae and Park 2014). This process can remove carbon over the oxidation of the organic materials by the microbial biomass (flocs). In this process, wastewater is mixed with the air in the aeration tank. Mixing should be adequate to avoid sedimentation of the microbial flocs (Ginoris et al. 2007). Activated sludge process includes aerobic and anoxic reactors in order to transfer ammonium into nitrogen gas. However, in the nitrate transferring to gas, carbon source is required in the anoxic reactor (Ginoris et al. 2007). Moreover, phosphorus removal includes microbial flocs recycling in anaerobic and aerobic zones to promote the phosphate accumulation by microorganisms. Aluminum sulfate and ferric chloride as chemicals are used to decrease the phosphorous concentration (Hreiz et al. 2015). The hydraulic retention time (HRT), sludge retention time (SRT), nitrogen and phosphorous compounds, dissolved oxygen (DO), mixed liquor suspended solids (MLSS), chemical oxygen demand (COD) and some other conditions affect the result of treating process. Furthermore, activated sludge system produces solids which could settle easily by gravity.

Membrane bioreactor (MBR) is a combination of AS treatment and micro/ultra-filtration membrane applied to separate suspended solids from the liquid (Meng et al. 2010). MBR has some benefits such as: high quality effluent, small layout size of the plant and possibility of reuse treated wastewater for irrigation purpose (Di Bella et al. 2015). Since by lowering DO concentration, nitrification is inhibited, a sufficient level of DO is required to accomplish the nitrification process. Nitrosomonas and nitrospira-like bacteria attend in ammonium (AOB) and nitrate-oxidizing bacteria (NOB). However, the absence of oxygen damages the performance of these bacteria (Radjenović et al. 2008). Despite MBR advantage, there is always membrane-fouling problem in MBR system due to organic colloids and extrapolymer substances (EPS) which decreases the performance and increases the total cost of the system (Radjenović et al. 2008).

One of the innovative electrochemical skills created technique is the electrocoagulation (EC) process (Bani-Melhem and Elektorowicz 2010). Electrocoagulation is able to remove colloidal particles, refractory organics, suspended solids, heavy metals, and soluble inorganic compound. The electrocoagulation devices can be varied from a simple anode and cathode to the multiple electrodes. EC is an extremely effective method in contaminants removal from water by direct electric current DC (Elektorowicz et al. 2009). Alternatively, submerged membrane electro-bioreactor (SMEBR) was applied to increase nutrient removal and decrease membrane fouling (Elektorowicz et al. 2009). In SMEBR system, activated sludge treatment with membrane filtration is related to an electrical direct current (DC) field in one chamber. In a simple design of SMEBR, electrodes are placed around the membrane and aluminum hydroxides are produced by dissolution of the anode. SMEBR featured by biological process, membrane filtration and electrokinetics in a single reactor has showed high COD and nutrient removal (Bani-Melhem et al. 2010 and 2011).

Another novel nutrient removal process defined as anaerobic/anoxic ammonium oxidation (Anammox) has been applied to treat ammonium from wastewater. Anammox bacteria make a shortcut in the nitrogen cycle to remove nitrogen compounds. In the same way, ammonium is directly oxidized to nitrogen gas instead of going through nitrification and denitrification. In this process nitrite perform as an electron acceptor (Ali et al. 2013). High level of ammonia removal in the lack of oxygen made it a good option comparing to the other available methods. In anammox method, high level of aeration and external organic carbon (methanol) addition which were used in conventional biological nutrient removal (BNR) process is eliminated. Since anammox process only requires the transformation of 50% of the ammonium to nitrite in the lack of oxygen, energy consumption would be decreased (Abma et al. 2007). Anammox is able to fix inorganic carbon with electrons obtained from ammonium. In this process, NO_2^- is transferred to NO and then NO reacts with ammonium (NH_4^+) through the hydrazine oxidoreductase (HZO) enzyme to produce hydrazine (N_2H_4) intermediate. Next, N_2H_4 is broken down and forms N_2. Anammox replaces the conventional denitrification stage completely saving half of the nitrification aeration costs (Jetten et al. 2001).

As it is aforementioned, SMEBR system has had high nitrogen, phosphorous and carbon removal efficiency (99% ammonia, 99% phosphorous, 92% COD) while MBR's results were not comparable (97% ammonia, 59% phosphorous, 80.4% COD) (Hasan et al. 2012). Moreover Chen et al. (2009) proved the nitrogen removal efficiency by anammox was up to 94.68%. Considering the extensive advantages of anammox application, it is a necessity to develop SMEBR processes where nitrate is produced and ammonia is transformed directly to gas nitrogen. Such SMEBR-Anammox process might lead to better removal of TN and subsequently to an excellent performance with respect to nutrient removal.

1.2 Objective

The main goal of this study is to examine the performance of submerged membrane electro bioreactor for anammox growth in order to remove ammonia and nitrate simultaneously in addition to phosphorous and carbon. Since it is the first application of anammox into such reactor, the secondary objectives are: a) conduct a comparative study between MBR, SMEBR and SMEBR-Anammox with respect to the removal of ammonia, nitrates, COD and phosphorous, b) investigate adequate operating conditions, c) define relation between operating conditions and nutrient removal, e) investigate the influencing parameters on nutrient removal; d) conduct bioaugmentation of anammox bacteria simultaneously.

2 Chapter LITERATURE REVIEW

2.1 Introduction

SMEBR process has been recommended into wastewater treatment plants recently. In SMEBR process, electrical system (electrodes, an adequate power supply and exposure time) is applied into activated sludge with membrane module, which play crucial role to treat wastewater (Bani-Melhem et al. 2010). On other hand, anammox process is a very effective method to eliminate nitrogen from wastewater while saving energy (Jetten et al. 2001). Combining of these two new systems was considered in this study in order to create a novel approach to municipal wastewater treatment. This literature review provides background knowledge with respect to treatment of wastewater. Since nitrogen removal particularly was the principal goal of this study, firstly different methods to remove it is reported then the characteristics of the studied reactors is discussed.

2.2 Ammonia removal methods

One of the major environmental concerns is ammonium removal from wastewater, thus, several methods have been considered. The most popular technology is stripping tower, beside batch single-stage electrolytic process, activated carbon, formalin, aeration/oxidation, zeolite adsorption, catalytic regeneration or biological treatment. The biological removal includes nitrification, denitrification and Anammox processes. The above methods were examined for years and each of them has its own advantages and disadvantages. Some of the most applicable methods are discussed in this section:

2.2.1 Electrolytic process

In this method, ammonium is converted to nitrogen in a single-staged batch reactor while there is no need for huge amount of current to start the process. According to this process, ammonia is oxidized to several nitrogen compounds as displayed in the following equation:

$$NH_3 \rightarrow NH_2OH_{ads} \rightarrow NO_x \rightarrow N_2 \tag{2-1}$$

In de Lima et al. (2009) study, anode was titanium covered with a layer of RuO_2+ TiO_2 while cathode was made of pure titanium. Ammonia removal was more than 99.9 wt% when the 0.68A current was applied for approximately 75 minutes. However due to current fluctuation, chlorine evolution was observed (de Lima et al. 2009). Therefore, there should be a restriction for the current level application due to chlorine gas emission, which is a strong oxidant and harmful for humans and environment.

2.2.2 Activated carbon

Activated carbon is produced by coal's heating at 900°C and exposed with an oxidizer. Then it is reformed to a lattice with a high surface area. Activated carbon's adsorption depends on the surface chemistry and pore structure of porous carbons. Many researches showed that H_2O_2, O_3 and HNO_3 increase acidic surface of activated carbon (Rivera-Utrilla et al. 2011). However, these treatments can reduce the activated carbon surface area, so the researchers tried to find alternative solutions with minor disadvantages. Rivera et al. (2011) concluded that nitrogenation or sulfuration can raise the ability of surface polarity and interplay with pollutants and heavy materials such as ammonium.

Noble metal catalysts on activated carbon can increase the removal of ammonia from wastewater by using wet air oxidation process in the liquid phase. According to Cao et al.

(2003), Pt/AC showed higher activity in comparison with RU/AC in all the temperatures whereas inductivity coupled plasma (ICP) analysis showed 0.6-4.2 mg/L of RU in the treated water. However, Pt dissolution did not happen due to high stability of Pt/AC than RU/AC. In this study, nitrate and nitrite were considered as electron acceptor. Elimination of ammonia at 200°C and pH 5.6 by Pt/AC was 88%. As a result, in specific amount of ammonia at certain pH and temperature, more than 50% of ammonia would be eliminated from highly polluted wastewater while it contains up to 1500 mg ammonia-N/L and up to 8000 mg COD/L.

AC method is mostly used for ornamental fish training by removing the percentage of ammonium in their living environment. However, activated carbon is very expensive and its formation is only appropriate for low capacity ions (Cao et al. 2003).

2.2.3 Chemical method

Formalin is a substance to remove ammonium in shrimp growing pool. Low dose of formalin is used for biofilter nitrification and microbial composition in small scale. In one study, formalin treatment consisted of six pilot scale recirculation aquaculture systems. However, this method was able to remove only 50% of ammonia (Pedersen et al. 2010). Formalin is very toxic, and kills phytoplankton, and it is not an appropriate method in comparison with other methods.

2.2.4 Aeration method

Aeration method can convert ammonium to nitrogen at high temperature. Pramanik et al. (2012) studied the methods to remove ammonium and manganese from drinking water at the same time. The biological aerated filter system showed that COD removal was increased by increasing aeration rate (AR). At the rate of 2.0 L/min, 99.3% ammonium was removed.

However, higher manganese removal (99.1%) was achieved at an AR of 0.3 L/min (dissolved oxygen = 2.94 mg/L). This method has not been tested in the industrial aquatic systems yet (Pramanik et al. 2012).

2.2.5 Ion exchange method (Zeolite)

Zeolite is a mineral used in ion exchange methods. Low price, high adsorption capacity and selectivity make zeolite (natural and synthesized) an efficient method to remove ammonium. Ammonium concentration, pH and contact time is considered during the experiments. Zeolite synthesized from fly ash by a fusion method resulted in 24.3 mg/g maximum ammonium removals (Zhang et al. 2011). Natural microwave-treated zeolite is described by pseudo-second order kinetic model. $AlSO_4$ and SiO_4 create negative charges then they react with positive charge such as Na^+, NH_4^+, Mg^{2+} and Ca^{2+}, Pb^{2+}. Therefore, this method is certainly usable in order to remove monovalents, heavy metals and toxic materials. The most considerable disadvantage of ion-exchange methods is that they cannot be used in salt water. The huge amounts of chloride (more than 19000 mg/L) do not let zeolite retain the ions while separate attained ions from resins (Maranon et al. 2006).

2.2.6 Nitrification and denitrification

Nitrification and denitrification are two important steps in biological process to convert ammonia to nitrogen gas. In nitrification process, ammonia nitrogen is converted to nitrate nitrogen and the level of nitrate in the effluent increases. In the nitrification process there are two important steps:

Firstly, *Nitrosomonas* microorganisms are responsible for converting ammonia to nitrite when O_2 is electron acceptor:

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

Then, *Nitrobacteria* transfer nitrite to nitrate:

$$2\ NO_2^- + O_2 \rightarrow 2NO_3^- \tag{2-3}$$

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \tag{2-4}$$

Therefore, complete oxidation converts ammonia to nitrate:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \tag{2-5}$$

Temperature, pH and chemical concentration in wastewater affect nitrification process (Grunditz and Dalhammar 2001). Also, when DO is high, ORP is high helping for autotrophic nitrification process.

Denitrification is defined as a step including growth of bacteria where nitrate is the terminal electron acceptor. In denitrification stage, nitrate acts as electron acceptor and nitrate is converted to nitric oxide, nitrous oxide and then nitrogen gas:

$$2NO_3^- + 4H^+ + 4e^- \rightarrow 2NO_2^- + 2H_2O \tag{2-6}$$

$$2NO_2^- + 4H^+ + 2e^- \rightarrow 2NO + 2H_2O \tag{2-7}$$

$$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O \tag{2-8}$$

$$N_2O + 2H^+ + 2e^- \rightarrow N_2 + H_2O \tag{2-9}$$

Nitrification and denitrification can use bacterium *Pseudomonas Stuszeri YZN-001* and remove 275.08 mg/L NO_3^-_N and 171.40 mg/L NO_2^-_N under suitable condition; 95% of ammonium will be eliminated in this method. In the same way, control of carbon and nitrogen ratio increases the level of ammonium removal (Zhang et al. 2011). *Acinetobacter sp. HA_2* is a newly isolated bacterium at 10°C, outline 3.03 NH_3- and 1.88 NO_3-mg /Lh for ammonium and nitrate removal (Yao et al. 2013).

2.2.7 Anammox

One of the most advanced progresses in biological wastewater treatment in recent years has been achieved through growth of anammox (anaerobic ammonium oxidation) bacteria. Mulder and his co-workers had discovered this bacteria impact in 1990s (Trigo et al. 2006). It is a microbial process which eliminates nitrogen species following the subsequent reactions:

$$NH_4^+ \rightarrow NH_3 + H \quad (2\text{-}10)$$

$$NH_3^+ + NH_2OH \rightarrow N_2H_4 + H_2O \quad (2\text{-}11)$$

$$N_2H_4 \rightarrow N_2 + 4H^+ + 4e^- \quad (2\text{-}12)$$

$$NO_2^- + 4e^- + 5H^+ \rightarrow NH_2OH + H_2O \quad (2\text{-}13)$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (2\text{-}14)$$

Anaerobic transformation of ammonium and nitrite to dinitrogen gas is defined as Anammox process. In this method, nitrifying bacteria converts ammonium to nitrite and in the second step nitrite is converted to dinitrogen while ammonium is an electron donor (Trigo et al. 2006). The Anammox process was noticed in marine and freshwater environment, and then it was studied for the BNR in wastewater treatment. Anammox process was applied to reduce operating costs and greenhouse gas emissions compared to a conventional BNR process (Jetten et al. 2001). A long biomass residence time (due to anammox slow growth rate) should be considered in designing Anammox process.

In the past, anammox was used in specific temperatures with some limitation but recently the studies have been extended to high and low temperature ranges (Lotti et al. 2014). As an example, anammox was tested in a continuous stirred tank reactor (CSTR) feed by ammonium and nitrite. At 35°C, pH of 8-8.2 and 0.4-0.6 mg/L dissolved oxygen (DO), ammonium removal efficiency was reached 79% after 46 days. After 272 days, 60% of the biomass was removed and after 511 days, sample color was significantly changed so that 90% of ammonium was removed (Chen et al. 2009).

Anammox can be found in wastewater treatment processes and in soils, such as sorption, volatilization, nitrification, denitrification process. Anammox can do complete transformation of ammonium to nitrogen gas. Shalini and Joseph (2012) indicated that in Anammox process, 100% less biodegradable organic carbon and 50% less oxygen is required rather than conventional methods. Various probable electron acceptors are nitrite, nitrate, iron III, sulfate and bicarbonate. Between these electron acceptors, nitrite is displayed as a suitable electron acceptor. Besides, ferrous iron, carboxylic acids (acetate, propionate, methylamines) and ammonium is possible electron donor which ammonium is chosen in this method (Shalini and Joseph 2012).

Anaerobically oxidizing ammonium bacteria is recognized as lithotrophs missing from nature, which is a type of bacteria *plantomycete*. Variety of anammox bacteria which has been discovered are: *anammoxidans*, *brocadia fulgida*, *kuenenia stuttgartiensis*, s*calindua brodae*, *scalindua wagneri*, *scalindua sorokinii*, *scalindua arabica*, *Jettenia asiattica* and *anammoxglobus propionicus*. *Brocadia, kuenenia* and *scalindua* are three types of anammox bacteria, which are monophyletic with some division inside the planctomycete group. Three of them are with the same metabolism and structure. Desired temperature for variable habitats is 37°C but it can be varied in environmental situations such as sediment of different places which can be reached 12°C. Possible pathways for Anammox process are (Shalini and Joseph 2012):

1- Oxidization of ammonium by hydroxylamine to form hydrazine. Then, reduction of nitrite to hydroxylamine and nitrite reduction to hydroxylamine and N_2 take place (eq.2-10 to 2-14).

2- Nitric oxide production from nitric oxide reduction. Then, produced hydrazine is oxidized to dinitrogen gas (eq. 2-14).

2.2.7.1 Description of anammox bacteria

Diameter of coccoid anammox bacteria is normally less than 1100nm and has a production time of 10-30 days. These bacteria are classified in Planctomycetes and anaerobic chemolithoautotrophs. Besides, removing anammox bacteria from the environment is very hard. Anammoxosome, which is a tight membrane-bound section, is placed in the cell bounded by a single membrane including ladderane lipids (Figure 2-1). Ladderane lipids have a strange form made of rigid lipids. Tight characteristic of membrane prevents protons to pass through the membrane. ATP is synthesized by anammox bacteria over anammoxosome membrane-bound determined by proton-motive force with the cytochrome oxidoreductase system. Also, energy metabolism takes place in extremely folded membrane of anammoxosome (Yin et al. 2015).

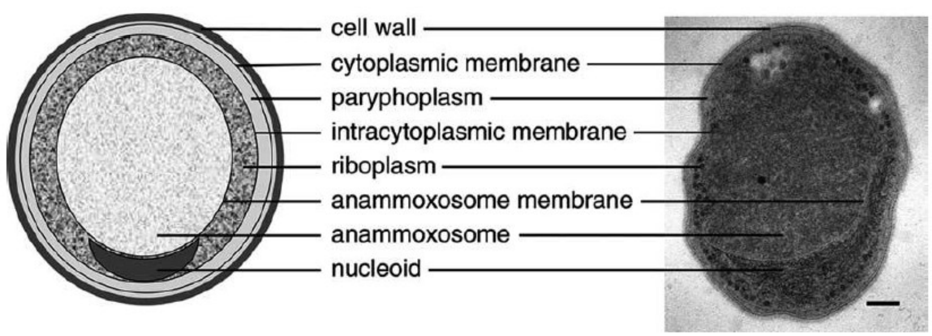


Figure 2-1. Left: schematic drawing of anammox bacteria. Right: photograph taken from a transmission electron microscopy of Cadidatus “Brocadia Anammoxidans” (van Niftrik et al. 2004)

2.2.7.2 Energy production of anammox bacteria

As it is depicted in Figure 2-2, when nitrite is reduced to hydroxylamine by an enzyme, hydroxylamine is joined to hydrazine. Then N_2 and liberates protons are formed. Proton gradient in this mechanism can produce energy in the system.

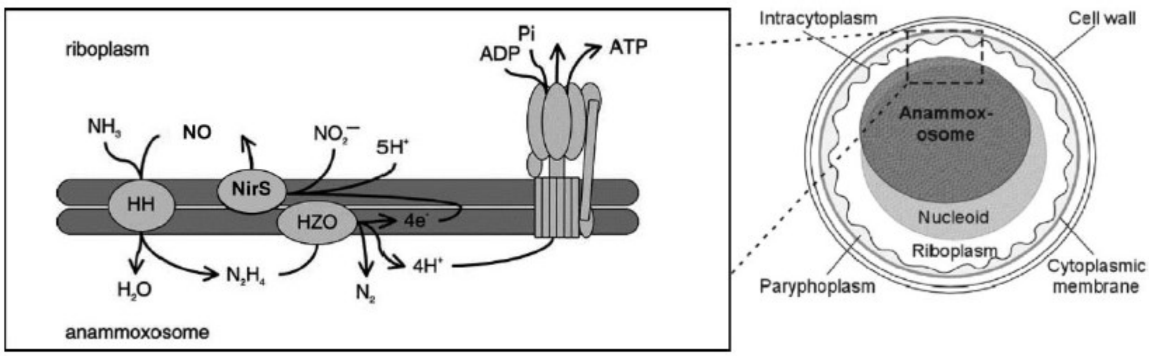


Figure 2-2. Right: Drawing of anammox cell. Left: Demonstration of the anammoxosome membrane with the enzymes involved in the Anammox process. HH: hydrazine hydrolase; HZO: hydrazine oxidizing enzyme; NIR: nitrite reducates (Brandes et al. 2007)

2.2.7.3 Anammox different sources

Sediments are the favorable place for the anammox bacteria even in the places with high or low water depth. One study proved that amount of anammox has reverse ratio with water depth. In fact, in deep sediments containing less organic compounds, total anammox importance will be decreased (Dalsgaard and Thamdrup 2002). Besides, larger distance with the inflow of water treatment plants reduce the importance of anammox because inflow consists of high amount of anammox bacteria, which flow to the river and increasing the distance from the source decreases its concentration (Trimmer et al. 2003). Another place for anammox is water columns. Anoxic basins of the Black sea, anoxic water column of Golfo Dulce in Costa Rica are the resources of anammox (Dalsgaard et al. 2003). In addition, Arctic sea ice is a place with high level of NH_4^+ where algae and detritus are degraded. To some extent, subtropical mangrove sediments and fresh water contain anammox in different levels (Meyer et al. 2005)

2.2.7.4 Anammox identification

Anammox bacteria are recognized by the following methods (Hertach 2008):

1) Incubation with $15NH_4^+$ and $15NO_3^-$

This method is used for distinguishing between denitrification and anammox which can happen simultaneously.

2) Lipid analysis (ladderane lipids)

The evidence of these lipids in the sample shows the existence of anammox bacteria. In this method the sample is filtered in large volume of water (up to 500 mL), then all lipids are extracted and separated into fatty acid and neutral lipid fractions which can be analyzed by gas chromatography (GC) which is later able to identify the ladderane lipids.

3) Fluorescence in situ hybridization (FISH)

This method is applied in different laboratories to identify the activity of microorganisms. Usually the oligonucleotide probes Amx-0820-a-A-22 and BS-820-a-A22 (MWG, Germany) are applied for anammox bacteria.

4) Phylogenetic analyses of partial 16S rRNA genes

This system is usually used for biomedical researches in the laboratories. For anammox identification, PCR is used in which the RNA of anammox is isolated and phylogenetic analysis with the maximum possible principle is performed.

2.2.7.5 Anammox process comparison with non-membrane methods

Recently, anammox has been taken in to consideration because of its lower sludge making, lower oxygen consumption, low carbon dioxide release, saving energy and satisfactory percentage of nitrogen and COD removal from wastewater. Moreover, it is a single reactor anaerobic autotrophic system, and there is no need to add extra carbon source (Siegrist et al. 2008). Since anammox does not have the extra costs for chemical addition and pre-treatment, it has been applied as a cost effective method in wastewater treatment technology (Dapena-Mora et al. 2004).

Anammox had more satisfactory effluent rather than nitrification–denitrification process (Trigo et al. 2006). Besides, autotrophic nitrifiers grow with heterotrophic biomass in nitrification-denitrification method and decrease nitrification activity but increase recycling requirement in the system and cost. However, applying anammox is associated with nitrification stage to oxidize ammonium to nitrate. Therefore, totally autotrophic nitrogen removal is attained. Another study illustrated that 90% CO_2 emissions reduction as well as 60% cost reduction was achieved using anammox instead of nitrification-denitrification reactor (Kartal et al. 2006). Besides, additional organic substance in denitrification process increases the operating cost (Trigo et al. 2006).

Slow growing bacteria increase the startup time. Anammox bacteria grow gently and their doubling time is long (Lotti et al. 2014). Besides, if the effluent washes out the sludge, biomass retention time will be decreased. According to one study in SBR reactors, anammox biomass grew without surrounding of nitrogen bubbles while the retention time was acceptable (Trigo et al. 2006). However, many researches stated that the rate of growing anammox bacteria in membrane including systems is higher than the other reactors (Tao et al.2012).

2.2.7.6 Anammox reactors

Anammox process in the systems with granular sludge such as up flow aerobic sludge blanket (UASB) has satisfactory results including low HRT and long and constant SRT. The problem of these systems is long start-up because of granular formation after passing the distributor part as well as clogging and floc forming on the pores which decrease the mass transfer. The created granules as a seed to the system can decrease the start-up time (Kartal et al. 2006).

Anammox bacteria grow in other types of reactors such as anaerobic biological filtrated reactor, continuous stirred-tank reactor, sequencing batch reactor (SBR), up-flow and biofilm reactor, biofilm reactors, fixed bed reactor, fluidized bed reactors, and gas lift reactor but anammox bacteria demonstrated a faster grow in membrane bioreactor. Ni et al. (2010) indicated that by connecting a non-woven membrane module with an anaerobic reactor the formation of Anammox was improved. The Anammox non-woven membrane reactor (ANMR) showed high biomass retention ability inside of the non-woven membrane. On the other hand, membrane sequencing batch reactor (MSBR) was tested to evaluate the anammox bacteria behavior. In this system, salt precipitation reduced nitrogen removal rate to ten times less. When the salt precipitation in the system decreased, efficiency of the system was greatly increased (Lotti et al. 2014). High energy consumption and high cost are other disadvantages of membrane in the system. The advantage of membrane is preventing wash out through the system while there is floating danger so it may be an appropriate reactor for nitrogen removal with anammox biomass.

In another type of study, nitrogen removal in SBR process was achieved to 99% efficiency (Dapena-Mora et al. 2004). High selective conditions in SBR reactor increased efficiency of the system. However, floating and wash out of colloidal sludge are the problems of SBR reactor so that shear forces relation to eliminate the negative effects was required to further studies (Dapena-Mora et al. 2004).

Various processes have been applied to culture anammox biomass (such as different seed sludge). 88% anammox bacteria were obtained in a rotating biological contactor (RBC) from treating a landfill leachate (Egli et al. 2001). Also, the culture improvement of anammox bacteria took place in the lab-scale reactor inoculated with marine sediments. Since anaerobic non-woven membrane reactor showed satisfactory results in domestic wastewater treatment, it was used to apply for anammox treatment systems (Fujii et al. 2002, Tsushima et al. 2007). One study illustrated the probability of using the SHARON (single reactor system for high-rate ammonium removal over nitrite) process in combination with anammox. SHARON was used for ammonium removal in the presence of nitrite. This system showed satisfactory results in the laboratory. Therefore, it was operated in the larger scale (2L to $1800m^3$) and removed ammonia to the lowest level. 53% of ammonium was oxidized to nitrite at 1.2 kg $N/m^3/day$. Then the effluent was transferred to an anammox SBR system and all nitrite and more than 90 percent of ammonium was removed (Jetten et al. 2001). However, this system needs two reactors to remove ammonia which requires a large footprint. This study proved that ammonium was removed in an oxygen-limited stage named CANON (completely autotrophic nitrogen removal over nitrite). Oland and deammonification are other oxygen-limited methods for one-step ammonium removal. They use the denitrification activity of conventional aerobic nitrifiers for ammonium removal (Kuai and Verstraete 1998, Helmer and Kunst 1998) while CANON is combined with anammox process. On the other hand, in laboratory-scale CANON sequencing batch reactors, comparatively low nitrogen conversion rates have been reported proving that gas-liquid mass transfer of oxygen is a limiting factor in these systems. Besides, CANON can remove ammonia more than SHARON from high-strength wastewater streams. However, nitrogen removal rate of CANON is lower than nitrification/denitrification process (Olav Sliekers et al.2003).

2.2.7.7 Anammox process inhibitors

One of the important concerns in the anammox reactor efficiency is how to supply anaerobic ammonium oxidation. Anammox bacteria in freshwater can even acclimate to salt concentrations but increasing the amount of salt in the system reduces efficiency of the bacteria. Therefore, salinity is a significant consideration for wastewater treatment (Kartal et al. 2006). Low concentrations of dissolved oxygen, organic compounds as well as nitrite or ammonium concentration are inhibitors in the system. Although nitrite plays an important role in this method, it can constrain the efficiency of the system. Successful application of anammox requires providing special conditions for i) temperature (above 20°C); ii) adequate amount of NO_2, iii) long time for growth, bio-augmentation (Trigo et al. 2006).

2.3 Membrane process

An innovative and cost effective technology for wastewater compounds' separation is membrane process. Membranes can be made of plastic, ceramic and metallic materials. Considering the pore size and way of operation, the membranes are divided in microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), electrodialysis (ED) and electro deionization (EDI). Each of these membranes has different application according to their pore sizes and separation range. For instance, UF membrane (as our chosen membrane) separation capacity is from 5 to 100 nm (Hasan et al. 2012). Membrane technologies are applied as secondary treatment in wastewater treatment plants designed side stream or inside the reactors (submerged) (Lobos et al. 2008). Figure 2-3 shows a simple scheme of a submerged membrane.

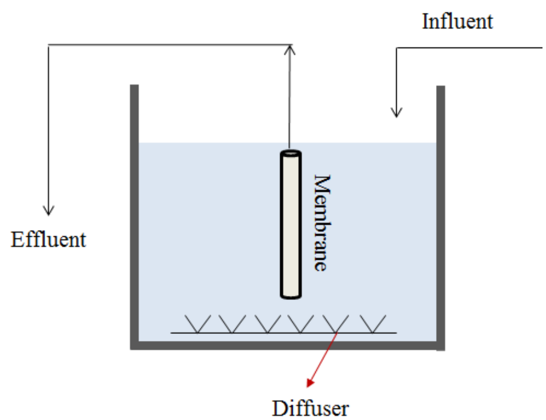


Figure 2-3. Submerged membrane with air diffusers

2.4 Activated sludge

Activated sludge is a biological process that eliminates BOD and suspended solids using suspended growth of organisms (Hreiz et al. 2015). While microorganisms grow, they form flocs (combined particles) resulting to water with the lowest amount of suspended solids or organic material (Metcalf and Eddy 1972). Activated sludge process includes production of an activated mass of bacteria, which are able to aerobic stabilization of organic materials. Formation of flocs, which contain aggregation of bacteria, is an important feature of activated sludge process. These bacteria are responsible for conversion of organic material and nutrients into water and carbon dioxide. Besides, the flocs are usually removed by gravity settling (Flemming and Wingender

2001). A carbon source as substrate is necessary to feed the microorganisms for their generation while its measurement is presented in COD (Zhang et al. 2014). In addition, removing nitrogen compounds and phosphorous and controlling the concentration of sludge require extra units in the activated sludge process (Ahn et al. 2003).

Temperature, pH, oxygen level, organic matter and wastewater toxicity affect activated sludge process efficiency (Barr et al. 1996) .On the other hand, extra-polymer substances (EPS) are organic components located at the surfaces or outside of the microbial cell surfaces that help the aggregation of cells into flocs. EPSs allow collecting enzymes and enabling the communication between cells (Laspidou and Rittmann 2002).

2.5 Electro-coagulation process (EC)

EC is one of the electrochemical processes which has been used in industrial wastewater treatment plants such as pulp and paper industries and synthetic detergent effluents (Mahesh et al. 2006). Alum [$Al_2(SO_4)_3 \cdot 18H_2O$] is a common coagulant in wastewater treatment. In coagulation process, surface charge is reduced as a consequence of decreasing repulsive potential of electrical double layer in presence of an electrolyte (Metcalf & Eddy 2003).

EC destabilizes the suspended or dissolved contaminants in an aqueous medium. It contains different mechanisms such as coagulation and flotation (Kobya et al. 2003). Electrolysis reactions around the anode area are the first step of electrocoagulation process. Since anode is made of perforated aluminum sheet, releasing Al compounds by electrocoagulation decreases zeta potential value and creates flocculation of organic sludge particles. Zeta potential is the potential at the plane of shear between the surface and solution (Greenwood 2003). Generating the bigger size flocs, better performance in comparison with electrochemical technology and decreasing the cost due to no chemical coagulants requirement, are some advantages of EC-based process (Kobya et al. 2006).

2.6 SMEBR

The electro-bioreactor is a novel technology for decreasing nutrients (nitrogen and phosphorus) in wastewater. SMEBR is a hybrid system which consists of a complete mixed bioreactor with a submerged ultrafiltration or microfiltration membrane and a system of electrodes. Combination of biodegradation, membrane filtration and electrokinetics were considered by Dr. Elektorowicz' research group, where nearly all carbon, nitrogen, and phosphorus were removed in a single reactor (Bani-Melhem et al., 2011, Ibeid 2011 and Hassan et al. 2011, Ibeid et al. 2013, 2015). Since biological, electrokinetic and membrane filtration processes taking place in SMEBR can be controlled, it can be also suitable for hard biodegradable compounds. In view of that, electro-coagulation and biodegradation are provided between anode and the cathode area while membrane filtration and biodegradation occur between the cathode and the membrane module.

SMEBR includes treating a mixture containing the wastewater and an activated sludge in a single reactor, with an electric current obtaining an adequate level of current density. This system contains at least one anode and one cathode that define an electrical zone for treating the mixture. In addition, an intermittent ON/OFF electrical exposure mode is adjusted to the electric current. Ibeid (2011) showed that with 5 min ON, 15 min OFF exposure time, SMEBR obtained better results for nutrient removal than other modes (Ibeid 2011). The aerobic activated sludge reactor is by far the most widely applied method to remove carbon over the oxidation of the organic materials by the microbial biomass.

Wastewater characteristics, biological parameters and operating conditions (a good temperature, pH and an adequate hydraulic/solid residence time or HRT/SRT, uniformly distribution of oxygen into the system, height and diameter of container, flowrate and the distance between anode and cathode) influence electro bioreactor's efficiency (Bani-Melhem and Elektorowicz 2010). Minimum space requirement and high quality effluent are the characteristics of SMEBR system (Elektorowicz et al. 2009).

SMEBR system might consist of a cylindrical polyethylene container while one membrane and activated sludge was inside of the bioreactor (Bani-Melhem and Elektorowicz, 2010). A number of air diffusers (to distribute air equally inside and outside of electrodes as well as for membrane) at the bottom of SMEBR, and two immersed circular perforated electrodes were connected to a power supply and timer to apply a direct current mode. He then proved that by connecting the current, sufficient amount of Al^{+3} was produced and nutrient removal was improved (Bani-Melhem et al 2011).

In conventional wastewater treatment systems, high energy is required to reduce nitrogen compounds level due to number of operation units. The SMEBR reduces additional units using low current density and low voltage requirement but oxygen supply to aerate the system for nitrification process can increase the costs. Removing environmental hazardous nutrients and sludge characteristic enhancement in one reactor is another advantage of this system. The operation of this system is very simple because the electrodes can be applied in parallel or circular and the membrane can be placed in the middle of electrodes or other place (Ibeid 2011).

In contrary to the conventional MBR system wherein fouling problem is an obstacle to obtain efficient nutrient removal, in the SMEBR system direct current (DC) changes the properties of mixed liquor suspended solids (MLSS), decreases membrane fouling and may enhance the removal efficiencies of nitrogen to 97% (Ibeid et al. 2013, 2015). Furthermore, when the system faces unexpected shocks, recovery of the process is faster than in MBR system (Ibeid 2011). Applying electrokinetics into wastewater decreases the plant footprints in addition to decrease fouling problem (Ibeid et al. 2015, Hasan et al. 2012). Although SMEBR is a very effective system, the sensitivity of microorganisms in this system is very high caused by applying current into the system (Bani-Melhem and Elektorowicz 2011).

Figure 2-4 shows SMEBR's schema, where a complete mixed activated sludge reactor is equipped with submerged membrane located in center and surround with perforated cathode and anode. Electrodes play an important role by applying constant current density into the system. In addition to these important parts, an appropriate aeration into the system is very essential; then, diffusors should be equally distributed inside of the reactor, since they are used for mixing wastewater and providing electron acceptors to microorganisms. In this study, anammox bacteria were applied in a submerged electro bioreactor to profit from the advantages of both processes.

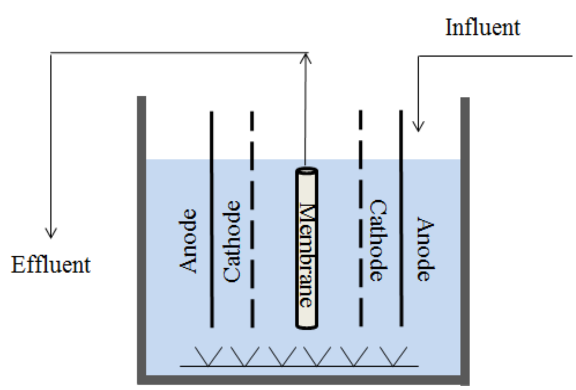


Figure 2-4. SMEBR schematic demonstration

2.6.1 Nitrification and denitrification

Nitrogen (N) removal requires sequential aerobic and anoxic biological reactions to attain complete conversion of the influent ammonium to nitrogen gas. Nitrification and denitrification process happens with biodegradation of compounds in aerobic and anoxic conditions, respectively. Firstly, in aerobic condition, nitrifiers transfer ammonia to nitrite and then to nitrate (equation 2-2 to 2-5). Next, in anoxic condition, denitrifying bacteria produce nitrogen gas (equation 2-6 to 2-9). The main advantage of SMEBR system is applying aerobic and anoxic conditions alternately. However, it needs full control of both electrical current and oxygen distribution. Carbon source is applied into the anoxic reactor to keep the heterotrophic denitrifiers responsible for transformation of nitrate into nitrogen gas, which is costly. However, nutrient removal in one single reactor is a challenging task. The mechanism of electrical process of nitrogen removal takes place in six steps (Ibeid 2015):

Step1: when direct current is activated, electrons will be released from the anode zone. These electrons react with DO to create hydroxide ions. However, DO concentration is decreased over time as long as there is current in the system because DO is consumed at the cathode surface.

Step 2: Although the entire electrons are consumed, ammonia oxidizers and nitrite oxidizer enhance the autotrophic nitrification process to convert ammonium into nitrate. When the current is still activated and the time is ON, DO level is reduced due to creating anoxic environment for denitrification process. When there is no current in the system, optimum aerobic concentration provides an appropriate situation for aerobic condition (equations 2-2 and 2-4).

Step 3: when discharged electrons react with DO and there is no sufficient DO to perform as electron acceptor in the aerobic condition, nitrate is introduced as electron acceptor while oxidation redox potential is decreased and anoxic condition is appeared. Then, heterotrophic nitrifiers will be activated and nitrate is converted to nitrogen gas.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2 \quad (2\text{-}15)$$

Step 4: in the lower oxidation reduction potential, the nitrification process can be improved. Likewise, in the lower ORP, anammox will be activated and started to nitrify the ammonium by nitrite (as electron acceptor). In the anammox process, ammonium and nitrate can be transformed to the nitrogen gas while the bacteria use an inorganic carbon source (CO_2) as substrate. Since the rate of anammox is higher than aerobic autotrophic nitrification, higher nitrification potential rather than conventional biological activated sludge will be achieved (equation 2-14).

Step 5: when the time is ON, sufficient electrons are produced to satisfy oxygen needs as electron acceptor. Next, DO is consumed and nitrate and then nitrite in anammox take this responsibility. However, when the current is deactivated and time is OFF, oxygen is recovered to support aerobic condition.

Stage 6: another pathway of nitrogen removal is the hydrogen trophic denitrification. In this step, bacteria can use hydrogen gas created at the cathode as electron donor and nitrate as electron acceptor to denitrify it into N_2 gas.

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O \quad (2\text{-}16)$$

Consequently, oxidation-reduction potential (ORP) level should be considered as an important parameter for acceptable removal of carbon, nitrogen and phosphorus from the wastewater in the single reactor. Maintaining ORP in an adequate level let the system fluctuate between the anaerobic, anoxic and aerobic conditions to create suitable operation conditions for the bacteria which participate in nitrogen transformation into gas.

Anammox bacteria are a suitable choice to be used in electro-bioreactor due to consuming less energy and reducing nitrogen level by making a shortcut in the nitrogen cycle. SMEBR system showed that 99% of ammonia was removed when the influent concentrations of ammonia were between 30 to 70 mg NH3-N/L (Elektorowicz et al. 2011 and Hasan et al. 2011).

2.6.2 Phosphorous removal

SMEBR system improves the chance of phosphorous (P) removal by electrocoagulation and deposition on electrodes (Hasan et al. 2012). When the direct current is applied, electrooxidation of the aluminum anode in SMEBR produces Al^{3+} (Mollah et al., 2004; Hasan et al., 2012), then $AlPO_4$ and Al(OH)s are produced. When the metal ions (Al^{3+}) are appeared on the anode side in the solution, they react with hydroxide ions (OH^-) which are produced on the cathode side. Then, aluminum hydroxide will be appeared according to the following reactions (Bani-Melhem et al. 2012):

at anode : $Al \rightarrow Al^{3+} + 3e^-$ (2.17)

at cathode : $3H_2O + 3e^- \rightarrow 1.5H_2 + 3OH^-$ (2.18)

in solution : $Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+$ (2.19)

The produced aluminum hydroxide is useful for the rapid adsorption of soluble phosphorus in bioreactor. Furthermore, excess aluminum ion (Al^{3+}) will react with phosphorous ions to create $AlPO_4$(s) according to the following reaction:

$Al^{3+} + PO_4^{3-} \rightarrow AlPO_4$ (2.20)

Different studies proved that nitrogen removal in one reactor (such as SBR) is possible while removing phosphorous requires additional units (Wang et al. 2009). However, SMEBR could remove phosphorous to 99% with the influent concentrations of phosphorous of between 2 to 10 mg PO_4^{-3}-P/L while there was no extra unit connected to the reactor (Hasan et al. 2011). Besides, Bani-Melhem et al. (2012) reported that electrocoagulation-submerged membrane bioreactor removed 94.3% of dissolved phosphate in raw grey water which was much higher than the submerged membrane bioreactor separately in terms of interactions with dissolved metal from the anode.

Phosphorous becomes part of suspended solids that could be recovered after solid-liquid separation by clarifier or membrane modules. In addition to electrochemical removal of phosphorous, biological removal is also expected because of the ORP changing from highly negative to highly positive range. Aluminum sulfate and ferric chloride are common P sources that are applied as replacements in the process of phosphorous removal (Hasan et al. 2011).

Biological removal of phosphorous takes place when wastewater is passing from anaerobic to aerobic conditions. Phosphorous accumulative bacteria (PAOs) are responsible for the biological phosphorus removal (Kortstee et al. 1994, BAO et al. 2007). Change in the amount of energy in the ATP battery of one phosphorus accumulating organism (PAO – an Acinetobacter is one example) cell moving from an anaerobic, VFA-rich environment, into the aerobic one. Note that in the anaerobic environment the phosphorus battery (ATP) is depleted (Polyphosphates, or Poly-P, breakdown and release orthophosphate) to give the bug energy to take up its favorite food – the VFA and convert it to storage material PHB – a fatty substance called polyhydroxybutyrate. These bugs are aerobes and they can't feed in anaerobic conditions. But they won't pass the VFA; it is just too good to pass even in the face of mortal danger of suffocation! They will fully consume (burn to CO_2) VFA later in the aerobic zone and this way gain energy or recharge their ATP battery increasing the storage of Poly-P, while depleting/oxidizing the stored PHB (Oleszkiewicz 2003)

2.6.3 Carbon removal

Oxidation of organic material by the biomass let the carbon (C) removal occurs in the SMEBR system. It seems that biodegradation is not the only pathway of carbon removal because Al releasing from anode surface and Al^{3+} production is a feature of SMEBR system in which Al^{3+} react with hydroxide ion and $Al(OH)^{2+}$, $Al(OH)^{2+}$ and $Al(OH)^{4-}$ and then more complex aluminum hydroxide compounds are produced. These cationic hydroxide complexes can adsorb negatively charged organic materials and carbon removal takes place. Dissolution of anode, discharges aluminum ions to wastewater, converts them to aluminum complexes (aluminum hydroxide long chains) and provides suitable conditions for phosphorous and carbon removal. Long chain of aluminum hydroxide with positive charge can adsorb the colloids and other organic compounds that have negative charge (Bani-Melhem and Elektorowicz 2011). For better performance of microbial activities, current density should be as low as possible, and also the time exposer mode (time OFF) should be as long as possible. Hasan et al. (2011) proved that SMEBR system can remove 92% of COD when the influent concentration of COD was 160 to 700 mg/L.

Generally in the systems including activated sludge, SRT control, the concentration of MLSS and HRT define the organic loading. Besides, long and short SRT affects the propensity of membrane fouling through changing sludge properties. In conventional activated sludge treatment, short SRT is applied to avoid accumulation of biomass (MLSS) and to fasten solid-liquid separation. Although long SRT in membrane bioreactors reduce sludge production and increases the concentration of MLSS to a dangerous level. Ibeid (2011) showed that the lower the organic loading the longer the SRT without fouling problem. In addition, the organic loading is directly related to the substrate concentration in the wastewater and indirectly to HRT.

3 Chapter EXPERIMENTAL WORK

Experimental work was conducted in four phases over 11 months (Figure 3-1). Anammox culture was developed during first phase in a separate reactor to reach acceptable ammonia removal. Phase 2 was conducted using a conventional membrane bio-reactor (MBR). Tests in phase 3 considered a design of the submerged membrane electro bioreactor (SMEBR). In phase 4 a novel design was investigated where SMEBR was redesigned to accommodate anammox.

Variable concentration of DO was applied in each phase. Nutrient removal efficiency in each phase was related to DO level and operating conditions, while temperature, ammonia (as ammonia nitrogen (NH_3-N)), nitrate (as nitrate nitrogen (NO_3-N)), dissolved oxygen (DO), oxidation reduction potential (ORP), pH and electrical conductivity (EC) were measured daily. The concentration of COD, phosphorous (as PO_4-P) and MLSS were measured once a week. Total nitrogen was measured whenever the ammonia and nitrate results were acceptable and MLSS was measured periodically in order to control biological process.

The main purpose of this study was to compare the capability of different systems for removal of COD and nutrients (nitrogen and phosphorous). Ammonia, nitrate and total nitrogen were the characteristics of nitrogen in the influent and effluent in the system.

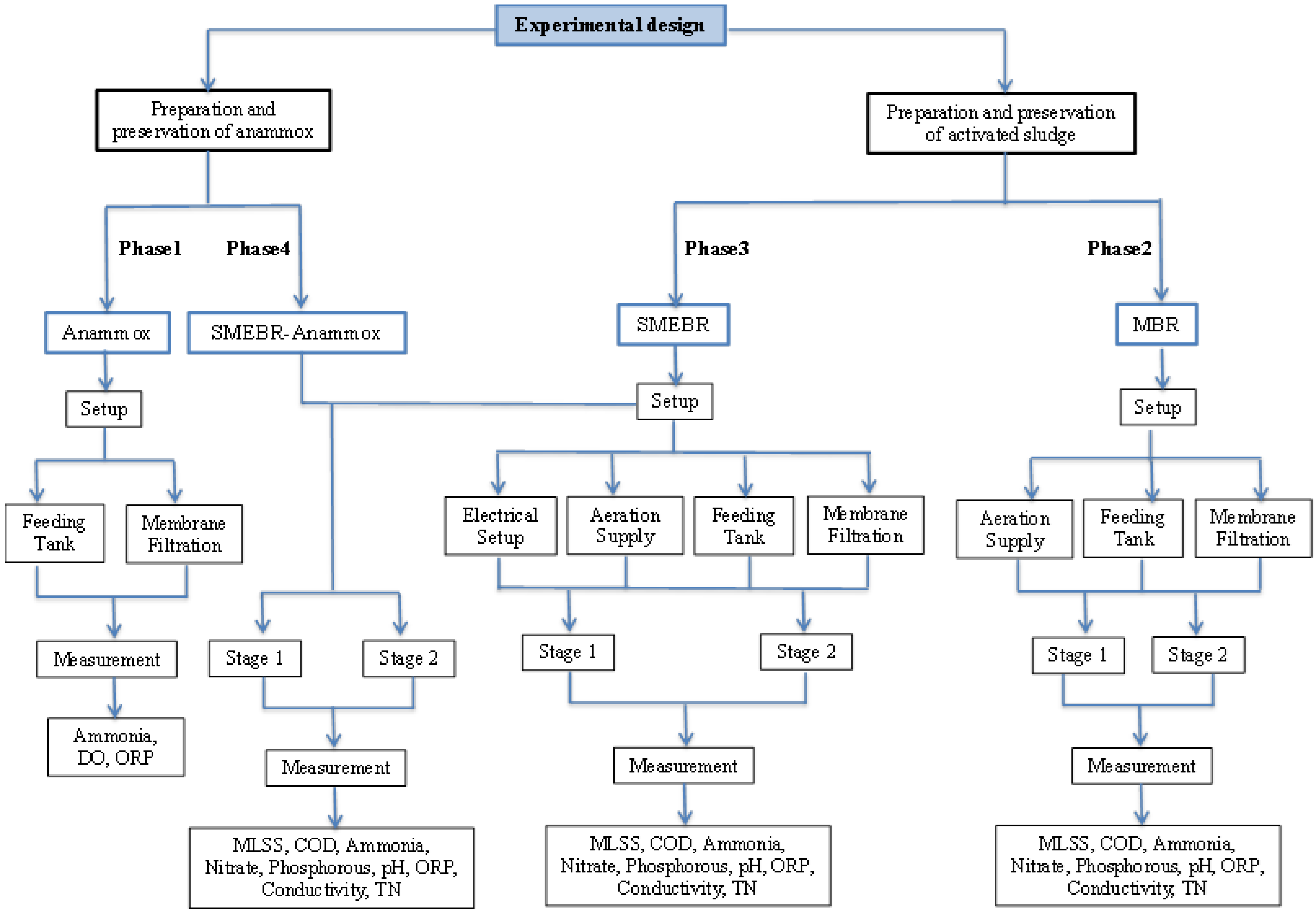


Figure 3-1. Work plan

3.1 Design of reactors

3.1.1 Phase 1: Anammox growth system

The objective of first phase was to conduct the augmentation of anammox culture using a bioreactor with 18 L effective volume fed with synthetic wastewater (Table 3-1) directly from a 40L feed tank. The content of ammonia was systematically increased in fed wastewater, in order to assess the capacity of the reactor in ammonia removal (converting to the nitrogen gas). The 18L-bioreactor stirred gently at 130-rpm, was appropriately subjected for anammox conditions (Figure 3-4a). For solid/liquid separation, an ultrafiltration ZeeWeed-1 (GE, Canada) membrane with a nominal pore size of 0.01 μm and 0.047 m^2 of filtration area was submerged in the bioreactor. No aeration was provided and NO_2 served as electron acceptor. The results of these investigations permitted to assess an adequate ammonia concentration in fed wastewater in next phases.

Table 3-1. Synthetic wastewater composition in first phase

Component	Concentration (mg/L)
Ammonium sulfate	200
Glucose	250
Potassium bicarbonate	200
Sodium nitrite	375
Potassium phosphate	50

3.1.2 Phase 2: MBR configuration

MBR system contained a membrane (ZeeWeed-1), feeding tank, pump, air diffusors, and effluent collecting tank. MBR system consisted of 2 stages: stage 1 included sludge with low MLSS and high concentration of DO. Stage 2 included sludge with higher MLSS and variable DO concentration. In the MBR system, air diffusors were responsible for mixing wastewater, providing oxygen as an electron acceptor for biological process, and preventing membrane fouling. Fouling is the main problem of this process so the membranes regularly as required to maintain a constant flowrate.

3.1.3 Phase 3: SMEBR configuration

The SMEBR included a hybrid reactor where biological processes (biodegradation), membrane processes (membrane filtration) and electrokinetic phenomena took place. The electrokinetic phenomenon was initiated due to electrical system consisting of two cylindrical perforated electrodes: an aluminum anode and stainless steel mesh as a cathode. The airflow rates were measured using Gilmont flowmeters (Model GV-2100). Electrical field by DC current has some advantages for SMEBR system: partially oxidizing organic compounds, helping to reduce the amount of sludge on the membrane as a cake layer, electrocoagulation, and oxidizing anode. Anode's material is an important factor in the electrokinetic process because releasing of the aluminum ions from the anode. These ions attend in the biological reactor to enhance phosphorous removal efficiency. During the experiment, the aluminum perforated anode had to be changed each month due to its dissolution. In the submerged membrane system, the cathode has never been changed. The composition of applied synthetic is given in Table 3-2. The concentration of ammonia was assessed based on the phase 1 – results from Anammox-reactor. Therefore, its amount was higher than usual concentration in the municipal wastewater.

Table 3-2. Composition of synthetic wastewater served in phases 2, 3 and 4

Component	**Concentration (mg/L)**
Glucose	420
Ammonium sulfate	140
Potassium phosphate	37
Magnesium sulfate	40
Manganese sulfate monohydrate	40
Iron sulfate	0.4
Calcium chloride	4
Potassium chloride	25
Sodium bicarbonate	25

Phosphorous source is from potassium phosphate (KH_2PO_4) which was added in wastewater to keep phosphorous influent around 23 mg/L. However, this amount was not constant for the whole experiment due to chemical mixing, error or its consumption in feed tank during the period (up to 6 days) between wastewater preparation (22 mg/L-18mg/L). Bacteria need carbon source in addition to other substrates to survive while carbon source is provided from glucose. 420 mg/L of glucose was added to the wastewater to increase influent COD to a specific level.

All of these compounds were mixed into 100L- tank and fed the microbial culture in the reactor. Effluents of SMEBR were removed by periplasmic suction pumps (Master flex, Cole-Parmer, USA).

The same type of membrane module for MBR was located at the center of the reactor to provide filtration and pump out the effluent. The SMEBR system is a complete mix reactor with submerged electrodes and membranes. The treated water was continuously filtered out through the membrane. Compressed air was injected into the bioreactor through 10 fine air diffusers, which were placed at the bottom of the container. To control of air and pressure in the system, an air flow meter and pressure regulator were used. In our experiment, hallow fiber (UF) membrane as an UF membrane was used (Figure 3-2). A pack of HF membranes were connected and the water was passed through the membrane by a pump for removing the effluent.

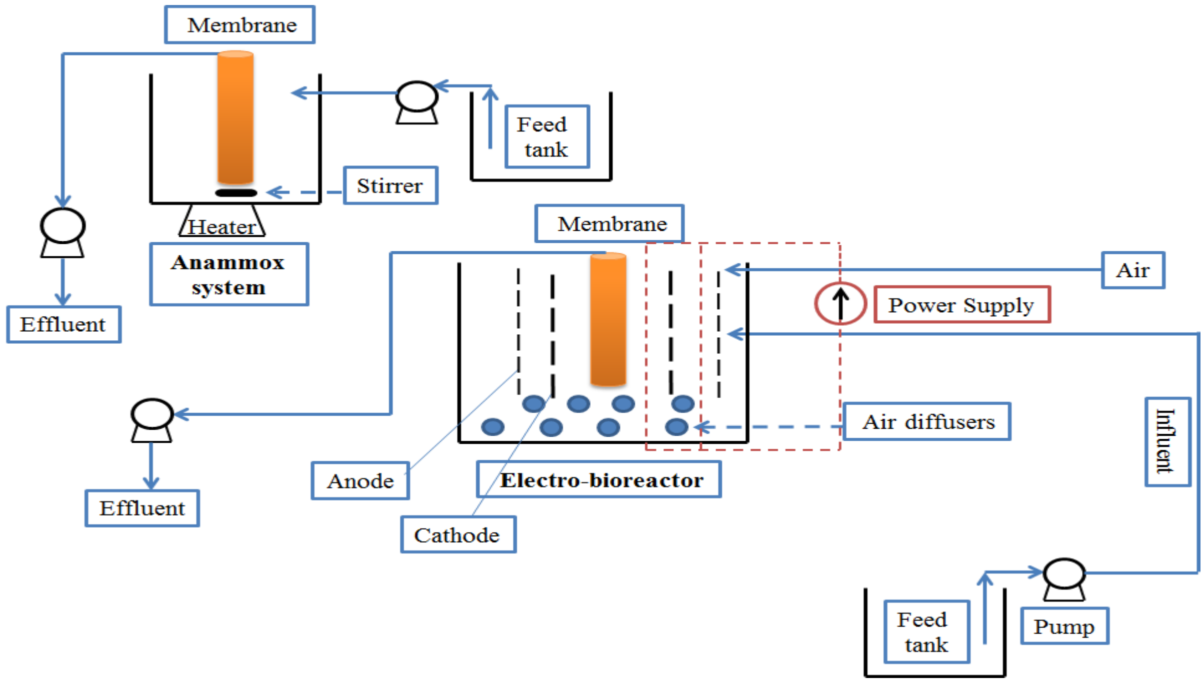
Figure 3-2. Laboratory used microfiltration membrane

The sacrificial anode was depleted over time because of dissolution. It was changed once a month. Air diffusors' ability was decreased through time, so it was required to be cleaned or changed to prevent the aeration reduction in the system. Furthermore, SMEBR system included two stages with different sludge. Although first and second stages had the same designs, nutrient and COD removal in these stages were totally different. It shows that little operational changes may affect the results. For instance, in the SMEBR first stage, after decreasing DO concentration, the bacteria lost their activity and the results were deteriorated. Therefore, in the stage 2, SMEBR was inoculated with a fresh sludge sample taken from activated sludge reactor at the wastewater treatment plant. Stage 1 included lower MLSS and lower DO variation than stage 2.

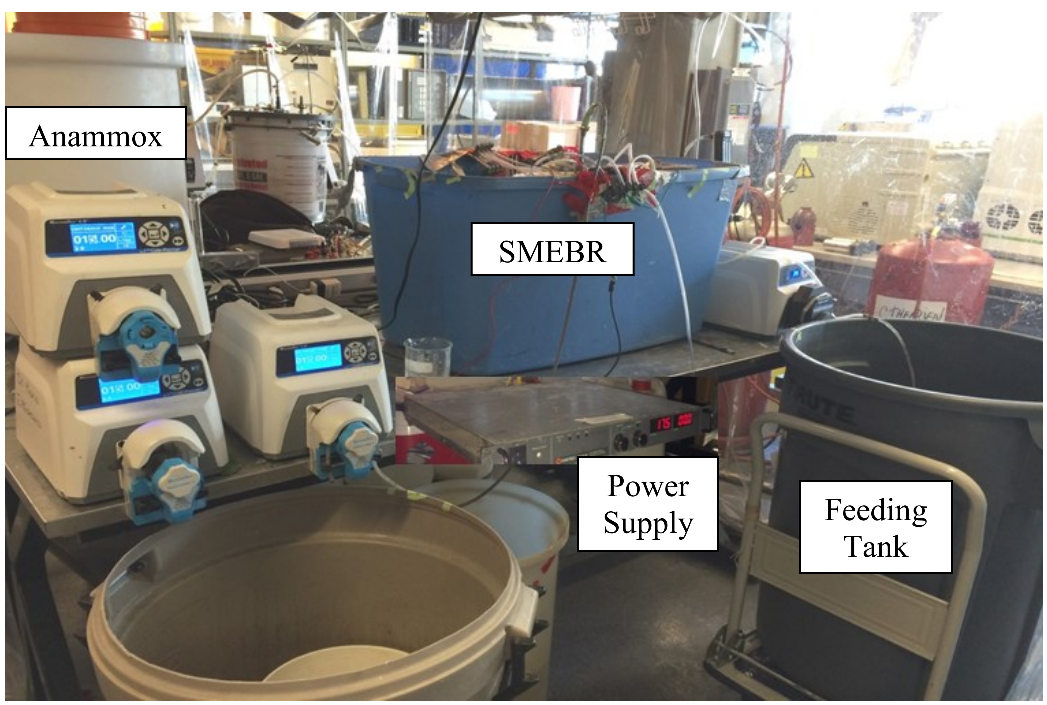
3.1.4 Phase 4: SMEBR-Anammox configuration

The system used in phase 4 was novel and never tested before. This system included SMEBR's components (Chap. 3.1.3); however, treatment of wastewater was expected to increase due to presence of an additional culture, anammox, which required new operating conditions. The composition of wastewater and flowrate, anode and cathode materials, as well as applied SRT, HRT, and exposure mode were similar to SMEBR. However, the MLSS concentration, current density, the number of air distributors, and the level of aeration were different, just adapted to anammox bacteria. The value of CD increased in phase 4 by anammox addition and voltage adjustment. Increasing the voltage could increase the current but it might negatively shock the bacteria. Moreover, the power of the current density defines the level of Al^{+3} and electrons created into the reactor and the level of hydrogen gas formed in cathode (Hasan et al. 2012). However, all the current cannot continuously induce to the system because microorganisms needs to be recovered in the time OFF when there is no current in the system. There are different exposure modes with 5'ON/5'OFF, 5'ON/10'OFF, 5'ON/15'OFF, 5'ON/20'OFF and each of them has different performance in the system. One of these exposure modes should be chosen to achieve appropriate results. Thus, change in each timer will affect the amount of Al^{+3}, electrons and H_2 gas production in cathode (reactions 2-17 to 2-19) (Ibeid et al. 2013).

This system was divided into two stages. In the first stage, 9L of wastewater were taken from a reactor providing augmentation of anammox (phase 1) for 10 months. Then, it was added to 5L of the sludge contained in the SMEBR system. After 3 weeks of operation, in stage 2, the additional amount of Anammox-reactor content was added to SMEBR-Anammox, then, it contained entirely sludge from Anammox-reactor, but now run under electrical field. Two more air distributors than SMEBR system was equipped to provide equal aeration because of decreasing DO concentration in SMEBR-Anammox system to prepare appropriate condition for anammox bacteria activity. The two 2 stages of SMEBR-Anammox system had different design and operational condition. Stage 1 included changing the culture of the system from SMEBR to SMEBR-Anammox. Also, MLSS alteration by anammox addition to SMEBR system was another characteristic of stage 1 of phase 4. In stage 2 of phase 4, the culture of reactor was changed by adding the whole amount of Anammox-reactor to the SMEBR system; therefore, different CD and MLSS were observed. Besides, in the last step the design of system was also improved and electron circuit was controlled. The schematic map and testing facility of SMEBR-Anammox in laboratory are illustrated in Figure 3-3.



(a)



(b)

Figure 3-3. (a) Schematic diagram of SMEBR-Anammox system (b) Laboratory facility

3.2 Operating conditions

3.2.1 Phase 2. MBR

MBR system was built in a continuous flow reactor of 14L effective volume (Fig. 3-4 b). It was fed with synthetic wastewater (Table 3-2) inoculated with activated sludge brought from WWTP of St. Hyacinthe, QC. Sludge initial concentration was around 3500-4000mg/L. MBR system operated with 4 to around 7 mg DO/L HRT and SRT were 24 h and 20 d, respectively and the effluent flowrate was 14L/d. In the first stages by decreasing DO to less than 4 mg/L and fouling problem (MLSS less than 3000 mg/L) there was low percentage of nutrient removal. However in stage 2, new sludge addition, MLSS increasing to around 3000 mg/L and control of fouling, COD and nutrient removal were improved. These operating condition variations are presented in Table 3-3.

Table 3-3. Operational conditions in stage 1 and stage 2 of phase 2

Operational condition	Stage 1	Stage 2
DO (mg/L)	4.09-6.77	4.68-7.02
MLSS (mg/L)	2500-3000	3000

3.2.2 Phase 3. SMEBR

SMEBR was built in the same continuous flow reactor as MBR was (Fig. 3-4 c). The same type of membrane was also used. HRT and SRT were similar to MBR i.e. 24 hours and 20 days, respectively. However, SMEBR was operated under around 0.5 to 3.5 mg DO/L to provide a suitable condition to SMEBR in order to satisfy simultaneous nitrification and denitrification conditions. The electrical system consisting of the cylindrical perforated anode and cathode was located in center of the reactor around the membrane. Intermittent DC at the beginning was 14.5A/m^2 current density. However, DC level was not constant during days and months for some reasons: firstly, anode part was gradually changed because of clogging; therefore for each time making new anode, there was a few percent error in height or diameter of anode. Also, different anodes with different size of openings were used so that it was impossible to make two similar anodes. Thus, in each time, anode surface area was varied. Secondly, according to fouling or decreasing the level of sludge in the system, current was changed. In the same way, the current level has a direct relationship with MLSS or level of sludge in the reactor. Thus, current will not be the same over the time and it even reached less than 10 A/m^2 during the SMEBR operation. In stage 1 of phase 3, CD reached 10.97 while in stage 1 in the last days of operation it reached 8.96 (it was around 14.5 A/m^2 in the first days of operation). In addition, sludge retention time (SRT) (20 days), hydraulic retention time (HRT) (24 hours), MLSS (3000-4000 in stage 1 and 2), air supply and its intensity were operational parameters. The influencing parameters in phase 3 are displayed in Table 3-4.

Table 3-4. Operating condition in stage 1 and 2 of phase 3

Operational condition	**Stage 1**	**Stage 2**
DO(mg/L)	2.78-4.5	0.5-4
MLSS (mg/L)	3000	4000
CD (A/m^2)	mostly around 9	10.97-13

3.2.3 Phase 4. SMEBR-Anammox

SMEBR-Anammox system had a design similar to SMEBR (Fig. 3-4d), however, some conditions such as CD, MLSS, design and DO concentration were different. The current was the same as in the third phase. It was provided by power supply by changing the voltage. Mode of operation was 5´ ON and 15´ OFF. Both stages of phase 4 had the same operation conditions. Furthermore, in stage 1, MLSS reached 4500 mg/L while in stage 2 it was 5000 mg/L. Besides, current density in stage 1 was around 13 A/m^2 and it reached 14.5 A/m^2 in stage 2 (Table 3-5).

Table 3-5. Operating condition in stage 1 and 2 of phase 4

Operating condition	**Stage 1**	**Stage 2**
DO (mg/L)	3.5-0.9	mostly around 0.7
MLSS (mg/L)	mostly 4500	mostly 5000
CD (A/m^2)	13-14	14-14.5

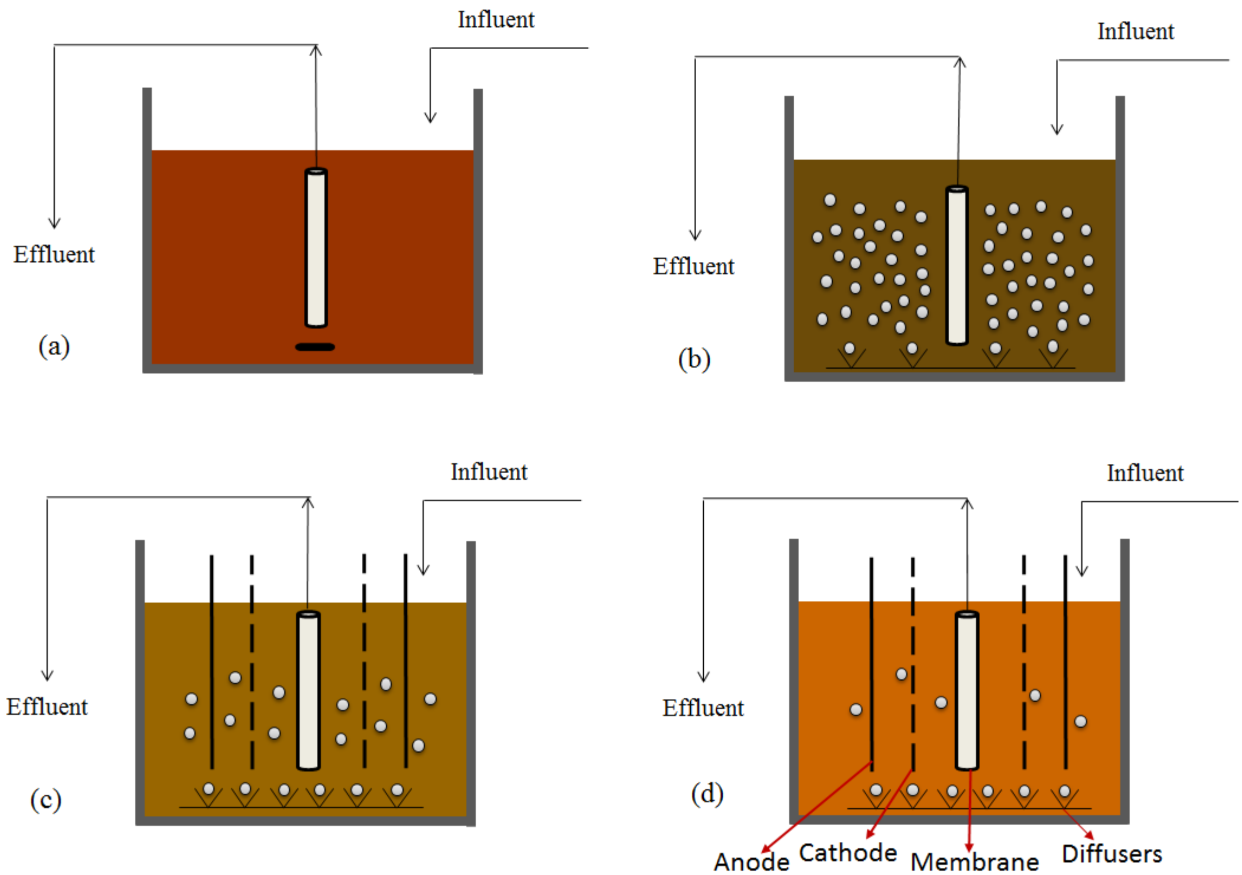


Figure 3-4. Laboratory design of a) Anammox (Phase1), b) MBR (Phase 2), c) SMEBR (Phase 3), d) SMEBR-Anammox (Phase 4)

3.3 Fouling prevention

To decrease the effects of fouling, the membrane in all reactors was cleaned every day. Through cleaning, the membrane module was removed from the reactor and washed with tap water to remove the sludge cake particles on the surface of membrane. However, the membrane was cleaned with a bleach solution once a week to remove bioflocs attached into the membrane module.

3.4 Activated sludge in different phases

SMEBR and MBR system used the same amount of activated sludge in the reactor but this amount was changed over the time to reach the appropriate amount of MLSS. Firstly, 13 L of sludge was prepared (6 L of activated sludge and 7 L water). However, when the results were not satisfying, 7 L of activated sludge was used, so the volume of sludge in the reactor was increased to 14 L. This amount in phase 4 (SMEBR-Anammox) was changed to 5 L of sludge in the SMEBR system and 9 L sludge with anammox. The MLSS level was randomly checked in SMEBR system. The preserved sludge in refrigerator had MLSS concentration around 7500-8000 mg/L. To use this sludge, it was aerated for one day and then applied into the system. After mixing with 7 L of water, MLSS reached around 3000 mg/L while this level was increased again during the experiment to around 4000 mg/L in order to achieve better result at low amount of DO.

3.5 SRT, HRT

The SRT is a well-known and usable functional factor for the activated-sludge process (Pollice et al. 2002). In this study, SRT was kept constant at the level of 20 days. HRT which is defined as reactor volume to flowrate was 24 hours. SRT affects the efficiency of reactors, so the systems had to be monitored in terms of decreasing or increasing SRT value. SRT and HRT are interrelated. If the reactor volume decreases, SRT and HRT are decreased in the same MLSS (Han et al. 2005).

3.6 Regular maintenance

The current density was controlled every day because of the biological and physicochemical phenomena in the reactor. Contact points of electrodes to power supply were also required a regular maintenance. In addition, the tubes connections with pumps and in the reactor were controlled daily to prevent clogging. Overall, the systems in all phases needed day-to-day monitoring to avoid irregularities.

3.7 Daily measurements

3.7.1 pH measurements

The pH value of the influent and effluent of all reactors was kept around 6-8 because of optimal conditions for microorganisms' growth. This parameter was measured by pH probe (HQ30d, multi- parameters meter, Hach, SA) by inserting the probe inside the influent and effluent sample. Before and after using the probes in each sample, it was rinsed with deionized water. Each pH probes needed a short time to be stabilized. Similar to all probes, the results were reported as an average mean of several tries.

3.7.2 Electrical conductivity

Electrical conductivity in wastewater is mostly related to the amount of dissolved ionic species or in other word the ability of conveying electrical current (Morrison et al. 2001). Conductivity was measured using a probe (IntelliCAL™ CDC401 Standard Conductivity Electrode, Hach) inside of the WAS sample. The result was appeared right after standing probe inside the solution. Thus, the tests required to be repeated to achieve the accurate results.

3.7.3 Dissolved oxygen (DO)

The most important parameter in this study was dissolved oxygen (DO) since its concentration was crucial for various microorganisms. When DO increases, the system is in aerobic condition and when it decreases it may change to anaerobic or anoxic condition. Subsequently, in each phase, different DO was supplied. In this study, the lowest level of DO was preserved for SMEBR-Anammox system, while the highest level was for MBR. DO concentration was measured by DO probe (IntelliCAL™ LDO101 Standard Luminescent Dissolved Oxygen electrode) by holding the probe inside the reactor. In the SMEBR and SMEBR-Anammox system, DO was measured in different times to figure out the relationship between DO and current ON and OFF. The concentration of DO was continuously adjusted in each reactor. At high concentration of DO, nitrification took place and nitrate level in the effluent increased. In 3 different reactors, DO showed variable behaviors. Although in SMEBR system DO was controlled by aeration supply, this level could be decreased or increased with or without current existence in the system. For example, when the current was ON, it seemed that denitrification happened because DO and ORP were decreased, but when the timer was OFF and there was no current in the reactor, DO and ORP levels were increased.

There should be some restriction for DO level to avoid producing much variable amount of ORP in to the system**.** DO decreasing with connecting the current is because of DO consuming by discharged electrons which were activated (Elektorowicz et al. 2013).

3.7.4 ORP

Oxygen reduction potential (ORP) has a direct relationship with DO (Elektorowicz et al. 2013). Likewise, ORP illustrates the accuracy of dissolved oxygen probe (Schön et al. 1993).

There are different probes to measure ORP but the one that was used in this study is IntelliCAL™ MTC101 Standard Gel-Filled ORP electrode (HACH). It should be stood inside the reactors to show the result continuously. In SMEBR system, it has to be between anode and wall of the container. If it stands between anode and cathode or inside of the cathode, the results are not acceptable. ORP can vary between low minus and high positive range depends on aerobic or anoxic condition. Similarly, in the SMEBR and SMEBR Anammox, DO and ORP were measured over time with or without electricity in the system. It was observed that ORP probe shows better results when it is kept in the reactor for several hours.

Nitrification and denitrification process are necessary to convert ammonia to nitrate and nitrite and at the end producing nitrogen by denitrification process. Thus, ORP of the system should be changed from low negative to high positive level. Low level of ORP happened when the timer was ON and sufficient electron was produced. In the time OFF this amount of oxygen was recovered and nitrogen gas was produced.

3.7.5 Ammonia nitrogen and nitrate nitrogen

Ammonia nitrogen and nitrate nitrogen were measured in influent and effluent in two different ways: using the chemical vials (TNT832, HR for ammonia nitrogen and TNT 835 LR for nitrate nitrogen). According to the measurements, these vials are varied for high range and low range measurements. The ammonium and nitrate electrodes can be also measured with the second method in which calibration is the principal factor. For calibration, three samples of 1, 10, 100 mg/L of ammonia nitrogen and nitrate nitrogen standard solution and a specific ionic strength adjustor (ISA) for each of them are needed. Besides, they were compared with the first method's results (chemical vials) to ensure the accuracy of standard solution and to verify the membrane functionality on the top of each probe.

3.8 Weekly measurements

3.8.1 Total nitrogen (TN)

TN was measured to achieve the overall amount of nitrogen composites. This measurement was only for phase 3 and phase 4 (SMEBR and SMEBR- Anammox system, respectively) in the best outcome, when a constant satisfied amount of ammonia nitrogen and nitrate nitrogen removal happened. The analysis was done based on TNT plus vials (Hach USA).

3.8.2 Phosphorus

There was always a need for weekly measurement to ensure the system's performance with respect to phosphorous removal. TNT Hach method (method 8178, USA) was applied to measure the orthophosphate-phosphorous concentration in effluent samples.

3.8.3. Chemical oxygen demand (COD)

COD analysis was used to investigate the level of organic compounds in water. Hatch vials (TNT 822, 20–1500 mg/L) measured COD content. If the level of COD in the reactor dropped too low, glucose as a carbon source was used to increases its level. Influent and effluent results comparison illustrated the COD removal efficiency and reactor performance.

4 Chapter RESULTS AND DISSCUSION

The results from the four phases were successfully recorded during the experiments. Each measurement is separately discussed including the results of current density, electrical conductivity, pH, DO, ORP, COD and nutrient removal. Moreover, the relationship between operational condition variation and nutrient removal is also deliberated in this chapter.

4.1 COD removal

COD (chemical oxygen demand) is one of the significant factors illustrating the mass of organic carbon in wastewater. COD removal explains the ability of three systems (MBR, SMEBR and SMEBR-Anammox) to biodegradate organic compounds. Carbon can be removed with biodegradation and flocculation in activated sludge process. Aluminum ions in anode area are transformed to aluminum hydroxide and then to more complex hydroxide chains. Organic compounds are adsorbed on these chains.

SMEBR-Anammox and SMEBR had better removal of COD than MBR. COD removal in SMEBR was performed in two stages with different MLSS. In stage 2, SMEBR was inoculated with a fresh sludge sample taken from activated sludge reactor in the wastewater treatment plant. The best results in MBR system was around 91.3% while in the SMEBR system these results reached 93.9%. In phase 4, anammox presence in SMEBR system increased the removal efficiency to 99.87%. High removal efficiency illustrates the good control of pH, temperature and electrical conditions in novel system, which allow for biomass growth. In addition, the differences between minimum and maximum COD input were due to incomplete dissolution of carbon sources in synthetic wastewater. According to Figure 4-1, maximum and minimum COD removal in MBR design were 91.3% and 69.91% in days 45 and 31, respectively. A good removal required an acclamation period for bacteria.

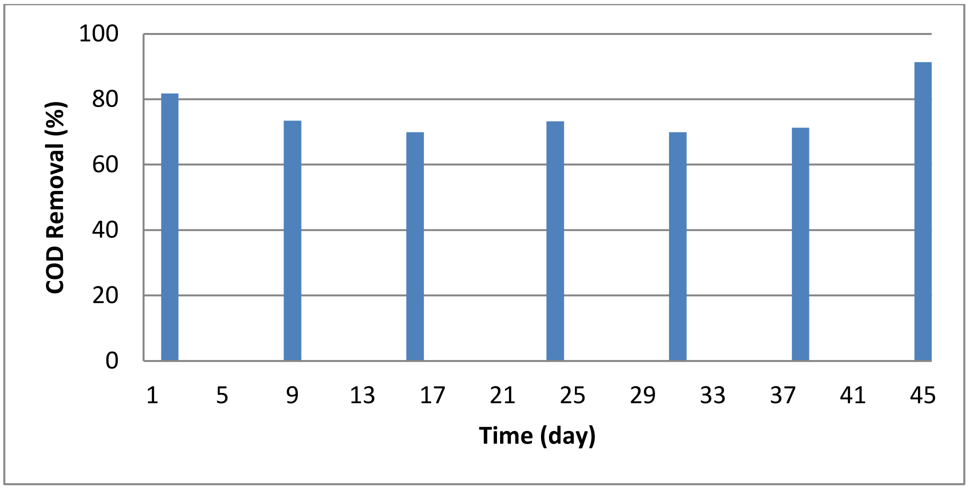


Figure 4-1: COD removal in MBR

However, SMEBR results proved the benefit of applying an electrokinetic system comparing to the conventional methods. According to Figure 4-2 and Figure 4-3, SMEBR system could provide coagulation process in addition to biological, which resulted in higher COD removal of 93.3% (day 24) while the minimum removal was in day 56 (67.2%).

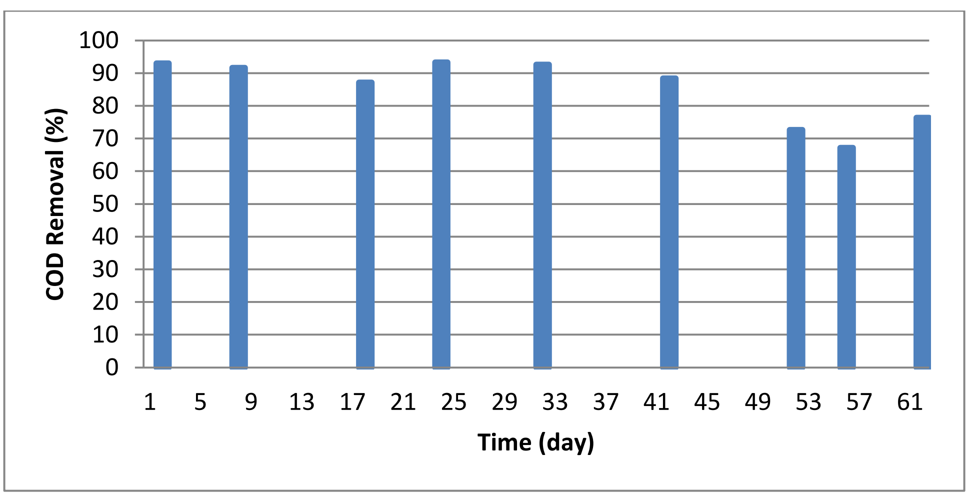


Figure 4-2: COD removal in SMEBR first stage

The second stage of SMEBR included a fresh sludge which achieved averagely higher results in comparison to SMEBR first stage (minimum and maximum COD removal were 86% and 93.90%) (Figure 4-3).

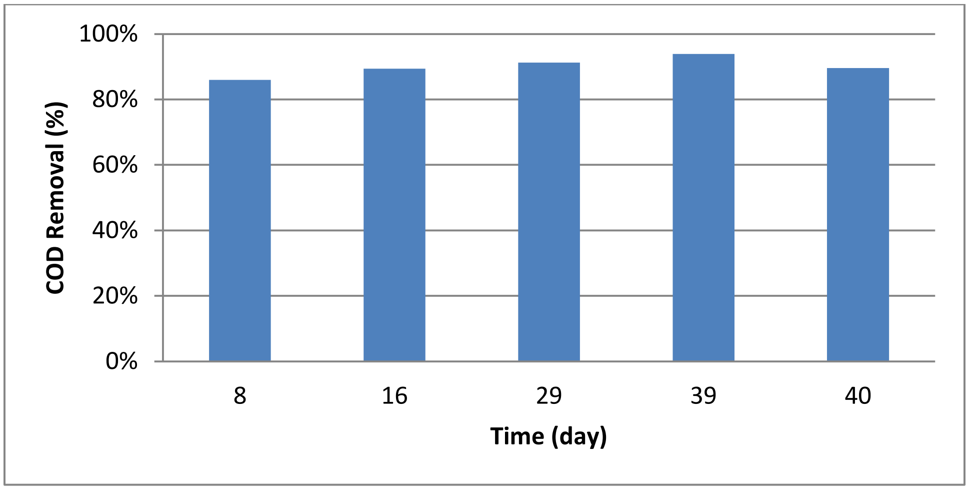


Figure 4-3. COD removal in SMEBR second stage

The last phase consisting of the SMEBR-Anammox system, showed almost complete removal of COD while minimum and maximum were 98.1 in stage 1 and 99.87% in days 2 and 45 (stage 2), respectively.

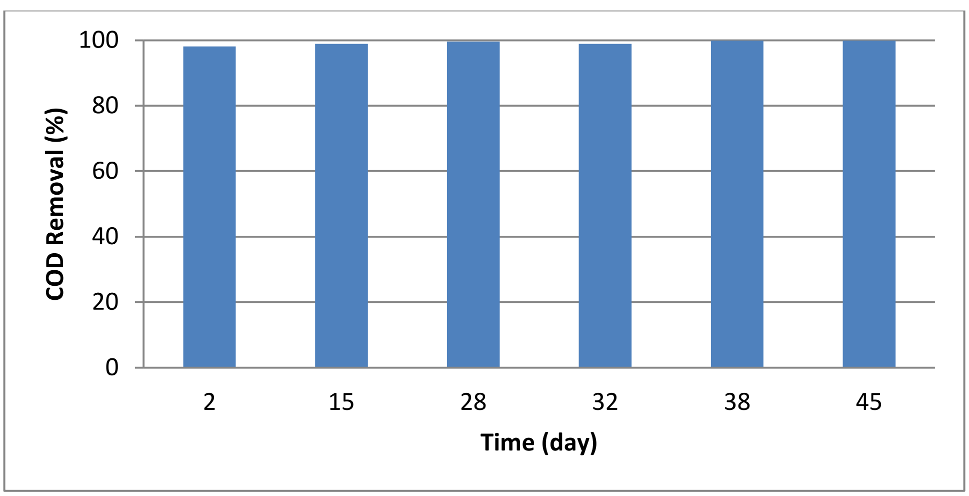


Figure 4-4. COD removal in SMEBR-Anammox

4.2 Current Density (CD)

Current density is defined as the current (in A) per anode surface area (m^2). In this study the maximum current density was 14.5 A/m^2 and the voltage was around 16-18 V. The current density was kept constant by adjusting the voltage. Table 4-1 illustrates the comparison between two stages of SMEBR and SMEBR-Anammox with diverse current densities.

Third week after SMEBR installation (stage 1), the current density reduced to around 9 A/m^2 so the voltage was the parameter which should be changed to increase the level of current input. However, increasing the voltage to more than 20 V negatively affected the nutrient removal.

Table 4-1. Average current input and current densities for SMEBR and SMEBR-Anammox

Stage	**Current density (A/m^2)**
SMEBR stage 1	8.96
SMEBR stage2	10.97-13
SMEBR-Anammox stage 1 reactor: 64% of Anammox reactor	13-14
SMEBR-Anammox stage 2 reactor: 100% of Anammox reactor	14-14.5

The current density was changed over the time even though the submerged area was constant. This fluctuation certainly affected the nutrient removal efficiency. Decreasing the current density to lower than 10 A/m^2 decreased the amount of Al^{3+} releasing which was not sufficient especially for phosphorous removal.

4.3 Dissolved oxygen

Dissolved oxygen has an essential influence on wastewater treatment. This parameter leads the system to perform nitrification, COD and phosphorous removal. Considering of 4 phases of this study, different DO concentration was required; therefore a DO adjustment to increase and decrease of aeration level was provided. SMEBR-Anammox system needed the lowest aeration level than SMEBR and MBR system, because anammox bacteria grow in anoxic conditions.

During the experiment, DO was controlled based on two parameters: 1) air supply 2) current density in the electro-bioreactor. Operating mode connecting and disconnecting the current with the timer significantly affected the ORP and DO. Subsequently, dissolved oxygen in all phases (Anammox, MBR, SMEBR, and SMEBR-Anammox) fluctuated between 0 and 7.0 mg/L. In phase 4, it was kept around 0.69-0.7 mg DO/L for more than two weeks to ensure the best nutrient removal at equilibrium conditions. As it is depicted in Figure 4-5, Anammox reactor (phase 1) provided the lowest amount of DO to grow anammox bacteria.

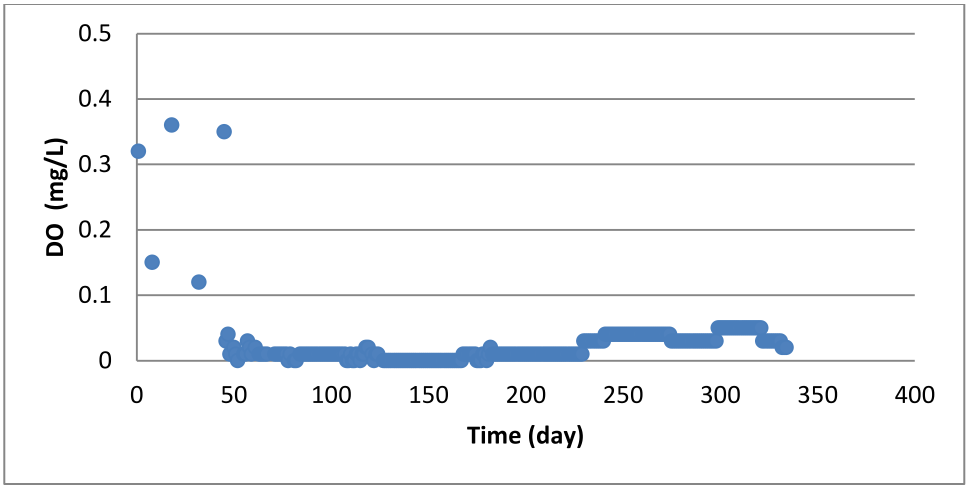


Figure 4-5. DO concentration in Anammox system

According to Figure 4-5, dissolved oxygen was changed from 0.36 to 0 mg/L in Anammox. DO was higher in the MBR phase to promote microorganisms growth in activated sludge. The level of oxygen was flocculated considerably in MBR system (Figure 4-6). In stage 2 of phase 2, new activated sludge was added in day 25 and recovering the bacteria achievement was observed which was more uniformed in phase 3. Therefore DO concentration flocculated around 4 to 7 mg/L.

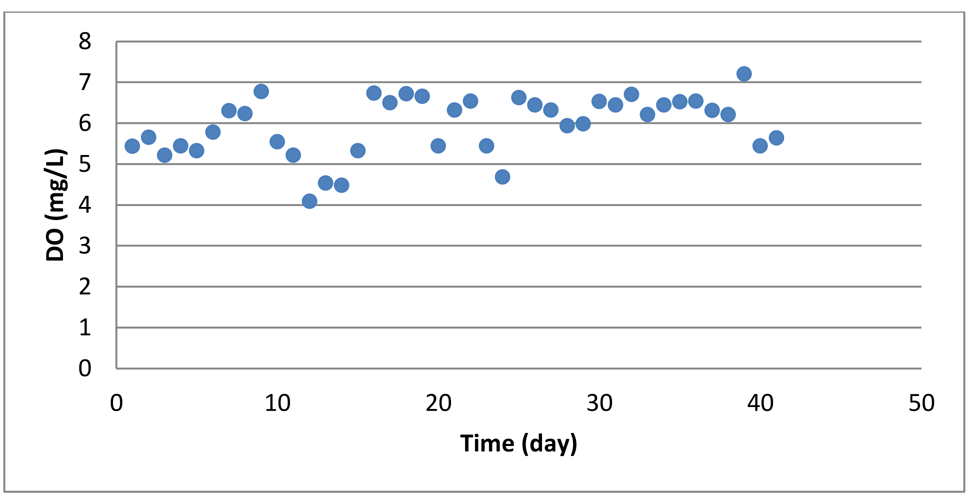


Figure 4-6. DO concentration in MBR

In SMEBR system, the low level of DO was needed to conduct denitrification process. The principal goal of this study was to achieve highest ammonium removal by the lowest level of DO. Therefore, DO was decreased to some extent so that the ammonium removal was increased in the SMEBR system.

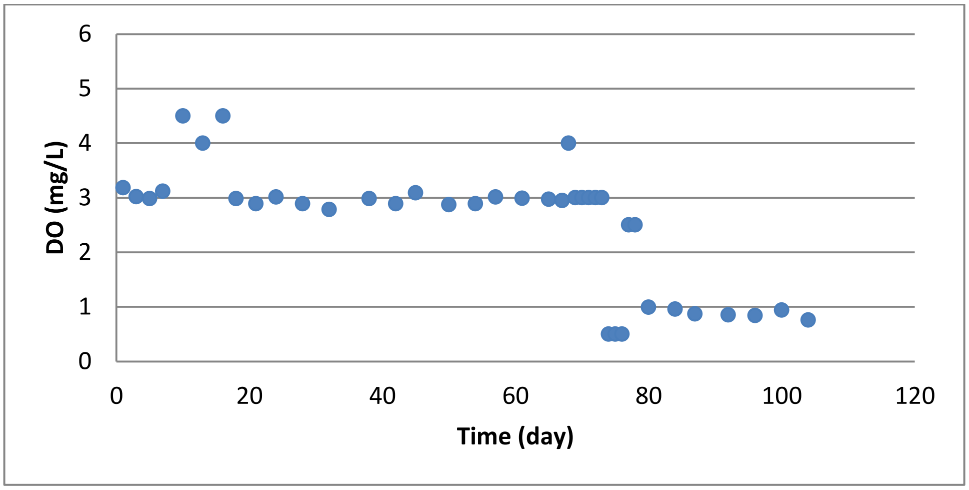


Figure 4-7. DO concentration in SMEBR (stage 1 and 2)

SMEBR-Anammox system needed less amount of aeration than SMEBR system. In fact, decreasing the DO level increased the efficiency of anammox bacteria.

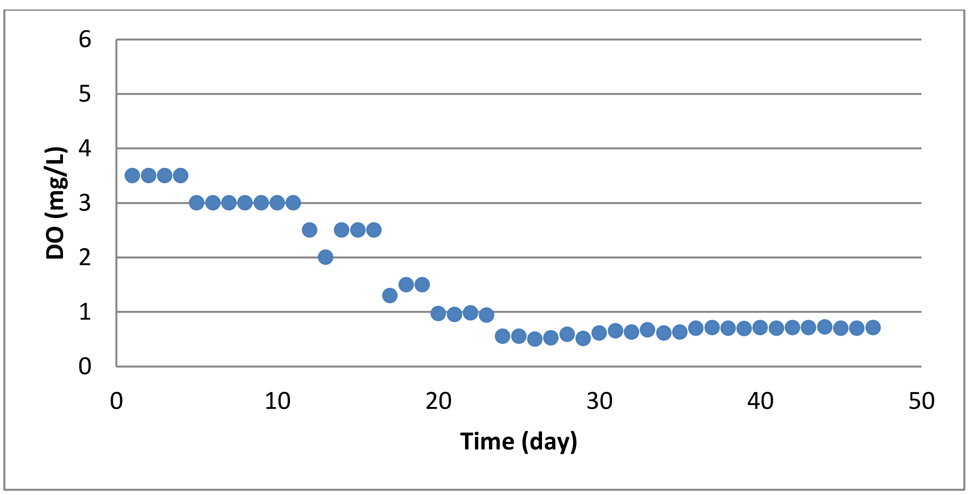


Figure 4-8. DO concentration in SMEBR-Anammox

Moreover, the average aeration level in SMEBR-Anammox was 0.7 mg/L. The experimental work permitted to calculate:

- Increasing MLSS decreased the level of DO to the lowest level; as the number of bacteria was increased, the oxygen consumption became greater.
- In the first days after the activated sludge addition, DO fluctuation was significant; but, when the bacteria were fully adapted to the new conditions, DO level was consequently increased.
- When the timer was ON, the dissolved oxygen level was decreased while with disconnecting the current (timer OFF), the level of DO was increased.

Figure 4-9 shows an example of direct relationship between DO and ORP when the electrical mode is ON / OFF = 5min/15min exposure time.

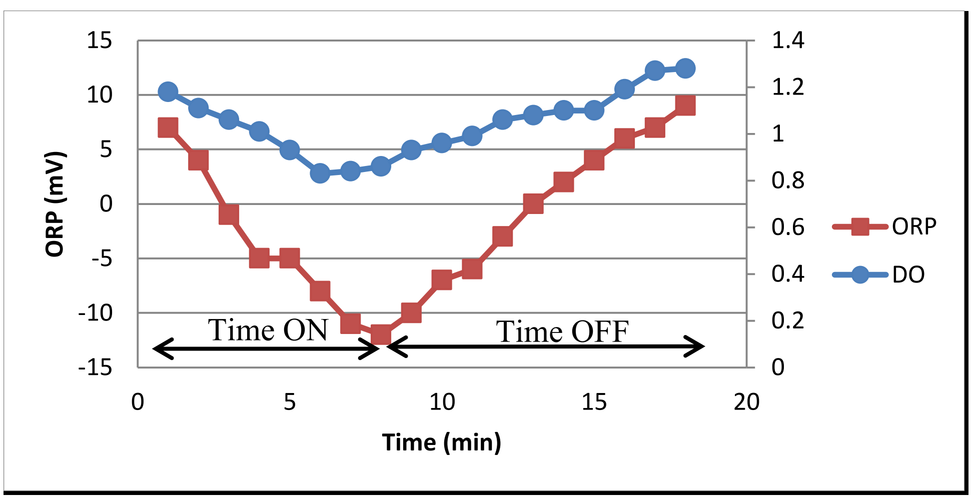


Figure 4-9. DO vs ORP in SMEBR-Anammox

- At the laboratory scale design, there were some unexpected changes such as unequal air distribution in the small container and problem in controlling of current due to timer inaccuracy. However, conducting the larger scale tests could solve most of problems.

- The small difference in DO might create a shock affecting the results in some days since the system could not return to its initial conditions. Therefore, the dissolved oxygen had to be maintained steadily to have a satisfactory nitrification/denitrification process at the same time.

4.4 Oxidation Reduction Potential (ORP)

Generally ORP shows oxidation condition at high DO values. Anoxic condition close to zero and anaerobic condition in negative values .Variation of the ORP between the aerobic and anoxic condition permitted to create reciprocal nitrification and denitrification process. The ORP measurements were frequently repeated when the timer was ON and OFF to evaluate the reactors' performance. For instance, in the Anammox system with 0.01 mg/L DO, ORP was negative while in the MBR system with the high level of DO, ORP was positive. Here are some remarks:

- ORP was changed directly by DO and current variation in aerobic, anoxic and Anammox systems.
- ORP measurement was a reliable method to recognize the changes of dissolved oxygen use in the systems.
- Variations of current input in the SMEBR and SMEBR-Anammox system affected the ORP under a constant aeration condition.

According to Figure 4-10 at DO=4.5 mg/L, CD= 9.98 A/m^2, and exposure mode of 5 min ON/ 15 min OFF, the ORP level was 16.6 mV, while at the higher concentration of DO there was not big difference between ORP levels. Likewise, by decreasing DO to 3 mg/L at the 5min

ON/15 min OFF exposure mode and HRT=24h, ORP level was decreased to 15 mV. On the other hand, decreasing DO to 2.5 mg/L had a sharp effect on ORP changing it to a negative level. Figures 4-10 to 4-16 illustrates the average ORP and DO fluctuation due to current input.

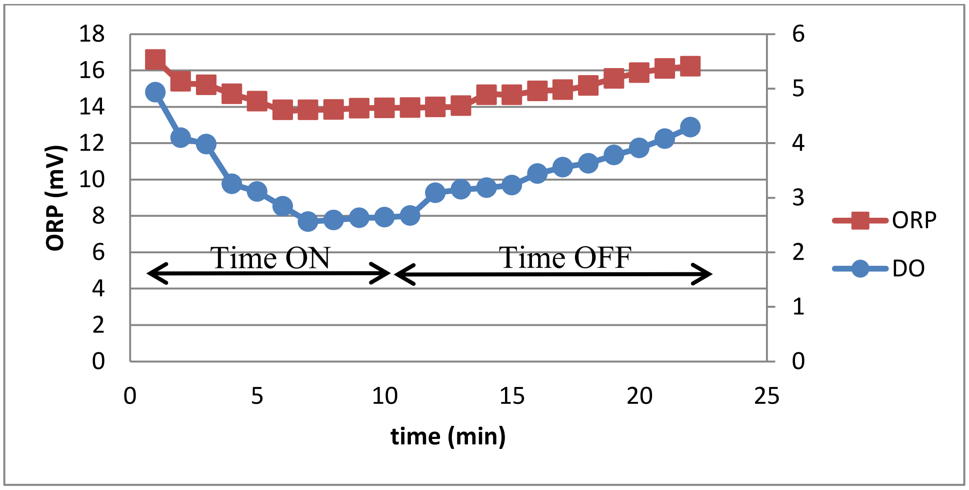


Figure 4-10. ORP flocculation at DO=4.5 mg/L, MLSS= 3000 mg/L, HRT=24 h, CD=9.98 A/m^2 in SMEBR

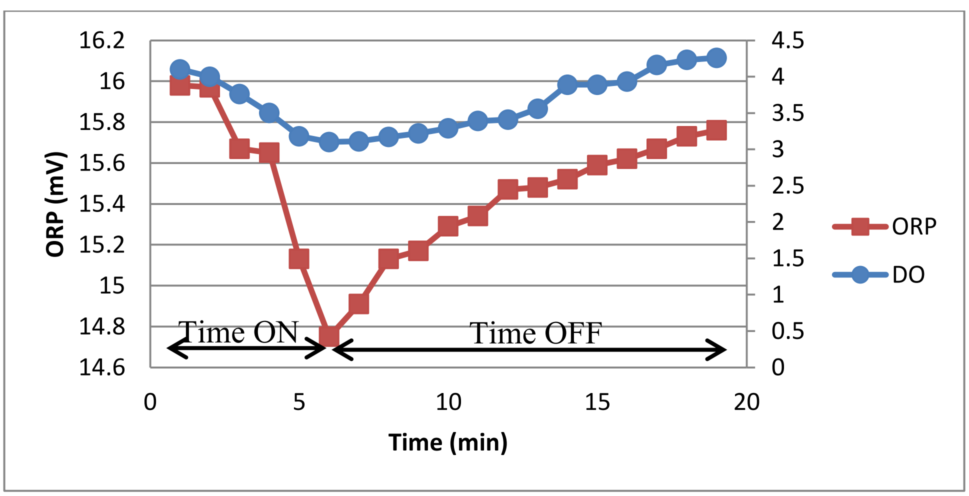


Figure 4-11. ORP fluctuation at DO=4 mg/L, MLSS =3000 mg/L, HRT=24 h, CD= 10.2 A/m^2 in SMEBR

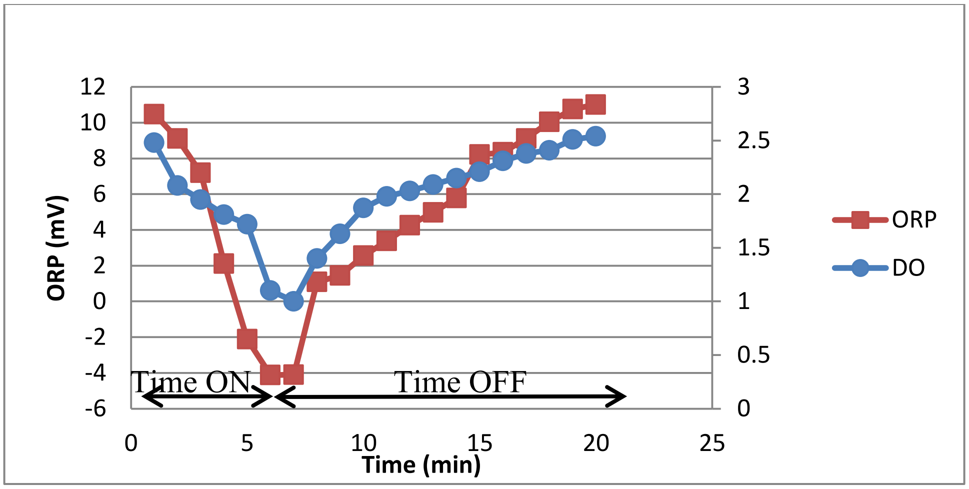


Figure 4-12. ORP fluctuation at DO=2.5 mg/L, MLSS=4000 mg/L, HRT=24 h, CD= 12.3 A/m^2 in SMEBR

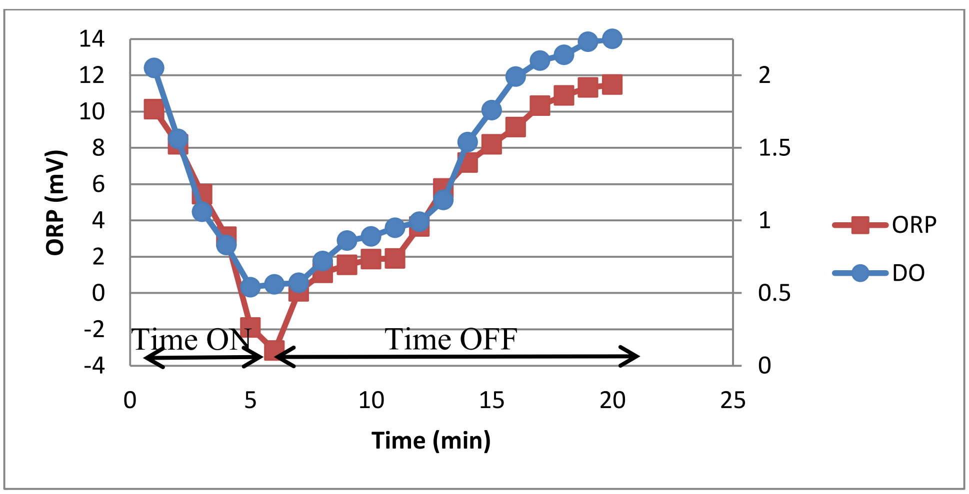


Figure 4-13. ORP fluctuation at DO =2 mg/L, MLSS=4000 mg/L, HRT=24 h, CD=12.5 A/m^2 in SMEBR

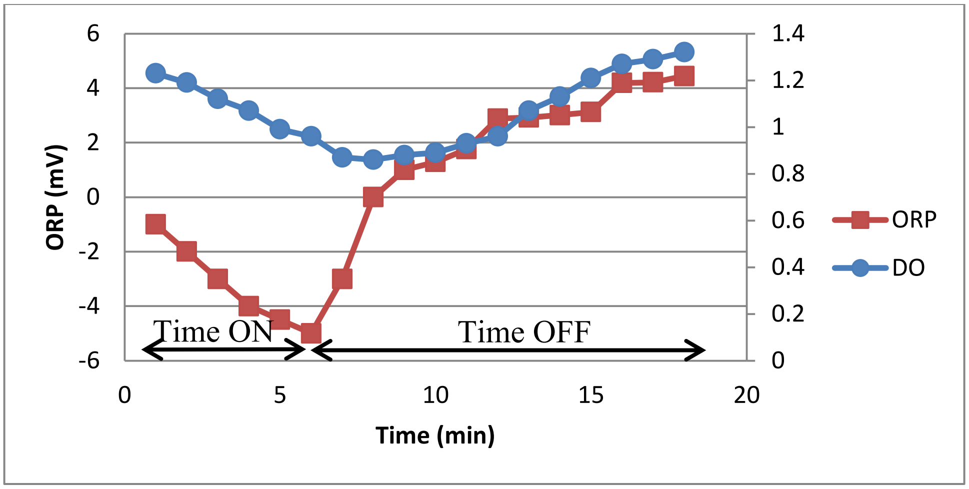


Figure 4-14. ORP fluctuation at DO =1.5 mg/L, MLSS=4000 mg/L, HRT=24 h, CD=13.1 A/m^2 in SMEBR

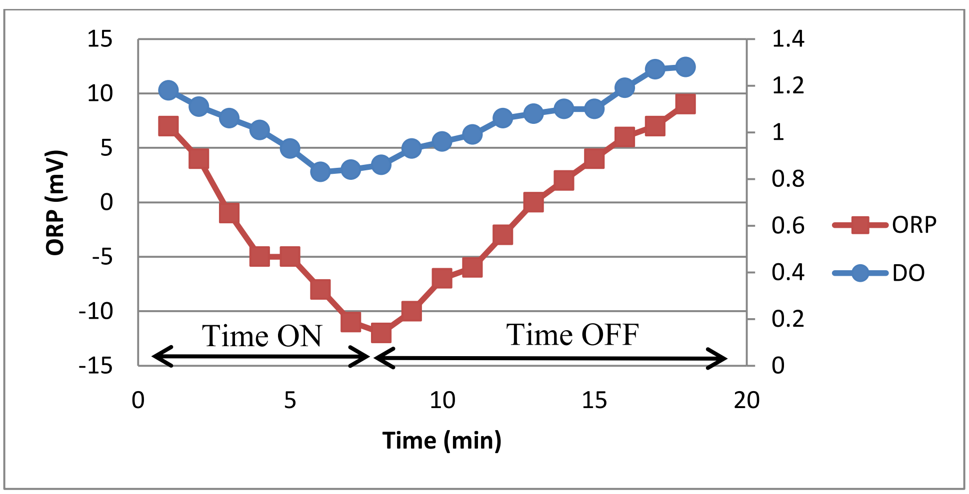


Figure 4-15. ORP fluctuation at DO = /L, 1.30 mg MLSS=4500 mg/L, HRT=24 h, CD=14 A/m^2 in SMEBR-Anammox

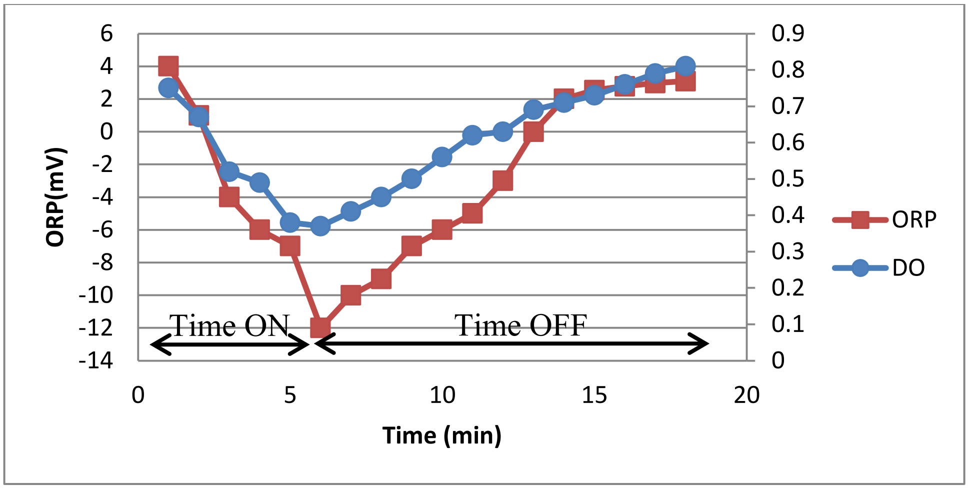


Figure 4-16. ORP fluctuation at DO=0.5-0.7mg/L,MLSS=5000mg/L,HRT=24h, CD=14.5A/m2 in SMEBR-Anammox

Figures 4-10 to 4-16 are the examples of SMEBR and SMEBR-Anammox system which could alter the ORP and DO in 5 min ON/15 min OFF exposure mode. According to Figure 4-16 in SMEBR-Anammox system with the lowest range of DO (0.45 to 0.8mg/L), lowest range of ORP was observed (4 to -12 mV) in comparison with SMEBR.

4.5 Electrical conductivity

Electrical conductivity in SMEBR and SMEBR- Anammox was lower in comparison to MBR process. Although MBR, SMEBR and SMEBR-Anammox were fed with the same synthetic wastewater, electro conductive removal in SMEBR and SMEBR-Anammox was around 20% more than MBR process. Table 4-2 shows the comparison between SMEBR-Anammox, SMEBR and MBR system in stage 2. However in stage 1 the electrical conductivity reduction for SMEBR and MBR was close together (around 10% reduction) while SMEBR-

Anammox had around 20% reduction. Electrical field conducting in the SMEBR and SMEBR-Anammox let the charged particles deposited on electrical surface. Therefore, they will not affect the suspended sludge.

Table 4-2. Electrical conductivity in different reactors

	Influent (μS/cm)	MBR (μS/cm)	SMEBR (μS/cm) CD=12-13A/m^2	SMEBR-Anammox (μS/cm) CD=14-14.5 A/m^2
Average	644	569	449	453
Maximum	654	619	652	542
Minimum	543	496	390	519

4.6 pH

Though pH is not a determining factor in the efficiency of wastewater treatment reactors, vary high or very low pH might affect the results to some extent. Besides, hydroxide ion production at the cathode by electrochemical reactions created higher pH in SMEBR than MBR. Likewise, SMEBR was capable to avoid the risk of acidic condition by hydroxide ion generation and increasing pH in the reactor. Figure 4-17 demonstrates the comparison between MBR, SMEBR and SMEBR-Anammox in different pH and variable DO.

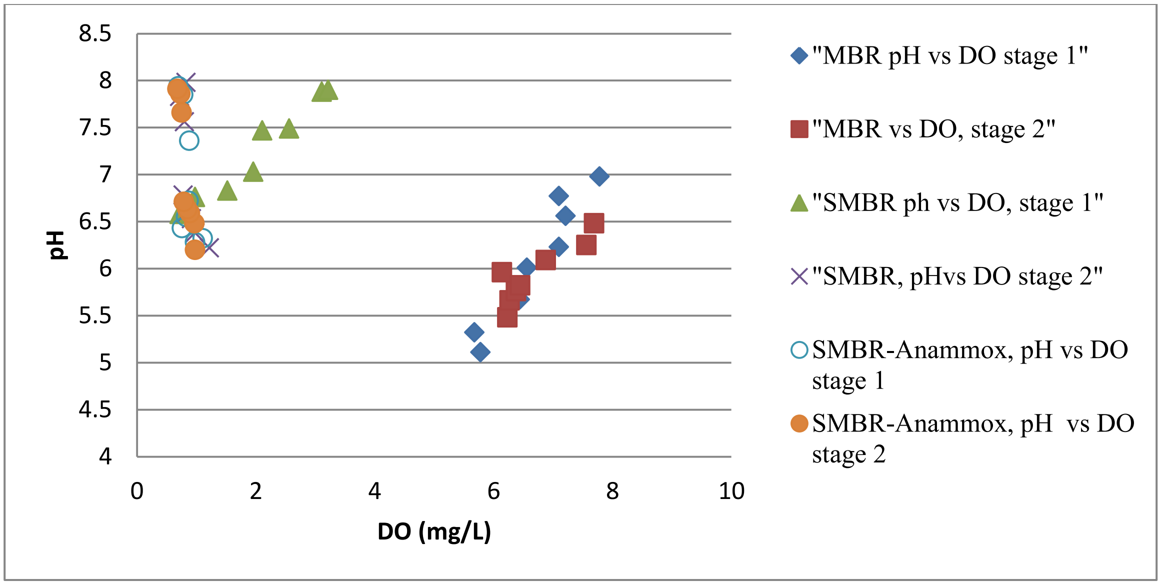


Figure 4-17. pH vs DO in MBR, SMEBR and SMEBR-Anammox

Figure 4-18 compares the MBR and SMEBR-Anammox system at 14 A/m^2 current density during 8 days. As it was aforementioned, higher amount of pH in SMEBR-Anammox indicates the production of hydroxide ion.

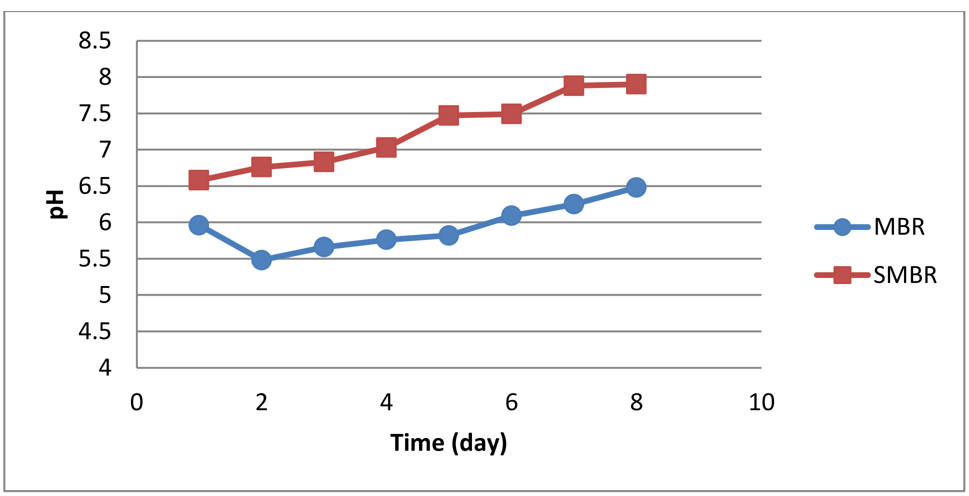


Figure 4-18. pH vs time in SMEBR-Anammox and MBR, CD=14 A/m2, 5min ON/15 min OFF

4.7 Nutrient Removal

4.7.1 Phosphorous removal

Decreasing phosphorous to the lowest level is another advantage of SMEBR system. Formation of aluminum hydroxide and the complex aluminum hydroxide ions indicated that a great phosphorous removal happened when SMEBR system was applied. On the other hand, production of aluminum hydroxide complex zones increased the total suspended solid in the reactor.

Having compared different reactors in the year of working, SMEBR and SMEBR-Anammox system achieved much better results than MBR system in phosphorous removal. In all of these studies, phosphorous influent ranged between 18 to 22 mg/L. Figure 4-19 shows that average PO_4-P concentration in effluent from MBR was around 9 mg/L and removal average was 46%.

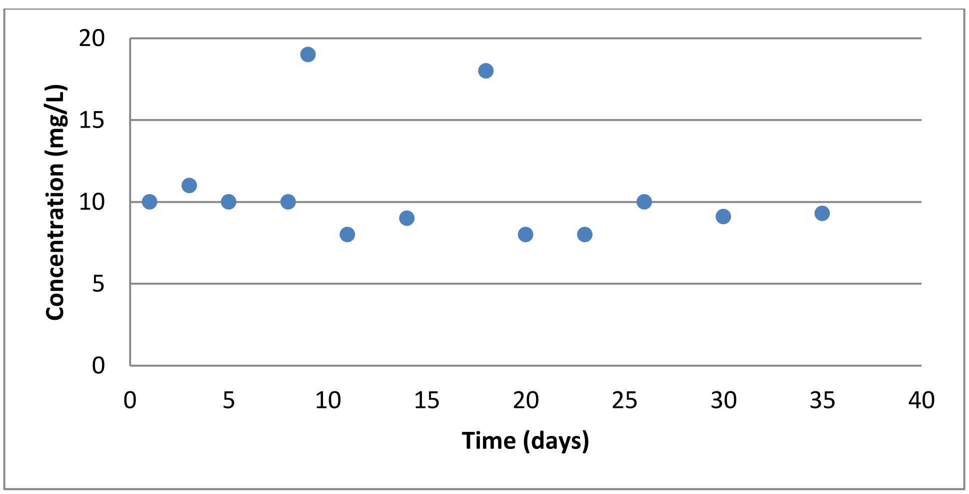


Figure 4-19. PO_4-P concentration in MBR effluent (stage 1 and 2)

As Figure 4-20 shows, the maximum amount of phosphorous removal was (65%) in day 11. However, this much phosphorous removal in the MBR was resulting to the addition of coagulant in the wastewater treatment plants which increased the removal of phosphorous to this level.

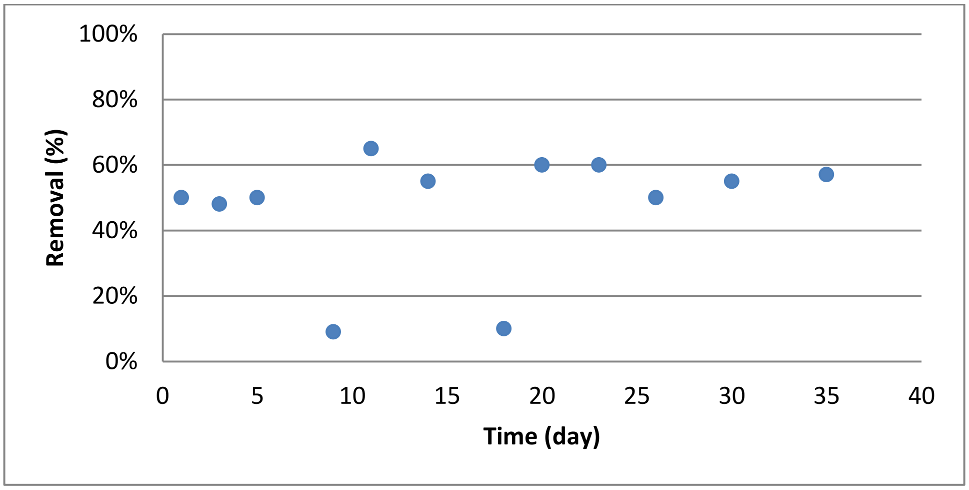


Figure 4-20. Phosphorous removal in MBR (stage 1 and 2)

Moreover, based on the above observations, laboratory scale MBR system needs extra treatment units/processes to remove the persisting phosphorous.

SMEBR system with different stages was applied to reach the highest nutrient removal in the effluent. In the first SMEBR stage, electrocoagulation due to electrokinetic process was the major reason of phosphorous removal (Figure 4-21).

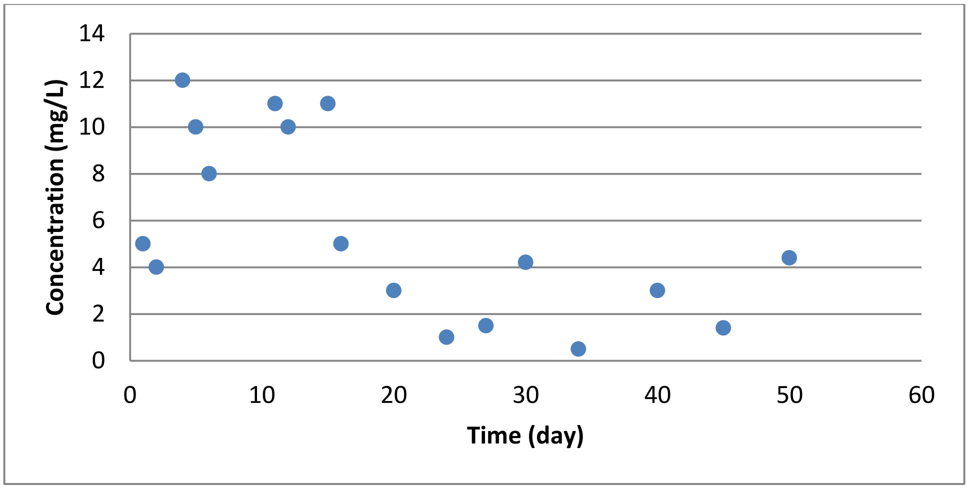


Figure 4-21. PO_4-P concentration in SMEBR effluent (stage 1)

According to Figure 4-22, the average phosphorous removal was 73.82 % while in MBR system the average removal was 46.54%.

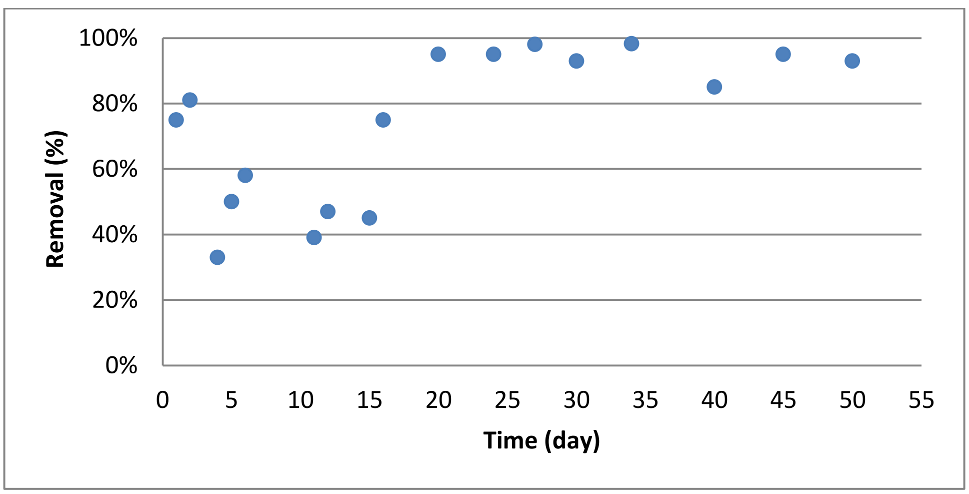


Figure 4-22. Phosphorous removal in the SMEBR first stage

Therefore, in the first stage of SMEBR, by electrokinetic process, electrocoagulation was the reason of phosphorous removal in addition to bio flocs growing and $AlPO_4$ formation.

Second stage of SMEBR included fresh sludge addition including coagulant significantly changed the results.

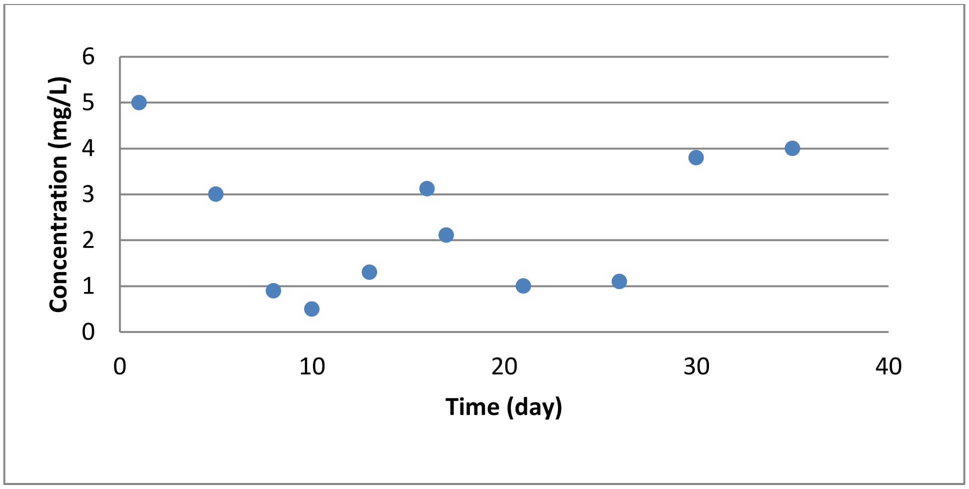


Figure 4-23. PO_4-P concentration in SMEBR stage 2

However, SMEBR second stage could increase phosphorous removal to 98.1% in day 17 (Figure 4-24).

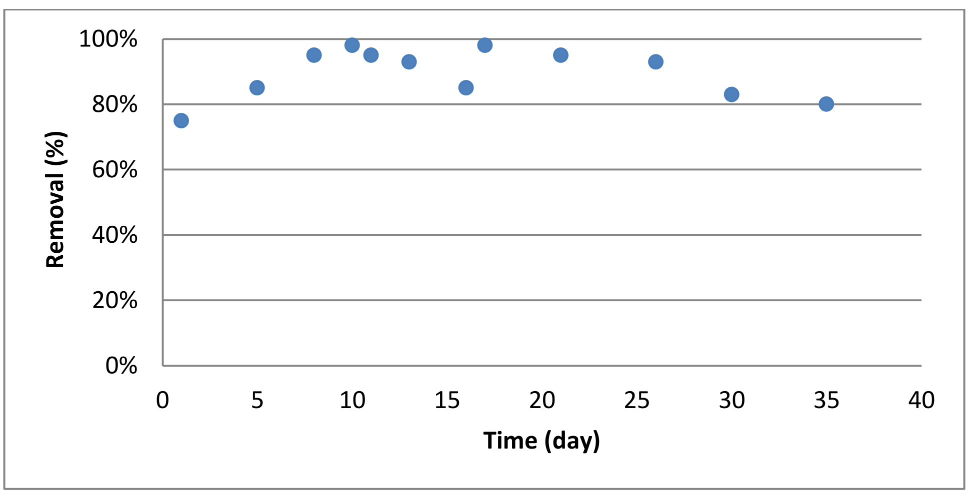


Figure 4-24. Phosphorous removal in the SMEBR second stage

In the SMEBR-Anammox, operating condition changed considerably and positively influenced on phosphorous removal. Due to the sensitivity of this system, in addition to design, operational condition such as pH and temperature adjustment, current density, air distribution and power supply control needed to be closely monitored.

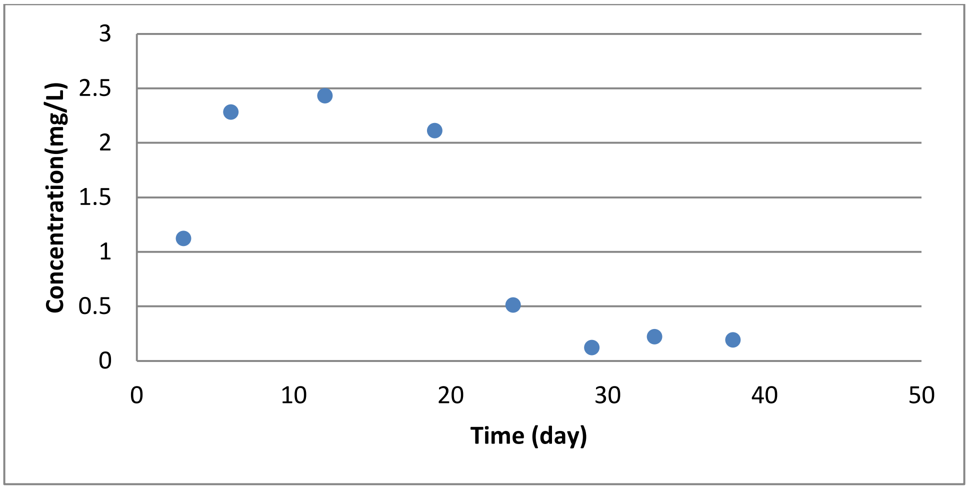


Figure 4-25. PO_4-P concentration in SMEBR- Anammox (stage 1 and 2)

Based on Figure 4-26, with improvement of the electrical circuit, phosphorous removal reached 99.91 percent in the last days of SMEBR-Anammox operation.

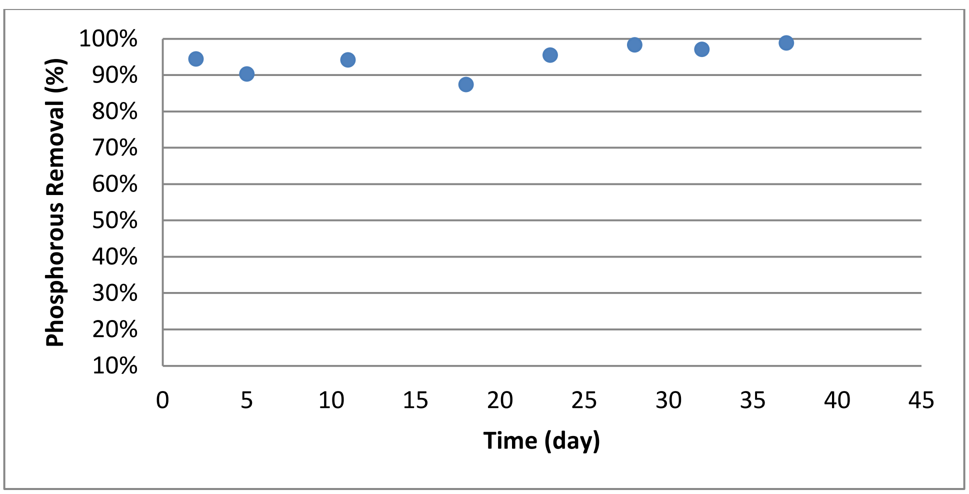


Figure 4-26. Phosphorous removal in the SMEBR- Anammox (stage 1 and 2)

In SMEBR-Anammox system, the bioflocs, electrocoagulation and PAO growth were responsible for phosphorous removal. In was possible that the last reactor provided adequate conditions (anoxic/aerobic) for PAO growth which dropped phosphorous concentration to around 0.1 mg/L in SMEBR-Anammox second stage. By changing the design of SMEBR-Anammox in the second stage more aluminium ion and more electrons were released, thus, phosphorous removal was increased. The standard deviation of PO_4-P measurements was 0.74.

The best results appeared in the SMEBR-Anammox system with 24 h HRT, 14.5 A/m^2 CD, 5 min ON/15 min OFF exposure time and DO less than 1 mg/L in week 3. The main difference between SMEBR (or SMEBR-Anammox) and MBR system was aluminum ions existence on the anode surface area. These ions reacted with phosphorous and made new bond to remove phosphorous from the treated water. Consequently, aluminum phosphates played an important role in decreasing the level of phosphorous in the SMEBR system. Although, during the SMEBR tests, sludge concentration, current density and dissolved oxygen were changed and influenced the results, a high phosphorus removal happened in SMEBR and SMEBR-Anammox system because of ability of SMEBR system to remove nutrients even in unsteady situations. As the matter of fact, the electrokinetic process could reduce the effects of undesirable conditions significantly.

4.8 Nitrogen removal

Nitrogen removal was assessed based on three tests including ammonia nitrogen, nitrate nitrogen and TN measurements. Ammonia nitrogen and nitrate nitrogen were measured at least once a day while TN measurement was limited to once a week or once in two weeks. Nitrogen removal was varied in different stages of the experiment since various operation conditions and MLSS improvement was applied.

4.8.1 Ammonia nitrogen removal

Ammonium decreasing is the most important goal of nutrient removal from wastewater. Ideally, the wastewater treatment facilities go for nitrification/denitrification process where ammonia is transformed into nitrogen gas. Each of these processes requires different operation conditions to separately grow nitrifiers and denitrifiers. In this study, the SMEBR's design permitted to grow both culture in the same reactor (phases 3 and 4). Usually, an additional device is required to support anammox bacteria growth; in this study a hybrid SMEBR-Anammox system permitted to grow anammox, nitrifiers and denitrifiers in the same reactor. As ammonia nitrogen removal was not more than 65% in the first stage of MBR and SMEBR, the reactors' operational condition was changed two or three times in order to increase the removal efficiency.

In phase 1, Anammox reactor was applied to grow anammox bacteria without oxygen injection. Since the anammox growth is slow, after 50 days of reactor's operation, the ammonium concentration in effluent varied between to 0.1 to 4 mg/L. Such fluctuation was related to investigation of the reactor maximum capacity of ammonia removal. Therefore the fluctuation of results was expected. Figure 4-27 shows the influent and effluent of ammonium in Anammox reactor.

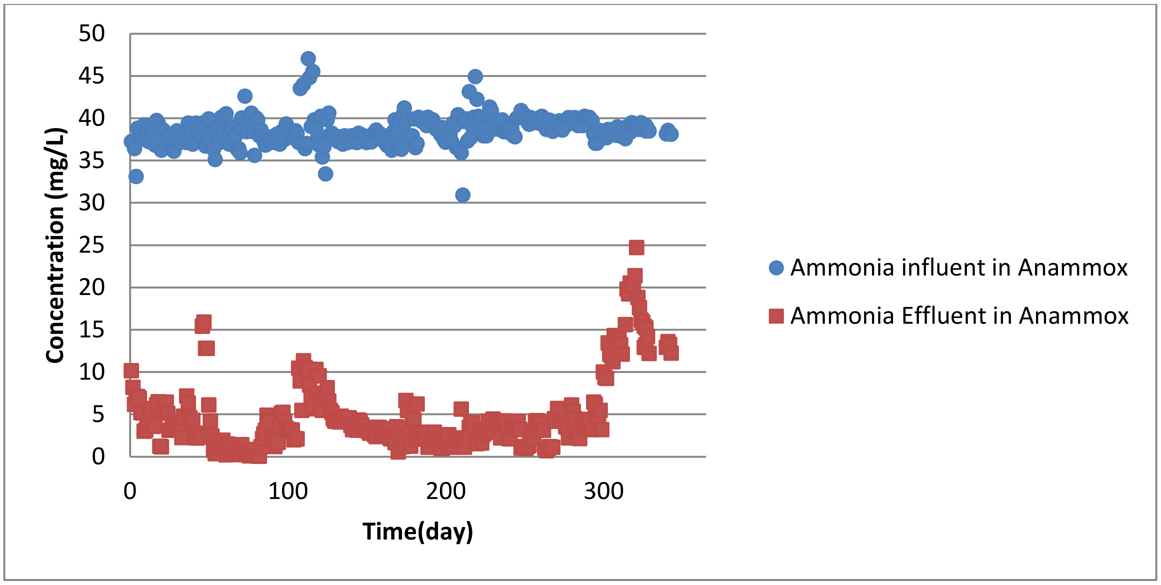


Figure 4-27. Ammonia nitrogen influent and effluent in Anammox growing reactor

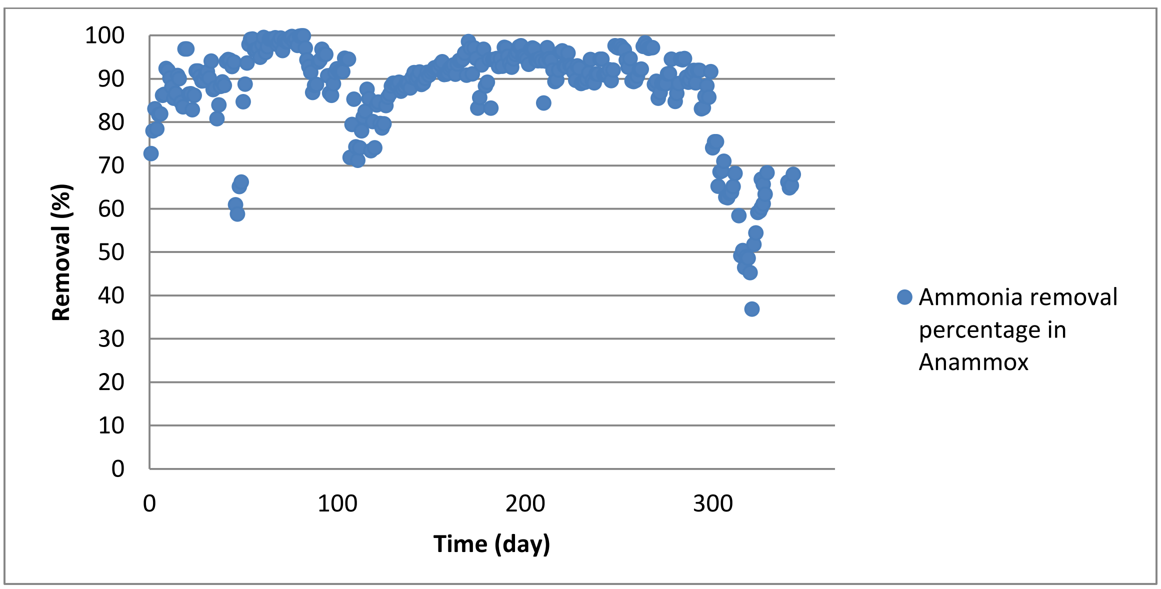


Figure 4-28. Ammonia removal in Anammox reactor

Anammox system resulted in a satisfactory outcome over 10 month-period when ammonia nitrogen removal reached the reasonable level of more than 99% (Figure 4-28). The specific reddish color of anammox was easily noticeable in the reactor.

In the first stage of phase 2 (MBR system), ammonia nitrogen removal level reached 73% at the DO concentration around 6.5 mg/L (Figure 4-29 and Figure 4-30). However, fluctuation of the DO level, negatively affected ammonia nitrogen removal in MBR system; for example, after reducing the level of DO from 6.5 to less than 4 mg/L in the first stage of MBR, ammonium removal efficiency was decreased to 50%.

When the level of aeration was increased to 6.5 mg/L, the results were not changed even after two weeks. Then, in second stage, when new activated sludge was applied to the MBR system, maximum level of ammonium removal reached 75%.

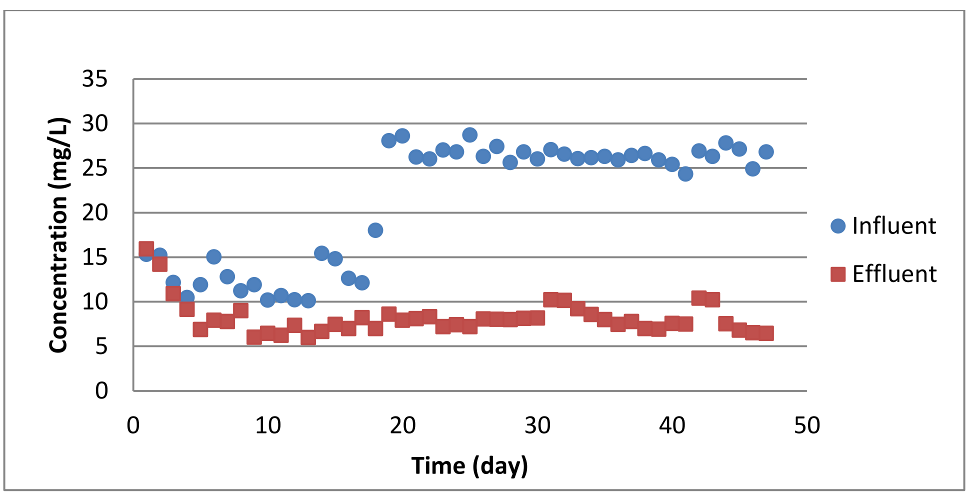


Figure 4-29. Ammonia nitrogen influent and effluent in MBR (stage 1 and 2)

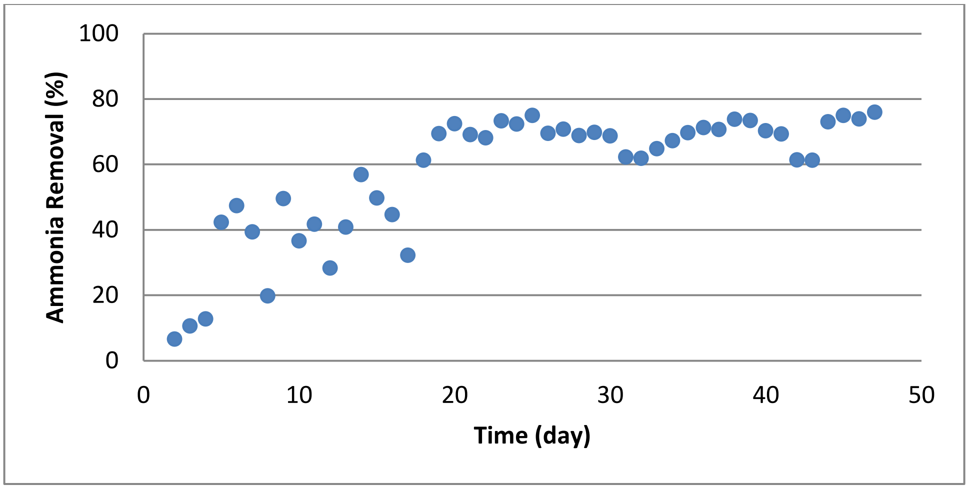


Figure 4-30. Ammonia removal in MBR

At high amount of oxygen (DO around 6 mg/L), the MBR system was reached 75.9% ammonium removal. However, in MBR design, ammonium level was decreased at least to 6.45 mg/L while the influent was around 27 mg/L.

In phase 3, DO around 3.5 mg/L was supplied to SMEBR with 5 min ON/ 15 min OFF exposure mode at the beginning. The results of stage 1 in phase 3 show that the minimum and maximum level of ammonium removal was 29.21% and 66.7%, (19.21 mg/L and 8.65 mg/L), respectively. Usually, the SMEBR has much higher removal rate of ammonia, however in the study, the influent contained much higher ammonia concertation that in a conventional wastewater.

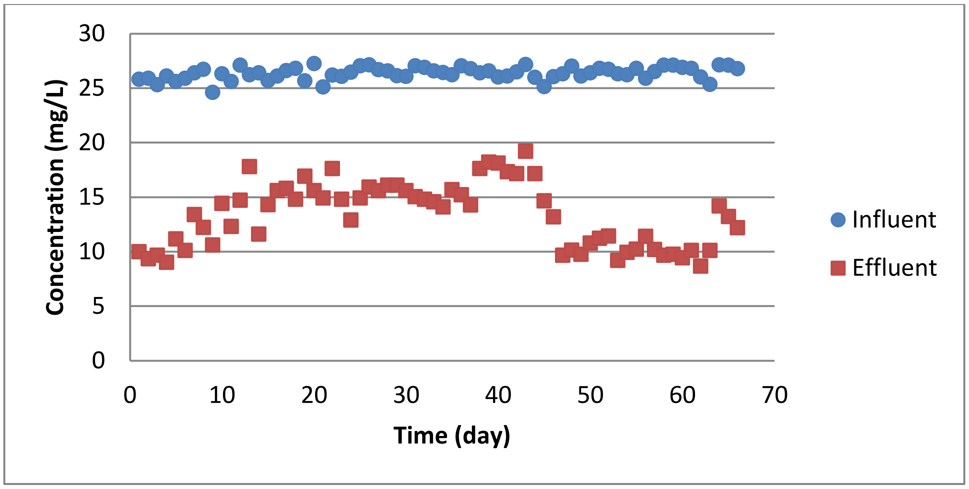


Figure 4-31. Ammonia influent and effluent in SMEBR, stage 1

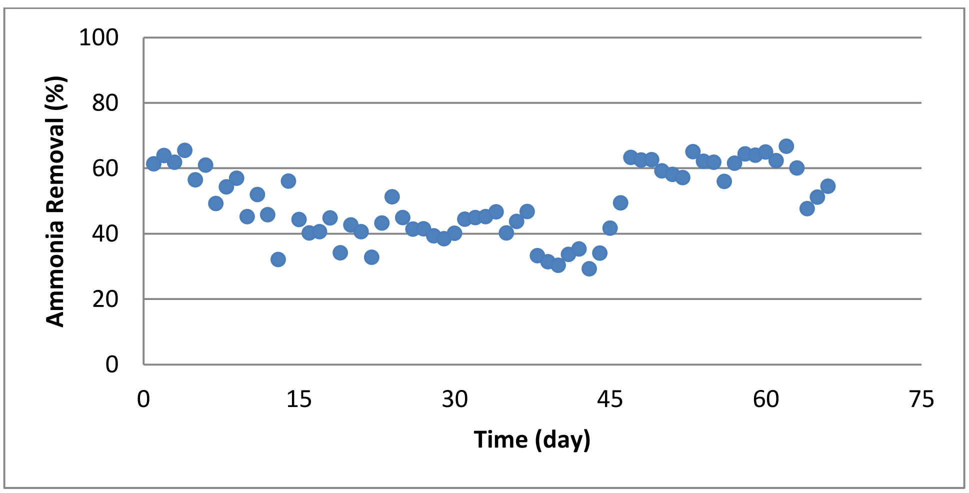


Figure 4-32. Ammonia nitrogen removal in SMEBR, stage 1

In the second stage of phase 3, a fresh activated slugged was imported from the activated sludge facilities in WWTP in St Hyacinte, QC.

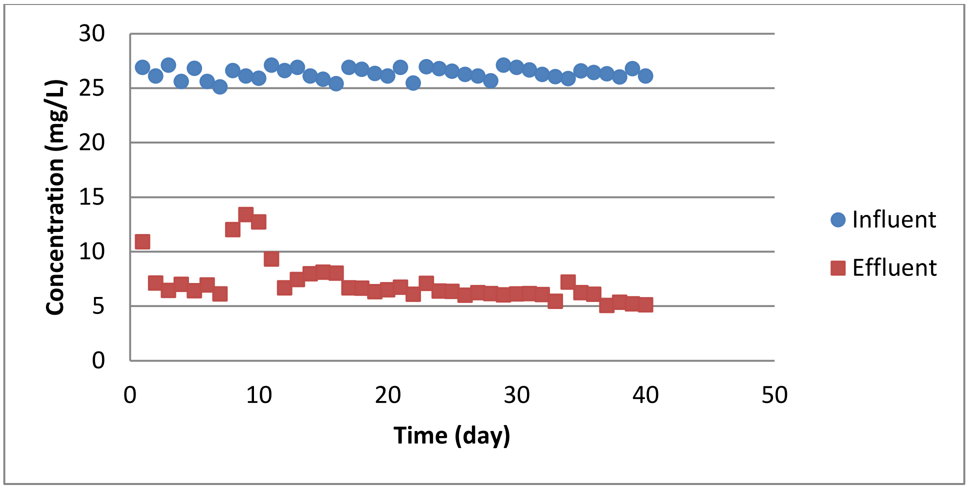


Figure 4-33. Ammonia nitrogen influent and effluent in SMEBR stage 2

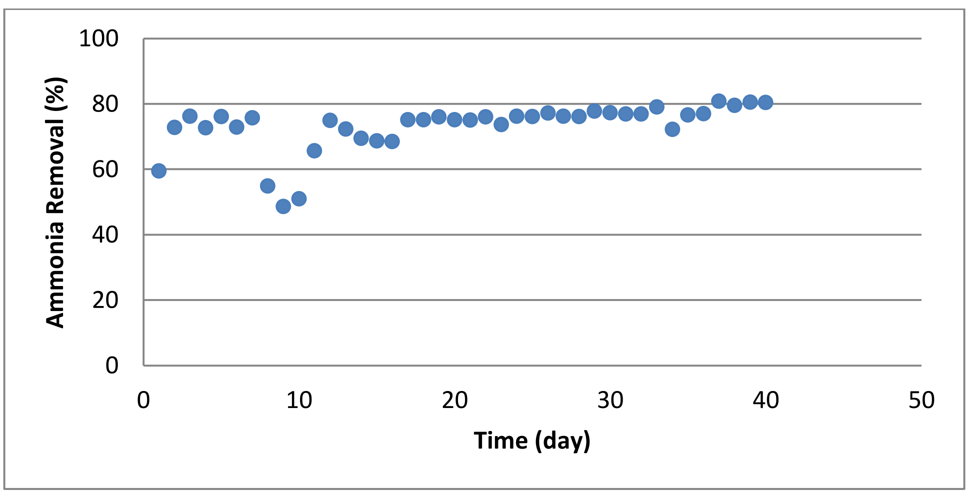


Figure 4-34. Ammonia nitrogen removal in SMEBR stage 2

As it is demonstrated in Figure 4-33 and Figure 4-34, average ammonia nitrogen removal was improved in SMEBR second stage 75.85% comparing to first stage 47.2%.

Phase 4 testing SMEBR-Anammox system consisted of two stages:

-First stages of phase 4 started after transferring 9L (64% of reactor volume) of Anammox-reactor to SMEBR.

-Second stage of phase 4 started after the whole SMEBR reactor was filled Anammox-reactor content.

All of these steps improved ammonia nitrogen removal. Firstly in stage 1, 64% of Anammox reactor addition decreased the ammonia nitrogen concentration in effluent to 4 mg/L. Then in stage 2, ammonia nitrogen was decreased to 0.89 mg/L (Figure 4-35), reaching 97% removal of ammonia nitrogen (Figure 4-36).

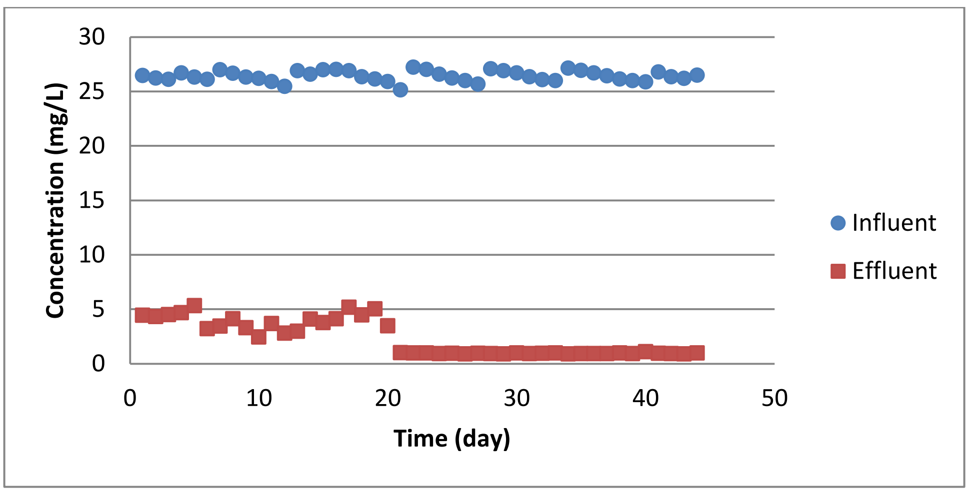


Figure 4-35. Ammonia nitrogen influent and effluent in SMEBR-Anammox

Figure 4-36 illustrates a considerable effect of changing design on the results.

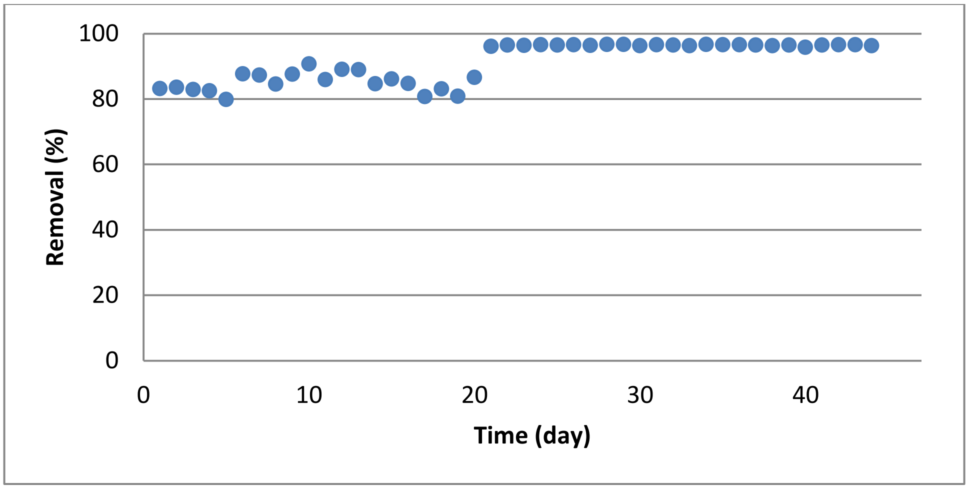


Figure 4-36. Ammonia removal in SMEBR-Anammox

Here are some important remarks for ammonia nitrogen removal in all phases:

- SMEBR system worked much better than MBR system especially when the anammox was added to the reactor.
- DO played a very important role to attain this conclusion. However, anammox bacteria could not work in high amount of DO since their ability had a direct relation with DO level.
- At DO less than 0.7 mg/L, SMEBR-Anammox system could not work well and ammonium removal was reduced.
- In the last phase, a complete nitrification and denitrification took place and the results were satisfactory.

4.8.1.1 Statistical analysis of ammonia nitrogen removal

One of the commonly used measurements of variation that account for how all the values are distributed is the standard deviation. This statistic shows how large values fluctuate above the average value and how smaller values fluctuate below it. Standard deviation is the square root of the sample variance. Ammonia nitrogen, as the major factor to examine performance of submerged membrane electro bioreactor for anammox growth, was measured several times a day to investigate the range of deviation and possible errors. Therefore, to calculate standard deviation, multiple measurements of ammonia nitrogen were conducted. To calculate the standard deviation, conducting formula:

$$\sigma=\sqrt{\Sigma(x-\gamma)^2/n-1} \quad (4\text{-}1)$$

where x is the measured value, n is the number of measurements, and γ is the mean of the values, same formula as average calculation are used for calculation of mean.

Table 4-3. Results of standard deviation in ammonium measurement

Ammonia nitrogen influent	Ammonia nitrogen effluent (MBR)	Ammonia nitrogen effluent (SMEBR)	Ammonia nitrogen effluent (SMEBR-Anammox)
0.71	0.76	0.39	0.14

4.8.2 Nitrate nitrogen removal

Ammonia is converted to nitrate in the presence of oxygen due to nitrification process. Nitrate is another type of nitrogen compound which is a big risk for the organisms.

Biological nitrate removal due to denitrification takes place in anoxic conditions while it has been highly affected by amount of DO. MBR with a high DO content does not provide adequate condition for nitrate removal; subsequently denitrification takes place in a separate reactor. In contrast, both SMEBR and SMEBR-Anammox systems create aerobic and anoxic conditions in one vessel for ammonia and nitrate nitrogen removal.

In addition, the biomass activity in the reactors should be able to use DO in both types of reactors. Since operational condition played an important role for nitrate nitrogen removal in the reactors, a good control of pH, temperature, CD, and electrode distance was necessary.

At the concentrations from 7 to 4 mg DO/L, a low nitrate nitrogen production during the first 20 days (stage 1) of tests was observed, demonstrating both lack of microbial activity and reactor equilibrium.

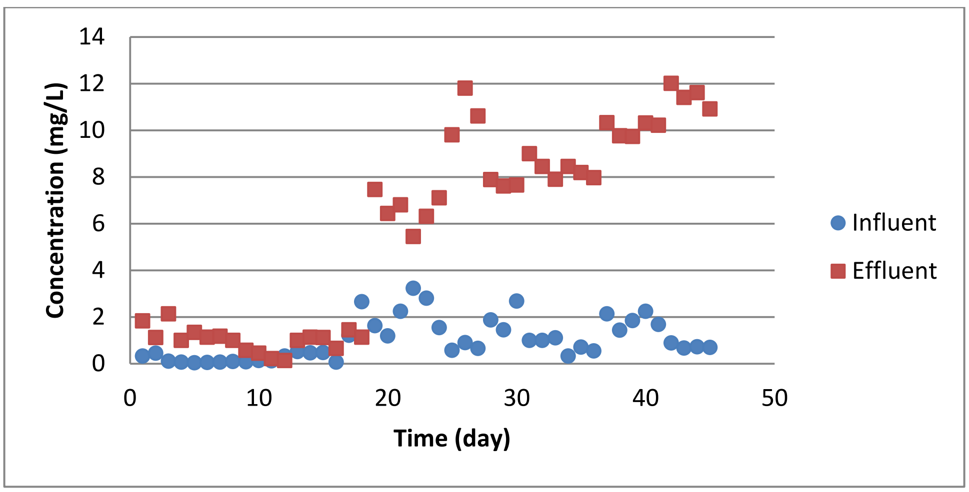


Figure 4-37. Nitrate nitrogen concentration in effluent in the MBR

The second stage was started from day 20 including fresh activated sludge (higher MLSS), then, an increase of nitrate nitrogen production was observed due to nitrification process. Then, in MBR phase stage 2, maximum nitrate nitrogen production of 12 mg/L was demonstrated. Furthermore, the average of nitrate nitrogen concentration in the effluent of stage 2 of phase 2 was 7.8 mg/L while in stage 1 this average was less than 2 mg/L (Figure 4-37).

The nitrate nitrogen concentration in first stage of SMEBR (phase 3) is shown in Figure 4-39, where the introduction of an electrical system into membrane reactor changes nitrate nitrogen fate.

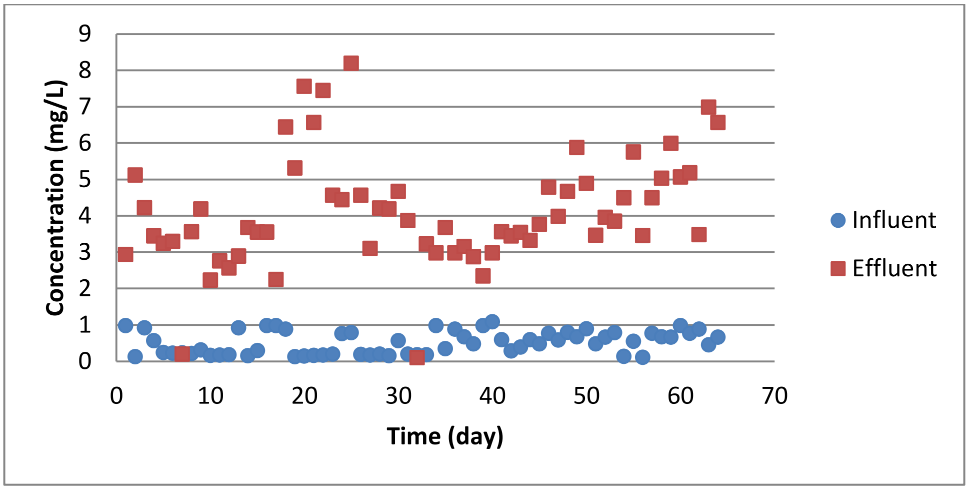


Figure 4-38. Nitrate nitrogen concentration in influent and effluent in SMEBR, stage 1

In SMEBR first stage, the production of nitrate nitrogen was related to fluctuation of aerobic/anoxic conditions. The amounts of nitrates nitrogen were decreased (comparing to MBR) due to nitrification process, however, it was still lower than it was reported by Ibeid (2011). The disturbing factors were membrane fouling, supernatant rising level, and power supply damage.

Figure 4-39 illustrates that DO had a high influence on ammonia nitrogen and nitrate nitrogen removal. When DO was decreased to a very low level (less than 0.7 mg/L), ammonia nitrogen and nitrate nitrogen concentration was increased. However, keeping DO around 1 mg/L increased nitrate nitrogen and ammonia nitrogen removal in SMEBR system.

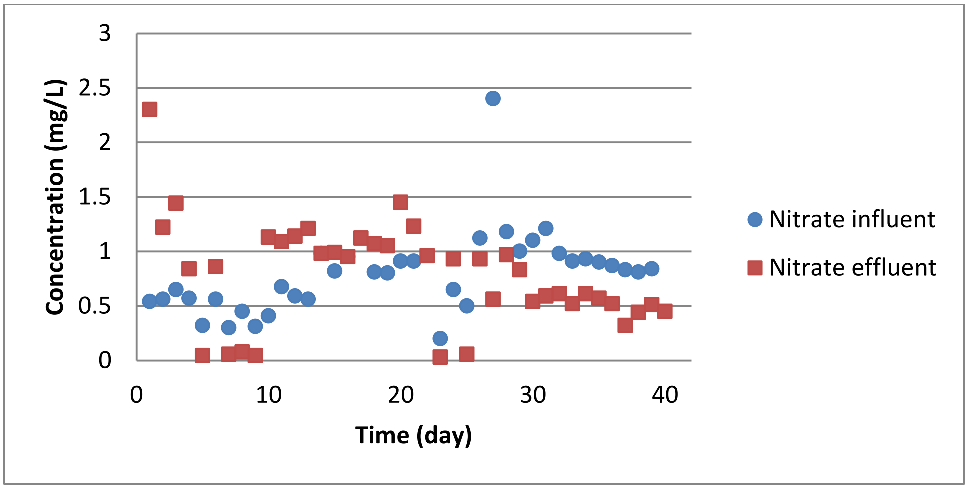


Figure 4-39. Nitrate nitrogen concentration in influent and effluent in the SMEBR, stage 2

The fourth phase (stage 1) SMEBR-Anammox, consisted of around 64% volume of anammox biomass. An immediate addition of biomass unbalanced the bioprocesses in the reactor, then, a decrease of nitrate nitrogen removal was observed during the first days (Figure 4-40). In the SMEBR-Anammox when DO was close to zero, nitrate nitrogen concentration was decreased even more reaching 0.04 mg/L concentration, while ammonia nitrogen concentration was increased. Then, decreasing DO to a very low level was not an adequate due to the increase of ammonia nitrogen level (poor nitrification performance). Therefore, finding the best amount of DO in which ammonia nitrogen and nitrate nitrogen removals reach their highest level was necessary.

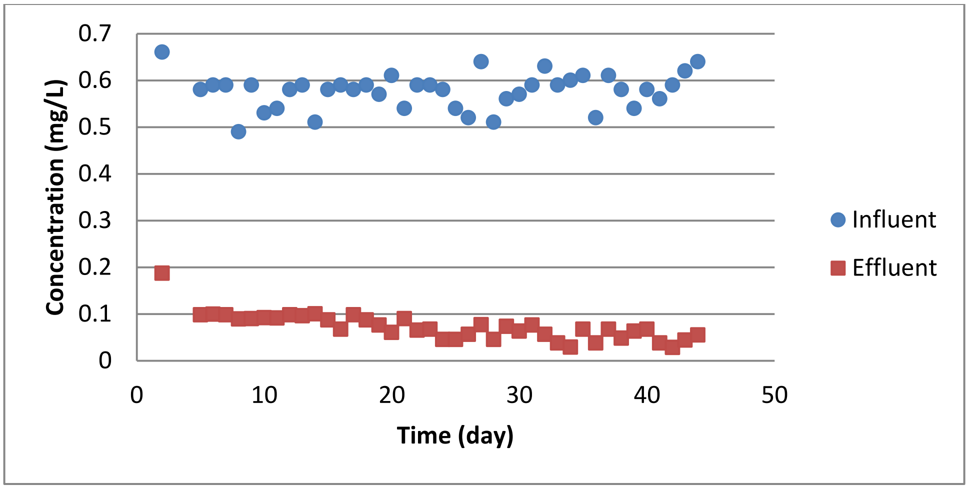


Figure 4-40. Nitrate nitrogen concentration in influent and effluent in SMEBR-Anammox (stage 1 and 2)

Afterwards, a trend in nitrate nitrogen decrease was observed until to the end of tests. The lowest concentration in the stage 1 was 0.06 mg/L. The second stage of SMEBR-Anammox lying on improvement of the electrical circuit, microbial culture and optimization of DO supply ended in the lowest nitrate nitrogen concentration of 0.02 mg/L (Figure 4-40).

Finally, the best results showed in 33 day, when DO was around 0.7 mg/L and current density was 14.5 A/m^2. This result also proved that SMEBR-Anammox system had a great denitrification capacity with DO around 0.70 mg/L. Furthermore, standard deviation of nitrate nitrogen in phase 4 was close to zero.

4.8.3 Total Nitrogen (TN)

TN expresses all forms of nitrogen in the system. In this study, TN measurement was performed when MBR, SMEBR and SMEBR-Anammox system had their best results.

TN removal tests proved that SMEBR-Anammox had better result than MBR and SMEBR (particularly at stage 1). Figure 4-41 shows that the highest TN removal in MBR reached the max amount of 24%. Furthermore, TN concentration in the effluent were 34.4 mg/L, 33.2 mg/L and 30.8 mg/L in days 12, 18 and 25, respectively.

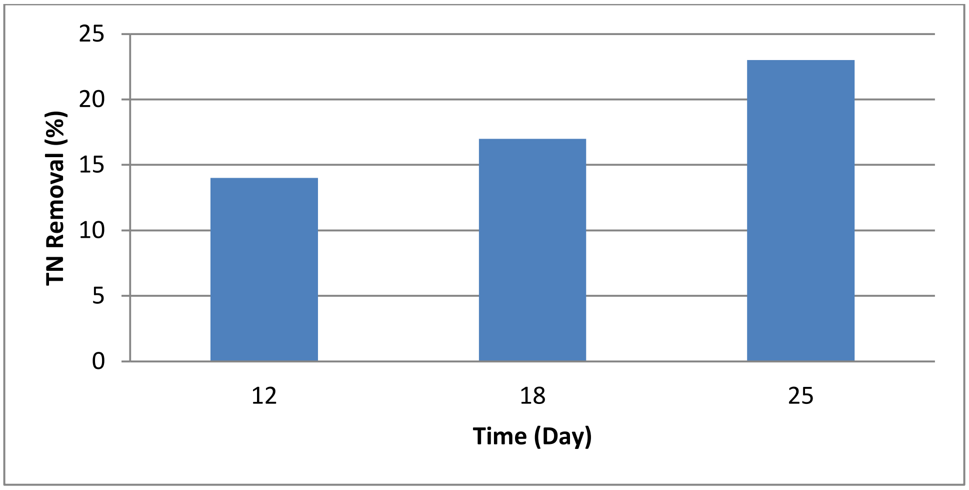


Figure 4-41. TN removal in MBR system

SMEBR having nitrification/denitrification process showed better results since in the SMEBR second stage nitrogen removal was 47% more than MBR second stage (Figure 4-42).

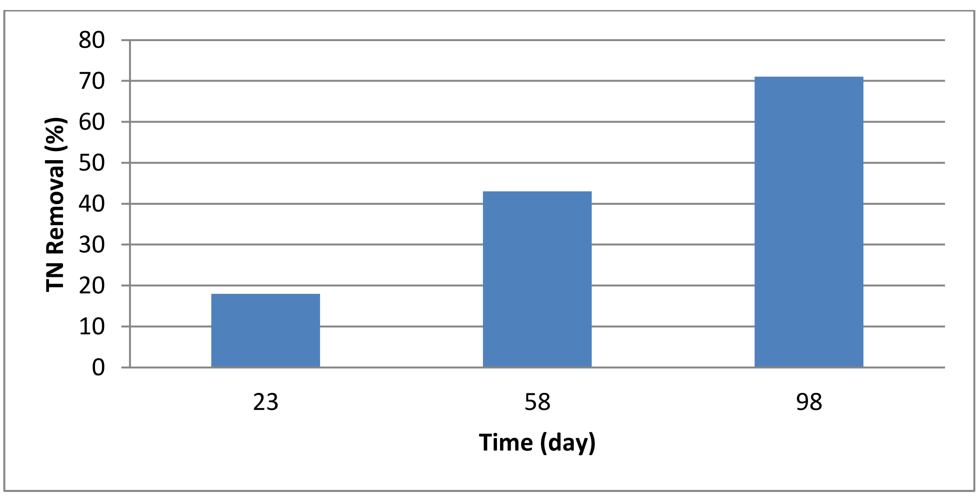


Figure 4-42. TN removal in SMEBR

In the first days of SMEBR system operation, the results were significantly affected by DO fluctuation. When the system was switched from MBR (with high amount of aeration) to SMEBR system, the microorganisms needed time to be adapted in the new condition. In the first week, SMEBR worked with DO level around 3.5 to 3 mg/L. Since SMEBR system did not show adequate improvement in stage 1 (43%), activated sludge was added in stage 2 in conjunction to DO adjustment (2mg/L). Stage 2 showed the best results of TN removal at the level of DO between 1 to 1.5 mg/L (around 70%). Similarly, the TN concentration reduced from 32.8 mg/L (phase 3, stage 1) to around 11 mg/L in phase 3, stage 2.

Last phase (SMEBR-Anammox) showed the best results of TN removal when DO was around 0.7 mg/L. However, keeping the DO concentration at the very low level (e.g. 0.5 mg/L) made the TN removal in SMEBR similar to MBR. Figure 4-43 indicates that SMEBR-Anammox was capable to remove more 83% of TN comparing to 23% in MBR and 70% (second stage) in SMEBR.

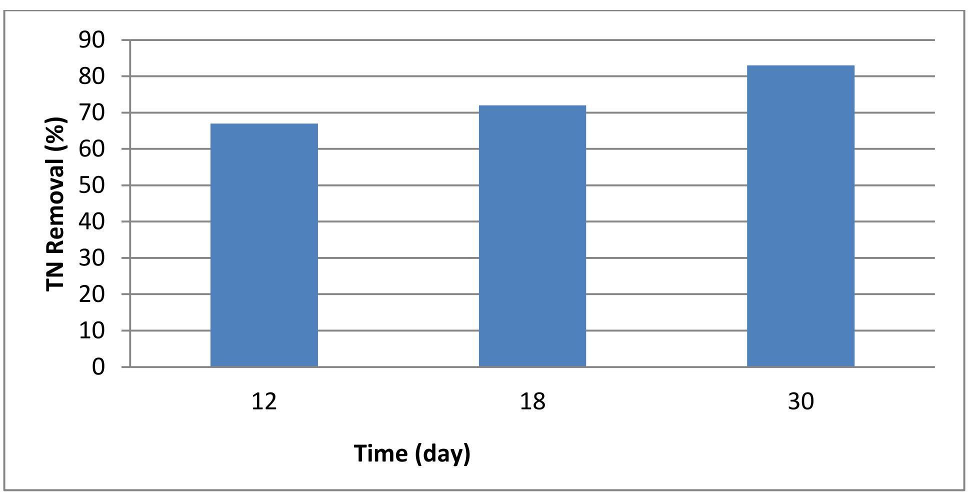


Figure 4-43. TN removal in SMEBR-Anammox

When DO level was kept between 3.5 and 0.7 mg/L, maximum TN removal of 83% in SMEBR-Anammox was detected in day 25, while the influent contained around 43 mg TN/L. However, by decreasing DO level to less than 0.7 mg/L the removal of ammonia nitrogen and TN was decreased. In the large laboratory scale a better condition would be provided for more uniform aeration in the reactor. Figure 4-43 included SMEBR- Anammox stage 1 (67% and 70%) and stage 2 (83%). Consequently, phase 4 in day 30 (stage 2) reached around 6 mg TN/L in the effluent. Therefore, an improvement around 80% took place in phase 4, comparing to phase 2 when 31 mg TN/L was generated in effluent.

4.8.4 Ammonia nitrogen versus nitrate nitrogen in phase 3 and 4

Increasing nitrate concentration is the indicator of nitrification process while decreasing in the concentration of nitrate and ammonia nitrogen in the effluent shows that denitrification takes place in anoxic conditions. The most important SMEBR characteristic which differentiates it from the other reactors is having simultaneous nitrification and denitrification.

This research evaluated the capability of SMEBR system combining by anammox bacteria, to remove nitrogen compounds by the lowest amount of DO. The relationship between ammonia nitrogen and nitrate nitrogen was another considerable factor according to nitrification and denitrification process. Figure 4-44 illustrates the level of ammonia nitrogen and nitrate nitrogen removal by SMEBR first stage.

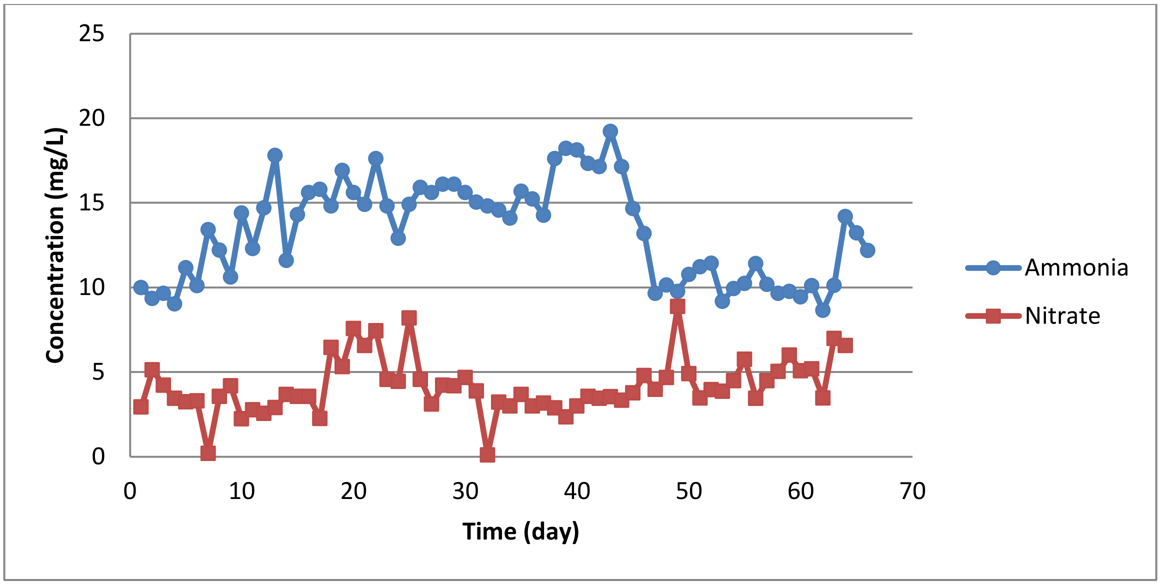


Figure 4-44. Ammonia nitrogen vs nitrate nitrogen effluent concentration in SMEBR, stage 1

In the first stage of SMEBR system, the average ammonia nitrogen and nitrate nitrogen effluent were around 14.14 and 4.1 mg/L at DO around 3 mg/L. Although the level of aeration was around 3mg/L, DO level was increased to more than 4 mg/L to motivate the inactivated bacteria in. However, in stage 1, the results required improvement since in such conditions, nitrification and denitrification were not pronounced strongly. Since ammonia concentration in SMEBR was higher than in conventional conditions, the removal was lower than usual (Hasan et al. 2012).

In SMEBR second stage, the ammonia nitrogen concentration was decreased from average 14.14 mg/L in first stage to 6.42 mg/L. Due to decreasing aeration level to 2 mg/L, nitrate nitrogen effluent became lower and denitrification process was more pronounced (Figure 4-45). At day 24, ammonia nitrogen concentration was less than 6.5 mg/L while in day 37 it reached

5.04mg/L. Moreover, nitrate nitrogen concentration was decreased to 0.32 mg/L at day 37 of SMEBR second stage.

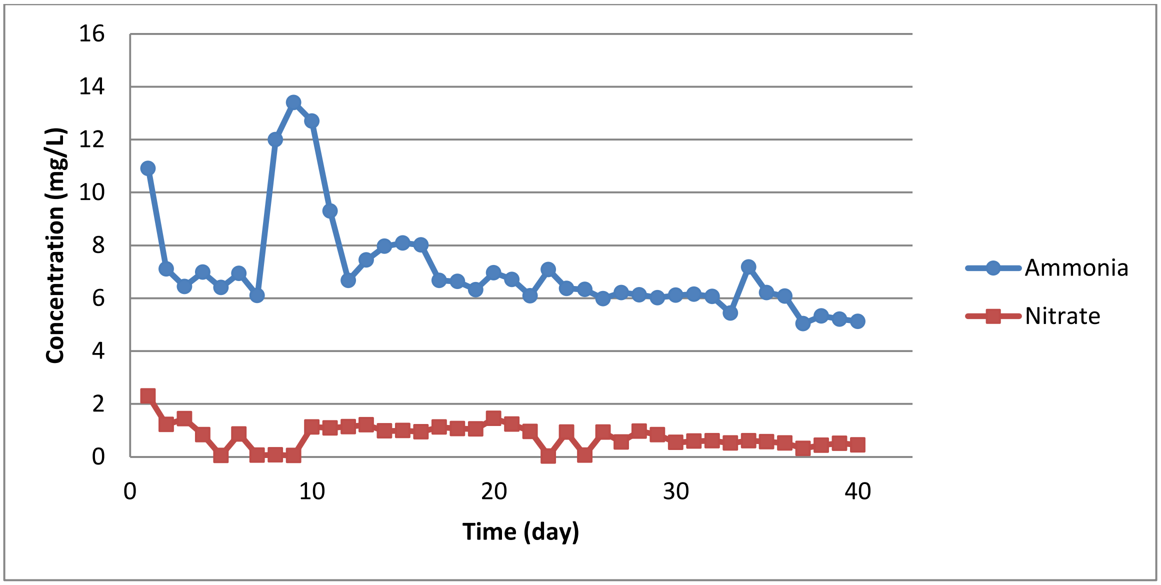


Figure 4-45. Ammonia nitrogen vs nitrate nitrogen effluent concentration in SMEBR, stage 2

Phase 4 included anammox bacteria in the SMEBR system. Anammox bacteria showed a great ability to remove ammonia nitrogen in the Anammox process. Since anammox bacteria need a low amount of DO in the SMEBR system, firstly, DO level was tested between 3.5 to 0.5 mg/L to assess better removal of ammonia nitrogen. In 3 mg/L DO, the results were still the same as SMEBR stage 2. It showed that anammox bacteria have not pounced their activities yet. In the stage 2 of SMEBR-Anammox, with increasing anammox populations and decreasing the level of aeration, better removal of ammonia nitrogen was observed. After day 22 of SMEBR-Anammox system set up, ammonia nitrogen and nitrate nitrogen effluent reached 0.99 and 0.06 mg/L by decreasing DO to around 0.7 mg/L. However, in the lower amount of DO, ammonia nitrogen concentration was increased. Consequently, the curves of ammonia nitrogen and nitrate nitrogen concentration in SMEBR-Anammox system were closer (better TN removal) than SMEBR in stage 1 and 2. In this SMEBR-Anammox system, DO was kept between 0.7 to 0.80 mg/L and ammonia nitrogen and nitrate nitrogen was decreased at the same time specifying suitability for nitrifiers, denitrifiers and for ammonia oxidizing bacteria. Figure 4-46 shows that SMEBR-Anammox system could decrease ammonia nitrogen and nitrate nitrogen to the acceptable level (0.89 and 0.028 mg/L, respectively).

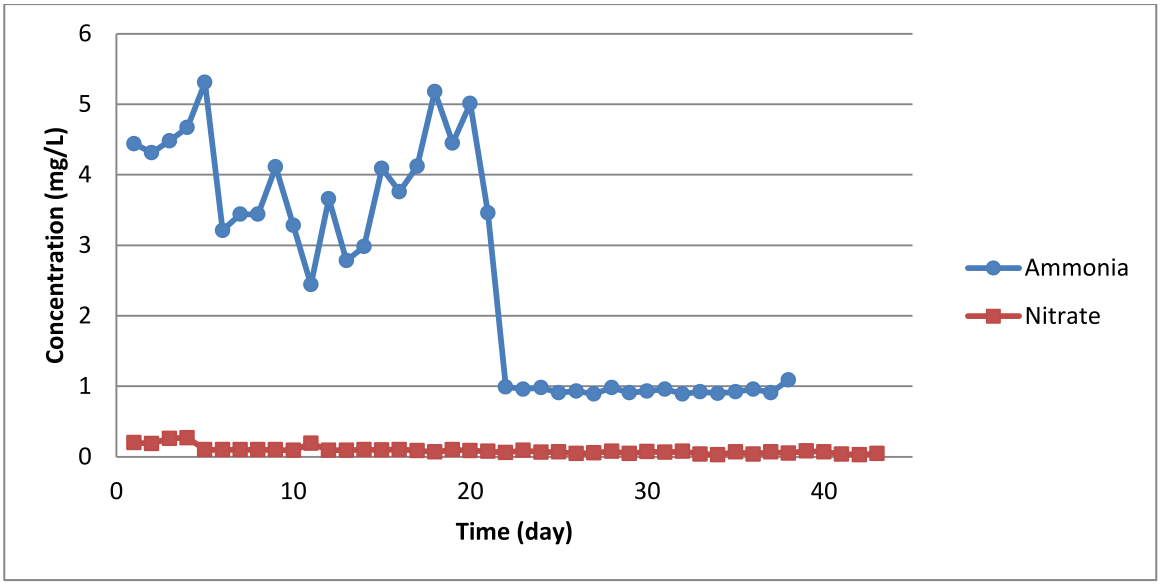


Figure 4-46. Ammonia nitrogen vs nitrate nitrogen effluent concentration in SMEBR-Anammox

Keeping DO level in the SMEBR-Anammox almost constant at 0.7-0.8 mg/L and improving the electrical circuit helped to increase ammonia nitrogen and nitrate nitrogen removal.

4.8.5 Nitrogen removal enhancement

The analysis of both ammonia nitrogen and nitrate nitrogen showed enhancement of nitrogen removal in a new design of SMEBR comparing to MBR. This study demonstrated that

MBR could remove ammonia nitrogen compound to around 76%. However, SMEBR-Anammox system increased ammonia nitrogen removal percentage to around 97%. This level of improvement was related to electrically controlled DO.

By decreasing the ammonia nitrogen concentration in the SMEBR effluent, the nitrate concentration in effluent might be increased if no denitrification process is pronounced. However, TN removal can be still decreased. Besides, increasing nitrate level in the effluent is the indicator of increasing of DO level leading to nitrification process well pronounced. In this experiment, sometimes DO or ORP probe was maintained inside of reactor and after 5 minutes, DO and ORP levels were decreased very slowly meaning that the bacteria could not reduce DO but a stream of electrons in electrical field did (eq. 2.17)

In the SMEBR first stage, the TN concentration in effluent reached 22.8 mg/L, which was a period that ammonia nitrogen and nitrate nitrogen reached their lowest level in the effluent. In SMEBR system when DO level became lower than 0.5 mg/L in the absence of DO, there was a partial nitrification (at the high concentration of ammonia in influent) and ammonia was a dominant form of nitrogen in the SMEBR effluent.

The stage 2 of SMEBR system provided better conditions for TN removal. Nitrate concentration in the effluent was decreased to an acceptable level by decreasing DO. Although ammonia nitrogen concentration in the effluent was still more than 5 mg/L, the results were improved significantly. TN concentration in phase 3, stage 2 was decreased to around 11 mg/L which showed 65% improvement comparing to MBR system.

However, by decreasing DO level to 0.5 mg/L the nitrate nitrogen concentration was decreased while ammonia nitrogen concentration was increased to 13.4 mg/L. Consequently, at a sufficient level of DO, better ammonia nitrogen and nitrate nitrogen removal resulted in better total nitrogen removal (70% in SMEBR process).

Finally, anammox bacteria were applied in a SMEBR-Anammox in an anoxic condition (at different DO concentrations) to enhance ammonia removal. In the last phase of SMEBR set up, 9 liters of Anammox-reactor content was added to 5 L of activated sludge. Besides, the DO concentration was adjusted between 3.5 to 0.5 mg/L; however, satisfactory results were achieved when DO was decreased to 0.7 mg/L only.

According to Figure 4-47, the dissolved oxygen after day 22 of SMEBR-Anammox system was set between 0.7-0.8 mg/L. This DO level shows that the sludge was mostly in anoxic conditions. Therefore, the denitrification (eq.2-6 to 2-9) or even direct ammonia oxidation reaction (eq.2-14) was greater than nitrification with such low amount of DO.

In the first days of SMEBR-Anammox system, 0.1 mg/L nitrate nitrogen was achieved in DO around 3 mg/L. However, the ammonium effluent was still more than 3 mg/L in the effluent at ammonium influent of 27 mg/L achieving an average ammonium removal of around 89%.

Ammonia nitrogen removal and nitrate nitrogen removal curve were continually changing even at the same level of aeration. This result confirms that the sludge has been also affected by other parameters such as DO control by electrical field. Figure 4-47 shows the nutrient removal improvement to reach ammonia nitrogen and nitrate nitrogen of 0.89 and 0.02 mg/L, respectively.

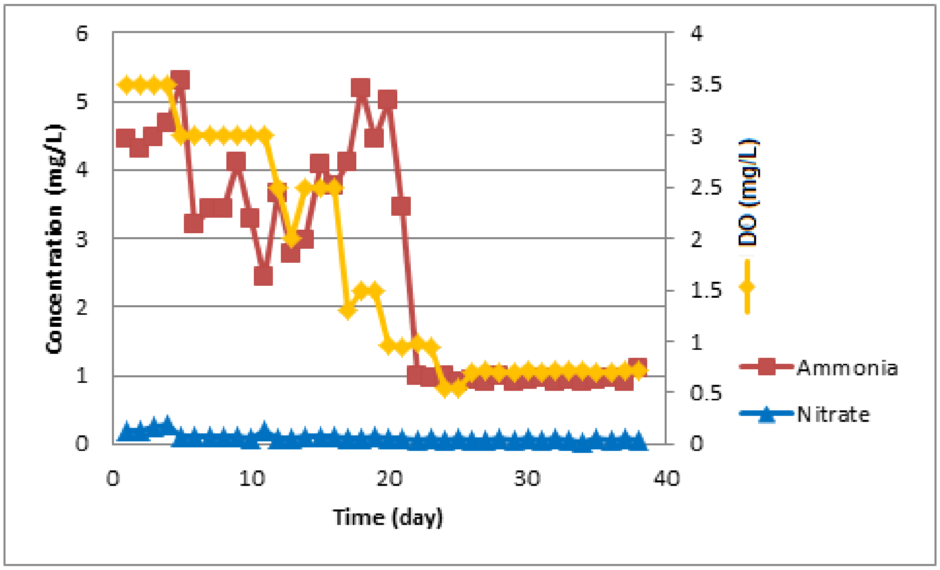


Figure 4-47. Improvement of ammonia nitrogen and nitrate nitrogen with DO level alteration in the SMEBR-Anammox

According to Figure 4-43, TN removal reached the best results (83%) when entire SMEBR-Anammox with filled with a content of Anammox-reactor (in addition to improvement of the electrical circuit) while 1.5 mg DO/L was enough to provide nitrification process in the laboratory scale reactors. SMEBR-Anammox concentration was decreased to 6 mg/L in effluent comparing to 11 mg/L in SMEBR and 31 mg/L in MBR. Therefore, TN removal improvement in SMEBR-Anammox was around 80% which proved decreasing ammonia nitrogen and nitrate nitrogen in the effluent.

TN concentration of SMEBR-Anammox system in stage 1 reached 11 mg/L, when CD of around 14 A/m^2 and DO between 1.5- 2 mg /L were applied. However, in stage 2 when more anammox bacteria were added to the system, at around 0.7 mg DO/L, the nitrate nitrogen improvement was increased to 99.95%.

The best result of ammonia nitrogen removal happened on SMEBR-Anammox system, 22 days after SMEBR- Anammox installation at DO around 0.7 mg/L. In this condition, ammonia nitrogen removal was around 97%.

The important goal of this experiment was to reach the lowest amount of ammonia nitrogen in the effluent at the lowest amount of oxygen requirement. It is assumed that the conditions generated in electro-bioreactor were adequate for all microorganisms participating in nitrification, denitrification and ammonia oxidation bacteria. The observation showed that the system is sensitive to sudden changes in DO supply; it required two to three days to recalibrate the reactor. From the economical point of view, this system seems to be the most cost-effective technology for nutrient removal at this moment.

A comparison between MBR, SMEBR and SMEBR-Anammox indicates that nitrogen removal enhancement was defined by adjustment in DO, ORP, electrical density, electrical mode of operation and pH levels. Anammox addition in the SMEBR system permitted to better ammonia nitrogen removal due to its oxidation to gas nitrogen, giving finally the best total nitrogen removal. SMEBR-Anammox achieved high simultaneous removal of ammonia nitrogen, (97.0%), nitrate nitrogen improvement (99.95%), phosphorous (99.91) and COD (99.87) representing a novel cost–effective technology for wastewater treatment.

5 Chapter COMPARATIVE ANALYSIS

5.1 Comparative COD removal

A comparative study between conventional membrane bioreactor (MBR), SMEBR and SMEBR-Anammox was performed.

Referring to Figure 5-1, COD removal after one week of operation was 73.4%, 91.6% and 96% in MBR, SMEBR and SMEBR-Anammox, respectively. The reactors were able to achieve the average concentrations of around 120, 25, and 3 mg/L in effluent for MBR, SMEBR and SMEBR –Aammox, respectively.

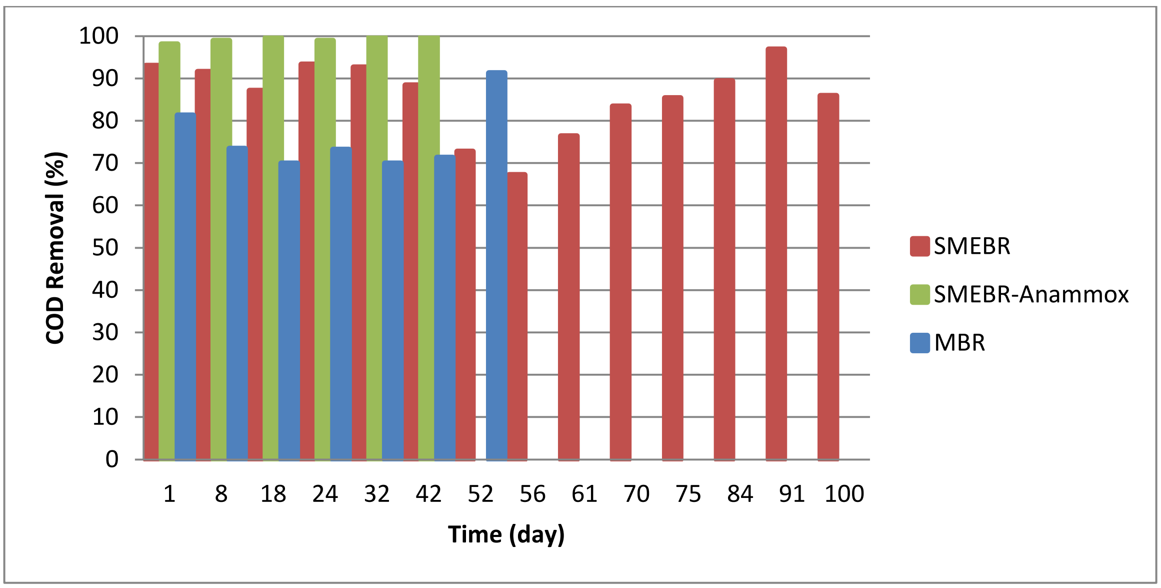


Figure 5-1. COD removal in MBR, SMEBR and SMEBR-Anammox system

The results in Figure 5-1 showed how COD removal was strongly dependent to bioreactor design and operation conditions. The concentrations of COD in MBR effluent fluctuated between 48.8 and 166.7 mg/L, while in the SMEBR and SMEBR-Anammox COD reached less than 30 and 10 mg/L in average. Considering that COD contains from 60% to 80% of BOD_5, the BOD_5 concentration could reach 1.8 mg/L, which is much lower than local regulations. The best results in COD removal were achieved in SMEBR-Anammox stage 2, when removal of nutrients was also the best. It was assumed that this design and operation condition were the best for simultaneous removal of carbon, ammonia nitrogen, nitrate nitrogen and phosphorous by 99.87, 97%, 99.95% improvement and 99.91%, respectively.

The experiment also showed that the system might be sensitive to dramatic changes within MLSS (SMEBR days 42 to 70). Moreover, the level of COD removal was changed when the MLSS of the system was increased. The system required some times to reach steady state conditions.

5.2 Comparative phosphorous removal

The highest phosphorous removal (65%) in MBR process took place in the first stage of phase 2. Average phosphorous removal efficiency was increased in SMEBR and SMEBR-Anammox to 81.71% and 95.06%, respectively. The highest removal of 99.91 % was observed in phase 4 showing that the concentration of 0.1 mg /L is much lower than acceptable limit for such type of wastewater treatment plants. The results prove that in the MBR system, the phosphorous removal was the result of some ions reaction in the presence of membrane filtration while in the SMEBR and SMEBR-Anammox system, electrokinetic process played more important role. Besides, DC voltage in SMEBR and SMEBR-Anammox process conducts precipitation of $AlPO_4$ due to electrocoagulation. According to Hassan and Elektorowicz (2011), in addition to electrocoagulation process in SMEBR, a high level of phosphorous removal is a consequence of the deposition of some phosphorus on the surface of the electrodes. Figure 5-2 shows much higher removal of phosphorous in SMEBR and SMEBR-Anammox than in MBR.

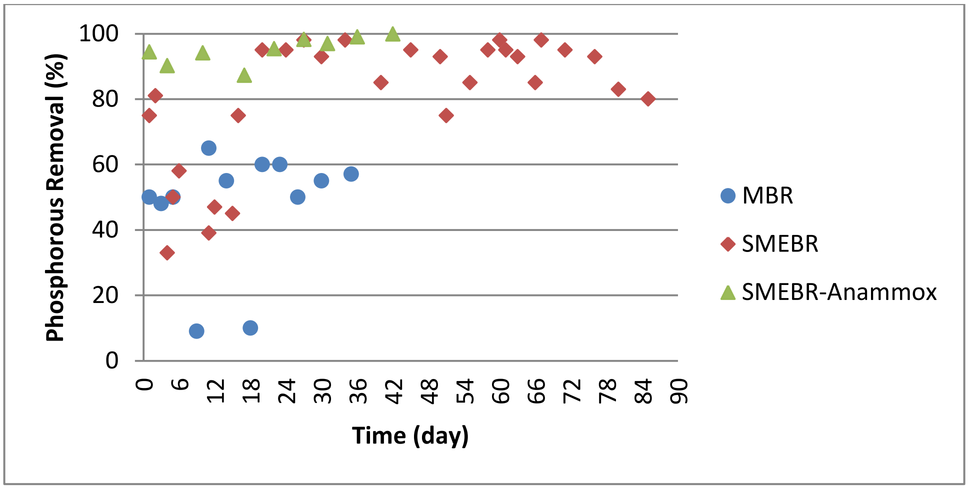


Figure 5-2. Phosphorous removal in MBR, SMEBR and SMEBR-Anammox

The fluctuation of phosphorous removal was strongly related to variation in design and operation conditions of reactors, which is the most visible in stage 1 of each design system (Figure 5-3). The main objective of the research was nitrogen removal, then, the priority was to adjust adequately the conditions for this purpose. However, since PO_4-P concentration in the SMEBR-Anammox reached 0.1 mg/L, it was provided an adequate condition to grow some other bacteria (PAO) to remove phosphorous.

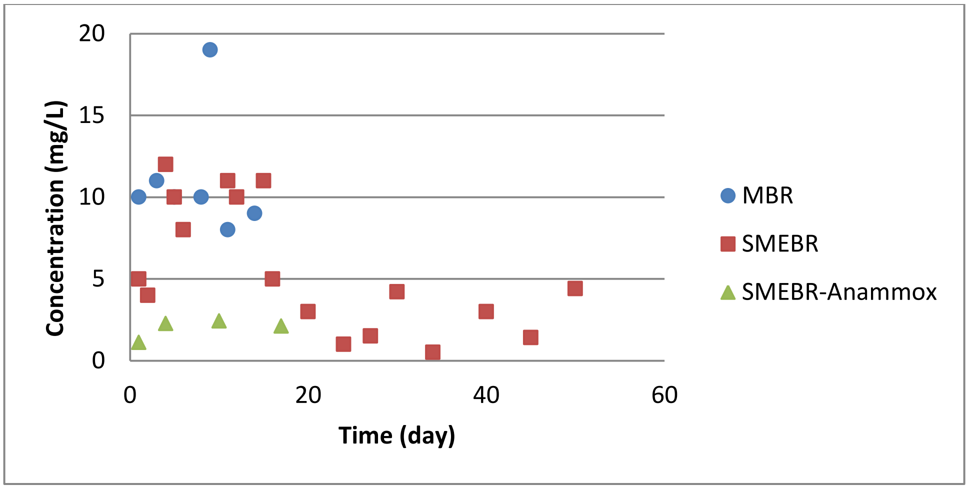


Figure 5-3. PO_4-P in MBR, SMEBR, SMEBR-Anammox, stage 1

Not all reactors were in steady state conditions. In stage 2 with the new and fresh sludge in MBR system, the phosphorous concentration decreased in effluent from 19 to 8 mg/L. To increase the activity of bacteria, while level of DO was decreasing, more fresh activated sludge (different MLSS) was added to SMEBR achieving less than 0.5 mg/L in effluent (Figure 5-4). This positive response with regards to phosphorous removal was in conjunction with the removal of other impurities. As it was mentioned in chapter 4, better COD and nutrient removal in SMEBR-Anammox system in stage 2 was because of adjustment of the system design and electrical circuit.

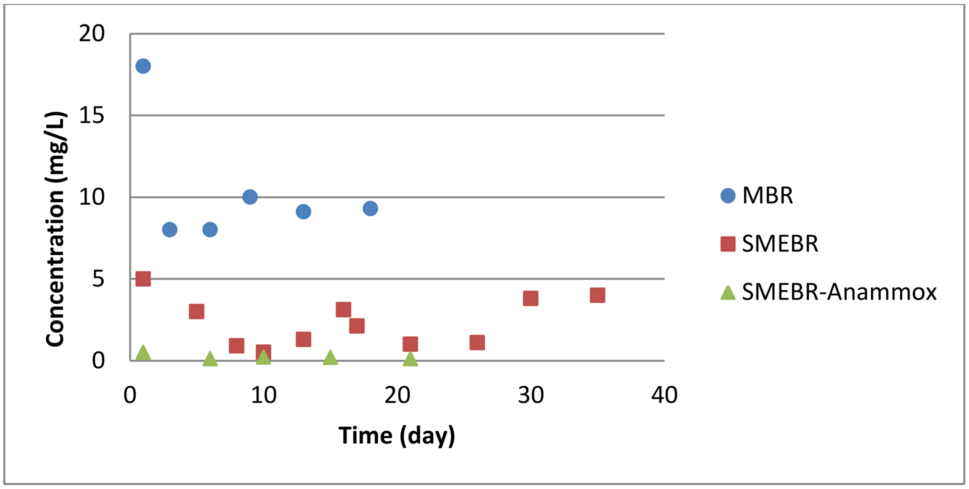


Figure 5-4. PO_4-P in MBR, SMEBR, SMEBR-Anammox, stage 2

5.3 Comparative nitrogen removal

5.3.1 Ammonia nitrogen removal

SMEBR-Anammox, being an innovative design system can thoroughly convert ammonia to nitrogen gas. Figure 5-5 shows that MBR decreased ammonia nitrogen concentration to around 6 mg/L in effluent in day 13. This level was further reduced to 5.04 mg/L and 0.89 mg/L in SMEBR and SMEBR-Anammox, respectively. SMEBR system has already illustrated the considerable improvement during the test.

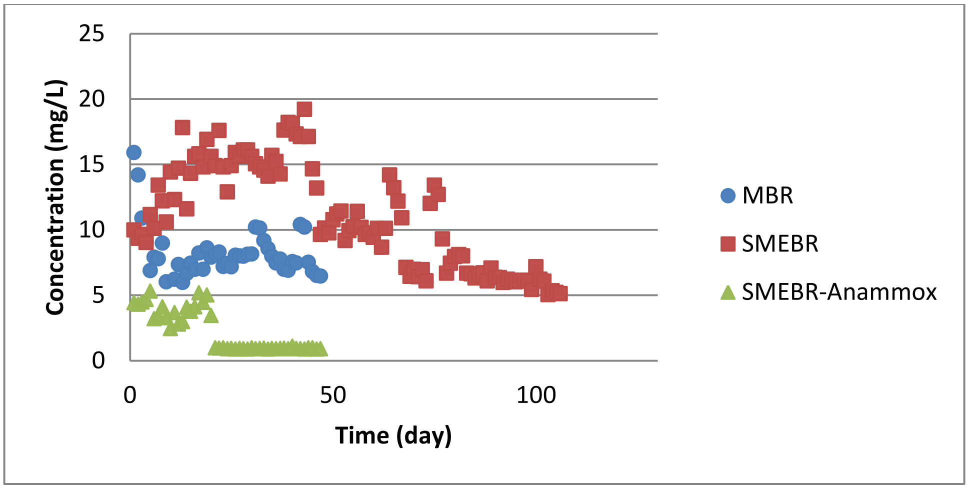


Figure 5-5. Ammonia nitrogen concentration in MBR, SMEBR, SMEBR-Anammox

In the first stage of MBR system, ammonium concentration in the effluent was the same as influent (in the wastewater); no removal of ammonia nitrogen was observed. It proves that the microorganisms did not have sufficient activity to convert the ammonia ions to nitrite and nitrate.

The objective of SMEBR system was to create aerobic and anoxic conditions in the same reactor; then, a decrease of DO was required. Subsequently, the first stage of phase 3 did not show immediate improvement in ammonia oxidation. The following remarks are with regards of the first stage of SMEBR system:

a) Adaptation of microorganisms at low DO might require a long period

b) Decreasing the level of aeration affected the process of ammonium removal

c) SMEBR system showed better results with higher level of oxygen in the first stage

However, after some period of exposure to the adequate conditions, SMEBR-Anammox system was able to decrease ammonia-nitrogen until 0.89 mg/L. At low DO nitrification, denitrification took place, but anammox used remaining nitrite to remove additional ammonia.

Then ammonia could be converted to the nitrogen gas by anammox bacteria in the electrokinetic process.

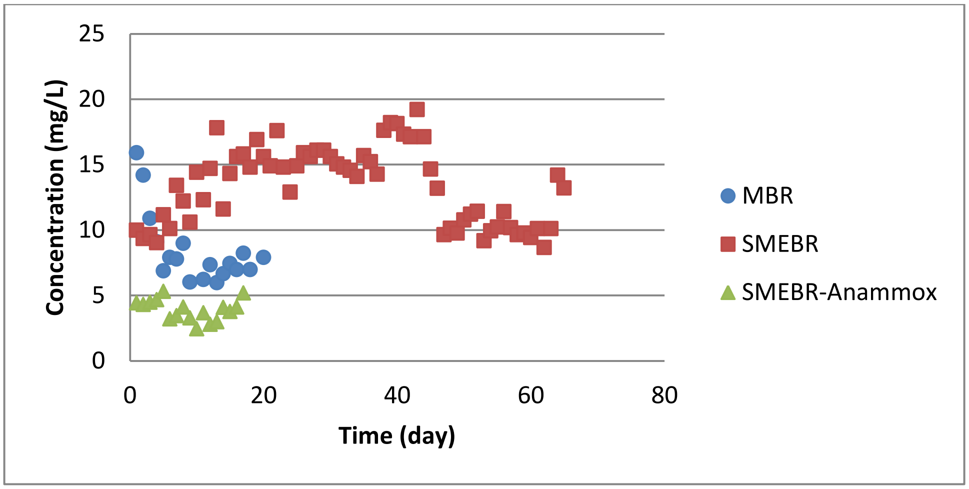


Figure 5-6. Ammonia nitrogen concentration in MBR, SMEBR, SMEBR-Anammox, stage 1

To accelerate process of microorganism's adaptation, fresh sludge was added to the rectors in the second stage. The second stage showed a better microbial activity. With better control of membrane fouling, tube clogging and aeration supply, the average ammonia nitrogen concentration reached around 6.5 mg/L in MBR system (Figure 5-7). In the second stage of SMEBR, ammonia degradation was exposed to optimal electrical field, and DO. Such conditions led to higher electron transfer, ammonia conversion on electrodes and some hydrogen peroxide production. Subsequently, the more pronounced activities on both electrodes permitted to better ammonia nitrogen removal (Figure 5-7). Figure 5-6 and Figure 5-7 indicates that the SMEBR-Anammox system's result in stage 1 was similar to the best result of SMEBR system in stage 2. It demonstrates the ability of anammox bacteria to remove ammonia nitrogen in the presence of electrokinetic process while the oxygen concentration in the SMEBR-Anammox was close to zero. However, it requires some time for an adequate anammox growth, subsequently an impact of these bacteria in the first stage of phase 4 was not evident. To accelerate the process, more anammox was added to SMEBR-Anammox from single anammox reactor in stage 2. This addition showed evident changes in the reactor performance and removal of ammonia nitrogen reached level of 0.89 mg/L in the phase 4 (Figure 5-7).

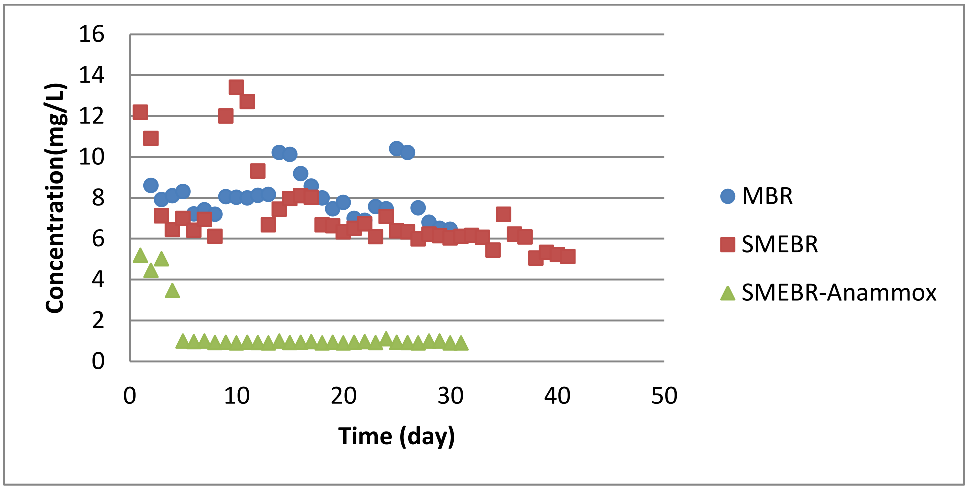


Figure 5-7. Ammonia nitrogen concentration in MBR, SMEBR, SMEBR-Anammox, stage 2

According to the results, additional contribution of anammox bacteria in SMEBR-Anammox stage 2 dropped the ammonia nitrogen concentration to less than 1 mg/L.

5.3.2 Nitrate nitrogen removal

According to section 4.9.2.2, SMEBR-Anammox illustrated a great denitrification ability to convert nitrite to nitrogen gas. Anammox used nitrite as a by-product of incomplete oxidation of ammonia to nitrate. Low DO controlled by electrical field did not permit on a complete oxidation nitrite to nitrate. Nitrate concentration in the SMEBR and SMEBR-Anammox pilot system was totally dependent on the DO concentration. In SMEBR, a small variation in DO level changed the nitrate effluent concentration quickly. For instance, when the DO concentration in the

SMEBR stage 1 was above 2.5 mg/L on days 20 to 22, nitrate concentration was more than 7mg/L (Figure 5-8). Though, when the DO was around 1-1.5 mg/L on days 30 to 40 in stage 2, nitrate concentration decreased to mostly around 0.5mg/L (Figure 5-9). Similarly, in the SMEBR-Anammox system, increasing denitrification capacity took place one week after SMEBR-Anammox installation when DO level was 0.7 mg/L (Figure 5-9).

DO concentration in SMEBR and SMEBR-Anammox system was reported at the end of the time-OFF mode of the electrical exposure time. SMEBR-Anammox results show that the system was working efficiently while the microbial activity was in the appropriate level.

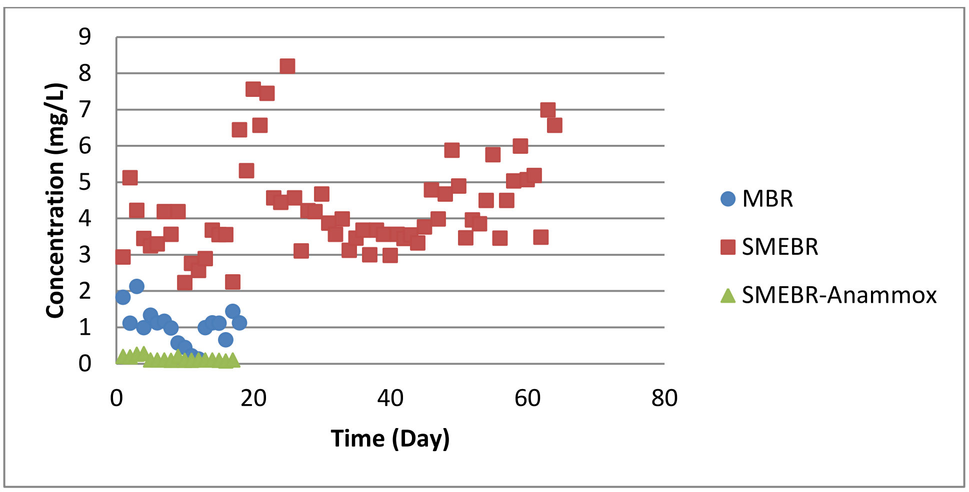


Figure 5-8. Nitrate nitrogen concentration in MBR, SMEBR, SMEBR-Anammox, stage 1

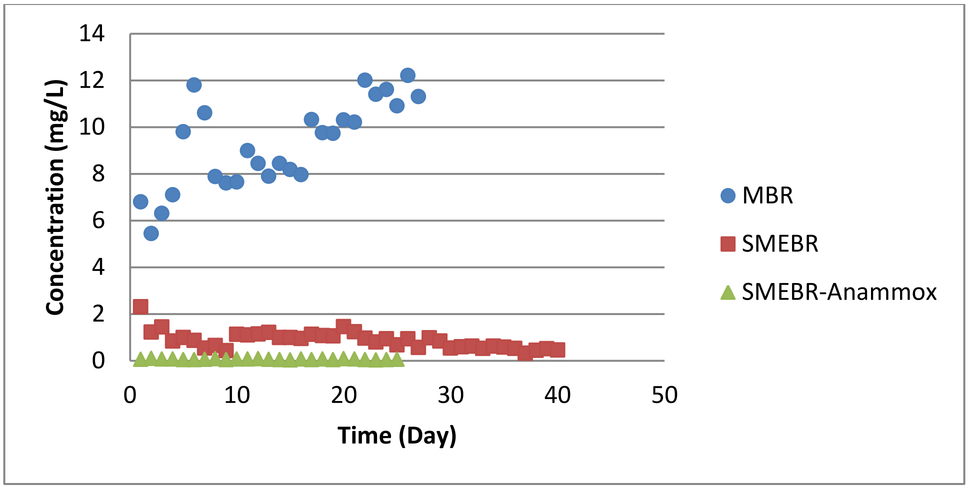


Figure 5-9. Nitrate nitrogen concentration in MBR, SMEBR, SMEBR-Anammox, stage 2

On the other hand, nitrate concentration in MBR was much higher than SMEBR-Anammox showing that there is highly improvement in nitrate concentration by SMEBR-Anammox. Likewise, averagely, 99.95% improvement took place in phase 4 more than phase 2 in stage 2.

Figure 5-10 shows cumulative results of three experimental phases for ammonia nitrogen and nitrate nitrogen concentration. The highest removal of ammonia nitrogen was observed in SMEBR-Anammox. The satisfactory results of 97.0% achieved in stage 2. The removal of ammonia nitrogen in MBR was around 76%. This amount of removal efficiency illustrates the good control of pH, temperature, reactor configuration, ON-OFF mode operation, current density, air supply versus conductivity of the sludge, concentration of MLSS, and anammox in SMEBR-Anammox.

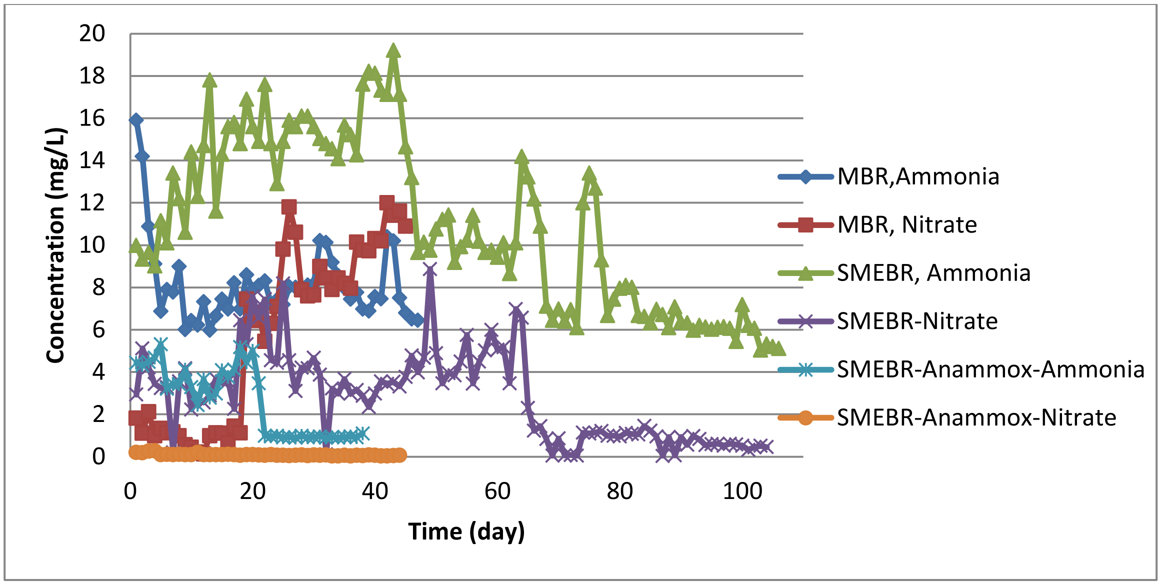


Figure 5-10. Nitrate and ammonia nitrogen concentration for different bioreactor types

5.4 Comparative dissolved oxygen

Figure 5-11 illustrates the variation in the aeration level in MBR, SMEBR and SMEBR-Anammox system. DO adjustment is an essential factor in all the reactors because it affects the ammonium and nitrate nitrogen concentration (Figure 4-47), pH (Figure 4-17), ORP (Figure 4-10 to Figure 4-16). Higher DO in MBR crated better removal of COD and ammonia nitrogen, while other parameters were poorly removed. The same conclusion was generated in SMEBR system (Figure 5-11). SMEBR-Anammox did not require a high DO concentration to activate the anammox bacteria. Moreover, anammox bacteria needed the lowest level of aeration in order to convert ammonia and nitrate to nitrogen gas (Figure 5-11).

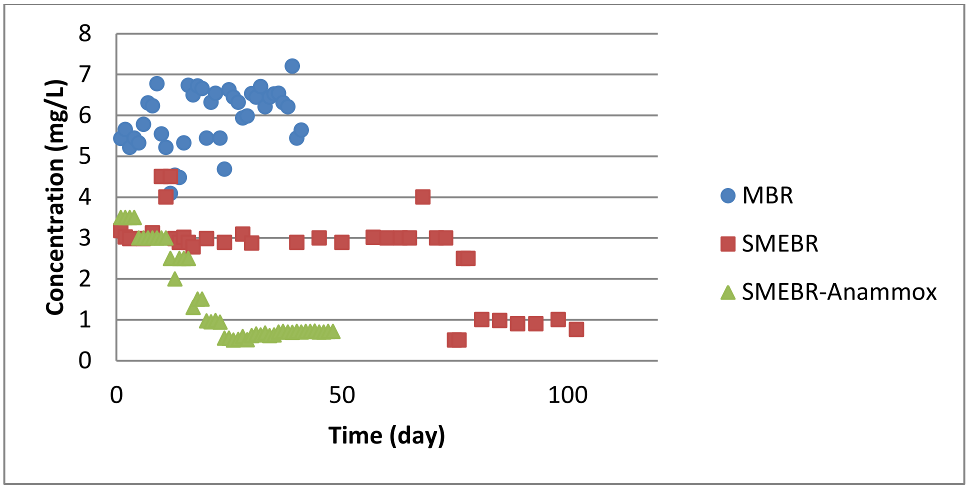


Figure 5-11. General DO concentration in different reactors

5.5 Comparative pH values

In this study, pH was measured by a probe-meter in 3 reactors (4 phases) over 8 days. In phase 2, there was no current in the system; therefore, pH in reactor was neutral (between 6 and 7). The pH monitored in phase 2, 3, and 4 is shown in Figure 5-12 and Figure 5-13. In case of SMEBR, as soon as the current was connected, CD=13A/m^2, pH was increased from 6 to 8; however, they decreased pH to 6 within a few days (Figure 5-12). In the SMEBR-Anammox the pH was increased slightly and decreased slightly later to a neutral level (around 7).

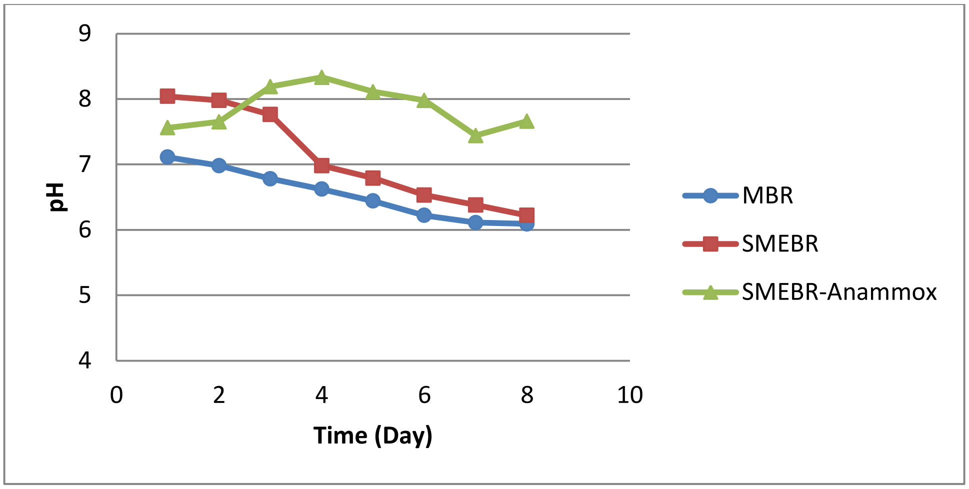


Figure 5-12. pH vs time in MBR, as well as in SMEBR, and SMEBR-Anammox where CD = 13 A/m^2 was applied

By increasing current to 1A (14.2 A/m^2), pH was increased to 8 in SMEBR and SMEBR-Anammox and stabilized (Figure 5-13). The pH value in MBR was slightly decreased (5.7) at the beginning and increased to neutral range later.

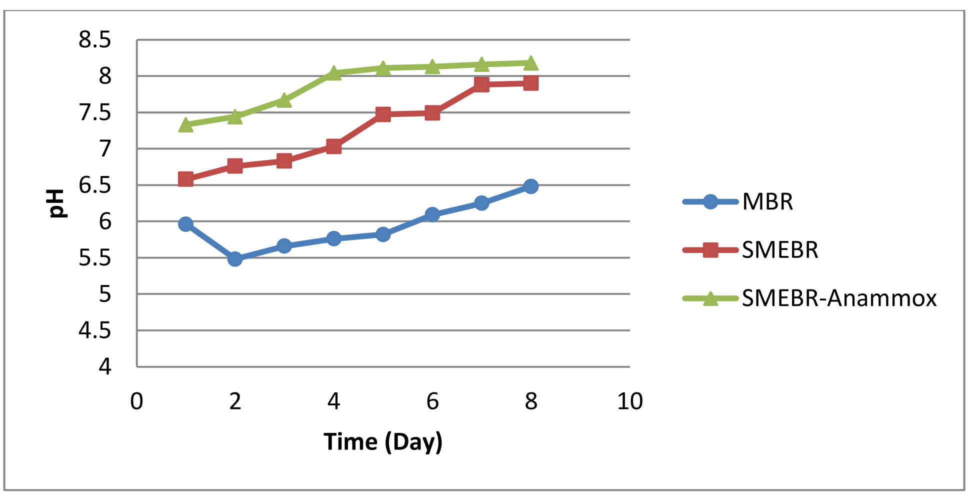


Figure 5-13. pH vs time in MBR, as well as in SMEBR, and SMEBR-Anammox where CD = 14.2 A/m^2 was applied

Increasing the current level in SMEBR and SMEBR-Anammox, produces more hydroxide ions which prevents acidic condition which is not favorable for the microorganisms' activity and therefore enhances biological processes.

5.6 Comparative performance of Anammox processes

The comparison between SHARON-Anammox, CANON-Anammox and SMEBR-Anammox systems as new and promising microbial processes to remove ammonia shows that:

- SHARON and CANON as the conventional treatment processes, require extra units for nitrite addition while in SMEBR-Anammox system all the procedures happen in the same reactor.

- SHARON and CANON need more pumps to connect different units which increase cost and footprint in comparison with SMEBR-Anammox.

- Unlike SHARON and CANON, SMEBR-Anammox always follows the same algorithm as a biological system.

- Nitrogen conversion of SHARON-Anammox is limited by the maximal strength of the wastewater being treated.

In the novel SMEBR-Anammox reactor, bacteria activity was improved by electrical field. Morphological change of anammox biomass under electric field might be the reason of high nutrient removal in SMEBR-Anammox. In the presense of hydrazine oxidoreductase (HZO) enzyme as an intermediate, N_2 is produced in anammox process (Yin et al. 2015).

Anammox bacteria addition in the biological system including activated sludge exp. SMEBR decreased oxygen demand approximately to more than 20% less oxygen demand than SMEBR separately and more than 50% than MBR system (Eini 2012).

Since carbon is needed for nitrification/denitrification processes, using the anammox from nitrite and ammonia instead of full denitrification from nitrate will save a large amount of carbon source.

In the same volume of reactor, energy cost in the biological reactor-Anammox is less than nitrification-denitrification process because nitrification process needs a huge amount of oxygen to convert ammonia to nitrate. Beside, mixing power in the nitrification process is much more than anammox.

Using an anammox process \$460,739 per year in 185,867 m^3 volume and 174,154 m^2 footprint will be saved, while reaching a high level of total nitrogen removal in comparison to nitrification-denitrification process (Eini 2012).

The addition of the anammox bacteria for the SMEBR system improved ammonia nitrogen to from 80.82% to 97%.

The net energy consumption in membrane combined process (anaerobic digestion and nitritation-anammox) was estimated around 0.09 kWh/m^3. However, in conventional activated sludge process, this energy is changed to 0.3-0.6 kWh/m^3 which will be used for oxygen consumption (Dai et al. 2015).

Finally, oxygen requirements of nitrification section in nitrification-denitrification processes increase the power consumption which is achieved by $P=VI$, where V is electrical potential (voltage), I is electrical current (amperes), $V=IR$ and R is resistance (ohms). This power depends on mechanical mixing which is reduced in the anammox- including reactors that do not need huge facilities. In the second stage of SMEBR-Anammox, power required was 0.016 kWh and the energy consumption was 1.14 kWh/m^3 considering 14 L reactor in stage 2 (best removal). In SMEBR system separately, this energy consumption was 1.30 kWh/m^3.

SMEBR-Anammox is a new system with totally different operational conditions and ratio of microbial community, in which, there are adequate conditions for simultaneous growth of nitrifiers, denitrifiers, anammox and PAO in order to participate in the process of nutrient removal. This system by decreasing the cost of chemical additions, oxygen requirement, energy consumption for pumping to different units (anoxic and aerobic), extra units for nitrate (denitrification process) or nitrite addition (anammox process) and increasing COD and nutrient removal, can be considered as an efficient system even at a large scale.

6 Chapter CONCLUSIONS

6.1 Conclusion

This study showed that a novel SMEBR-Anammox technology was able to provide adequate conditions for anammox activity in electrical field and to achieve an excellent performance with respect to removal of nitrogen, phosphorous, and COD from municipal wastewater. Anammox reactor as a separate system was applied to grow anammox and achieve acceptable capacity of ammonia nitrogen removal in anammox culture (more than 99%) at a high ammonia load.

A comparison between MBR, SMEBR and SMEBR-Anammox with respect to removal of ammonia nitrogen, nitrate nitrogen, COD and phosphorous was performed by applying various operation conditions. Here are the main outcomes:

-The simultaneous removal of ammonia nitrogen was 97.0, 80.82 and 75.9% in SMEBR-Anammox, SMEBR and MBR, respectively

-The simultaneous removal of nitrate nitrogen was 95.2% and 85% in SMEBR-Anammox, SMEBR respectively. No removal of nitrate nitrogen was observed in MBR since a separate reactor should be constructed for denitrification process for MBR system. Improvement by percentage in SMEBR-Anammox was 99.95% in comparison with MBR.

-The simultaneous removal of phosphorus was 99.91, 98.22 and 65% in SMEBR-Anammox, SMEBR and MBR, respectively. The phosphorous removal in conventional MBR is usually much lower if coagulants are not used.

The simultaneous removal of COD by 99.87, 96.9 and 91.3% was observed in SMEBR-Anammox, SMEBR and MBR, respectively.

Relationship between operation conditions and nutrient removal showed that decreasing DO and increasing CD level increased the removal rate of ammonia to more than 97% in SMEBR-Anammox. The best results were achieved when HRT=24 h, SRT = 20 days, DO=0.7 mg/L and intermitted CD=14.5 A/m^2 considering mode of operation ON/OFF = 5min:15min.

Nitrification, denitrification and anammox process were responsible simultaneously for nitrogen removal in SMEBR -Anammox.

Besides electrocoagulation in SMEBR and SMEBR-Anammox, PAO growth in SMEBR-Anammox was responsible to achieve superior phosphorous removal.

It was also concluded that SMEBR system with time under adequate conditions can be transformed into SMEBR-Anammox since anammox bacteria require a long time for growth. Subsequently, it is expected that the performance of the SMEBR system will be higher with time due to the anammox and PAO growth.

6.2 Contribution

This study developed and provided for the first time an assessment of a novel reactor combining SMEBR and anammox process in one reactor.

The particular operation conditions, which satisfied aerobic and anoxic type of microorganisms in one vessel, were defined.

Research also demonstrated that electrical field can generate nitrite due to partial oxidation of ammonia, which is adequate to growth anammox in one reactor without additives, subsequently, the reactor shows a superior performance of SMEBR-Anammox over MBR and SMEBR (for a high ammonia load) .

6.3 Future works

- Since DO control is difficult in the small scale facilities, the effort should be concentrated on application of this methodology at pilot/full scale wastewater treatment plants. It is expected to have less disruption in large scale reactors in terms of equal air distribution.
- SMEBR-Anammox system should be tested under variable temperature to evaluate the ability of this system to adapt to different environmental conditions. Testing anammox temperature was around 21° C. Increasing temperature to more than 21° C will positively affect the results of SMEEBR-Anammox system.
- Tests at pilot tests should be conducted with SMEBR –Anammox where daily variation of COD, ammonia nitrogen and phosphorous is observed.

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