Nitrogen Removal by the Combined Partial Nitrification and Anammox Processes in the BioCAST Technology

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ABSTRACT

Nitrogen Removal by the Combined Partial Nitrification and Anammox Processes in the BioCAST Technology

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Nitrogen removal from synthetic wastewater through combined partial nitrification (PN) and anammox processes in a new integrated multi-environment wastewater treatment technology called BioCAST was investigated. Based on the design and operation strategy of BioCAST technology, it was assumed that this technology was suitable for nitrogen removal by the combined nitrification and anammox processes. This is due to the fact that the BioCAST technology contains several zones with different environmental conditions that are required for the growth and activity of both ammonium oxidizing bacteria (AOB) and anammox bacteria. Moreover, the three zones are in physical contact with each other, implying that the byproduct of nitrification; i.e. nitrite will be readily converted by the anammox bacteria to nitrogen gas via the anammox process. The system operated with ammonium concentration in the range of 10 to 350 mg/l for 120 days at hydraulic retention times of 2 and 4 days. The nitrogen loading rates (NLR) during the reported operation period changed in the range of 0.0021 to 0.17 $kg/m^3.d$. The most favorable dissolved oxygen (DO) concentration inside the aerobic, microaerophilic and anoxic zones of the first BioCAST bioreactor was found to be in the range of 0.9-1.2 mg/l, 0.1-0.4 mg/l and 0.0 mg/l, respectively. The most favorable pH during partial nitrification and anammox processes was found to be in the range of 7.5-8.1 in aerobic and microaerophilic zones and 7.8-8.1 in the anoxic zone of the bioreactor. The BioCAST technology demonstrated ammonia-nitrogen as well as total nitrogen (TN) removal efficiencies up to 85.6% and 81.2%, respectively, through PN and the anammox processes. Furthermore, scanning electron microscopy (SEM) revealed the presence of irregular cauliflower structure of anammox bacteria inside the BioCAST bioreactor. In conclusion, the BioCAST technology with unique characteristics for combined partial nitrification (PN) and anammox processes is suitable for the removal of nitrogen from a synthetic wastewater without the need for organic carbon.

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Dedication

To my mother for all her love, support and strongly being with me through all steps of my life.

To my lovely and kind brothers; Behnam & Behzad.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degrees Celsius
ANAMMOX	Anaerobic Ammonium Oxidation
AOB	Ammonia Oxidizing Bacteria
CANON	Completely Autotrophic Nitrogen Removal
DO	Dissolved oxygen
HRT	Hydraulic retention time
NH_3-N	Ammonia-nitrogen
NLR	Nitrogen loading rate
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
NOB	Nitrite Oxidizing Bacteria
OLAND	Oxygen Limited Autotrophic Nitrification Denitrification
PN	Partial Nitrification
SBR	Sequencing Batch Reactor

SHARON	Single reactor system for High-rate Ammonium Removal Over Nitrite
SEM	Scanning Electron Microscopy
TN	Total nitrogen
TSS	Total suspended solids
VSS	Volatile suspended solids

CHAPTER 1: INTRODUCTION

1.1 Background

Nitrogen is a vital element that plays an important role in the global nutrient cycle. It is an essential compound in the protein synthesis as well as the biological wastewater treatment processes. Nitrogen is also an important pollutant in municipal, agricultural and industrial wastewater that has to be removed for safe discharge of wastewaters into the surface waters. Wastewater treatment has been classified as primary, secondary and tertiary wastewater treatment that depends on the contaminant concentration and disposal limits. Wastewater treatment can be performed with a variety of processes depending on the contaminants concentration and disposal limits (Tchobanoglous et al., 2003). In wastewater, the most common forms of nitrogen are ammonia, ammonium, nitrite and nitrate. Urea and proteinaceous matter in freshwater are considered the main sources of nitrogen. The nitrogen in protein and urea is organic nitrogen. Organic nitrogen can enter septic systems as bodily wastes, discarded food material, or as components of cleaning agents. (Tchobanoglous et al., 2003).

Nitrogen compounds in wastewater play an essential role in eutrophication and nitrite enrichment that reflect the high amounts of chemical nutrients (i.e., compounds containing nitrogen or phosphorus) in an ecosystem. Eutrophication reflects in an increase of algae, aquatic plants, decrease in the amount of dissolved oxygen, and increase of taste and odor (National academy of sciences, 1969).

There are various methods for nitrogen removal, including physical, chemical and biological processes. Nitrification, denitrification and the anammox process are considered as biological techniques for ammonia removal. In the biological process, there are specific methods to remove ammonia from high nitrogen concentration wastewaters with low organic carbon. Nitrification, denitrification and anammox process can happen by using different processes such as SBR (Strous et al., 1998), SHARON (Dongen et al., 2001), CANON (Hendrik & Strous, 2002; Hanaki et al., 1990; Kuai & Verstraete, 1998) and OLAND (Li et al., 2008; Khin & Annachhatre, 2004; Zhang et al., 2008).

In this project, a feasibility study for the occurrence of anammox process in a new multi-environment technology called BioCAST has been conducted. BioCAST includes two interlinked reactors with four different zones of aerobic, microaerophilic, anoxic and anaerobic.

It also has two clarification zones and a filtration unit in order to separate solids from liquid (Behzadian, 2010). The first reactor contains three biological zones of aerobic, microaerophilic and anoxic. The aerobic zone contains fixed film and suspended microorganisms, contributing to the increase of biomass retention capacity of this technology. The support medium for the fixed-film biomass is fabricated from stainless steel and is wrapped in a geotextile material. The second bioreactor includes an anaerobic zone, a clarification zone, a filtration unit and an outlet for the effluent (Yerushalmi et al., 2011).

The BioCAST technology is developed for simultaneous removal of organic and inorganic compounds (i.e. nitrogen and phosphorous) (Yerushalmi et al., 2011). The unique characteristics of the BioCAST technology, including the presence of both suspended and immobilized biomass as well as the multiplicity of zones with various environmental conditions of aerobic, microaerophilic, anoxic and anaerobic produce a favorable environment for the growth and activity of the anammox bacteria, leading to the removal of nitrogen by the combined nitritation and anammox processes instead of nitrification and denitrification processes that were previously shown to contribute to the removal of nitrogen by this technology (Yerushalmi et al., 2011; Alimahmoodi et al., 2012).

1.2 General Goal

The main objective of this project was to design and conduct a feasibility study for the establishment of the anammox process and the removal of nitrogen by the combined partial nitrification and anammox processes in a new multi-environment technology named BioCAST.

1.3 Specific Objectives:

1. To set up the laboratory-scale multi-environment BioCAST bioreactors as well as the monitoring and control systems.
2. To determine favorable operating conditions for the growth of anammox bacteria and the continuous operation of anammox process. The pertinent operating parameters included the DO concentration, pH, air flow rate and HRT in various bioreactor zones according to the requirements of PN and the anammox process.
3. To characterize the anammox bacteria by using optical microscope and scanning electron microscope (SEM) images.

1.4 Organization of the Thesis:

Based on the specific objectives of this project, this thesis is organized as follows:

Chapter 1:

A brief introduction of different methods of nitrogen removal in wastewater treatment systems and introduction of the BioCAST technology.

Chapter 2:

Literature review, focusing on the importance of ammonia removal in the wastewater treatment, physical, chemical and biological methods of ammonia removal, different biological methods of nitrogen removal and presentation of the anammox process as an energy-efficient process, comparison of BioCAST technology with the existing technologies for the anammox process.

Chapter 3:

Description of materials and methods used in this study.

Chapter 4:

Results and discussion of the BioCAST operation with the anammox process, presentation of removal efficiencies for ammonia and the total nitrogen, presentation of growth data on AOB , NOB and anammox bacteria in response to different operation conditions, and characterization of the anammox bacteria with SEM images.

Chapter 5:

Conclusions.

Chapter 6:

Recommendations for future work.

CHAPTER 2: LITERATURE REVIEW

2.1 Sources of Ammonia

Ammonia is a colorless gas with a strong pungent odor. Ammonia dissolves readily in water to form aqueous ammonium ion. This is a reversible equilibrium reaction shown in equations 2.1 and 2.2:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad 2.1$$

$$\frac{[NH_3]\,[H^+]}{[NH_4^+]} = 5.7 \times 10^{-10} \qquad 2.2$$

The ammonia solubility in water is affected by the temperature, atmospheric pressure and dissolved or suspended materials (Ramalho, 1983). The various forms of nitrogen for domestic wastewater include 50-60% of NH_4^+ nitrogen, 40-59 % organic nitrogen (a nitrogen compound that had its origin in living material), 0-5% nitrites and nitrates. Therefore, the major forms of nitrogen in domestic wastewater consist of organic nitrogen, nitrate, nitrite, ammonia and ammonium ion (Ramalho, 1983), According to the World Health Organization (1986), some of the physical and chemical properties of ammonia are presented in Table 2.1 (UNEPA, 1986).

Table 2.1 - Physical and chemical properties of ammonia.

Properties	Values
Boiling point	-33.42 °C
Melting point	-77.74 °C
Density of Vapor	0.6 g/L at 20 °C
Solubility in water	529 g/L at 20 °C ; at 0 °C 895 g/L
Vapor Pressure	857.1 kPa at 20 °C

Ammonia can be easily found in the environment resulting from natural processes and through industrial activities (UNEPA, 1986). Most wastewater streams and contaminated groundwater require the removal of organic and inorganic pollutants. Wastewater may contain high ammonia concentrations of up to 2000 mg/l (Jetten et al., 1998). Aerobic nitrification and anoxic denitrification are two biological processes which have been commonly used for the removal of nitrogen in ammonium-rich wastewaters. However, they are not considered as the best option due to their inherent difficulties and limitations (Rittmann & McCarty, 2001).

A new process called anammox "Anaerobic Ammonium Oxidation" has recently become a significant player in the global nitrogen cycle for the removal of nitrogen (den Camp et al., 2006). The anammox process is the oxidation of ammonia to nitrogen by utilizing nitrite as an electron acceptor (den Camp et al., 2006). Ammonium compounds consist of a large number of salts such as ammonium chloride, ammonium nitrate, and ammonium sulfate as well as organic nitrogen that can breakdown and present potential ammonia sources. Moreover, ammonium is one of the major substances that enters the environment through the use of agricultural fertilization, coal gasification, synthetic fiber plants, meat and milk processing, cleaning operations, ice plants (Barnes & Bliss, 1983). Ammonia is released into the aquatic environment from a variety of man-made point source and non-point sources.

2.1.1 Point Sources of Ammonia

The main man-made point sources of ammonia discharge into the watershed include conversion of coal to coke in coke plants, metallurgic operations, ceramic production, strip mining, chemical synthesis (nitric acid, synthetic monomers, and plastics), waste gas treatment, sewage treatment plants, production of household cleaners, oil refineries, and food processing (Oram, 1999).

2.1.2 Non-Point Sources of Ammonia

Non-point sources of ammonia are extremely complex and are not easy to estimate their magnitude in comparison with the point sources. Non-point sources include fertilizer runoff, animal wastes spread on the soil, urban runoff, and precipitation (UNEPA, 1986).

2.2 Harmful Effects of Ammonia

2.2.1 Side Effect on Human Beings

Two general types of ammonia such as gas and particulate form show side effects on human. The particulate form is able to be respired and diffused deep into lung tissue to the alveoli causing a diversity of respiratory ailments such as bronchitis, asthma, and coughing. Ammonia gas can combine with water to generate ammonium hydroxide, that can injure and burn the respiratory tract. Ammonia can also alter the uptake of oxygen by hemoglobin due to the increase of pH in the blood, which leads to decreased oxygenation of tissues, and decreased metabolic function. Moreover, spilled liquid containing ammonia on the skin can lead to the entrance of ammonia in the body through the skin (ATSDR, 2006).

2.2.2 Effect on Aquatic Environment

Ammonia concentrations in the aquatic environment are variable. The ammonia toxicity on the aquatic system is mainly related to NH_3 (free ammonia) rather than NH_4^+. High ammonia levels result in foliar injury and growth effects that may lead to changes in the plant community composition (Department of Health and Human Services, Public Health Service, 2004).

Ammonia is recycled naturally; therefore, it does not last very long in the environment. Generally, plant and microorganisms consume ammonia very fast. As a result, the amount of ammonia in soil drops to low values in a few days. Basically, ammonia can be found in air, soil, ponds, and water samples at hazardous waste sites (Department of Health and Human Services, Public Health Service, 2004).

2.3 Microbial Conversion Processes in Activated Sludge

Based on the reports on numerous microbial processes in wastewater treatment plants, it is clear that microbial conversion processes in activated sludge are very complicated due to the various reactions and different types of bacteria which are involved in different parts of nitrogen removal in activated sludge (Jetten et al., 1998). The possible microbiological conversions in nitrogen removal are presented in Figure 2.1. This thesis describes the overview of possible microbiological nitrogen conversions in different processes.

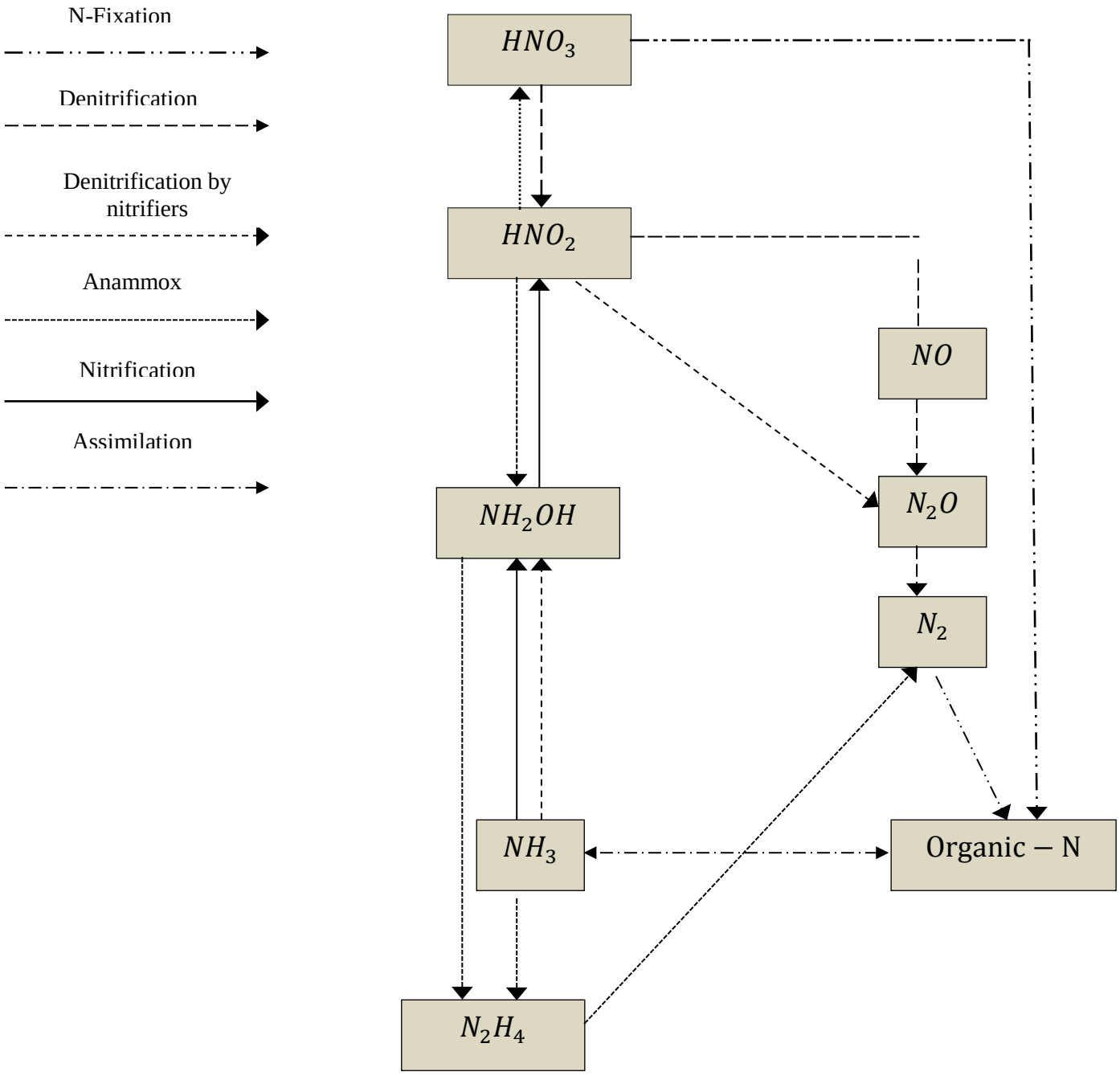


Figure 2.1 -Possible microbial nitrogen conversions (Van Loosdrecht & Jetten,1998)

Three major conversions are involved in the nitrogen removal process including nitrification, denitrification, and anammox. In the next part of this review, each method is described.

2.3.1 Nitrification

Nitrification is a two-step microbiological process during which ammonia is first oxidized to nitrite by ammonia oxidizing bacteria (AOB) such as *Nitrosomonas* and then nitrite is oxidized to nitrate by nitrite oxidizing bacteria (NOB) such as *Nitrobacter* (Tchobanoglous et al., 2003). This process is performed with autotrophic bacteria (Viessman, 2009) which use CO_2 as a carbon source for biosynthesis. These bacteria convert ammonia to nitrate through a two-step process with nitrite as an intermediate compound (Viessman, 2009) as shown below:

$$NH_3 + CO_2 + 1.5\,O_2 \xrightarrow{\textit{Nitrosomonas}} NO_2^- + H_2O + H^+ \qquad 2.3$$

$$NO_2^- + CO_2 + 0.5\,O_2 \xrightarrow{\textit{Nitrobacter}} NO_3^- \qquad 2.4$$

Based on the above equations, *Nitrosomonas* oxidizes ammonia to nitrite with oxygen (equation 2.3), then *Nitrobacter* oxidizes nitrite to nitrate using oxygen derived from water molecule (equation 2.4) (Horan, 1990).There are several key factors that show a significant effect on nitrification, including temperature, pH and dissolved oxygen concentration.

2.2.1.1 Temperature dependency

Nitrifying bacteria, like other kinds of microorganisms, are sensitive to sudden variations in the temperature. The temperature dependency of the biological processes is related to the Van't Hoff exponential expression as shown in equation 2.5 (Horan, 1990).

$$\mu_{max}(\boldsymbol{T}) = \mu_{max}(\mathbf{20}^{\circ}\mathrm{C}).\,\boldsymbol{e}^{(\boldsymbol{k}(\boldsymbol{T}-\mathbf{20}))} \qquad 2.5$$

This equation applies to the temperature range of 10-22 °C. At higher temperatures between 30-40 °C the growth rate is constant and at 45 °C, it approaches zero. The nitrifying process cannot take place at thermophilic temperatures of 50-60 °C (Horan, 1990).

2.2.1.2 pH

The optimal pH range for the nitrification process is 7.8 - 8.9. The growth rate will be reduced to 50% at pH values below 7.0 and above 9.8 (Viessman, 2009).

2.2.1.3 Dissolved Oxygen Concentration

Nitrification requires high dissolved oxygen (DO) concentrations. They need free dissolved oxygen to oxidize ammonia. The nitrifying bacteria are extremely sensitive to low oxygen concentration. Therefore, the DO concentration of 2 mg/L is the critical value for nitrifying bacteria (Barnes & Bliss, 1983).

2.3.2 Denitrification

In the absence of dissolved oxygen, many heterotrophic microorganisms use nitrate ion for their respiration based on the reduction of nitrate to atmospheric nitrogen. This can be done by a variety of denitrifying bacteria such as *Alcaligenes, Achroomobacter, Micrococcus* and *Pseudomonas* (Horan, 1990). The denitrification process also needs an electron donor which can be provided by organic matter or a reduced compound such as hydrogen or sulfide (Jetten et al., 1998). Denitrification process is effective only at low dissolved oxygen concentrations (Barnes & Bliss, 1983). An important concern in the denitrification process is the release of intermediate compounds such as HNO_2, NO and N_2O from the treatment process into the environment (Schulthess et al., 1994) because, all of those intermediate compounds are toxic or undesirable (Horan, 1990).

2.3.3 Anammox

The anammox process is the oxidation of NH_4^+ to N_2 by using NO_2^- as an electron acceptor under anaerobic conditions (equation 2.6).

$$NH_4^+ + NO_2^- \longrightarrow H_2O + N_2 \qquad 2.6$$

It is assumed that first nitrite is reduced to hydroxylamine that is coupled to ammonium to produce hydrazine which will be subsequently oxidized to atmospheric nitrogen (Van de Graaf et al., 1997). So, the hydrazine and hydroxylamine are both catabolic intermediates.

Anammox was discovered in a denitrifying pilot plant for the wastewater treatment from the Gist-Brocades yeast factory in Delft in the 1990s (Mulder et al., 1995). With the development of the anammox process, a new opportunity has been created for the biological removal of nitrogen from industrial wastewater (den Camp et al., 2006;Van de Graaf et al., 1997).

One of the main concerns with the anammox bacteria is their very low growth rate. The maximum specific growth rate of these bacteria is 0.0027 h^{-1} and their doubling time is 11 days (Jetten et al., 1998., Strous et al., 1999). Due to the very low doubling time, this organism is difficult to grow and maintain. The removal of total nitrogen through the anammox process requires less oxygen and has a low sludge production (Mulder et al., 1995). Therefore, the application of anammox process in the wastewater treatment can reduce the operating cost by 90%. The next chapter of this thesis focuses on physical, chemical and biological processes for ammonia removal and describes in more detail the anammox process.

2.4 Ammonia Removal Process

There are a number of physical, chemical and biological processes available for removing ammonia from wastewater according to the US Environmental Protection Agency (USEPA).The physical processes for ammonia removal are air stripping while the chemical processes include ion exchange as well as breakpoint chlorination. In contrast, biological processes use nitrification, denitrification and the new technique that is called “anammox” or Anaerobic Ammonia Oxidation. In the following parts, each process will be explained briefly.

2.4.1 Physical Process

2.4.1.1 Air stripping

The air stripping process with relatively low cost and simple equipment is widely used to remove ammonia from wastewaters. In this process, high elimination rates of ammonia can be achieved (Ozturk et al., 2003) Ammonia stripping is suitable for wastewater with ammonia concentration levels of 10-100 (mg/l). Higher ammonia content (i.e., more than 100 mg/l) may need alternative removal techniques such as steam stripping and biological methods.

The air stripping method involves the passage of large quantities of air over the wastewater that causes the conversion of liquid ammonia to ammonia gas. The process requires a pH of higher than 10.8 based on the following reaction:

$$NH_4^+ + OH^- \longrightarrow NH_3 + H_2O \qquad 2.7$$

To obtain high removal efficiency, the process usually occurs in a packed tower since it can provide a large mass transfer area (Reynolds & Richards, 1996). Air stripping in packed towers usually leads to scaling and fouling on packing, because of reactions between CO_2 in the air and some metal ions in wastewater. To reduce costs, slaked lime is usually used to adjust the pH value of wastewater. A packed tower is not suitable for air stripping of this kind of suspension because of the presence of solid particles that are seen in the suspension. In addition, air stripping is a time-consuming process, especially when it is operating by using traditional equipment. The reason for that is low ammonia transfer from the liquid phase to the gas phase. Moreover, air stripping method is effectively limited to wastewaters with temperatures greater than 10 $^{\circ}$C to avoid freezing. Therefore, it cannot operate in subfreezing weather (Canada, Hydromantis Inc, & AXOR Experts-Conseils Inc, 2003). Finally, noise and air pollution are considered as other practical problems with air stripping.

2.4.2 Chemical Processes

2.4.2.1 Ion Exchange

This process uses resins, commonly made out of zeolites, for ammonia removal. The favored zeolite for ammonia removal is clinoptilolite, which is able to select ammonia ions in the presence of divalent metallic ions (Reynolds & Richards, 1996). In this process, the secondary effluent is treated by ion exchange using fixed bed columns. The ion exchange technology consists of feeding filtered wastewater through a packed column or a fixed bed of clinoptiolite. Wastewater passes through the column and exchanges ion with the zeolite resin. The column operates until exchange sites on the resin are completely exhausted. This can be determined by a high effluent residual ammonium. Ion exchange is more expensive than the air stripping process (Reynolds & Richards, 1996).

2.4.2.2 Breakpoint chlorination

Another chemical process is the breakpoint chlorination method. Chlorination by chlorine gas oxidizes ammonia to produce intermediate chloramines. This process forms nitrogen gas and hydrochloric acid. The reaction of this process is shown in equation (2.8).

$$2NH_4^+ + 3Cl_2 \longrightarrow N_2 + 6Cl^- + 8H^+ \qquad 2.8$$

The reaction depends on pH, temperature and contact time (Canada et al., 2003).

Some undesirable side reactions may occur to form dichloramine, trichloramine, and nitrate gas. These reactions can be prevented from taking place if the pH is controlled around 7 to 8. A base like NaOH should be added to control the pH value around 7 once the alkalinity becomes insufficient. The disadvantages of breakpoint chlorination are increased dissolved solids and increased operating cost twice as much of those in ammonia stripping. In addition, due to the adverse health effects of trihalomethanes that are formed during the chlorination, the use of breakpoint chlorination may decline in the future (Reynolds & Richards, 1996). The advantages and disadvantages of air stripping, ion exchange, and breakpoint chlorination are summarized in Table 2.2.

Table 2.2 - Comparison of available technologies for the physical and chemical removal of ammonia from wastewater.

Process	Advantages	Disadvantages
Air Stripping	♣ Low cost ♣ Removal ammonia with the minimal addition of dissolved solids ♣ Simplicity ♣ Reliability	❖ Poor efficiency in cold weather ❖ Needs pH adjustment ❖ Carbonate scaling of packing
Ion Exchange	♣ High efficiency ♣ Insensitivity to temperature fluctuation ♣ Removal ammonia with a minimal addition of dissolved solids ♣ Ability to eliminate any discharge of nitrogen to the atmosphere other than nitrogen gas	❖ High cost ❖ Complex operation ❖ Requires better control and highly skilled operator
Breakpoint Chlorination	♣ Low capital cost ♣ High degree of efficiency and reliability ♣ Not affected by toxic compounds	❖ Produces high chlorine residuals which are toxic ❖ Need pH control ❖ High operation cost

2.4.3 Biological Treatment

Biological treatment processes use microorganisms for biodegradation of organic contaminants in wastewaters from municipal, industrial and agricultural activities (Rittmann & McCarty, 2001). Biological processes consist of aerobic, anaerobic or a combination of both. The selection of the process depends on the nature of wastewater. For example, activated sludge process is widely used for biological treatment of wastewaters. This process has been developed in 1914 in England by E. Arden and W.T. Lockett. Simply, in this method an aeration tank, a settling tank, solids recycle from settler to the aeration tank and sludge wasting line are utilized. The aeration tank is a suspended-growth reactor that has aggregated microorganisms called the activated sludge. The sludge and the wastewater together are called the mixed liquor (Rittmann & McCarty, 2001).

In biological processes, the microorganisms consume organic pollutants. The major characteristics of wastewater define the kind of microorganism in activated sludge. Generally, prokaryotes like bacteria, eukaryotes like fungi, protozoa, algae, rotifers and bacteriophage called bacteria virus are present in activated sludge (Rittmann & McCarty, 2001).

2.5 Aerobic and Anaerobic Treatment

There are two major types of systems used for wastewater treatment: aerobic and anaerobic systems. The operation principle of both treatment systems is to use organic contaminants as the energy sources. In aerobic processes, the bacteria utilize oxygen as an electron acceptor to convert organic material to carbon dioxide. In contrast, anaerobic treatment is a process in which microorganisms convert organic matter into other products, including biogas in the absence of oxygen. The biogas produced in anaerobic processes is a source of renewable energy that can be used to replace fossil fuels such as oil and natural gas or to generate electricity (Rittmann & McCarty, 2001). The advantages, disadvantages, and applications of each biological treatment method are summarized in Table 2.3 (Rittmann & McCarty., 2001).

Table 2.3 – Comparison of treatments by aerobic and anaerobic biological processes.

Process	Advantages	Disadvantages	Applications
Aerobic Treatment	♣ Simply start up for the growth of microbes ♣ Low capital and operation cost ♣ Minimum odor when properly loaded and maintained	❖ High operational and waste biomass disposal costs ❖ Higher maintenance requirements	Suitable for treatment of organic chemical, textile, municipal sewage, and petrochemical industry
Anaerobic Treatment	♣ Low energy process, making it more environmentally friendly ♣ Lower running costs as a result of the low energy inputs ♣ Low sludge production ♣ Production of methane gas for energy purposes	❖ Long and complicated start up ❖ Higher temperatures are needed, usually around 35 °C ❖ More odor generation	Suitable for the treatment of low to high concentration organic chemical, textile, petrochemical, food and pulp and paper industry

2.6 Microorganisms

Microorganisms used in wastewater treatment systems are divided into two groups according to their cellular structure: prokaryotes and eukaryotes. Prokaryotic cells are simple cells of smaller than 5 microns; they do not contain a distinct nucleus or other membrane-bound organelles. Prokaryotes include bacteria and archaea. Conversely, eukaryotic cells contain membrane-bound organelles, including a nucleus. Eukaryotes can be single-celled or multi-cells. They are more complicated than prokaryotes and they are larger in terms of the cell size which is approximately 20 microns (Mulligan, 2002; Rittmann & McCarty, 2001).

2.6.1 Prokaryotes

2.6.1.1 Bacteria

Bacteria are present in the land, water, and air. This kind of microorganism plays an important role in the environment. They can convert different types of organic and inorganic pollutants into harmless materials that which can then recycle back to the environment. Bacteria can oxidize different types of synthesized industrial as well as nature products via the biological process. They have the ability to transform waste organic material into methane gas that is useful as an energy source. In addition, they can convert inorganic materials like ammonia and nitrate into nitrogen gas. Bacterial reproduction is based on binary fission by dividing into two cells (Mulligan, 2002).

2.6.1.1.1 Bacterial Morphology

The morphology of bacteria includes shape, size, structure and their spatial relationship to one another. Bacteria have three general shapes including coccus, bacillus, and spirillum (Mulligan, 2002). The main component of the bacterial cell is the cytoplasm. It is the place for many cellular functions (Rittmann & McCarty, 2001). Cytoplasm consists of water, dissolved nutrients, enzymes, and nucleic acid (DNA or RNA). The cell membrane is a phospholipid bilayer. It is semipermeable and it controls the nutrients passage into and out of the cell. The composition of the cell wall varies among species and it is an important character for identifying and classifying bacteria. Most of the bacteria have quite a simple cell membrane, but it becomes more complex in autotrophic bacteria and much more complicated in phototrophic bacteria (Rittmann & McCarty, 2001).

Bacteria can be classified based on the energy and carbon sources that they need. Organisms which use energy from light are called phototrophs. Bacteria that obtain energy through chemical reactions are called chemotrophs (Mulligan, 2002). These kinds of bacteria are divided into two types of organisms. The first group is chemoorganotrophs, which acquire organic chemicals for growth and activities. The second group is chemolithotrophs, which obtain inorganic chemicals for growth and activities. Autotrophs are another type of bacteria that use inorganic carbon like CO_2 for growth. Generally, chemolithotrophic bacteria are considered autotrophic and chemoorganotrophs are heterotrophs (Mulligan, 2002; Rittmann & McCarty, 2001).

Bacteria can also be divided according to their requirements for oxygen. Those that need oxygen for respiration in their metabolic process are termed aerobic; the other group that does not require oxygen and uses other chemicals in the absence of oxygen for respiration process called anaerobic. Moreover, some bacteria can live either in the presence of oxygen and absence of oxygen is called facultative bacteria (Mulligan, 2002; Rittmann & McCarty, 2001).

2.6.1.1.2 Environmental conditions for growth

Some factors are necessary for appropriate physical and chemical growth environment, including temperature, pH and oxygen availability (Rittmann & McCarty, 2001). The bacterial growth rate increases with temperature approximately twice for each 10 °C rise until a maximum level that depends on the type of bacteria (Reynolds & Richards, 1996; Rittmann & McCarty, 2001). Table 2.4 describes briefly the bacteria normal temperature range for growth.

Table 2.4 - Normal temperature range for the growth of bacteria.

Temperature classification	Normal temperature range for growth (°C)
Psychrophilic	5-20
Mesophilic	8-45
Thermophilic	40-70
Hyperthermophilic	65-110

2.6.1.1.3 Acidogenic Bacteria

These types of facultative anaerobic bacteria can consume important organics in wastewater includes proteins, lipids as well as hydrocarbons. Proteins are hydrolyzed into amino acids. The cell walls and membrane of bacteria utilize these amino acids. Lipids include glycerin and fatty acids. Glycerin can be used for anabolic reactions. Acidogenic bacteria cannot consume fatty acids; therefore, they are excreted from the cell (Viessman, 2009).

2.6.1.1.4 Acetogenic Bacteria

Few cultures of acetogenic bacteria such as *Synthrophomonas wolfie* are capable of acetate formation. The rest of acetate is formed directly during fermentation (Viessman, 2009).

2.6.1.1.5 Methanogenic Bacteria

The methanogens are very old microorganisms. Two kinds of methanogenic bacteria are *Methanosarcina* and *Methanothrix* which are able to use acetate for catabolism and generate 70% methane during the digestion process. Also, methanol and methylamine are two intermediate products that are biodegraded to methane and carbon dioxide (Viessman, 2009).

2.6.1.2 Archaea

Archaea are also considered as prokaryotic microorganisms. This kind of microorganisms is similar to bacteria in many aspects; however there are some differences between Archaea and bacteria. For instance, archaea are different in terms of cell wall material composition and membrane lipids (Mulligan, 2002; Rittmann & McCarty, 2001).

2.6.2 Eukaryotes

2.6.2.1 Fungi

Fungi are chemoheterotrophic, aerobic, unicellular and multicellular organisms. They can tolerate a variety of environmental conditions. These kinds of microorganisms are useful in the biological treatment and require a pH range of 2 - 9. Most fungi prefer temperatures between 22-30 °C while some can live at temperatures of up to 60 °C (Rittmann & McCarty, 2001). Molds are among the fungi that can reproduce by spores in both sexual and asexual reproduction. Yeast is another type of fungi which can be used in bread and wine production. Fungi are distributed in three major groups described in the following table (Rittmann & McCarty, 2001).

Table 2.5 - Three major groups of fungi.

Fungi class	Characteristic
Ascomycetes	Largest class of fungi, sources of many types of antibiotics, highly active in soil rather than water
Basidiomycetes	Responsible for wood destruction, some of them are parasitic to destroy fruit, highly active in soil rather than water
Deuteromycetes	Useful for human applications, appropriate for cheese production, good sources for important antibiotics such as penicillin

2.6.2.2 Protozoa

Protozoa are single-cell heterotrophic organisms which commonly reproduce asexually. The most common type of asexual multiplication is binary fission, in which the organelles are duplicated and the protozoan is divided into two complete organisms (Mulligan, 2002; Rittmann & McCarty, 2001), *Sarcodina*, *Mastigophoran, Ciliophora* and *Sporozoa* are different types of protozoa. Protozoa feed on bacteria and other types of organisms. This kind of microorganism is identified in fresh water and marine habitats. Optimum pH and temperature for protozoa are between 3.2-8.7 and 15- 55 °C, respectively (Rittmann & McCarty, 2001).

2.6.2.3 Algae

Algae are autotrophic, single-cell and multicellular microorganisms. These kinds of organisms show a significant impact on water quality. Algae are photosynthetic; they convert light into the cellular organic material. There are so many types of algae have been classified based on their size, shape and color. One of the most important types of algae is green algae which can be found in fresh water. Despite the great advantages of algae, there are some problems with their growth such as generation of tastes and odors in water, and filter clogging in water treatment plants (Mulligan, 2002; Rittmann & McCarty, 2001).

2.6.2.4 Rotifers and crustacean

Rotifers and crustacean are multicellular and aquatics microorganisms that can be found in freshwater and moist soil. These kinds of microorganisms are very small and have soft bodies. Only their hard parts can be preserved in the fossil record. Their diets mainly consist of dead organic materials and unicellular algae. Moreover, they are useful to maintain balance population in the marine food chain (Rittmann & McCarty, 2001).

2.7 Anaerobic Ammonia Oxidation (Anammox) Bacteria

2.7.1 Anammox History

Hamme and Thompson (1941) stated that the dissolved nitrogen sink in the oceans could be related to the bacteria in the oceans, which is now known as anaerobic ammonia oxidation (Randall & Thompson, 1941). Later, Richards (1965) showed that ammonium was unexpectedly eliminated under anoxic conditions (Richard, 1965) Also, Broda (1977) showed the lack of two kinds of lithotrophs based on Gibbs free energy calculation. From there, the first possible anammox source was hypothesized (Broda, 1977). In 1990, the anammox process was discovered in a denitrifying pilot plant for wastewater treatment from Gist-Brocades yeast factory (Mulder et al., 1995). In 1998, Strous identified nitrite as a key electron acceptor in the anammox process (Strous et al., 1998). In 1999, Strous et al. purified the anammox bacterial cells from laboratory enrichment culture. This purified cell was able to convert ammonia and nitrite to nitrogen gas in the absence of oxygen. Based on the Strous report, *Brocadia anammoxidans* was chosen as the name for the discovered anammox bacteria, and it was given the status of "candidatus" since it was not pure based on classical microbiological standards. So far, five anammox species have been identified with 16S rRNA gene sequence identities of the species ranging between 87% and 99% (Jetten et al., 2009). Four "Candidatus" anammox species have been identified from activated sludge as named here:

- *Kuenenia* (Schmid et al., 2000; Strous et al., 1999).
- *Brocadia* (Kartal et al., 2008; Kuenen & Jetten, 2001; Strous et al., 1999).
- *Anammoxoglobus* (Kartal et al., 2007) and "Jettenia" (Quan et al., 2008).
- *Candidatus Scalindua* (Kuypers et al., 2003; Schmid et al., 2003; Van de Vossenberg et al., 2008).

The fifth anammox species has often been found in natural habitats, particularly in the sea floor and marine sediments under the minimum amount of oxygen. Moreover, research has shown that in the Black Sea, they are responsible for 30–50% of the nitrogen consumption on the planet (Dalsgaard et al., 2005; Van Niftrik et al., 2004; Penton et al., 2006; Schmid et al., 2007; Woebken et al., 2008).

2.7.2 Characterization of Anammox bacteria

The diameter of coccoid anammox bacteria is usually less than 1 µm and they have a duplication time of 10 - 30 days. Anammox bacteria are anaerobic chemolithoautotrophs. They convert ammonium to nitrogen gas using nitrite as the electron acceptor (Van Niftrik et al., 2004). During the nitrogen removal process, ammonia is partially oxidized to nitrite under aerobic condition. In the anammox process, nitrite is first reduced to hydroxylamine which is subsequently coupled to ammonium to produce hydrazine which will be then oxidized to atmospheric nitrogen (Van de Graaf et al., 1997). So, the hydrazine and hydroxylamine are both catabolic intermediates in the anammox process. There is no pure culture for anammox bacteria since the isolation process is very difficult, but enriched culture of anammox bacteria can be found from wastewater facilities (Dalsgaard et al., 2005).

2.7.3 Growth and metabolism of Anammox bacteria

2.7.3.1 The Anammox Cell

The enrichment culture of anammox bacteria usually has a red color derived from heam protein which constitutes around 20% of the cellular protein mass. Transmission electron microscopy has showed that the anammox bacterial cell is similar to prokaryotic cells. Anammox cells have three sets of the membrane system. The first set is the outer membrane called cytoplasmatic which is in direct contact with thin cell wall and provides the cell boundary. The second set consists of two inner-cell membrane systems, and the third set surrounds the central vacuole. The anammox riboplasm includes DNA cells, ribosomes, and storage materials (Kartal et al., 2008). Figure 2.2 illustrates the schematic of anammox bacteria cell. All types of anammox bacteria have a membrane-bound compartment in their cell, identified as the anammoxosome. 50-70% of the anammox total cell is anammoxosome that has rigid ladderane lipids (Kartal et al., 2008).

Based on the images from transmission electron microscopy and electron tomography, the anammoxosome compartment is transported to the daughter cells upon cell division (Jetten et al., 2009). The anammox process is occurring inside the anammoxosome (Van Niftrik et al., 2004).

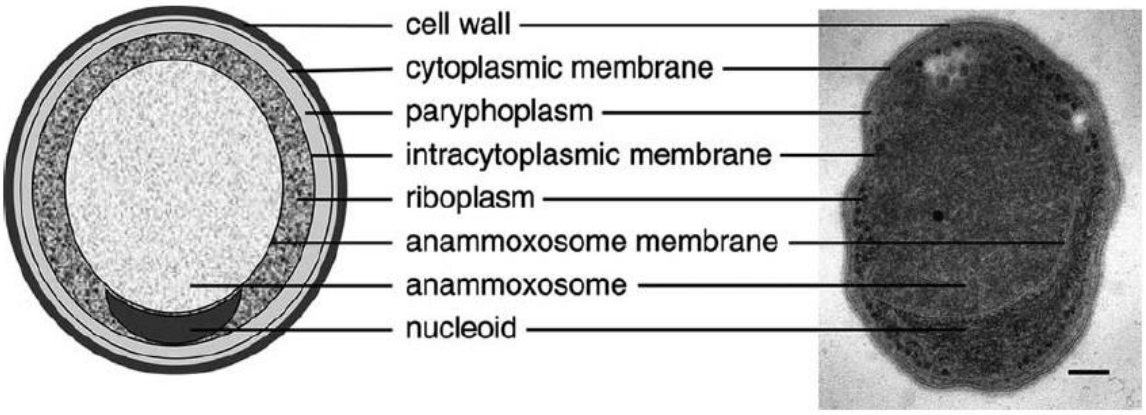


Figure 2.2 - Left: schematic drawing of anammox bacteria. Right: Photograph was taken from a transmission electron microscopy of Cadidatus *Brocadia anammoxidans* adopted from (Van Niftrik et al., 2004).

Anammox bacteria have three structures of ladderane membrane lipids which are unique in nature (Van Niftrik et al., 2004).

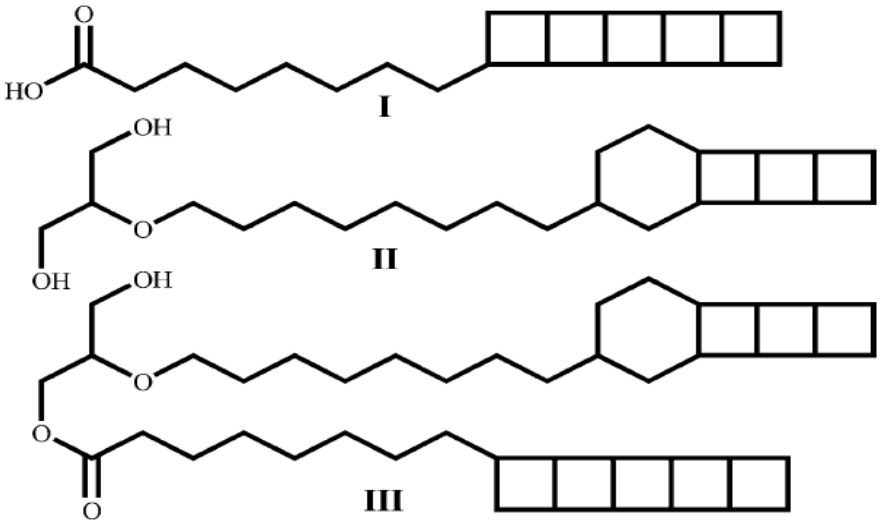


Figure 2.3 - Structures of three characteristic ladderane lipids adopted from (Van Niftrik et al., 2004).

Molecular modeling has determined that the anammoxosome is surrounded by highly packed rigid ladderane lipids. Without this rigid ladderane, lipids proton leakage and loss of intermediate metabolites could easily happen. As a result, this structure may reduce the damage (Jetten et al., 2009). In order to produce ATP in the cell, the annommoxosome membrane stores the proton motive forces and generates them as needed. Because of this importance, the impermeability of membrane is critical (Van Niftrik et al., 2004).

Moreover, this structure leads to restricting the diffusion of protons across the membrane and to enhance the ATPase efficiency (Jetten et al., 2009). It is hypothesized that in the membrane, nitrite is reduced to hydroxylamine (NH_2OH) by nitrite reducing enzymes (NR). In the next step, hydrazine forming enzyme HZF combines ammonium with hydroxylamine to produce hydrazine (N_2H_4). The hydrazine oxidizing enzyme, HZO (equivalent to hydroxylamine oxidoreductase-like protein) oxidizes hydrazine to nitrogen gas (Kuenen & Jetten, 2001).

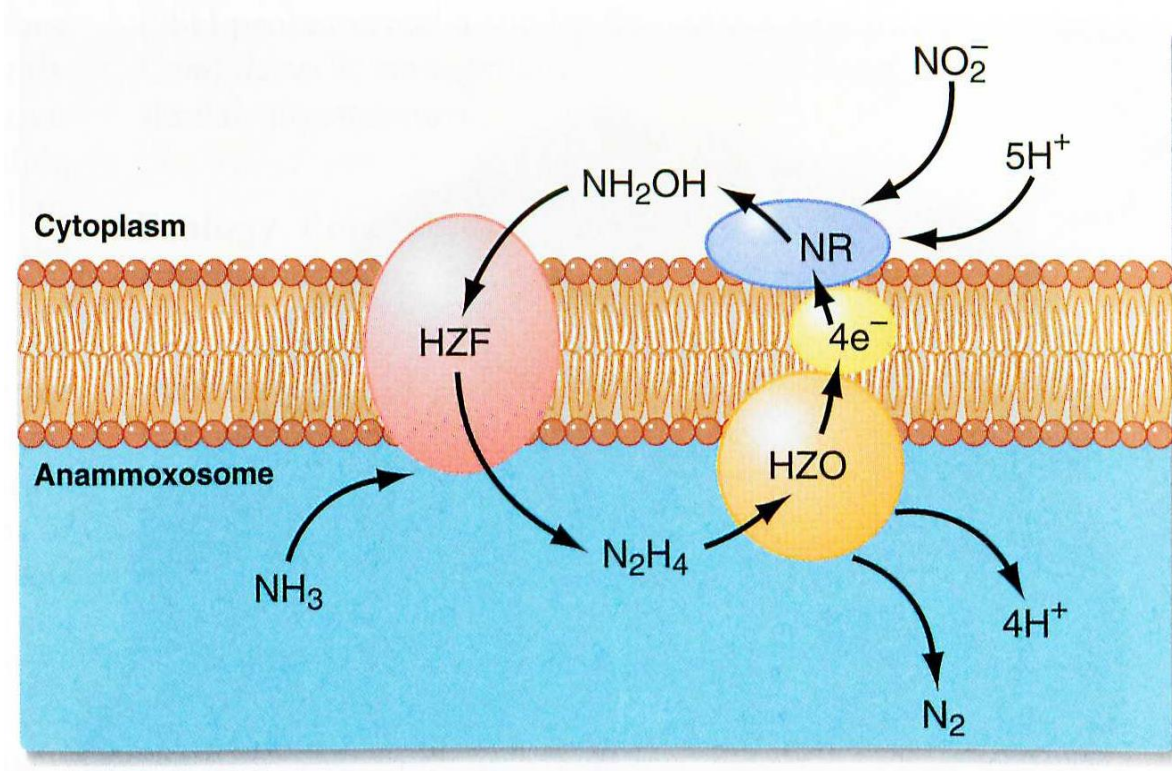


Figure 2.4 - Mechanism of anaerobic ammonium oxidation. NR is a nitrite reducing enzyme (NH_2OH is the product), HZF hydrazine forming enzyme (combining ammonium and hydroxylamine), HZO hydrazine oxidizing enzyme adopted from (Kuenen & Jetten, 2001).

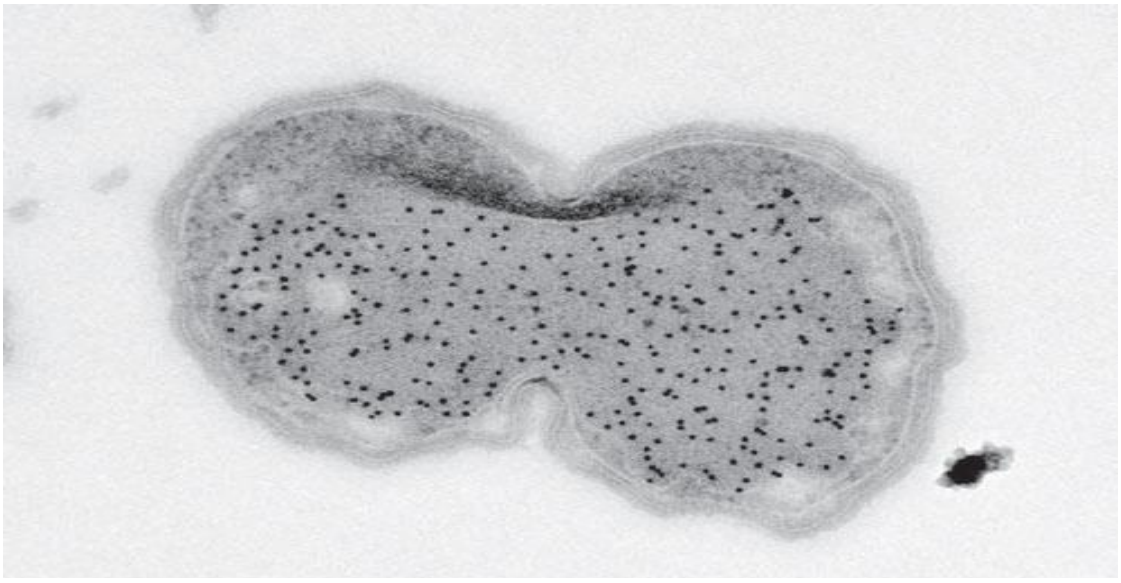


Figure 2.5 - Electron micrograph showing the immunogold localization of hydrazine hydroxylamine oxidoreductase (black dots) to the anammoxosome compartment in the anammox bacterium "Candidatus Kuenenia stuttgartiensis". Scale bar, 500 nm adopted from (Jetten et al., 2009).

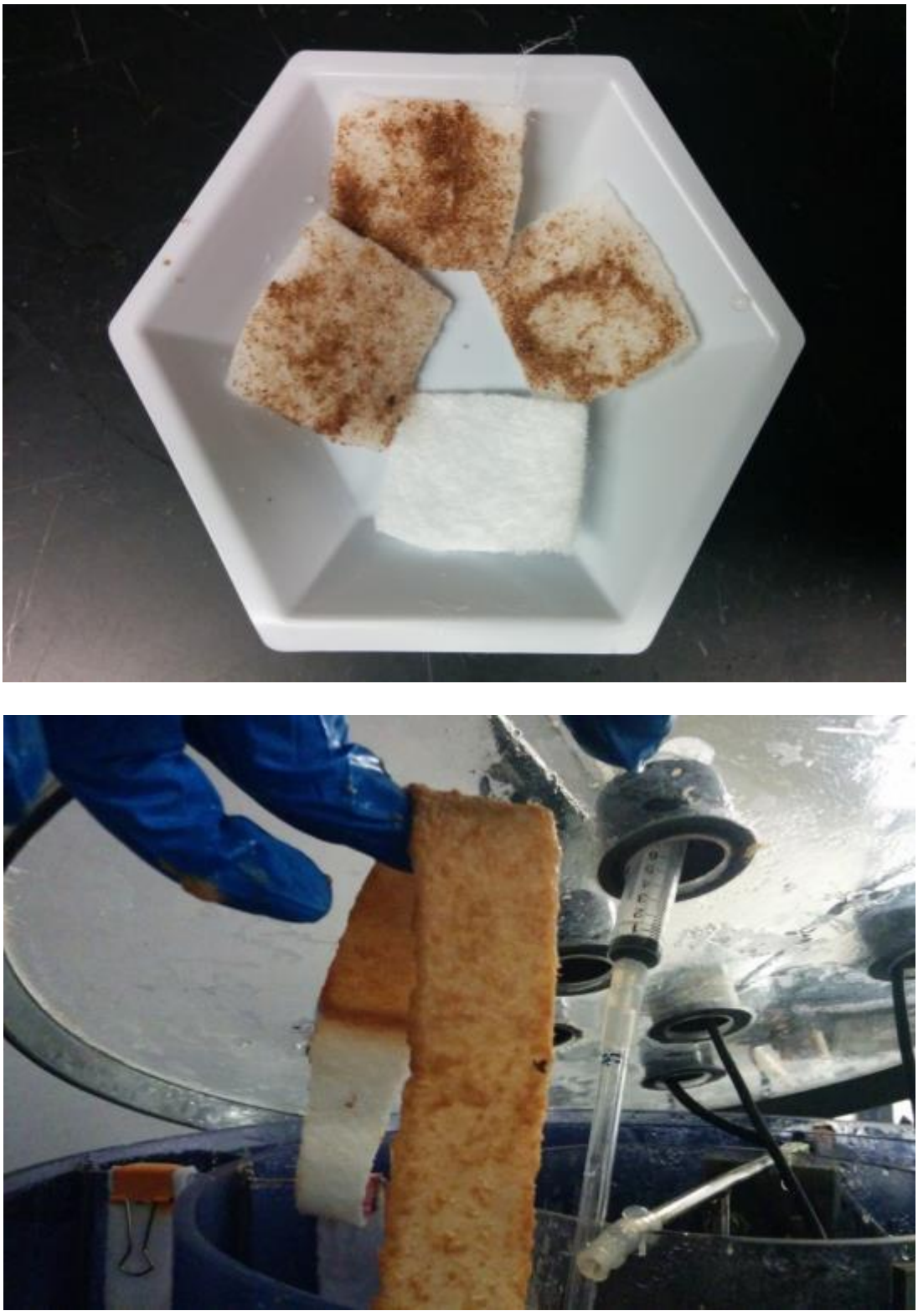


Figure 2.6 - Anammox bacteria enriched on the geotextile media at environmental lab (Concordia University), the color turned to reddish-brownish after 4 months.

2.7.4 Application of Anammox Technology in Wastewater Treatment Plants

Anammox is extremely effective as one of the biological technologies in achieving high level ammonia- nitrogen removal from wastewater effluent. To implement the anammox process, the selection of the type of reactor is very essential. In the last 20 years, several bench and full-scale applications of this technology have gained broad interest. This process has been investigated in a sequencing batch reactor SBR (Strous et al., 1998), in a suspended SHARON-ANAMMOX system (Dongen et al., 2001; Van Dongen et al., 2001) , in the CANON reactor (Hanaki et al., 1990; Hendrik & Strous, 2002; Kuai & Verstraete, 1998) and the OLAND system (Li et al., 2008; Khin & Annachhatre, 2004; Zhang et al., 2008).

2.7.4.1 Sequencing Batch Reactor (SBR)

Strous et al. (1998) investigated the use of sequencing batch reactors for the anammox growth. The research has shown that SBR is a powerful technique in comparison to other technologies. The anammox SBR culture was maintained in a 15-litre vessel. The operating pH and temperature were between 7.8-8 and 32-33 °C, respectively. Anaerobic conditions were controlled by mixed gas flushing of Ar/CO_2 (95/5%). The fresh medium was continuously fed over 11.5 h of gentle mixing. After the filling period, the investigators let the bacteria flocs settle for 15 minutes. Then, they removed the supernatant to measure process parameters such as nitrite, nitrate, and ammonia. Finally, part of the liquid was purged by the effluent pump. Figure 2.7 shows the experimental set up for SBR.

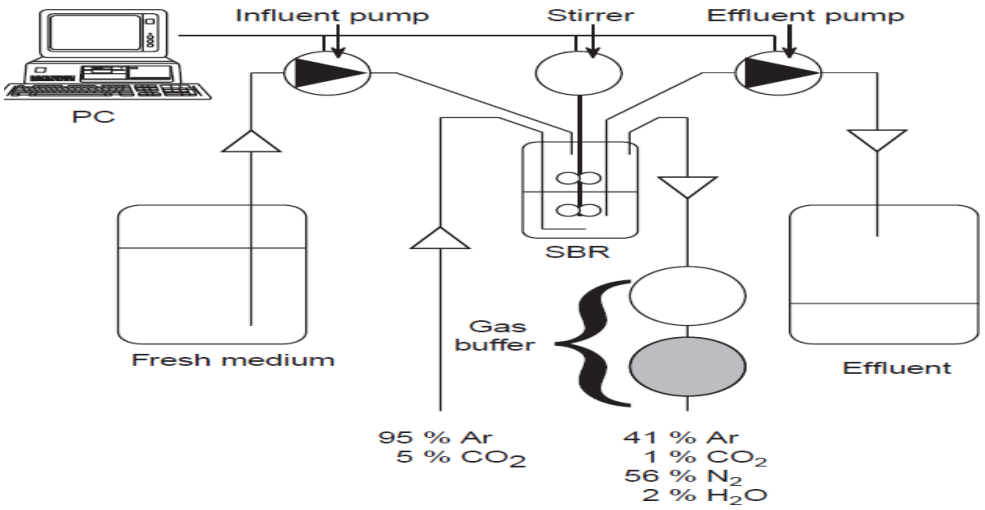


Figure 2.7 - Experimental set–up of the sequencing batch reactor (SBR) adopted from (Strous et al., 1998).

The SBR technology has shown efficient biomass retention, a homogenous distribution of substrate and stable conditions. This technique is useful for a large number of slow growing microorganisms (Strous et al., 1998).

2.7.4.2 The SHARON Process

SHARON (Single reactor system for High-rate Ammonium Removal Over Nitrite) process is considered as one of the oldest methods of converting ammonia to nitrite under aerobic conditions using ammonium oxidizing bacteria (i.e., nitrification). It can produce a 50-50 mixture of ammonium and nitrite in the system. The following reaction describes the chemical reaction in Sharon reactor (Dongen et al., 2001):

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad 2.9$$

In the presence of nitrification bacteria and ammonium oxidizing organism, the following reaction happens under aerobic condition where nitrite is oxidized to nitrate:

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \qquad 2.10$$

Both nitrite and nitrate can be removed under anoxic conditions in the Sharon reactor by the heterotrophic organism (denitrification). The carbon source is necessary for any conversion in this process (Dongen et al., 2001). Figure 2.8 illustrates the SHARON process.

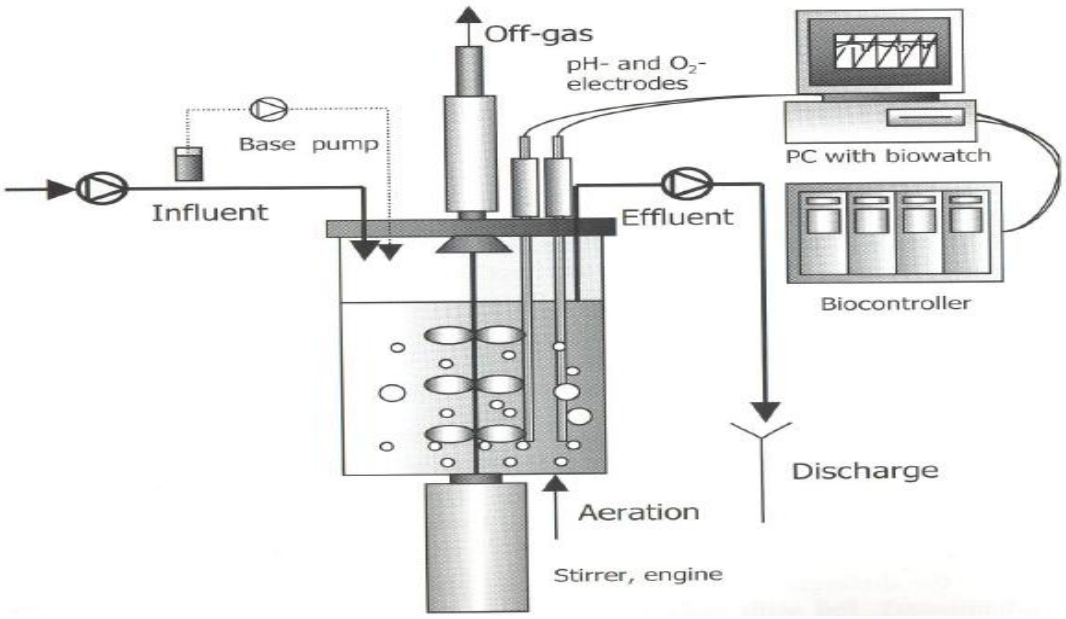


Figure 2.8 - Schematic presentation of the Sharon reactor adopted from (Dongen et al., 2001).

The SHARON-ANAMMOX process**:**

The first full-scale technique was built in Rotterdam WWTP Dokhaven. Jetten and Van Dongen (2001) introduced the combination of partial nitrification process and anoxic ammonium oxidation for sludge treatment. In the Sharon reactor, the partial nitrification of ammonia to nitrite occurs by fast growing nitrification bacteria named *Nitrosomonas eutropha*. Then, nitrite reacts with ammonium, serving as the electron acceptor. This process was operating for more than two years. Consequently, with DNA extraction and PCR amplification microbiology techniques, they could identify the presence of anammox bacteria (Van Dongen et al., 2001).

The combination of partial nitrification process and the anammox process has been tested successfully using sludge digester effluent. This innovative biotechnological method improved significantly the treatment of ammonium rich wastewater with minimal energy usage. Figure 2.9 demonstrates a fundamental flow scheme of the Sharon-anammox reactor (Van Dongen et al., 2001).

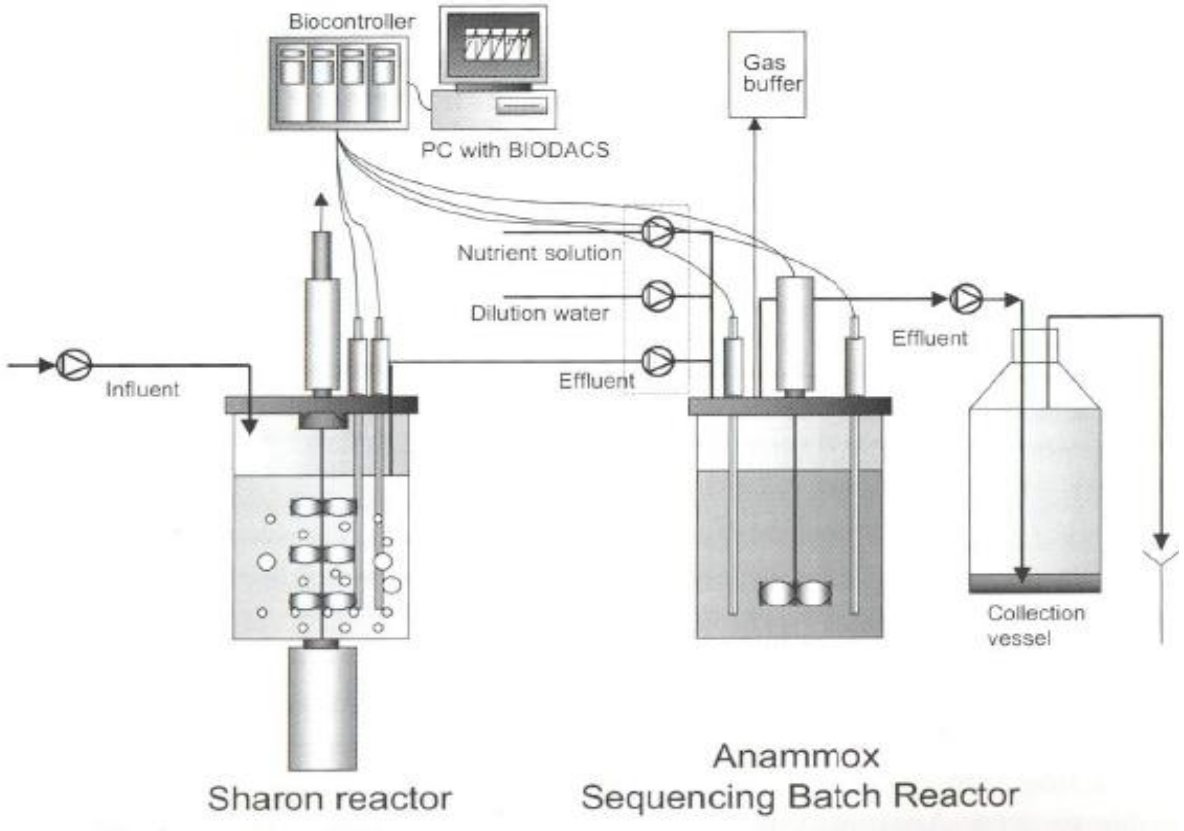


Figure 2.9 - Schematic representative of the anammox sequencing batch reactor using effluent from the Sharon reactor as feed staff adopted from (Dongen et al., 2001).

2.7.4.3 Completely Autotrophic Nitrogen removal Over Nitrite (CANON)

Hendrik and Strous (2002) described a novel technique for biological nitrogen removal called CANON (Completely Autotrophic Nitrogen removal Over Nitrite) process. In this process, ammonium removal can be achieved in a single reactor containing low amounts of organic materials under oxygen limited conditions (< 0.5% air saturation) (Hendrik & Strous, 2002). In the CANON process, two groups of autotrophic microorganisms are active: one is *Nitrosomonas*- such as aerobic bacteria and the other is *Planctomycete* –like anaerobic ammonium oxidizing bacteria. Based on reactions 2.11 and 2.12, these autotrophic microorganisms convert ammonia to nitrogen gas where nitrite is an intermediate chemical.
Under oxygen-limited conditions, ammonium is oxidized to nitrite by aerobic nitrifiers such as *Nitrosomonas* and *Nitrososira* (Hanaki et al., 1990).

$$NH_4^+ + 1.5\,O_2 \rightarrow NO_2^- + H_2O + 2H^+ \qquad 2.11$$

Consequently, anaerobic ammonium oxidizers *Planctomycete*-like anammox bacteria transform ammonium to dinitrogen gas (Strous, 2000).

$$NH_4^+ + 1.3\,NO_2^- \rightarrow 2H_2O + 1.02N_2 \qquad 2.12$$

The CANON process is economically efficient for wastewater treatment, especially for ammonium rich wastewaters devoid of organic carbon (COD). The CANON process is completely autotrophic, so it requires no COD addition. Moreover, the complete nitrogen removal can be achieved in a single reactor with a little aeration. This greatly reduces the space and energy requirements. The autotrophic process consumes 63% less oxygen and 100% less reducing agents than other conventional nitrogen removal processes (Kuai & Verstraete, 1998).

2.7.4.4 Oxygen Limited Autotrophic Nitrification Denitrification (OLAND)

A new process called OLAND is an acronym of oxygen-limited autotrophic nitrification-denitrification (Zhang et al., 2008). In this process, the dissolved oxygen concentration is maintained below 0.2 DO mg /L and can be carried out in a one-stage system or in two-stage systems. For one stage systems, in a single reactor, usually rotating biological reactor (RBC) is used. Partial nitrification and anammox process can take place simultaneously (Windey et al., 2005). For two stages systems, membrane bioreactor (MBR) can be employed where partial nitrification and anammox process take place separately (Wyffels et al., 2004). OLAND process can operate under low temperature conditions between 22-30^{o}C (Li et al., 2008).

2.7.4.5 Membrane Bioreactor (MBR)

Martinez et al. (1995) investigated a lab-scale MBR anammox bioreactor at the Technological University of Delft. In this study, the investigators achieved very high solid retention times for anammox bacteria which can provide a sufficient time for anammox growth. The MBR anammox bioreactor was determined as a promising technology for effluent treatment with high ammonia concentration and low organic carbon. The MBRs have been developed as an alternative for wastewater treatment with low sludge production. In addition, the MBR process maintenance and operation is much easier than the conventional activated sludge process. To avoid membrane biofouling due to the growth of attached biofilms, the membrane was replaced and cleaned biweekly (Martinez et al., 1995).

2.8 Multi-Zone Wastewater Treatment System- BioCAST Technology

The BioCAST technology consists of two separate but interlinked bioreactors (Yerushalmi et al., 2011). The laboratory-scale BioCAST bioreactors were made from PVC sheets (AC Plastic Inc, Quebec, Canada). Each bioreactor has different zones and a multiplicity of environmental conditions. The first bioreactor contains aerobic, microaerophilic and anoxic zones as well as a clarification zone. The volumes of various laboratory-scale reactor zones were 17, 61, and 22 L, respectively, while the volume of clarification zone was 85 L. The diameters of aerobic, microaerophilic and clarification zones were 16.7, 35.7 and 49.5 cm, respectively. The heights of aerobic and microaerophilic zones were 91cm and 100 cm, respectively. The second bioreactor contained an anaerobic zone at the bottom, a solid-liquid separation zone in the middle and a filtration unit at the top. The selected type of filter medium depends on the characteristics of the effluent and operation scale of the process. The diameter of the second bioreactor was 12 cm and it had a total volume of 12 L. The height of the bioreactors was 1.13 m.

The aerobic zone has been designed according to the principles of the air lift reactors. Mixed liquor flows upward in this zone (riser) and downward in the microaerophilic zone (downcomer) on a continuous basis. There are three custom-built air diffusers located at the bottom of the aerobic zone and above the anoxic zone which provide air into this zone. Aeration supplies oxygen for aerobic biological processes as well as proper liquid mixing. Moreover, aeration produces circulation of liquid between the three adjacent zones of aerobic, microaerophilic and anoxic, thus exposing the contaminants to three environmental conditions every few minutes. The aerobic zone contains suspended and attached-growth microorganisms.

There is a cylindrical structure of stainless steel wrapped in non-woven geotextile as microbial support which is installed in the middle of the aerobic zone, above the diffusers to support the attachment of microbial biomass and the formation of microbial biofilm. Because of the low growth rate of anammox bacteria, support media are required to attach the biomass and form immobilized culture or biofilm. Once there is sufficient amount of biomass accumulating in the system, complete conversion of ammonium may occur (Van de Graaf et al., 1996). In the present study, in addition to a cylindrical structure inside the aerobic zone, six strips of geotextile were placed inside the microaerophilic zone.

The operating parameters, including temperature, aeration rate and dissolved oxygen concentrations in the aerobic and microaerophilic zones were continuously monitored by a real-time control system developed by Behzadian et al. (2010). Figure 2.10 shows the schematic diagram of the integrated multi-zone wastewater treatment system.

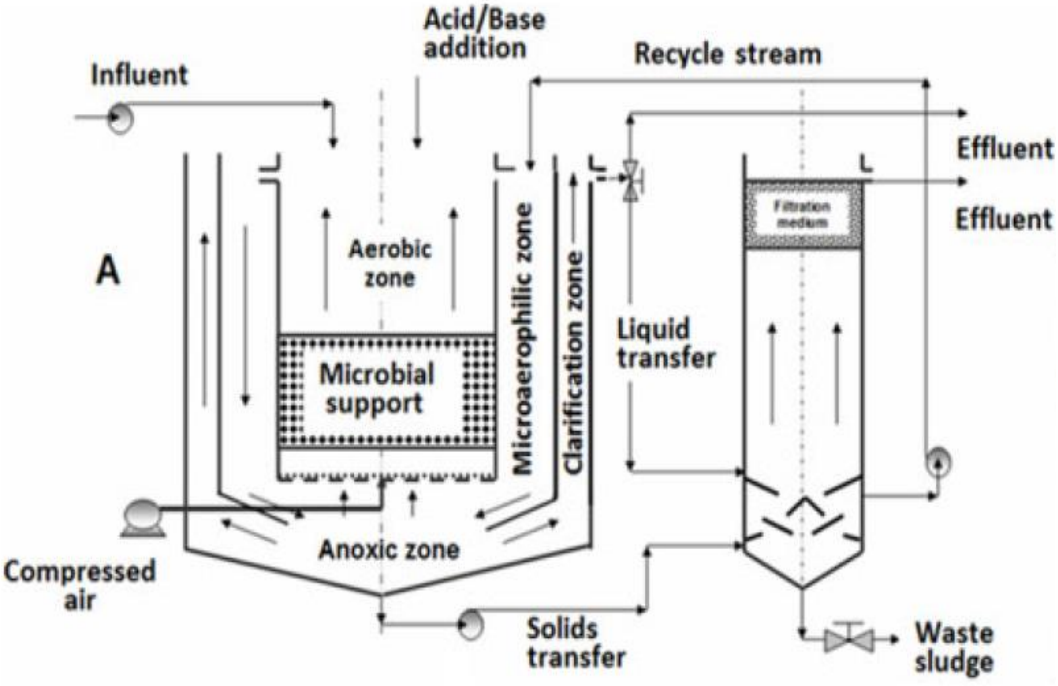


Figure 2.10 - Schematic diagram of the multi-zone BioCAST technology.

Figure 2.11 - Structure of the microbial support.

2.8.1 Key Factors for Controlling Anammox Process in BioCAST Technology

One of the important attributes of the BioCAST technology is its capacity to support PN and anammox processes. This is due to the various environmental conditions, inside the BioCAST bioreactors that favor the establishment of these processes. Operating parameters such as DO, pH, temperature, and nitrite accumulation need to be strongly controlled in order to facilitate the growth of AOB and anammox bacteria, while suppressing the activity of NOB.

2.8.1.1 Dissolved Oxygen

Under oxygen limiting conditions, both AOB and NOB compete for oxygen. Dissolved oxygen concentrations of less than 0.4 mg/L, with a high ammonium environment, favors the growth of AOB over NOB, as NOB gets washed out (Schmid et al., 2003). In addition to free ammonia, NO and hydroxylamine can inhibit NOB. However, the inhibiting mechanisms have not been known (Sundermeyer & Bock, 1981). The anammox activity is completely inhibited by the presence of oxygen (Strous et al., 1997). In BioCAST, the desirable DO for AOB in aerobic zone and the microaerophilic zone is 0.9-1.2 mg/l and 0.1-0.4 mg/l, respectively.

2.8.1.2 pH

AOB growth rate and activity over NOB is increasing at pH 7.5-8. In addition, at pH below 7.0, the nitrification growth rate decreases (Hellinga et al., 1999). Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams(Van Hulle et al., 2010). The optimal pH range for anammox was found to be 7- 8.3 (Strous, 2000). In BioCAST, the pH had to be maintained at 7.5 -8.1 in order to support both AOB and anammox bacteria.

2.8.1.3 Temperature

Temperature beyond 25 ºC increases AOB growth over NOB, whereas temperatures higher than 40ºC cause deactivation of bacterial activities (Hellinga et al., 1999). The optimal temperature for AOB and NOB was found to be 35ºC and 38ºC, respectively (Grunditz & Dalhammar, 2001). For the anammox process, the optimum temperatures of 30 to 40 ºC have been reported, with the highest activity at 37 ºC (Egli et al., 2001; Strous et al., 1999). In BioCAST, the optimum temperature was maintained in the range of 33-35 ºC.

2.8.1.4 Nitrite Accumulation

In the anammox process, nitrite is an important element. Nitrite produced by AOB is consumed along with ammonia, by the anammox bacteria to produce nitrogen gas. However, there are different studies reporting that the excess of nitrite in the system is toxic to the anammox bacteria. Short term and complete inhibition of anammox bacteria in the presence of excess amount of nitrite was reported by Bettazzi et al., (2010) and Strous et al., (1999) at 60 mg/l NO_2-N and 100 mg/l NO2-N, respectively. In contrast, 50% loss of anammox bacteria activity was reported at the high nitrite concentration of 350 mg/l NO_2-N (Cho et al., 2010; Dapena-Mora et al., 2007; Kimura et al., 2010). Lotti et al (2012) reported severe inhibition of suspended and flocculent biomass in comparison to granular biomass (probably because of the outer layer of the biofilm that protect the inner layer). Consequently, they found a higher level of nitrite tolerance, 350 mg/l NO_2-N based on the numerous manometric batch test in case of biofilm or granular sludge.

2.8.1.5 Ammonium Concentration

Different studies have determined the inhibitory effect of ammonium on the anammox process. Strous et al. (1999) reported that up to 1000 mg/l of ammonia showed no anammox inhibitory effects during SBR operation (Strous et al., 1999).While a different study using batch tests reported 50% inhibition of the anammox process at 770 mg $NH4^+$-N/L (Dapena-Mora et al., 2007). The observed differences in the ammonium inhibition effects might have been related to various operating conditions and experimental methods employed. Most studies have shown that ammonium had no inhibitory effects on anammox, however, free ammonia inhibited anammox bacterial activity at pH values higher than 7.6 (Puyol et al., 2014). The activity of NOB is limited when the free ammonia concentration in the range of 0.1- 1 mg/l inside the system since these bacteria are more sensitive to free ammonia compared to AOB (Anthonisen et al., 1976).

The BioCAST as a multi-zone treatment system with unique characteristics that may verify to be an ideal for nitrogen removal without the need for organic carbon by the combined PN-anammox processes. These characteristics include the presence of both suspended and immobilized biomass at high retention times, multiplicity of zones having aerobic, microaerophilic and anoxic conditions, and continuous circulation of wastewater between these zones.

CHAPTER 3: MATERIALS AND METHODS

3.1 Feed

The synthetic wastewater was composed of inorganic compounds including potassium hydrogen carbonate ($KHCO_3$), sodium di-hydrogen phosphate (NaH_2PO_4), magnesium sulfate ($MgSO_4.7H_2O$), calcium chloride ($CaCl_2.H_2O$), ferrous sulfate ($FeSO_4$) and ethylenediaminetetraacetic acid (EDTA). A trace mineral solution was also used in the synthetic wastewater to complete the nutritional requirements of microorganisms. In this study the ammonium carbonate NH_4 $(CO_3)_2$ was used as a nitrogen source. All chemicals were purchased from Fisher Scientific (Montreal, Canada). The influent concentration of nitrogen was in the range of 10-350 mg/l. The loading rate of nitrogen concentration and nitrogen concentration in feeding solution with time are shown in Table 3.3. The microbial seed was a mixture of AOB-NOB and anammox bacteria that was retained at 35 ºC obtained from the HRSD Company in the USA. Operation of the BioCAST bioreactor was done as follows. The bioreactor, containing 100 L volume, was fed with synthetic wastewater at a flow rate of 25 L/day with 4 days HRT. The synthetic wastewater fed flow rate was changed to 50 L/day with 2 days HRT at day 96. The synthetic wastewater was agitated with an electrical mixer to avoid precipitation. Sampling was done 4-5 times per week to analyze the nitrite, nitrate, ammonium, and total nitrogen in the effluent. The liquid pH inside the reactor changed in the range of 7.5-8.1 while the temperature was maintained at 33-35 °C. Aeration was provided by introducing air at the flow rate of 0.5-0.9 liters/min (LPM) in order to keep the oxygen level in the aerobic and microaerophilic zones between 0.9-1.2 mg/l and 0.1- 0.4 mg/l respectively. In this study only the first bioreactor was used because of the low sludge generation and clarity of the effluent emerging from the first bioreactor. Operational conditions of the BioCAST bioreactor such as DO, pH, temperature and inlet composition make the system a proper environment for PN-anammox process. Table 3.1 presents the composition of synthetic wastewater and Table 3.2 presents the composition of trace elements used.

Table 3.1 - Synthetic wastewater composition.

CHEMICAL	CONCENTRATION (g/l)
$KHCO_3$	1.25
NaH_2PO_4	0.05
$CaCl_2.2H_2O$	0.3
$MgSO_4.7H_2O$	0.2
$FeSO_4$	0.00625
EDTA	0.00625

Table 3.2 - Composition of trace elements in the synthetic wastewater.

CHEMICAL	CONCENTRATION (g/l)
EDTA	15
$ZnSO_4.7H2O$	0.43
$CoCl_2.6H2O$	0.24
$MnCl_2.4H_2O$	0.99
$CuSO_4.5H_2O$	0.25
$NaMoO_4.2H_2O$	0.22
$NiCl_2.6H_2O$	0.19
$NaSeO_4.10H_2O$	0.21
H_3BO_4	0.014

Table 3.3 - Nitrogen concentration in the feed solution and nitrogen loading rates.

Time (day)	Nitrogen Concentration (mg/l)	NLR(kg/m^3.d)
1	10	0.002
4	20	0.005
8	30	0.007
12	40	0.010
16	50	0.012
20	60	0.015
24	70	0.017
30	80	0.020
32	110	0.027
36	140	0.035
40	170	0.042
56	200	0.050
64	230	0.057
84	250	0.062
96	250	0.125
100	300	0.150
110	350	0.175

3.2 SEM Sample Preparation

Microbial samples were analyzed by SEM at Concordia University to confirm the existence of anammox culture. The samples were prepared by fixing them with 3% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2 for 2 h, followed by dehydration with 60%, 80% and 100% ethanol for 10 min (Wang et al., 2011). The glutaraldehyde in phosphate buffer solution was purchased from Cedarlene (Toronto, Canada). Then, the samples were dried and gold coated by a sputter (Chen et al., 2009). Finally, the samples were observed with SEM. Figure 3.1 shows the SEM sample preparation.

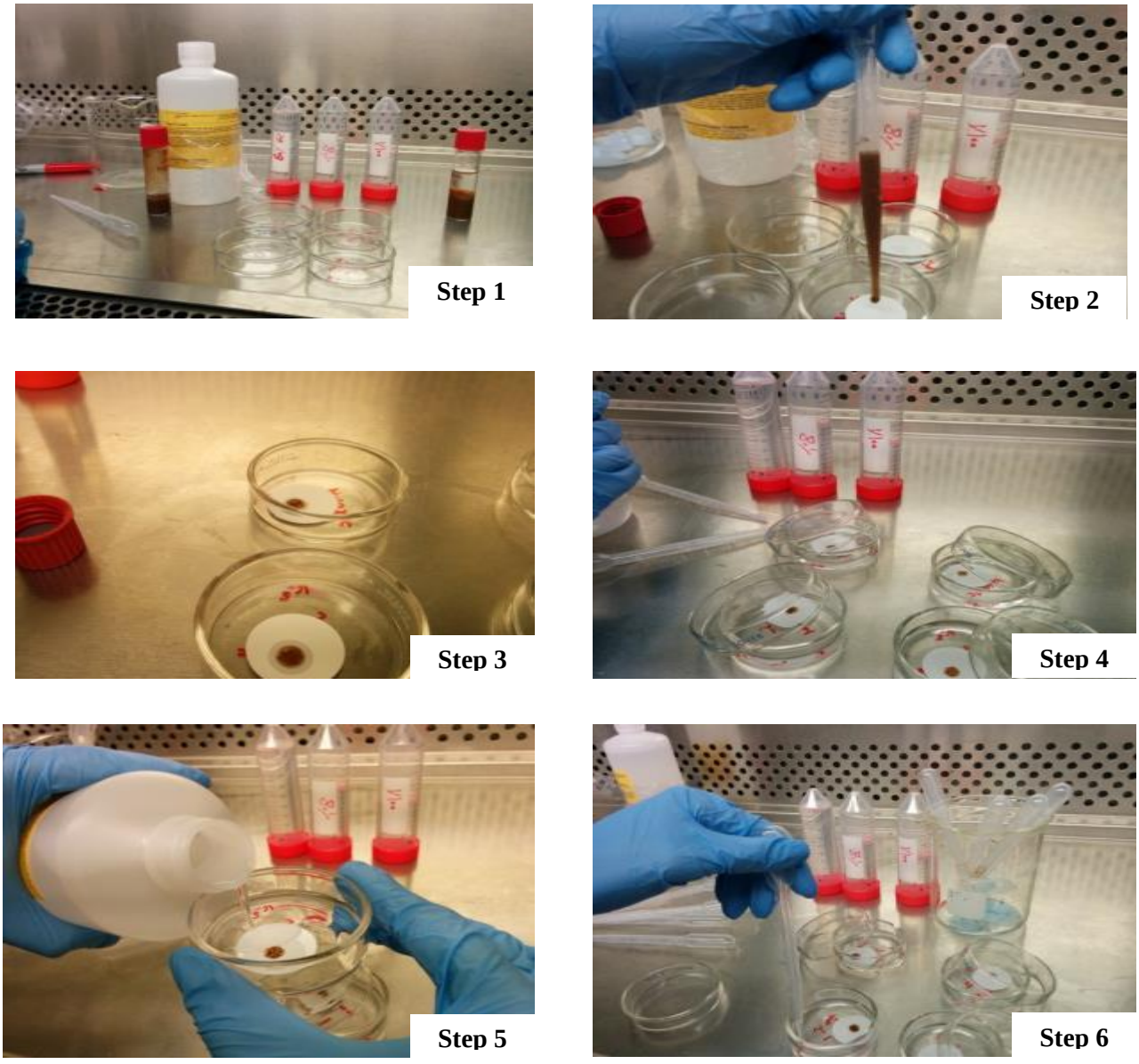


Figure 3.1 - Sample preparation for SEM

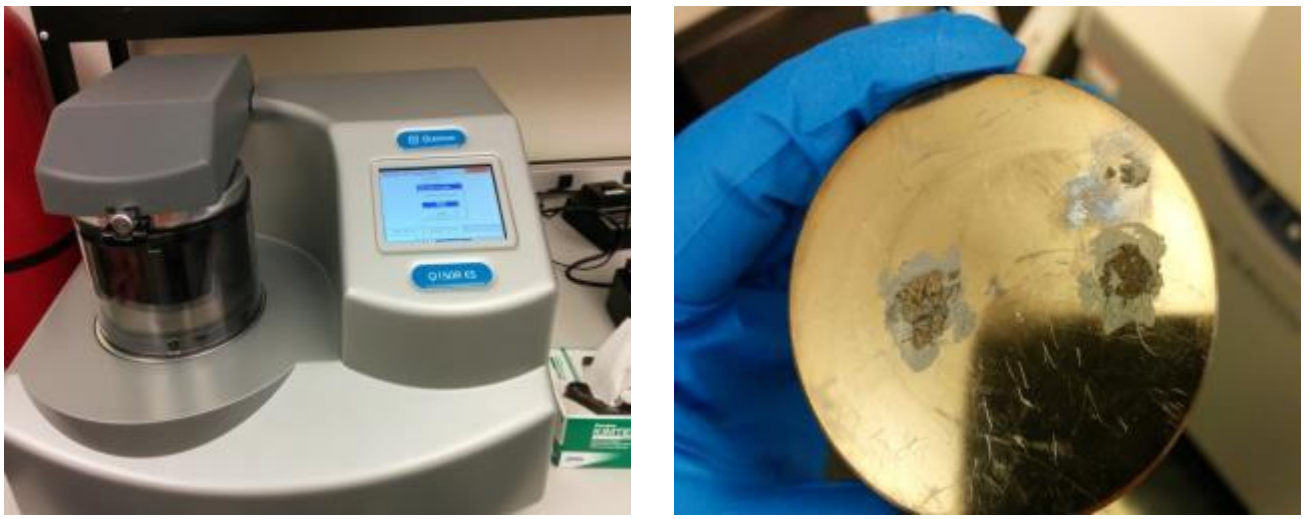


Figure 3.2 - Left image: gold coated machine, Right image: Sample after gold coating.

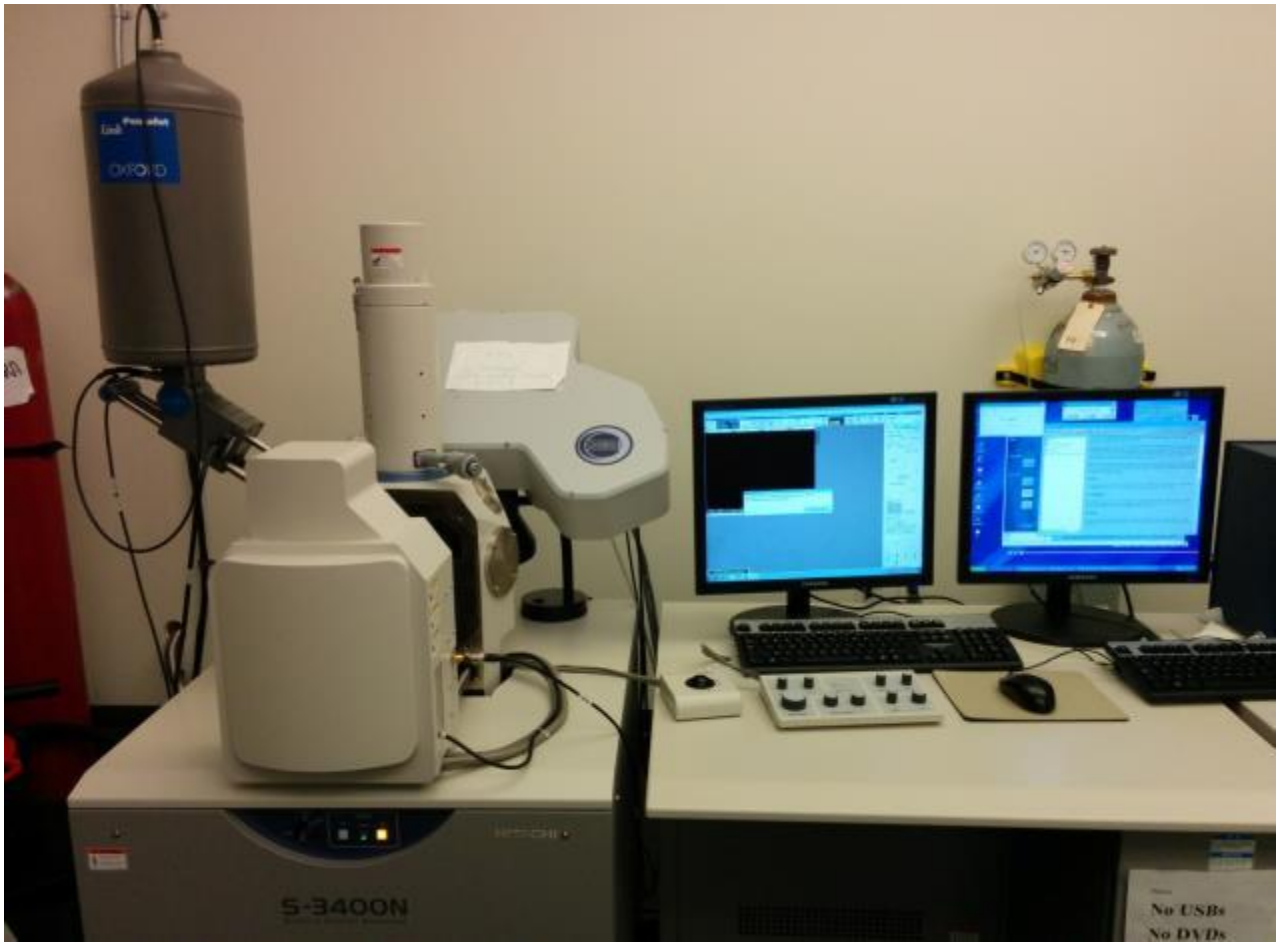


Figure 3.3 - Scanning Electron Microscope in Concordia University.

3.3 Operating Parameters

The environmental conditions in the different three treatment zones of BioCAST are presented in Table 3.4. The DO concentration was controlled by a DO controller (model alpha- DO2000W) to keep the oxygen level between 0.9-1.2 mg/l in the aerobic zone. The DO concentration in the microaerophilic zone was controlled by a different DO meter (OAKTON PD-650) between 0.1-0.4 mg/l.

Figure 3.4 - Microaerophilic DO controller connected with infrared adaptor.

The pH of the aerobic, microaerophilic and anoxic zones changed in the range of 7.5- 8.1, 7.5-8.1, and 7.8-8.1, respectively. pH was measured by submersible pH electrodes placed inside each zone (Model Cole-Parmer 27001-80). The pH in the feeding solution was adjusted in the range of 7.5-8 while the bioreactor pH was only monitored with a pH meter (OAKTON pH probe) and was not controlled. A heater was placed inside the anoxic zone to maintain the mixed-liquor temperature at 33-35 ºC. As anammox bacteria are sensitive to light; therefore, the BioCAST reactor was covered with black plastic as shown in Figure 3.5.

Table 3.4 - Environmental conditions in various zones of the BioCAST bioreactor.

Parameter / Zone	DO(mg/l)	pH	Temperature(°C)
Aerobic	0.9 - 1.2	7.5-8.1	33-35
Microaerophilic	0.1 - 0.4	7.5-8.1	33-35
Anoxic	0	7.8-8.1	33-35

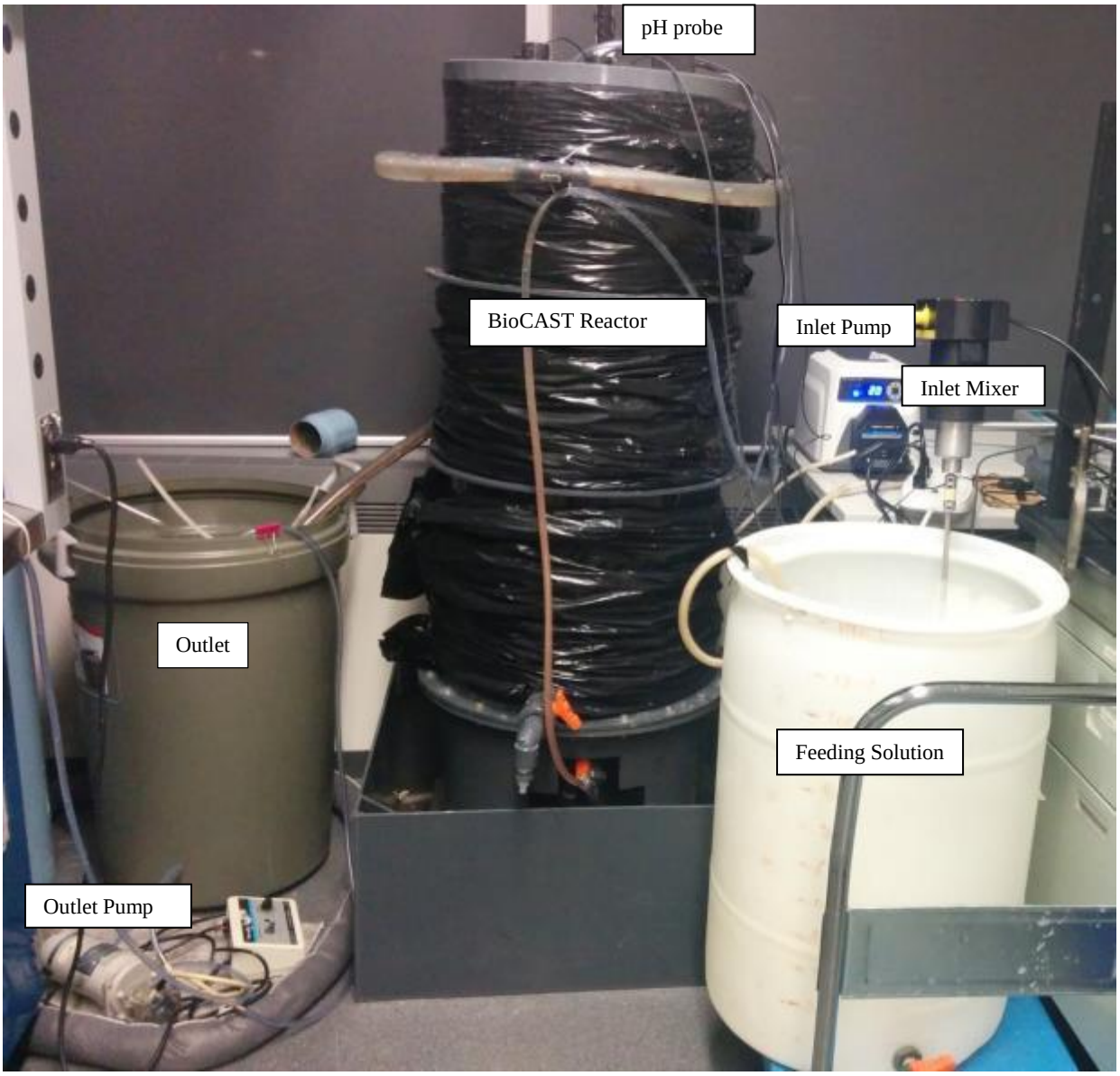


Figure 3.5 - Pilot- experimental setup for anammox process in BioCAST.

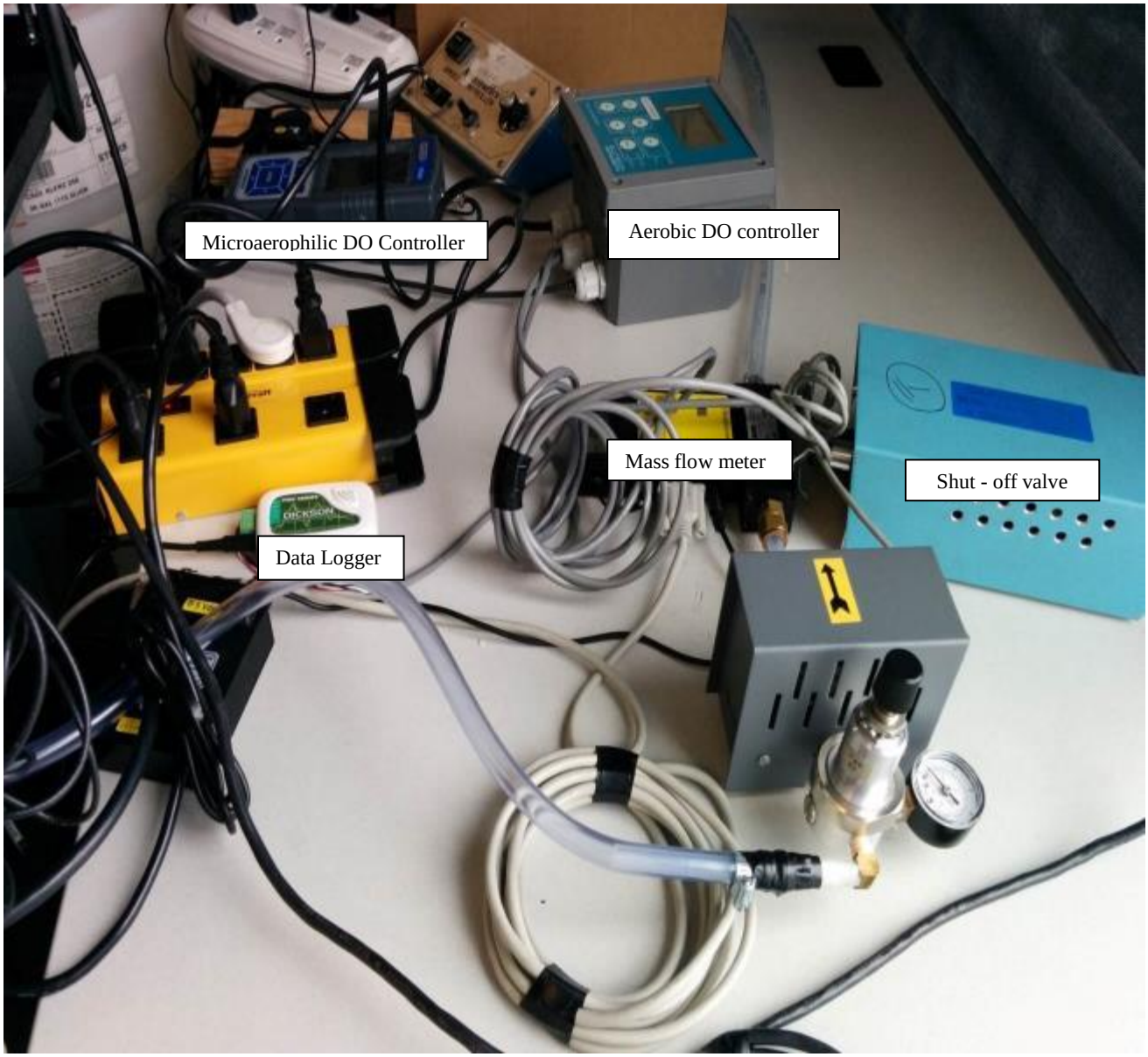


Figure 3.6 - Experimental setup of instrument connection for anammox process in BioCAST.

3.4 Monitoring, Control and Operation

The nitrogen content and pH of the bioreactor feed solution were regularly monitored and adjusted. The pH of the feeding solution was adjusted in the range of 7.5 –8. An electrical mixer was installed inside the feed tank to ensure the homogeneity of feed into the bioreactor. The temperature, pH, and DO concentration inside the different zones of the bioreactor were monitored regularly. A digital heater (True Temp titanium heating system) was used for heating the mixed liquor and for maintaining the temperature at the desired level. pH monitoring inside the bioreactor was performed with Oakton pH probe and meter. The pH meter was calibrated bi-weekly using a 3-point calibration with standard buffer solutions of pH 4, pH 7 and pH 10. Monitoring and control of the dissolved oxygen concentration were important as the DO level in the aerobic and microaerophilic zones had to be kept between 0.9-1.2 mg/l and 0.1- 0.4 mg/l, respectively. The monitoring of ammonium, nitrite, nitrate and total nitrogen concentrations in the mixed liquor and the effluent was conducted 4-5 times per week by the Hach test kits. The Oakton DO Meter for dissolved oxygen measurement was calibrated with an oxygen saturated solution. In order to have a stable result of oxygen measurement, the calibration solution inside the DO electrode was replaced every week. The pump tubes feeding were replaced every two weeks to avoid clogging during the feeding.

3.5 Sample Collection and Preservation

Samples of biofilm were collected every month and they were analyzed for the presence of anammox bacteria by using an optical microscope and scanning electron microscope (SEM). Samples were immediately preserved and stored at 4°C for further analysis. Figure 3.7 shows anammox granules under a microscope (20x).

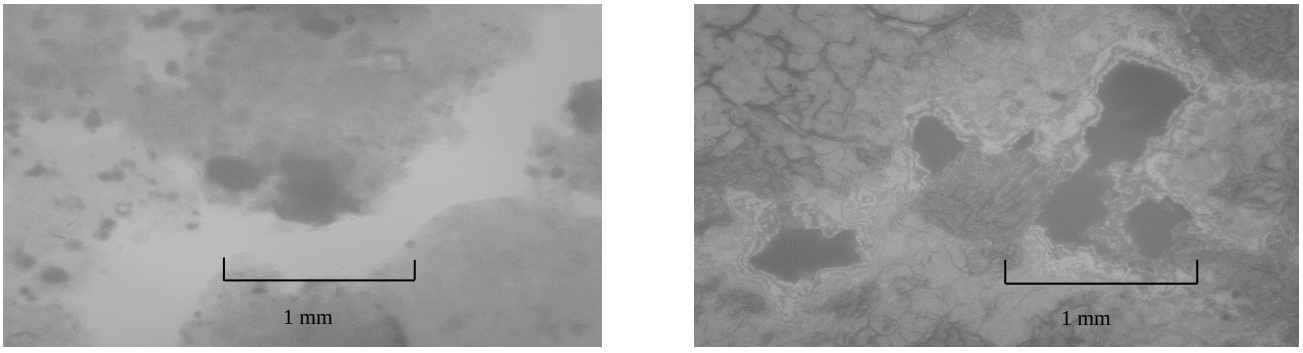


Figure 3.7 - Anammox bacteria under optical microscope (20x); left image at start up and right image after 90 days.

3.6 Sample Analysis

All chemical agents used for sample analysis were purchased from Fisher Scientific (Montreal, Canada). The concentration of ammonium, nitrite, nitrate and total nitrogen in the influent and effluent of the bioreactor were measured for at least 4 times every week. pH, temperature and DO concentration were monitored on a daily basis. All analytical measurements including the TSS, VSS, ammonia (TNT 832), nitrite (TNT 840), nitrate (TNT 836) and total nitrogen (TNT 827) were performed at the Environmental Engineering Laboratory of the Department of Civil Engineering, Concordia University. The test kits were manufactured by Hach Company (Colorado, US). For the purpose of daily monitoring of nitrite, nitrate, and ammonium, in an effort to prevent nitrite toxicity in the bioreactor, quick test kits were utilized. All test kits were purchased from Fisher Scientific (Montreal, Canada).

Figure 3.8 - Ammonium, nitrate, and nitrite test kits (Concordia Environmental Lab).

3.7 Total Suspended Solids and Volatile Suspended Solids (TSS and VSS)

Samples from the aerobic, microaerophilic and anoxic zones were collected, and then distilled water was added at the same volume to the plastic vials. Samples were centrifuged for 20 minutes to separate the solids from the liquid. Afterward, the samples were filtered through pre-weighed filter papers. They were dried overnight at 103-105 °C for TSS and at 550 °C for VSS for 1 hour in the oven. TSS and VSS were calculated from the following equations based on Standard Methods 2540 D/E (APHA, 2005).

TSS, mg/L = ([103-105 °C] dry weight - filter weight), mg / volume of sample, L

VSS, mg/L= (550 °C dry weight – [103-105 °C] dry weight), mg / volume of sample, L

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Operating Parameters

4.1.1 Ammonium Concentrations in Feed and the Loading Rate

The ammonium concentration of synthetic wastewater during the treatment by the integrated multi-zone treatment system is shown in Figure 4.1. At first, the ammonium-nitrogen concentration inside the feed was 10 mg/l; it was then increased gradually to reach 350 mg/l. As mentioned before, the ammonium carbonate was used as a source of nitrogen.

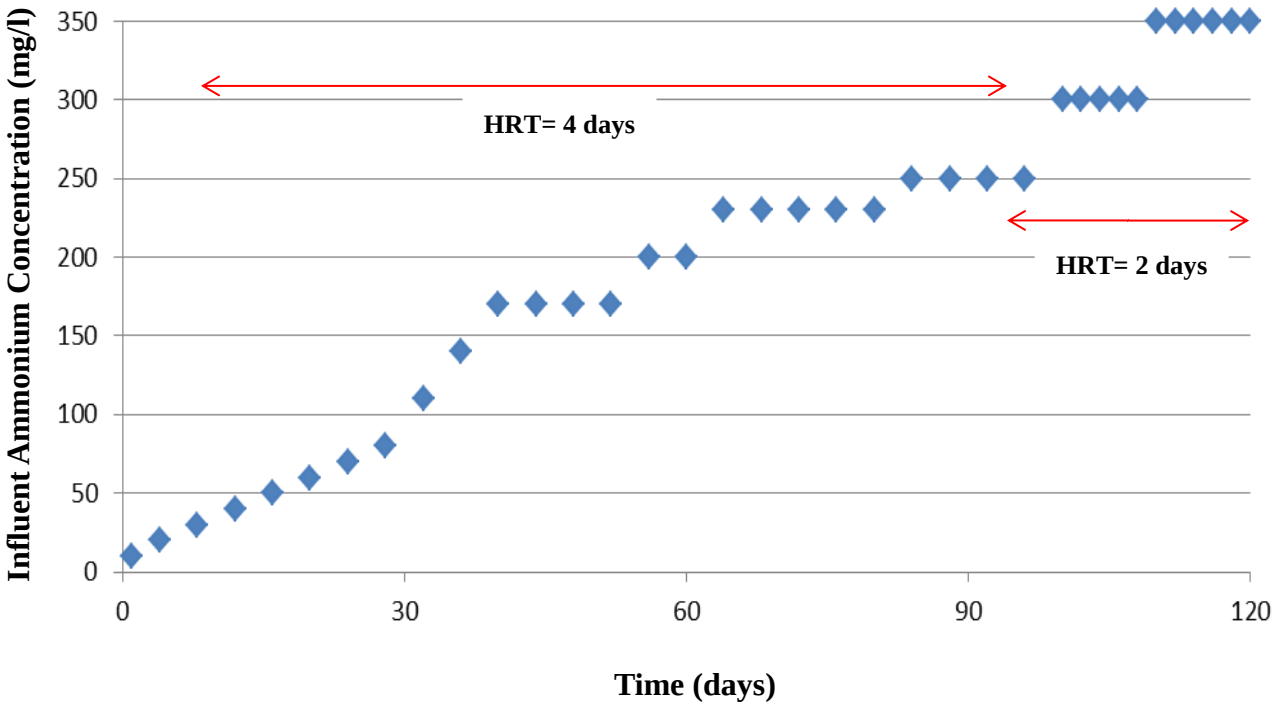


Figure 4.1- Ammonium concentration in the system during the treatment of synthetic wastewater.

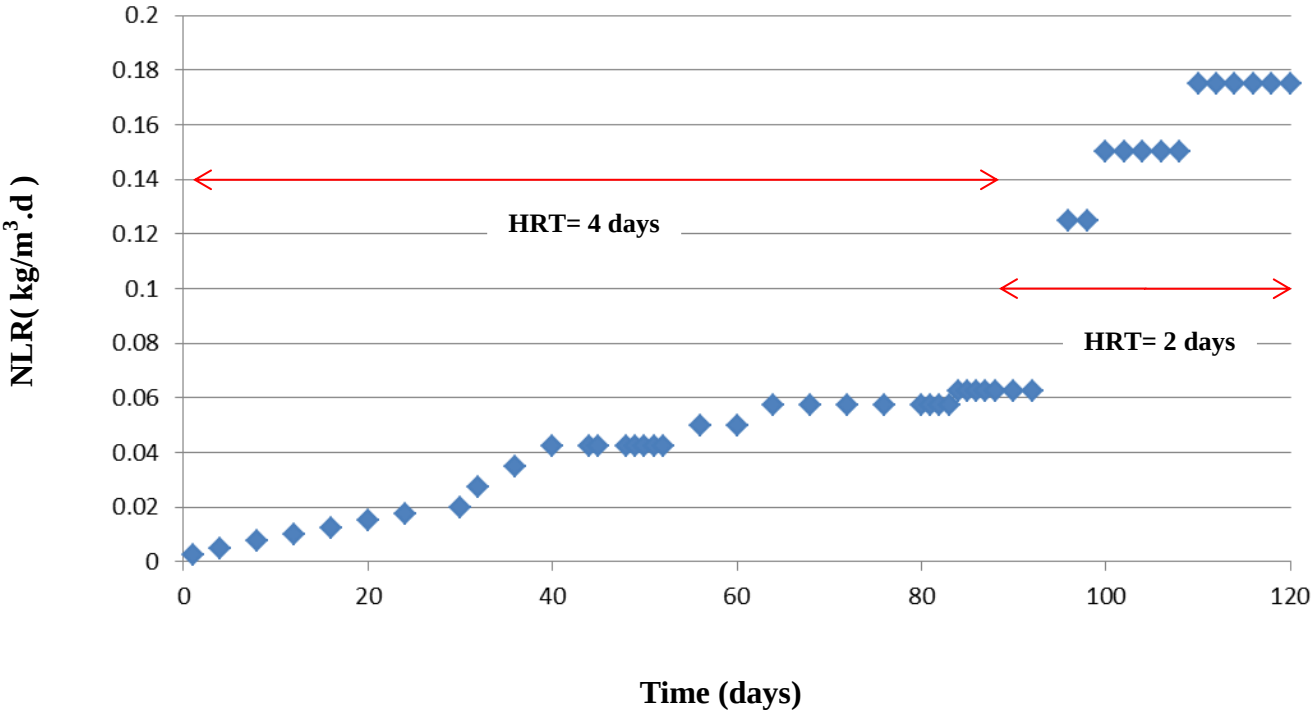


Figure 4.2- Variation of the nitrogen loading rate with time.

The loading rate of nitrogen for the treatment synthetic wastewater is shown in Figure 4.2. The nitrogen loading rate (NLR) was in the range of 0.002 to 0.175 kg/m^3.d.

4.1.2 Dissolved Oxygen

An adequate DO concentration was required in the aerobic zone for the proper conduct of the partial nitrification (PN) process. In the aerobic zone of BioCAST bioreactor, the optimum DO was between 0.9- 1.2 mg/l that was controlled by the air flow meter which was connected to the mass flow meter and the shut off valve. The dissolved oxygen controller controlled at the appropriate DO inside the bioreactor. Figure 4.3 shows the DO concentration in the aerobic, microaerophilic and anoxic zone.

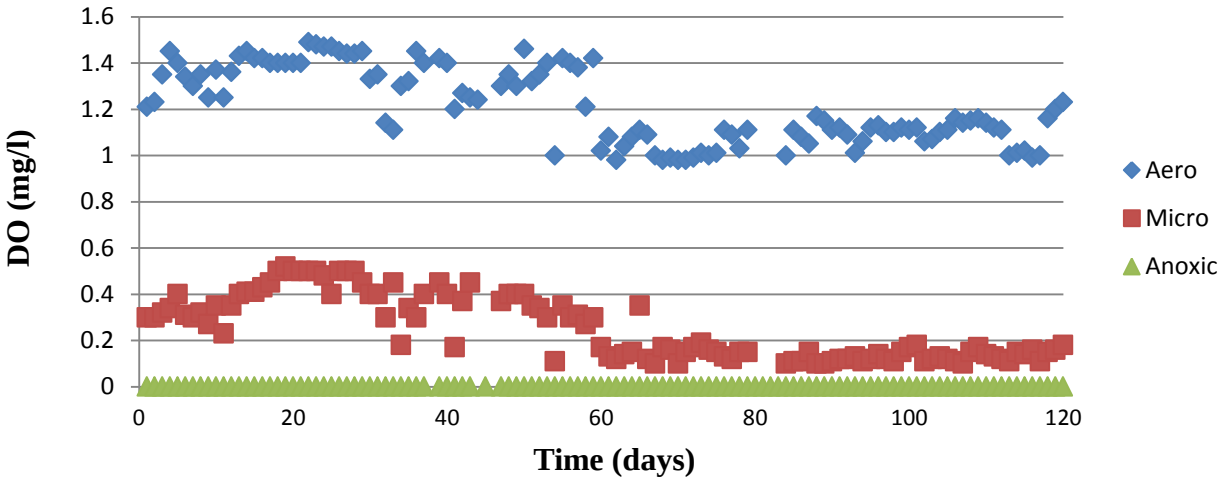


Figure 4.3- Dissolved oxygen concentration in three different zones of bioreactor during the operation.

The control of the level of DO concentration in the bioreactor is important since only the PN process should occur and not a full nitrification. PN operating in the BioCAST bioreactor at higher DO may raise the concern of nitrate production by the NOB, consequently, decreasing the anammox bacteria activity and efficiency. The AOB have been shown to have a higher affinity for oxygen in comparison to the NOB; therefore, high DO concentration may promote the AOB and NOB growth and activity (Schmidt & Bock., 1997; Jayamohan et al., 1988).

According to the literature, for treating wastewater with low ammonia concentration, the appropriate DO concentration is less than 1 mg/l that has to be maintained to reach successful PN (Guo et al., 2009; Wyffels et al., 2004). But, for treating wastewaters with high ammonia contents, the NOB growth at high DO is not a concern. At the DO level in the range of 0.9-1.2 mg/l in the aerobic zone, AOB successfully converted more than 85% of the ammonia to nitrite. At the same time, inside the microaerophilic zone, the DO level was kept between 0.1-0.4 mg/l which was determined as an ideal concentration in this zone.

4.1.3 pH

pH is one of the important operating parameters. The optimum pH inside the reactor was in the range of 7.5-8.1. This level of pH is appropriate for the activity and growth of AOB and anammox bacteria (Strous, 2000).The pH was monitored by an OAKTON meter PD-650. The variations of liquid pH in different zones of the treatment system are depicted in Figure 4.4.

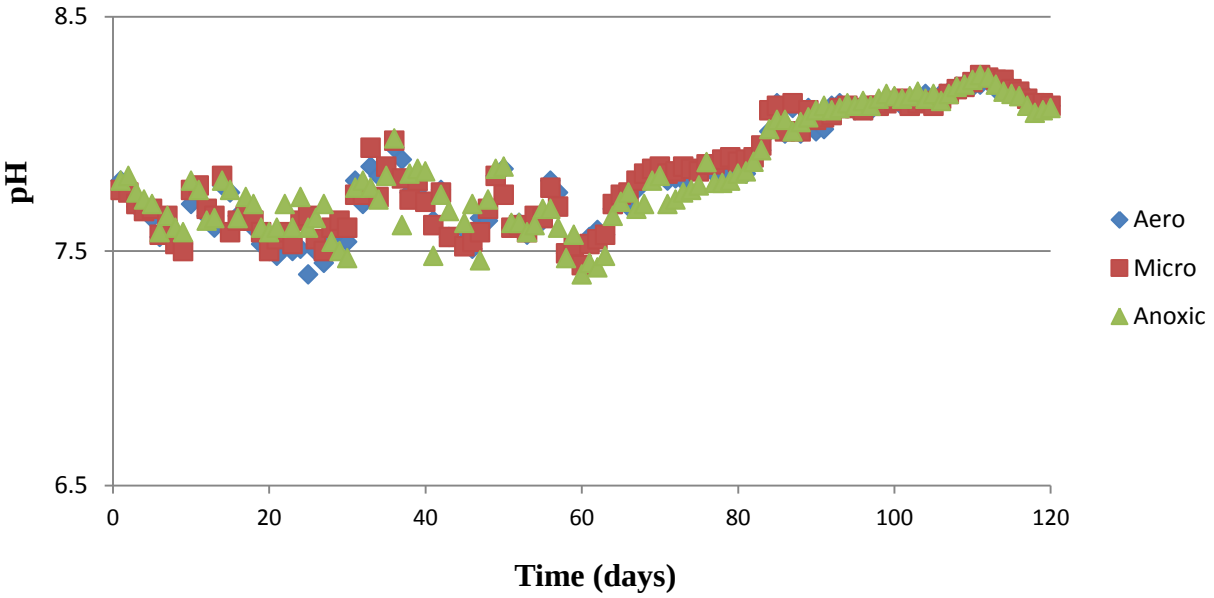


Figure 4.4- pH in aerobic, microaerophilic and anoxic zones of the reactor during the operation.

For nitrifying bacteria, the maximum specific growth rate depends on the pH. Previous studies reported the optimum nitrification pH in the range of 7- 8.2 (Anthonisen et al., 1976). At pH < 6.5 and pH >10 low activity or no activity was reported (Downing & Nere, 1964). According to the previous reports, AOB, growth and activity increases at the expense of the growth and activity of NOB because of the increase in ammonia concentration (Hellinga et al., 1999; Van Hulle et al., 2010). It was observed that in the BioCAST system, AOB growth rate and activity was increased at pH above 7.5 over NOB during the 120 days of the experiment. Moreover, the optimal pH range for anammox was found in the range of 7- 8.3 (Strous, 2000). In the BioCAST bioreactor, the optimal pH was between 7.5 -8.1 in the three different zones.

4.1.4 Temperature

The nitrifier growth rate depends on temperature. Downing et al. (1964) indicated that the raise of temperature by 10 °C increased the growth rate by three times (Downing & Nere, 1964). Temperature above 25 ºC increases AOB growth rate over NOB while temperatures higher than 40ºC causes deactivation (Hellinga et al., 1999). The desired temperature for AOB and NOB was found to be 35ºC and 38ºC, respectively (Grunditz & Dalhammar, 2001). Moreover, AOB grow faster than NOB above a temperature of 25 °C. In order to have a stable partial nitrification, it is important to enrich the AOB and limit, inhibit and wash out the NOB (Blackburne et al., 2006). Anammox bacteria are sensitive to temperature, the optimum temperatures of 30 to 40 ºC have been reported for anammox with the highest activity at 37 ºC (Egli et al., 2001; Schmid et al., 2003; Strous et al., 1999). The favorable temperature range for the anammox process was reported around 35-37°C (Schmid et al., 2003). Several studies have been reported on anammox activity in moderate to low temperature 11-28 °C (Egli et al., 2001; Kimura et al., 2010). In the BioCAST system, the optimum temperature was determined at 33- 35 °C.

4.1.5 Hydraulic Retention Time (HRT)

The hydraulic retention time is the measure of the average length of time that water remains in the reactor. In this study, the hydraulic retention time ranged from 4 to 2 days.

Hydraulic Retention Time (HRT)

HRT, days or min = $\frac{V_R}{Q}$

V_R= Volume of reactor, L

Q = Flow rate, L/d or ml/h Also, $Q = \frac{V_f}{T}$

V_F=Volume of feed consumed, L

T= time, days or min

In this study, the variations of ammonia, nitrite, nitrate, total nitrogen, DO and pH were investigated at the HRT of 4 days. During the first 60 days, the synthetic wastewater was made with deionized water; however in the following 60 days tap water was used and the HRT was decreased to 2 days on day 96.

4.2 Operating Conditions, Nitrogen Composition and Removal Efficiencies

4.2.1 Ammonia-Nitrogen Removal

In order to determine the ammonia- nitrogen concentration in the effluent, laboratory testing of ammonium is required. The ammonia Hach test kits were used 3-4 times per week to measure the ammonia concentration in the effluent. The influent concentration of ammonia-nitrogen was in the range of 10-350 mg/L. The ammonia – nitrogen concentration in effluent and ammonium removal efficiency are shown in Figures 4.5 and 4.6.

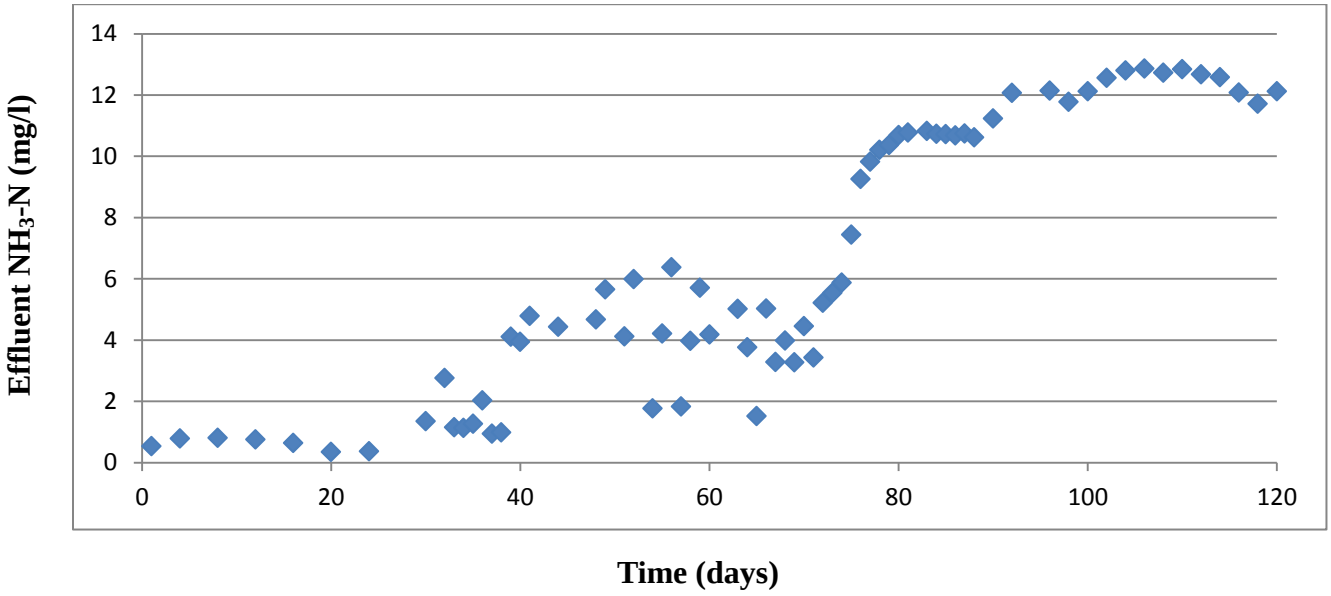


Figure 4.5 - Ammonia-nitrogen concentration in the effluent.

According to figure 4.5, while the ammonium concentration inside the feed was increased the ammonia–nitrogen concentration decreased inside the reactor. Ammonium has been consumed with AOB through partial nitrification process. The AOB convert ammonium to nitrite during partial nitrification process inside the bioreactor. Figure 4.6 shows the removal efficiency of ammonia- nitrogen inside the bioreactor.

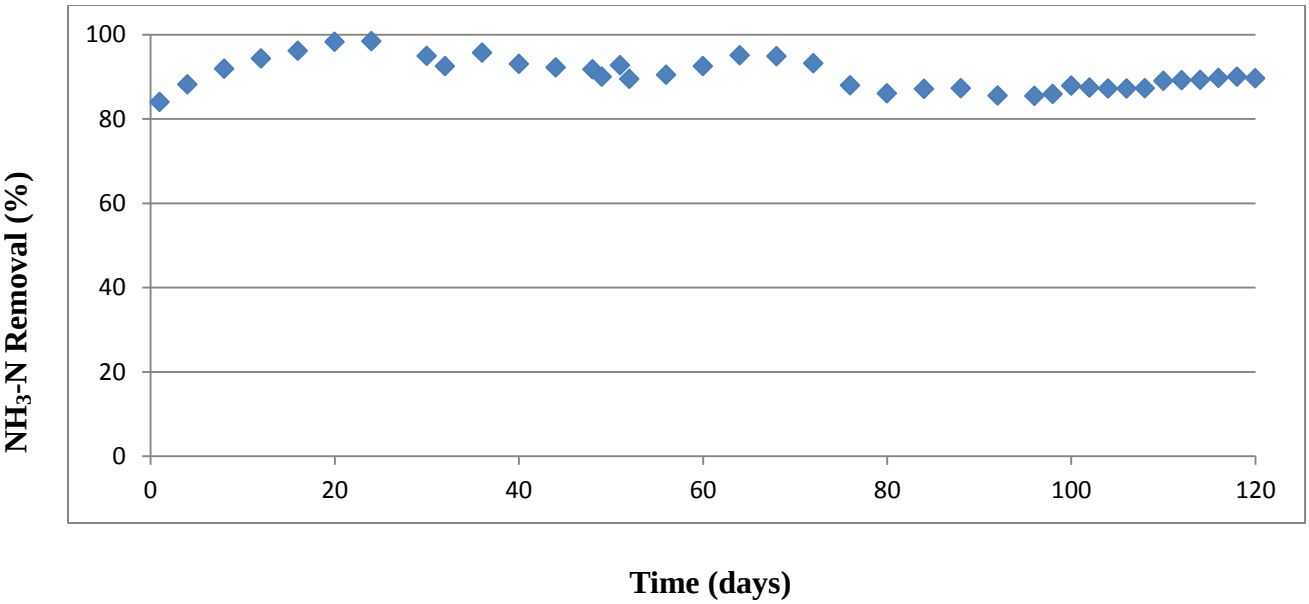


Figure 4.6 - Ammonia-nitrogen removal efficiency inside the bioreactor.

According to Figure 4.6, the ammonia-nitrogen removal efficiency is between 85.4 % - 98.4%. This removal efficiency shows that the AOB inside the bioreactor convert more than 85% of ammonium to nitrite in the influent with 0.9-1.2 mg/l of DO inside the aerobic zone. More than 80% of ammonium was converted to nitrite during PN process with the AOB while the influent ammonia-nitrogen concentration was in the range of 10- 350 mg/l during 120 days of the study. The percentage of total nitrogen removal was calculated according to the following equation:

$$\% \text{ Removal} = \frac{A_{in} - A_{eff}}{A_{in}} \times 100 \qquad 4.1$$

Where A_{in} (mg/L) = Ammonium concentration in the influent

A_{eff} (mg/L) = Ammonium concentration in the effluent

Figure 4.6 presents the ammonia-nitrogen removal with the increased ammonium influent concentration.

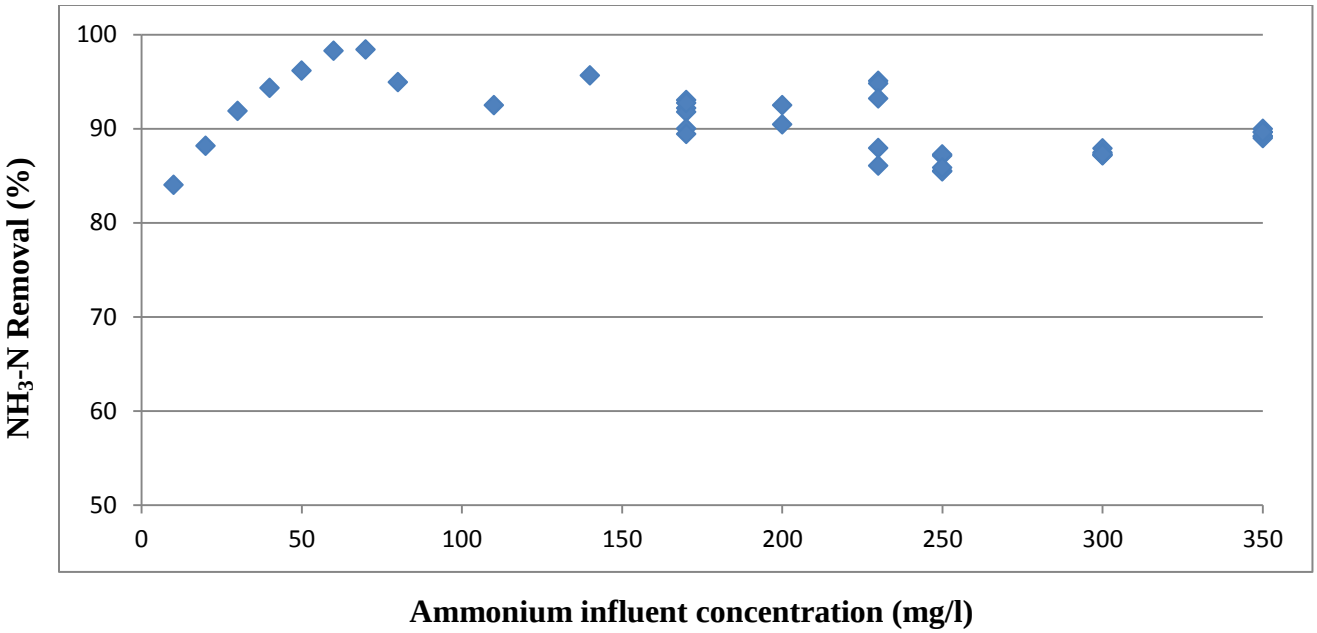


Figure 4.7 - Ammonia- nitrogen removal while increasing influent nitrogen.

Figure 4.7 presents that the increase in the removal of nitrogen with the increase of influent nitrogen which is due to the increase of biomass concentration (Figueroa et al.,, 2011). Figure 4.8 shows the bacterial growth on the attached group inside the aerobic zone. Also, the color of the biofilm turned red, as expected, as an indicator of anammox growth. At the start up, the reactor TSS and VSS were 576 mg/l and 487 mg/l, respectively. The TSS and VSS gradually increased to 1120 mg/L and 950 mg/L respectively (day 90). In this study, it was found that with the successful partial nitrification more than 80% of the ammonium was converted into nitrite.

In the first 30 days of the study, while the ammonia - nitrogen in the influent was between 10-110 mg/l, the ammonia - nitrogen concentration in the effluent reached 1- 2 mg/l and the total ammonia-nitrogen removal efficiency was between 84% - 98.4%, showing that the AOB converted around 90% of ammonium to nitrite. However, after day 30 with increasing the ammonia - nitrogen concentration inside the feed, the ammonium effluent concentration reached 9-12 mg/l and the total ammonia-nitrogen removal efficiency was in the range of 85.4% - 93.0%.

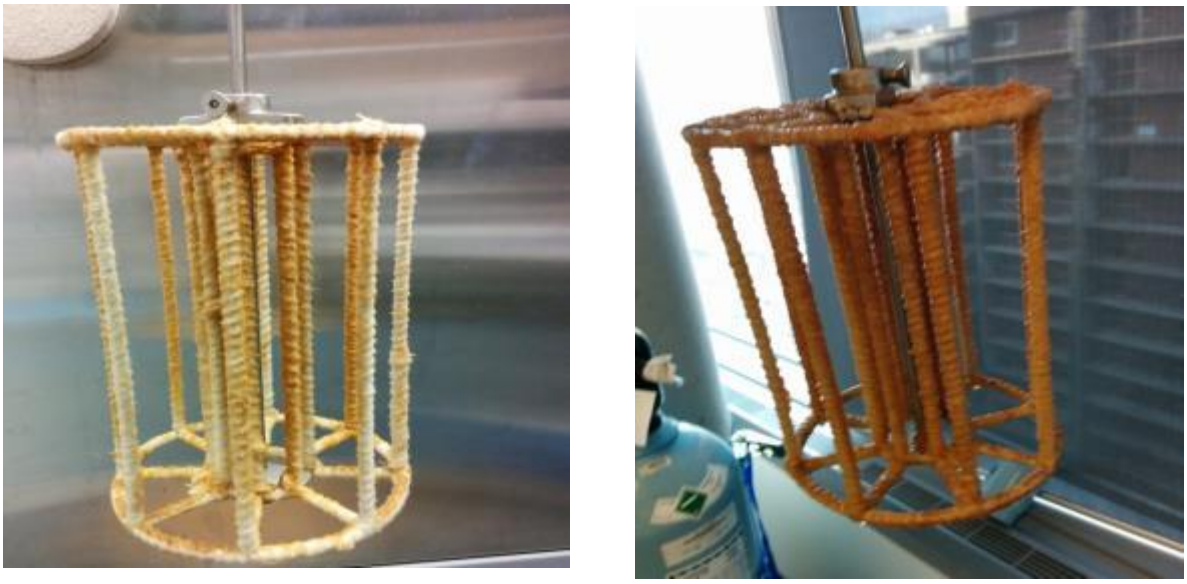


Figure 4.8 - Bacterial growth on the support medium group in the aerobic zone of BioCAST bioreactor left image on day 28 and right image on day 55.

4.2.2 Nitrite - Nitrogen Removal

During the 120 days of anammox enrichment, the reactor content gradually turned red resulted in the growth of anammox bacteria on geotextile fabric media as biofilm aggregates. Figures 4.9 and 4.10 show the anammox growth and its red color. Based on the results and color observation of the bacterial aggregation on the support medium and the observed flocs inside the mixed liquor, the anammox bacterial growth and activity increased. Anammox bacteria consumed nitrite and converted it into nitrogen gas. As such, nitrogen gas bubbles were observed during anammox culture collection inside the centrifuge tube, which was an indication of suitable anammox process.

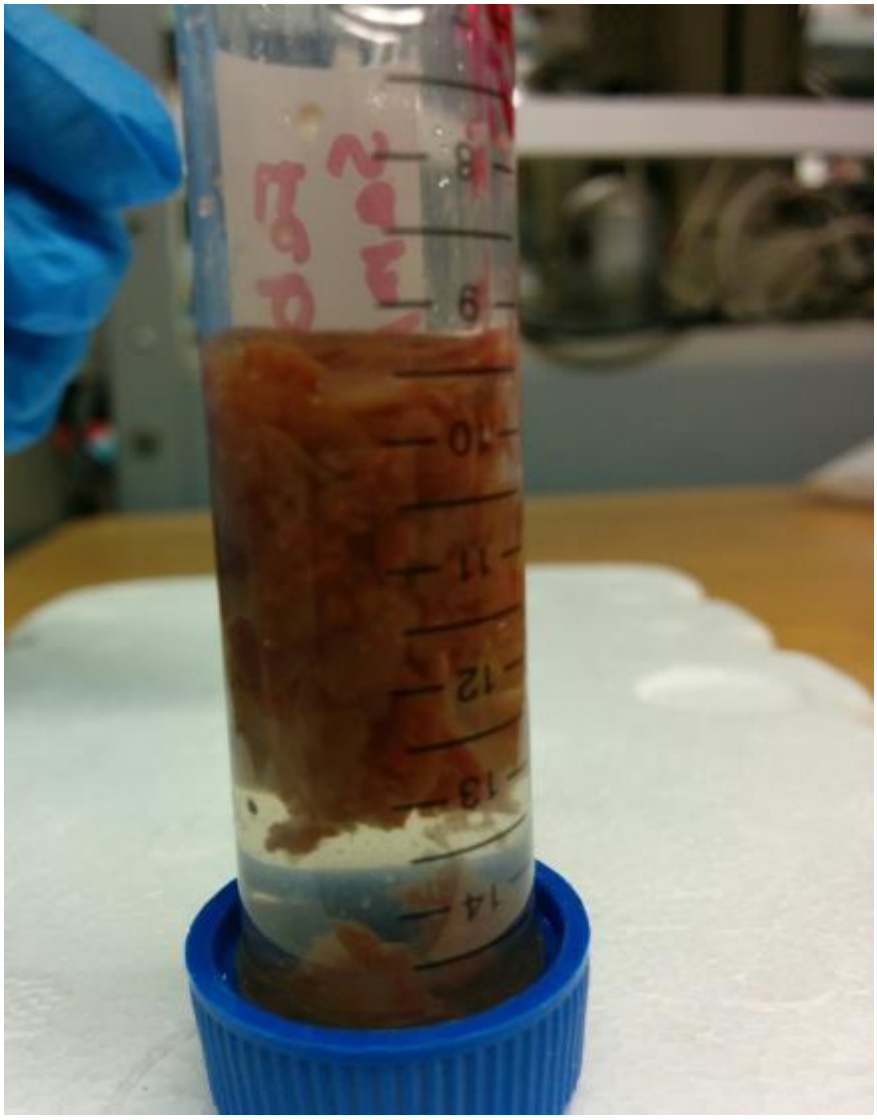


Figure 4.9 - Anammox bacteria on day 120 when the color of bacteria is red.

Figure 4.10 - Anammox bacteria aggregation on geotextile fabric media on day 90.

Figure 4.11 presents the nitrite- nitrogen concentration in the effluent of the bioreactor. According to this figure, the nitrite concentration in the effluent decreased due to the bacteria growth and activity. At first, partial nitrification happened and ammonium was converted to nitrite with AOB, then with anammox bacteria nitrite has been converted to the nitrogen gas. Moreover, in this study the NOB activity and growth were investigated since the first culture was a mixture of AOB-NOB and anammox. Therefore, it was necessary to limit or inhibit NOB activity and growth.

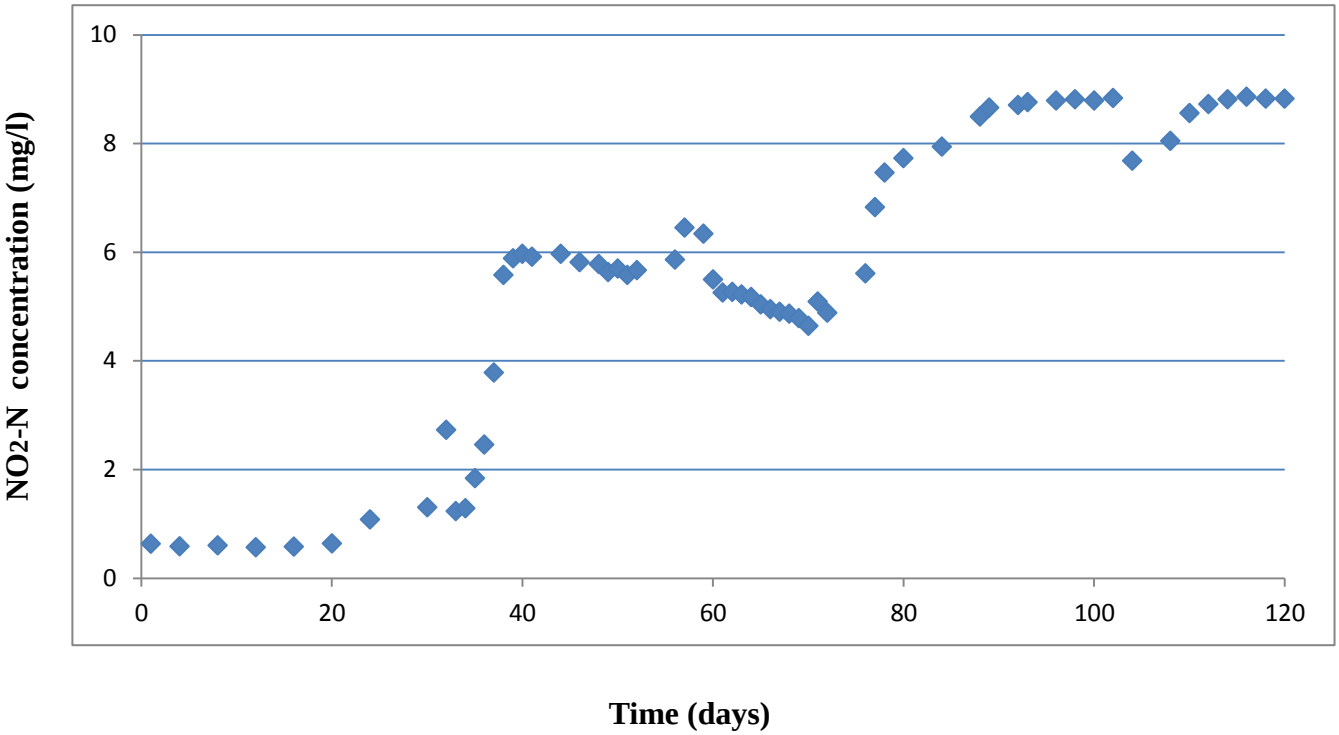


Figure 4.11 - Nitrite-nitrogen concentration in the effluent.

4.2.3 Nitrate- Nitrite

One of the important factors in this study was to have an ideal partial nitrification and limit or inhibit the NOB activity and growth. Figure 4.12 shows the nitrate- nitrogen concentration in the effluent. As mentioned before, The DO concentration is a key factor to overcome this problem. Operation at a higher DO concentration, in an effort to enhance the efficiency of PN, may also enhance the NOB activity and growth, which is not desirable. Therefore, the monitoring and control of the DO concentration is essential since both AOB and NOB compete for oxygen. In order to have a stable PN, it is required to enrich the AOB and limit, inhibit and wash out the NOB (Blackburne et al., 2008; Peng & Zhu, 2006). In this study the NOB existed along with AOB and anammox. Therefore, controlling dissolved oxygen concentration at a lower value was important, since NOB have a lower affinity for oxygen than AOB. Thus, DO concentration is the key parameter that can inhibit and wash out NOB. In the BioCAST bioreactor during the PN process, the appropriate DO concentration was determined to be in the range of 0.9-1.22 mg/l that perfectly controlled and limited the NOB growth and activity. Several operational

parameters such as the DO concentration, pH, temperature, and substrate concentration have been shown to eliminate or washout NOB (Aslan et al., 2009; Peng & Zhu, 2006).

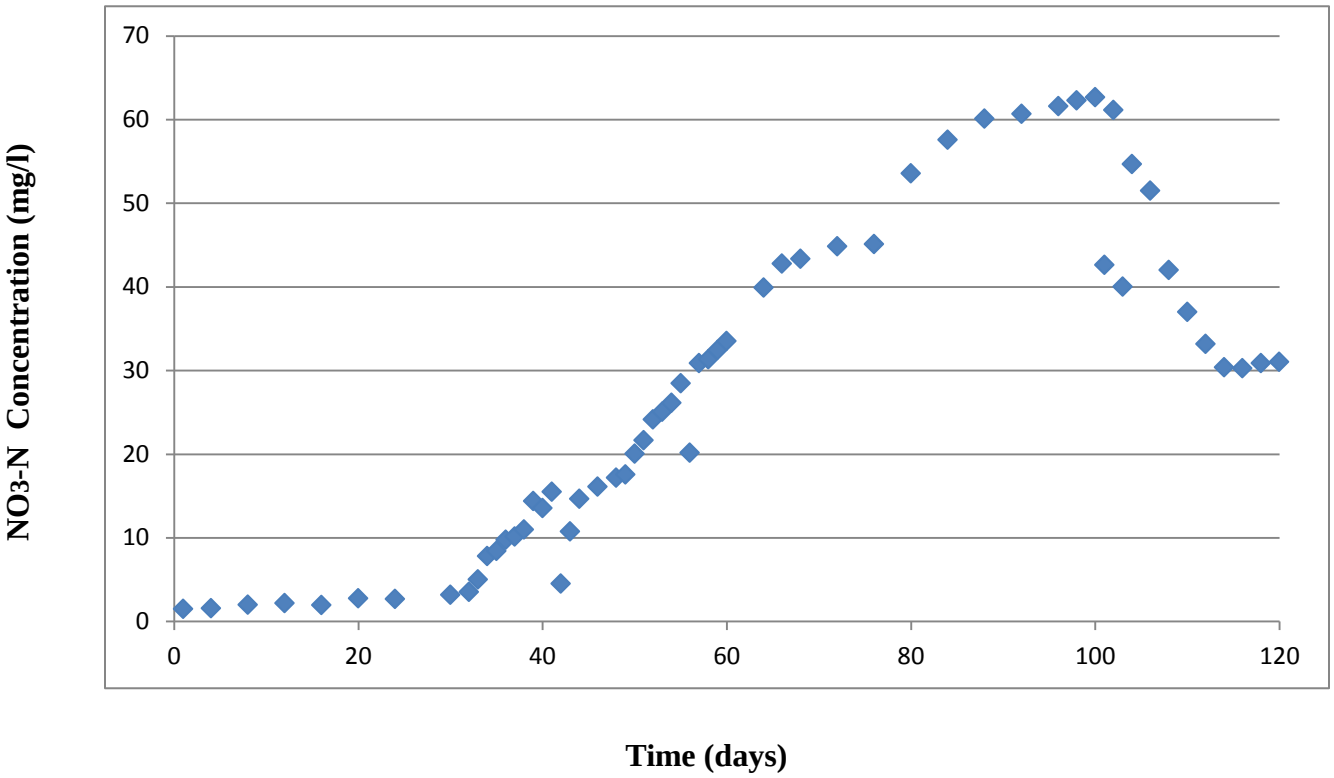


Figure 4.12 - Nitrate-nitrogen concentration in the effluent.

The effect of HRT on the activity of NOB was investigated. The NOB concentration increased to 63 mg/l during this study. This indicated the fact that the NOB activity and growth increase with the increase of nitrogen concentration in the feed. In fact, the HRT of 4 days that was applied at the beginning of the study until day 96 provided enough time for the NOB to grow, despite the controlled DO concentration inside the bioreactor. However, after day 96, the HRT of 2 days was applied when the ammonia nitrogen source had reached 250 mg/l. The results showed that with the decrease of HRT, i.e. increase of the feed flow rate, the NOB did not have enough time for growth and activity, and it caused them to be washed out through the reactor. In fact, the lack of time at this point helped the system to prevent NOB activity and growth. Finally, by the end of the study at day 120, the nitrate-nitrogen concentration reached the level of 30 mg/l. Free ammonia concentration is also an important parameter that may inhibit NOB growth even at high DO concentrations (Bernet et al., 2005). Anthosian et al. (1976) reported that once the free

ammonia concentration rises in the reactor and reached in the range of 0.1- 1 mg/l, NOB growth and activity will be limited because of the sensitivity of NOB to free ammonia. Moreover, the NOB sensitivity to free ammonia is much higher compared to the AOB. Another factor that affects the NOB growth is the liquid pH. At the pH in the range of 7.5-8, the AOB growth and activity is higher than those of the NOB. Therefore, at higher pH values, the AOB will out-compete the NOB.

4.2.4 Total Nitrogen Removal Efficiency

The nitrogen removal in the multi-zone integrated bioreactor system is achieved by the PN and the anammox processes. The PN in the mixed liquor (aerobic zone) transforms the ammonium nitrogen to nitrite by the AOB. The generated nitrite is subsequently converted to nitrogen gas by the anammox bacteria. Figures 4.13 and 4.14 present the change in the total nitrogen removal efficiency with time and with the feed ammonium concentration, respectively. The removal of total nitrogen and ammonia-nitrogen are presented in Figure 4.15.

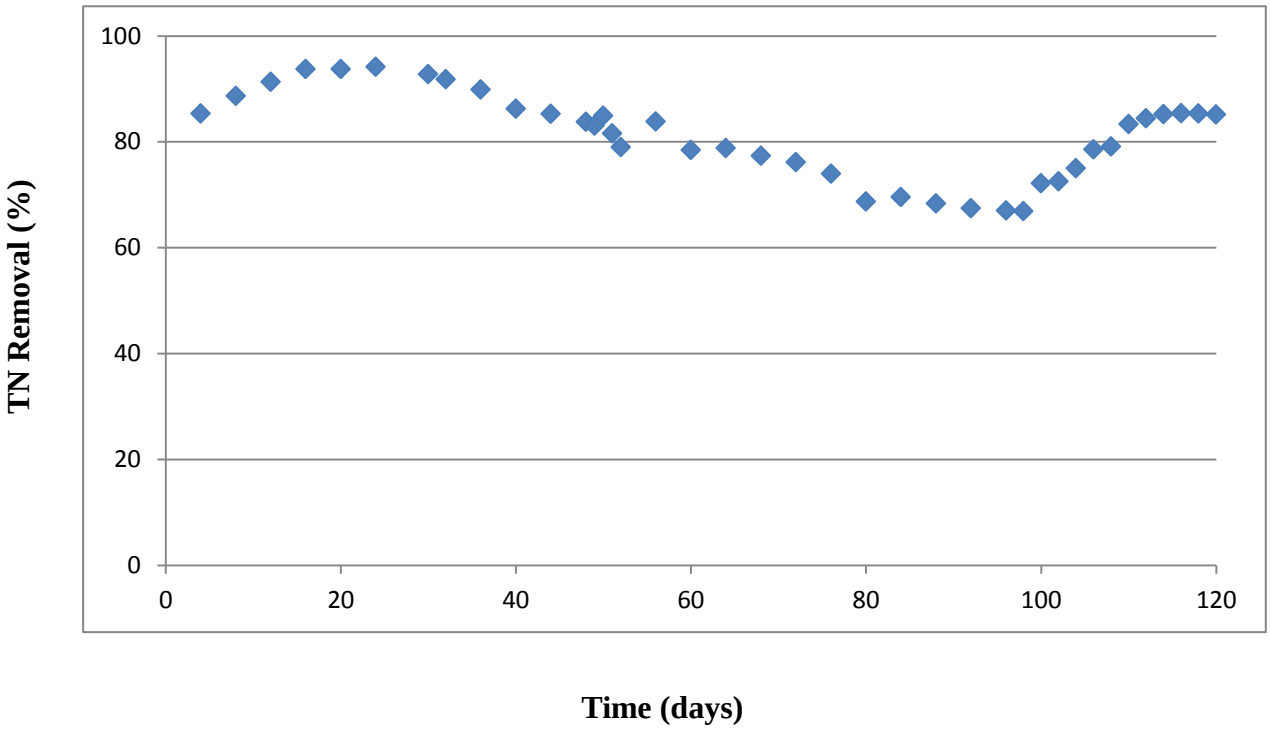


Figure 4.13- Variations of the total nitrogen removal efficiency with time.

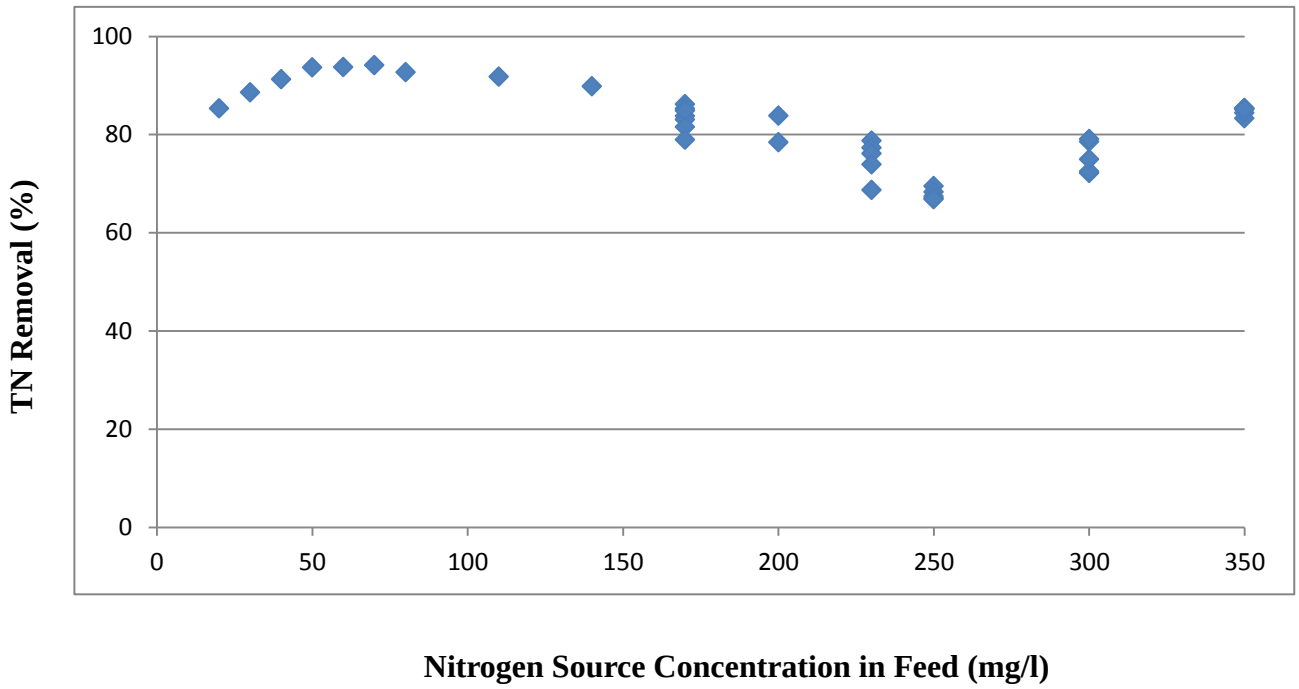


Figure 4.14 - Variations of the total nitrogen removal efficiency with the feed ammonia-concentration.

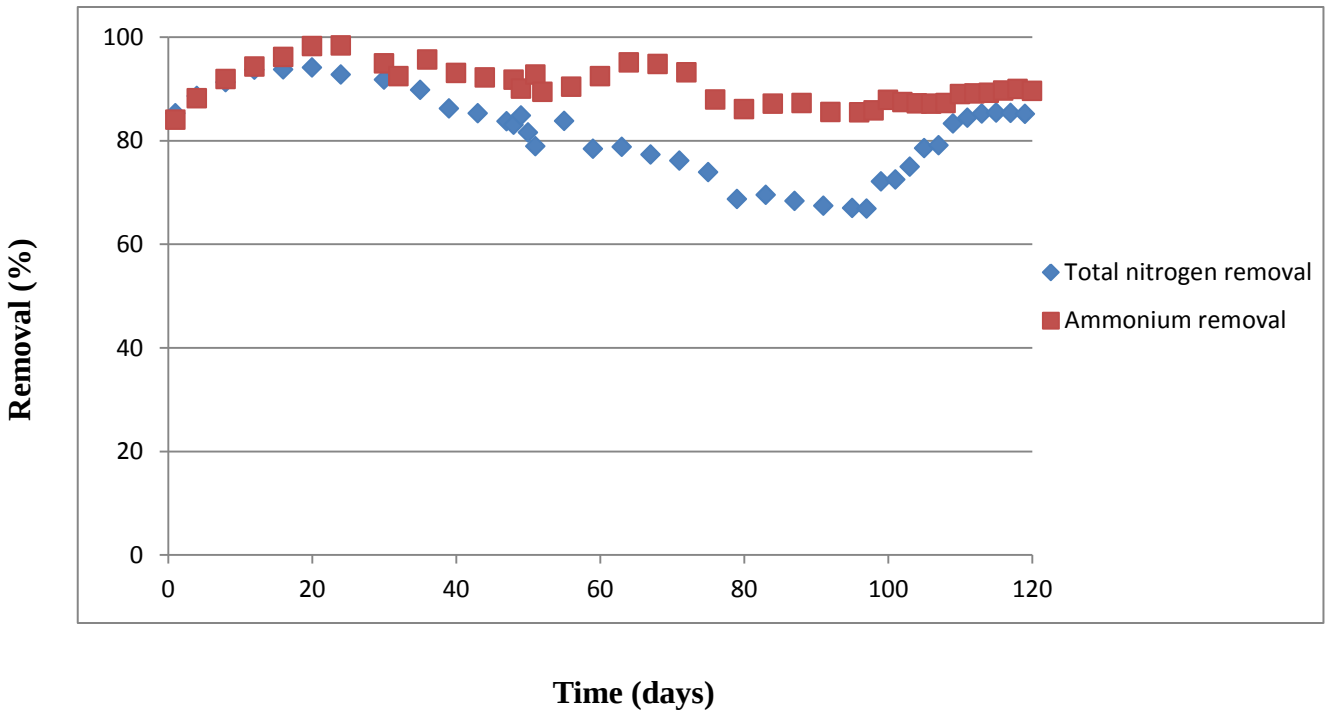


Figure 4.15 - Variations of the total nitrogen removal and the ammonium removal efficiencies with time.

Figure 4.15 shows that the percentage removal of total nitrogen and ammonia-nitrogen increased with the increase of ammonium concentration in the feed until day 60. After that, the total nitrogen removal efficiency decreased because the system started accumulating nitrate in the mixed liquor due to the NOB activity and growth until day 96. On the following days with the decrease HRT to 2 days, i.e. increase of the feed flow rate from 25 L/d to 50 L/d, the nitrate accumulation decreased and the total nitrogen efficiency increased. Figure 4.15 shows that optimum TN and ammonia-nitrogen removal occurred 30 days after the beginning of the study with the average of 96% removal efficiency for both parameters. Between days 45 to 90, the TN removal efficiency was reduced from 95% to 65% while the nitrogen source was in the range of 100-250 mg/l. This was related to nitrate accumulation in the system because of NOB activity and growth. However, by applying the HRT of 2 days and controlling the DO concentration between 0.9-1.22 mg/l, the TN removal increased to 85%. These results demonstrated the proper performance of ammonium removal and total nitrogen removal in the BioCAST system for the PN and anammox processes.

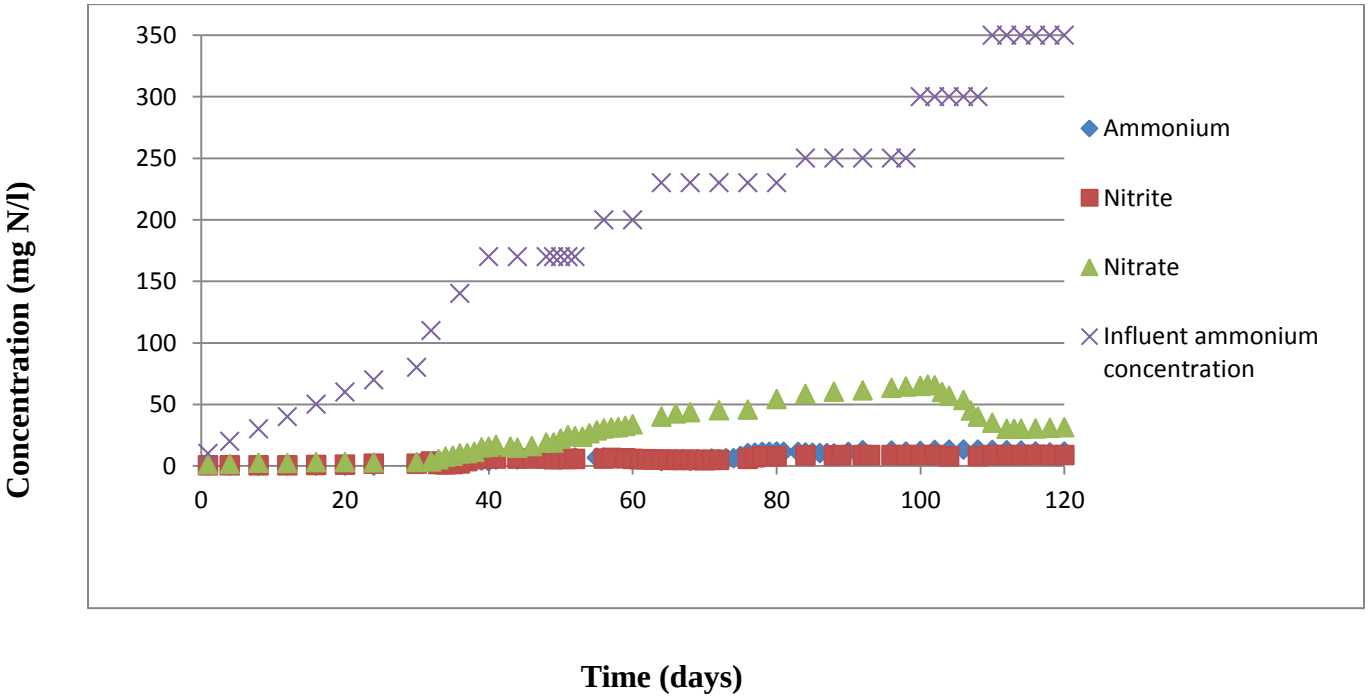


Figure 4.16 - Influent ammonia concentration and ammonium, nitrite and nitrate concentration in the effluent

Figure 4.16 presents the influent ammonium concentration and ammonium, nitrite and nitrate concentration in the effluent during the reported operation period. As discussed before, the AOB convert ammonium to nitrite in the presence of oxygen. The oxygen supply at this stage should be just enough to produce nitrite. However, the NOB are competing with the AOB since they both need oxygen, but the AOB are more efficient in the uptake and consumption of oxygen. Moreover, the AOB tend to grow slightly faster in comparison to the NOB in the presence of oxygen. Therefore, in the aerobic zone, the NOB are out-competed by the AOB. The AOB convert half of ammonium to nitrite, Therefore, the anammox bacteria serve as a sink for the generated nitrite. Also, the affinity of anammox bacteria for nitrite is much higher than the NOB affinity, So, the NOB are competing in two different ways with the anammox bacteria: competing for oxygen and for nitrite (Van Loosdretch, 2014). In the present study, with the increase of ammonium concentration in the feed, the NOB activity increased at the HRT=4 days since the NOB had enough time to grow and formed up to 63 mg/l of nitrate when DO was monitored and controlled in the range of 0.9-1.2 mg/l. The decrease of HRT to 2 days along with the increase of feed ammonium concentration inhibited the growth and activity of the NOB, resulting in the decrease of the effluent nitrate-nitrogen concentration to 30 mg/l.

4.3 Scanning Electron Microscopy

Microbial samples were analyzed by SEM at Concordia University to confirm the existence of anammox culture. The microbial samples were obtained from reactor biofilm on days 75 and 110 when there was a stable consumption of ammonia and nitrite.

4.3.1 SEM Observation

SEM observation revealed an irregular cauliflower structure for anammox bacteria. This cauliflower appearance has been shown before to be the characteristics of anammox enrichment culture (Arrojo et al., 2006; Trigo et al., 2006). The morphology and structure of the biofilm biomass are shown in Figures 4.17- 4.19.

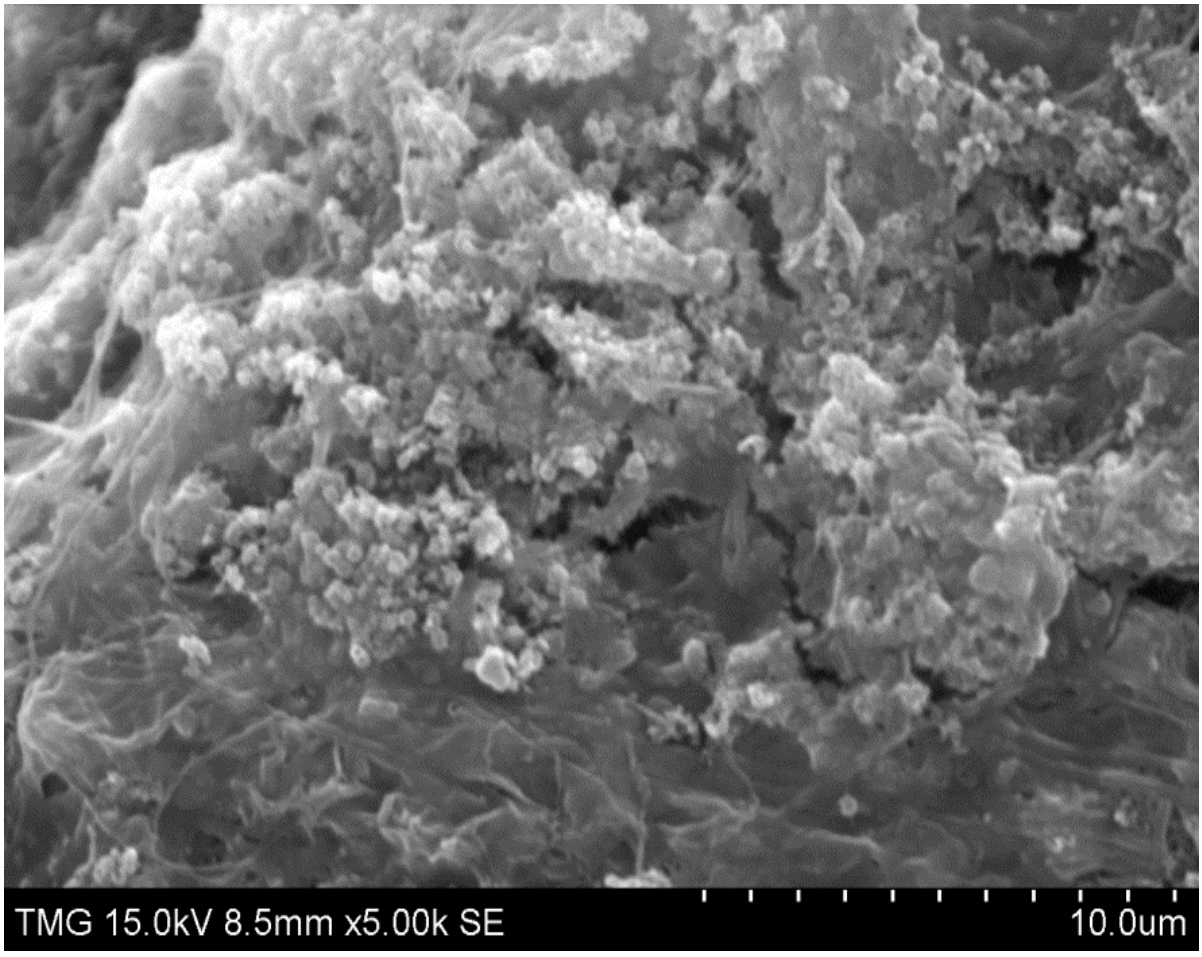


Figure 4.17 - SEM images of the anammox biofilm in BioCAST on day 75.

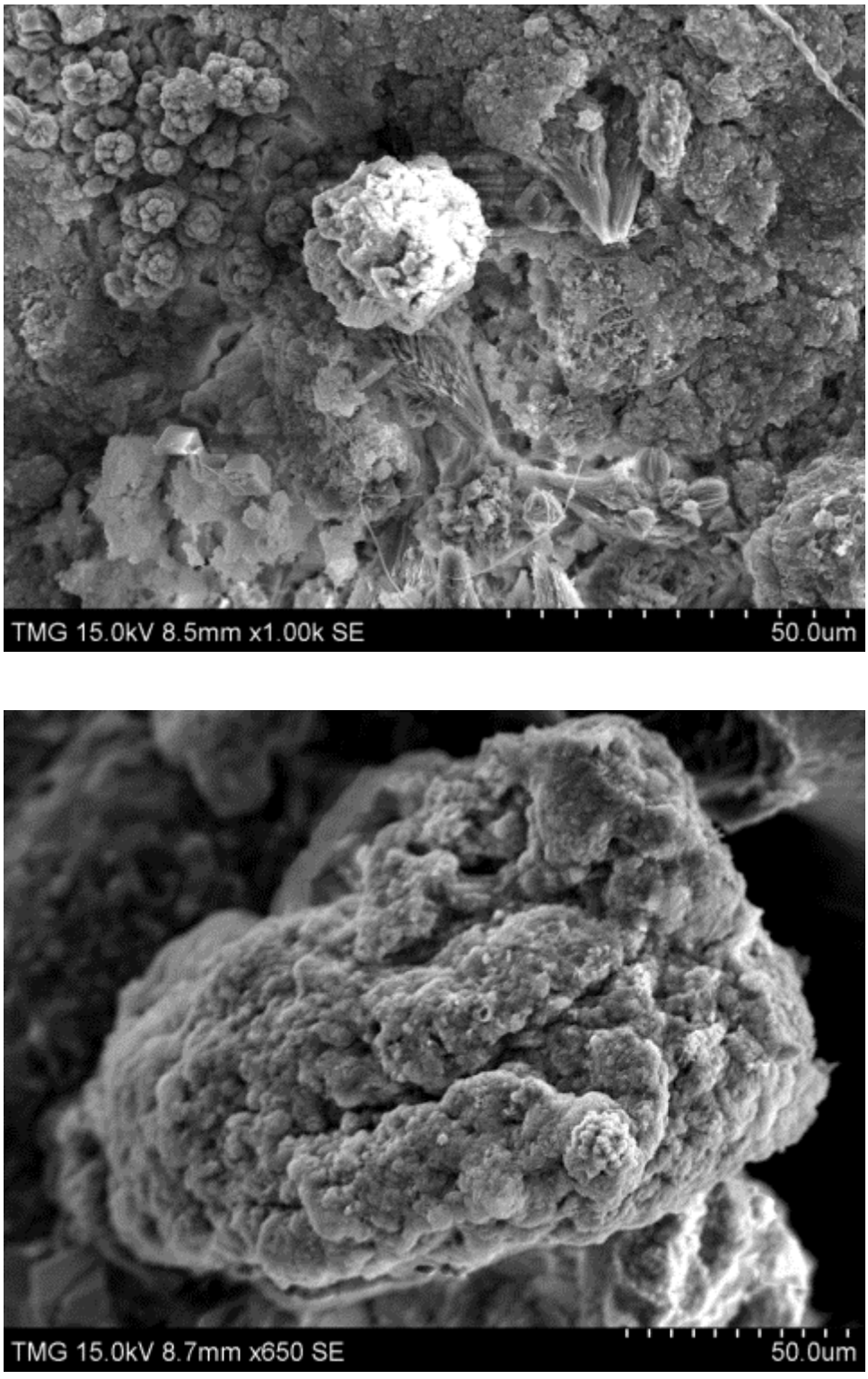
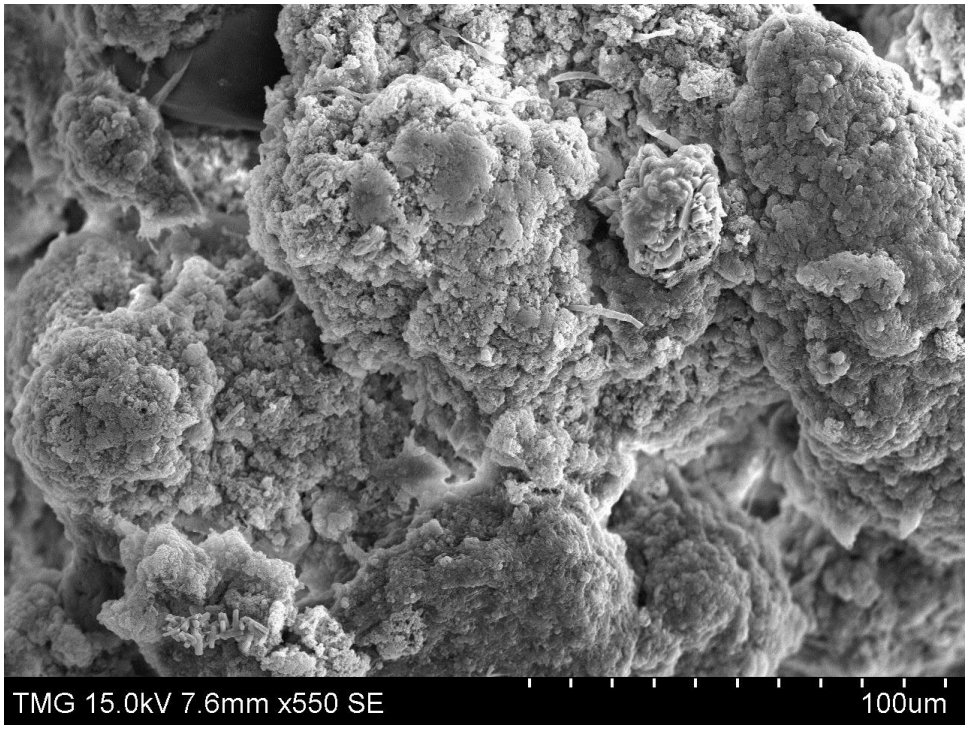


Figure 4.18 - SEM image, anammox granules with a cauliflower shape in BioCAST on day 75.



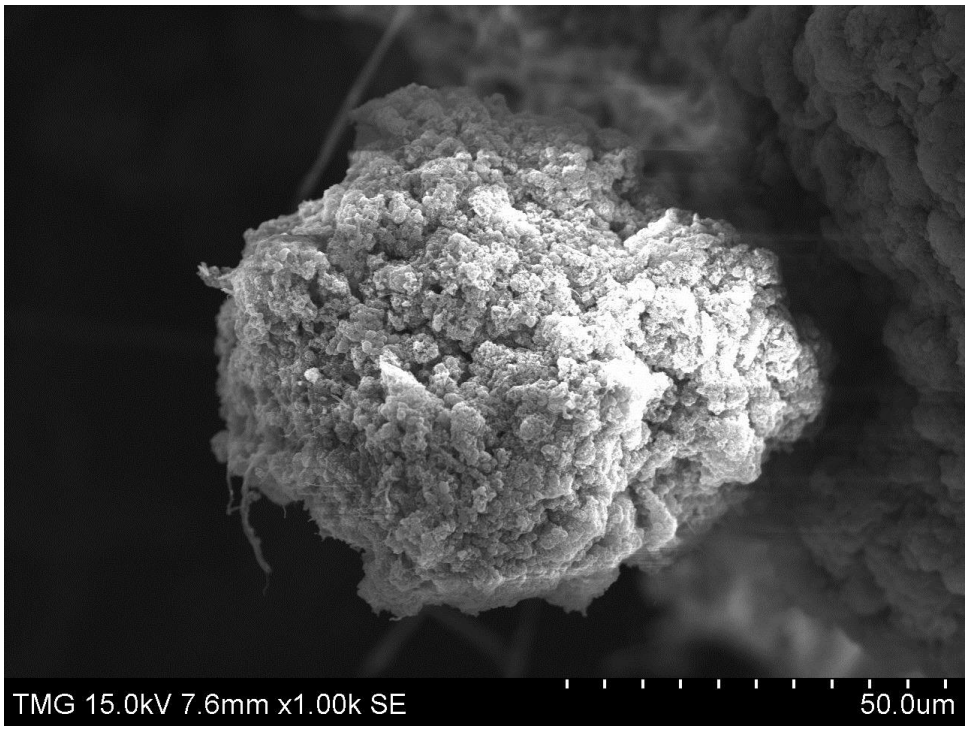


Figure 4.19 - SEM image, anammox granules with a cauliflower shape in BioCAST on day 110.

4.4 Comparison with Previous Work

Van de Graff et al. (1996) studied the anammox growth in a Fluidized Bed Reactor (FBR). The 2.5 l FBR reactor was operated at 36 °C and the investigators maintained the pH at 7. In this study, they found that acetylene, phosphate and oxygen were anammox bacteria inhibitors. The system achieved nitrogen removal rate of 3 kg N/m^3.d. The first full-scale gas-lift anammox reactor (in Rotterdam) achieved very high nitrogen removal rates (10 kg $N/m^3 \cdot d$) after a startup period of three years (Van der Star et al., 2007).

Qin and Zhou (2009) evaluated the application of anammox bacteria in an upflow anaerobic sludge blanket (UASB) bioreactor. They used landfill leachate wastewater treatment plant activated sludge. The reported optimal conditions were pH at 7.5–7.8 as well as temperature was maintained at 32–34 °C. The TN removal efficiency in this study was reported to be 90.3%. Also, the removal efficiencies of the ammonia- nitrogen and nitrite-nitrogen were 99.9% and 96.8%, respectively. Moreover, they discovered three new species of anammox bacteria in the ecosystem based on 16S rRNA and phylogenetic analysis. The sludge color from UASB reactor was reported to be salmon pink.

Hippen et al. (1997) reported the high ammonium removal efficiency up to 75% from the leachate treatment plant of the landfill in Mechernich. This study focused on the nitrification process but under oxygen limited conditions (DO=1mg/l). The process was named aerobic deammonification.

The performance of a sequencing batch airlift reactor was studied by Araujo et al. (2011). They used domestic wastewater treatment plant in order to enrich and identify two *Brocadia*-like anammox phylotypes in the SBR system. Temperature was controlled and maintained at 34–35 °C via a heat blanket, and the operating pH was controlled at 7.5. The Ar/CO_2 (95%/5%) gas mixture was used in the reactor and in the synthetic wastewater to maintain anaerobic condition. The average ammonia removal efficiency was 90% from the domestic wastewater with 24 h HRT.

Siegrist et al. (1998) showed high nitrogen losses (up to 70%) from treating high ammonium-rich leachate from a hazardous-waste landfill site in Kollikon in a nitrifying rotating biological contactor (RBC). In this study, the pH was reported to be in the range of 7–7.3. The investigators

hypothesized that the nitrite reduction might occur under two possibilities: combination of nitritation and anammox processes or only nitritation, i.e. *Nitrosomona*s activity.

Ni et al. (2010) proposed a new approach to combined innovative anaerobic reactor with a non-woven membrane module for anammox enrichment during 8 month experiment. They introduced the anammox non-woven membrane reactor (ANMR) and determined the performance of ANMR with high biomass retention ability on the non- woven membrane surface. The synthetic medium was fed to the reactor after deoxygenation by flushing with argon gas due keep anaerobic condition in the reactor. The pH was reported in the range of 7.5- 8.0. In this study, the HRT was initially 2.5 days and was gradually reduced to less than 1 day. The NLR was gradually increased from 40.6 to 1100 mg N/L. This innovative technology was effective for removing 90.9% of ammonium and 95% of nitrite. Consequently, scanning electron microscope and polymerase chain reaction (PCR) identified 97.7% anammox bacteria in the reactor.

The BioCAST technology presents an ideal system for nitrogen removal by the PN-anammox processes since it retains microorganisms in both suspended growth and immobilized forms, and contains a multiplicity of zones having different environmental conditions of aerobic, microaerophilic and anoxic to support the growth and activity of AOB and anammox bacteria that require different DO concentrations. The PN of ammonium will be achieved in the aerobic zone with low DO concentration and also in the microaerophilic zone that inherently contains low DO levels. In the BioCAST technology with mixed culture of AOB-NOB and anammox bacteria the favorable condition was achieved to control the activity and growth of AOB and anammox bacteria in order to suppress the NOB growth and activity with controlling the level of DO. In this system the lowest level of DO was applied for aerobic and microaerophilic zones that were 0.9-1.2 mg/l and 0.1-0.4 mg/l respectively. The TN removal efficiency and ammonium removal were 81.2% and 85.5% respectively.

Some of the parameters such as TN removal (%), total ammonium removal (%), NLR, HRT, pH and temperature of previous studies compared with the BioCAST technology treatment system are presented in Table 4.1. As demonstrated in able 4.1, the TN removal efficiency in BioCAST technology was 81.2% in comparison to the Qin and Zhou (2009) and Siegrist et al. (1998) studies. These amounts were reported as 91.8 % and 70%, respectively. The total ammonium removal efficiency in BioCAST technology was 85.5 % while in Qin and Zhou (2009),

Araujo et al. (2011), and Ni et al. (2010) were in the range of 99.8%, 90% and 90.9% respectively. In the BioCAST technology the favorable pH was in the range of 7.5-8.1 that was only monitored with a pH meter (OAKTON pH probe) and was not controlled. However, in the Qin and Zhou (2009) study, the pH value was adjusted in the range of 7.5-7.8 with $NaHCO_3$. Araujo et al. (2011), kept the pH to 7.5 while Siegrist et al. (1998) and Ni et al. (2010) maintained the pH in the range of 7- 7.3 and 7.5 - 8 respectively. Moreover, in the BioCAST technology, a digital heater (True Temp titanium heating system) was used to determine the favorable temperature in the range of 33-35 °C while in Araujo et al. (2011), the investigators controlled and maintained the temperature at 34–35 °C via a heat blanket. Qin and Zhou (2009) kept the temperature in the range of 32-34 °C that shows the optimum temperature for anammox bacteria.

Table 4.1-Comparison between BioCAST technology treatment System and previous work.

Parameters	BioCAST Technology (this study)	Qin & Zhou (2009)	Araujo et al., (2011)	Siegrist et al. (1998)	Ni et al. (2010)
TN removal%	81.2	90.3	-	70	-
Total ammonium removal %	85.5	99.8	90	-	90.9
NLR	0.002-0.175 $kg/m^3.d$	0.192-0.249 $kg/m^3.d$	-	-	4.6-1100 mg N/L
HRT	2-4 d	-	24 h	-	1-2.5 d
pH	7.5-8.1	7.5-7.8	7.5	7-7.3	7.5-8
Temp (°C)	33-35	32-34	34-35	15-24	-

The aerobic zone of the bioreactor contains both suspended-growth and attached (fixed-film) microorganisms, which has a profound effect on the stability of the PN-anammox processes. In addition to a cylindrical structure for the immobilization of microbial biomass and formation of microbial biofilm inside the aerobic zone, six strips of geotextile were also placed inside the microaerophilic zone to support the growth of anammox bacteria. The present study demonstrated that the BioCAST is a new technology for nitrogen removal from wastewaters by the combined PN-anammox processes at low DO concentrations. This study showed the possibility of achieving high removal efficiencies for TN and ammonia- nitrogen in a single bioreactor.

CHAPTER 5: CONCLUSIONS

In this study, BioCAST, a new integrated multi-zone wastewater treatment technology, was used for the removal of nitrogen by the combined PN-anammox processes. The treatment system was operated with synthetic wastewater for 120 days. The combined PN-anammox processes showed a great potential for nitrogen removal at low dissolved oxygen concentrations and without using any organic carbon. The synthetic wastewater used in this study was composed of 10-350 mg/l of ammonium carbonate as the nitrogen source that was successfully removed with the combined PN-anammox processes. The treatment system contained an aerobic zone operating at adequate DO concentrations in the range of 0.9-1.2 mg/l, and provided enough mixing for the activity and growth of nitrifying bacteria. The control of the ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and anammox bacterial growth and activity was challenging. This study showed that the successful function of the combined PN-anammox processes depends on the control of several parameters including the liquid pH, DO concentration, temperature, and ammonia and nitrite concentrations. Moreover, replacing deionized water with tab water on day 60 showed several variations of pH, DO as well as effluent nitrogen measurements. In this study, only the first bioreactor has been used. Therefore, BioCAST bioreactor showed great potential for using only the first reactor for combined PN-anammox process for nitrogen removal.

The results indicated that the BioCAST technology is suitable for nitrogen removal by the combined nitrification and anammox processes. The treatment system design and operation showed that the combined partial nitrification and anammox processes removed ammonia-nitrogen and total nitrogen with high efficiencies of up to 85.5% and 81.2%, respectively. Based on the obtained results and the SEM images, it can be concluded that BioCAST is a promising technology for nitrogen removal from wastewaters by the combined PN and anammox processes.

CHAPTER 6: RECOMMENDATIONS FOR FUTURE WORK

The following recommendations are suggested for future work:

- Establishment of the optimum operating conditions for the maximum growth of anammox bacteria and minimum activity of nitrite-oxidizing bacteria.
- Microbiological characterization of microbial biomass in the anammox process under various operating conditions.
- Investigation of the efficiency of nitrogen removal from nitrogen-rich industrial wastewaters by the BioCAST technology using the combined partial nitrification (PN) and anammox processes.
- Investigation of the impact of organic carbon on the activity of anammox bacteria and the overall nitrogen removal efficiency by the combined PN-anammox processes.
- Increase the size of openings between the aerobic and microaerophilic zones in order to increase the liquid circulation velocity between these zones to transfer the nitrite to the low oxygen zones with faster rate and have a shorter time in the aerobic zone.
- Evaluation of the impact of the hydrodynamic characteristics such as circulation rate and mass transfer coefficient by the BioCAST technology using a combined PN-anammox process.

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