DOKUZ EYLÜL UNIVERSITY THE GRADUATE SCHOOL OF NATUREL AND APPLIED SCIENCES

NITROGEN REMOVAL WITH ANAMMOX PROCESS IN AN UASB REACTOR

by

Gamze BAYRAM

February, 2011

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M.Sc. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"NITROGEN REMOVAL WITH ANAMMOX PROCESS IN AN UASB REACTOR"** completed by **GAMZE BAYRAM** under supervision of **PROF. DR. DELİA SPONZA** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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NITROGEN REMOVAL WITH ANAMMOX PROCESS IN AN UASB REACTOR

ABSTRACT

Anaerobic ammonia oxidation (ANAMMOX) is a novel process in which is a new powerful tool especially for remove the strong nitrogenous wastewaters. In this study, the anaerobic ammonia removal efficiencies was investigated in an UASB RECTOR and ammonia and COD removal kinetics was researched at six different hydraulic retention times (HRT). The COD concentrations were adjusted between 300 and 1000 mg/L and HRTs were decreased from 4,4 to 0,73 days by adjusting the up-flow rates from 0,5 to 3 l/d. Thought continuous operation of anammox - UASB reactor using synthetic wastewater for 294 days.

Ammonium, nitrit, nitrat and chemical oxygen demand (COD), removal efficiencies, total, nitrogen gas, hydrogen sulfide gas, metan gas productions and methane percentage ratios were investigated in UASB reactor at different Ammonium, nitrit, nitrat and chemical oxygen demand (COD) concentration, and decreasing HRTs. The maximum ammonium removal efficiency was found as fifty five percent when the influent nitrit, nitrat and chemical oxygen demand (COD)concentrations were 600 mg/l 131 mg/L, 0,1 mg/L, respectively in run 1 at a HRT of 4,4 days. The maximum nitrit removal efficiency was hundred percent when the influent COD/NH4-N/NO3-N/NO2-N ratios were between 20/2/2.6/1 and 15/1.25/1/2.5 in runs 3,4 and 5 at HRTs of 2, 1,1 and 0,73 days. The maximum COD removal efficiency was obtained as ninety percent when the COD/NH4-N/NO3-N ratios were 6:1:1,3 in runs 1 and 2 at a HRT of 4.4 days. The maximum nitrogen gas production was found as 130,4 l/day, in continuous operation of the UASB reactor indicating the anaerobic ammonia removal process (anammox process) occurred. The kinetic studies performed in the UASB reactor showed that Stover Kin-cannon and Grau kinetic models were meaningful for ammonia and COD removal in the UASB rector.

Keywords: Ammonia, Anammox, nitrogen removal,UASB reactor

UASB REAKTÖRDE ANAMMOX PROSESİ İLE NUTRİENT GİDERİMİ

ÖZ

Anaerobik amonyak oksidasyonu (Anammox) atıksulardan yüksek konsantrasyonlu azotun gideriminde yeni bir yöntemdir. Bu çalışmada, altı farklı hidrolik alıkonma süresinde (HRT) 'de ve anaerobik amonyak giderim verimleri ve giderim kinetiği incelenmiştir. KOİ konsantrasyonu 300 ve 1000 mg/l arasına, hidrolik alıkonma süresi 4,4 günden 0,73 güne azalan, debisi 0,5 l/günden 3 L/güne artacak şekilde ayarlandı.

UASB reaktörde farklı amonyum, nitrit, nitrat ve KOİ konsantrasyonlarındaki sentetik atıksuyun amonyum, nitrit, nitrat ve KOİ, giderim verimleri araştırıldı toplam azot gazı, hidrojen sülfür gazı, metan gazı üretimi ve metan yüzdesi oranları hesaplandı. Maksimum amonyum giderim verimi, KOİ konsantrasyonu 600 mg /L, nitrit konsantrasyonu 131 mg/ L, NO3-N konsantrasyonu 0,1 mg/L iken yüzde ellibeş olarak bulunmuştur. Hidrolik bekleme süreleri bu esnada 4.4 gündür. Giriş atıksuyu için COD/NH4-N/NO3-N/NO2-N oranları 20/2/2.6/1 ve 15/1.25/1/2.5 arasında iken Maksimum nitrit-azotu giderimi yüzde yüzdür. Hidrolik bekleme süreleri bu esnada 2.2 gün ,1.1 gün ve 0.73 gündür. Maksimum KOI giderimi yüzde doksan olarak bulunmuştur. Bu esnada giriş amonyum, nitrit, nitrat ve KOİ, oranları 6:1:1.3, hidrolik bekleme süresi 4.4 gündür. Sürekli çalışmalarda, maksimum azot gazı üretimi 130,4 l/gün, olarak bulunmuştur, bu durum bize UASB reaktörde anaerobik koşullarda amonyak gideriminin oluştuğunu (anammox) göstermiştir. UASB rektörde yapılan kinetik çalışmalar amonyak azotu ve KOI' nin Stevor Kin-Cannon and Grau Second order Kinetic modellerine göre giderildiğini göstermiştir. UASB reaktörde anammox prosesi için uygun bulunmuştur.

Anahtar Kelimeler: Amonyak Azotu, Anammox, Azot giderimi, UASB reaktör

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CHAPTER ONE
INTRODUCTION

1.1 Nitrogen Sources

Nitrogen is the most important components in wastewater which has to be removed before effluents can be discharged. Different forms of nitrogen are found globally in aquatic ecosystems. Nitrogen in the aquatic environment occurs in four forms (ammonium ion, ammonia, nitrite, and nitrate). The most toxic nitrogen to biota is ammonia, followed by nitrite and nitrate. Because ammonia and nitrite are quickly oxidized to nitrate by bacteria and algae in the aquatic environment, they are mainly problems when they originate in large volumes from point sources such as industrial effluents and livestock feed lots and slaughterhouses or areas that lack nitrification treatment of urban sewage. Although nitrate is the least toxic of the three forms, it occurs at the highest concentrations and is the most stable form of nitrogen in the aquatic environment.

Most of the nitrogen in domestic wastewater is the product of our eating habits and food preparation, body exudates washed off in the bath or shower and products washed from clothes. Cleaning chemicals also contribute organic compounds in varying amounts. These organic compounds require microbial activity to degrade them (Patterson, R.A., 2003).

Food preparation including washing of vegetables with small vegetable scraps entering the wastewater system through the sink, adding to the potential nitrogen load in the septic tank. In the bathroom wastewater is contaminated with perspiration (sweat) that contains neutral fats and volatile fatty acids, traces of albumen, urea ($CO(NH_2)_2$), sodium chloride, potassium chloride and traces of alkaline phosphates, sugar and ascorbic acid calcium and magnesium salts and nitrogenous compounds (organic N, ammonia-N, urea and amino acids), the latter which vary with diet (Osol, 1973). Other human wastes include skin, hair, body oils and greases and the ‘dirt’ from other sources. Hair shampoo, conditioners and other

personal care items contain large proportions of very complex organics, the fate of which is unclear (Patterson, R.A., 2003).

There are many different kinds of human activity that generate wastewater with large quantities of ammonium: petrochemical, pharmaceutical, fertilizer and food industries, leachates produced by urban solid waste disposal sites or waste from pig farms. Disposal of this type of waste is a serious environmental problem because free ammonia, diluted in water, is one of the worst contaminators of aquatic life.

1.1.1 Applications of Nitrogen

As more and more uses for the element have been found, the demand for nitrogen has increased dramatically over the past few decades.

The most important applications of nitrogen depend on the element's inertness. For example, it is used as a blanketing atmosphere in metallurgical processes where the presence of oxygen would be harmful. In the processing of iron and steel, for example, a blanket of nitrogen placed above the metals prevents their reacting with oxygen, forming undesirable oxides in the final productions.

Nitrogen is used in high temperature thermometers where mercury cannot be used. This is because mercury boils at 356.7^{o}C and hence cannot be used in such thermometers. A volume of nitrogen is enclosed in a vessel and introduced into the region of high temperature. Depending upon the temperature, expansion of the nitrogen volume takes place. Then applying the gas equation, the temperature is calculated (www.tutorvista.com).

Nitrogen is also used in the production of electronic components. Assembly of computer chips and other electronic devices can take place with all materials submerged in a nitrogen atmosphere, preventing oxidation of any of the materials in use. Nitrogen is often used as a protective agent during the processing of foods so that decay (oxidation) does not occur.

Another critical use of nitrogen is in the production of ammonia by the Haber process, named after its inventor, the German chemist Fritz Haber. The Haber process involves the direct synthesis of ammonia from its elements, nitrogen and hydrogen. The two gases are combined at temperatures of 932–1,292°F (500–700°C) under a pressure of several hundred atmospheres over a catalyst such as finely divided nickel. One of the major uses of the ammonia produced by this method is in the production of synthetic fertilizers (science.jrank.org).

Nitrogen mixed with argon is used in electric bulbs to provide an inert atmosphere. It helps in prevention of oxidation and evaporation of the filament of the bulb, giving it a longer life.

It is used to produce a blanketing atmosphere during processing of food stuff, to avoid oxidation of the food. It is also used when food is being canned, so that microorganisms do not grow.

Nitrogen in the air helps as a diluting agent and makes combustion and respiration less rapid.

It is used by the chemical, petroleum, and paint industries to provide inactive atmosphere to prevent fires or explosions.

It is used in the industrial preparation of ammonia, which is converted into ammonium salts, nitric acid, urea, calcium cyanamide fertilizers etc.

Liquid nitrogen is used as a refrigerant for food, for storage of blood, cornea etc. in hospitals. Meat, fish etc., can be frozen in seconds by a blast of liquid nitrogen, which can provide temperatures below -196^{o}C.

Liquid nitrogen is used in scientific research especially in the field of superconductors.

Nitrogen is essential for synthesis of proteins in plants. Proteins are essential for synthesis of protoplasm, without which life would not exist.

1.2 Biological Nitrogen Removal Process

Nitrogen can be removed from wastewaters by a variety of physico- chemical and biological processes. Because biological nitrogen removal is effective and inexpensive, it has been adopted widely in favor of the physico-chemical processes (EPA, 1993).

The biological process is the most widely practiced approach for nitrogen control in wastewater treatment. For many years, the traditional method for nitrogen removal from wastewater has been the combination of nitrification-denitrification processes. With the aim to obtain better process stability, some researchers have been focusing on combinations of anaerobic and aerobic processes. Different reactor configurations and systems working with one or two reactors can be used. Simultaneous removal of nitrogen and COD can be achieved using the conventional nitrification and denitrification systems. However, conventional methods for the biological removal of these compounds involve two discrete steps namely nitrification and denitrification. Firstly, nitrification is an energy demanding process for aeration and due to low growth rate of nitrifiers, large nitrification volumes are required. Secondly, denitrification requires organic carbon as electron donor. If the carbon content in the wastewater is not sufficient, an extra carbon source has to be supplied which causes an increase of overall treatment costs (Güven&Sözen, 2010). Over the past few years, new technologies for nitrogen removal have been developed mainly because of the increasing financial costs of traditional wastewater treatment technologies. Recently, a novel process named Anammox (anaerobic ammonium oxidation) was discovered in which ammonium ion could be converted to nitrogen gas under anoxic conditions with nitrite as the electron acceptor. This innovative Anammox process, as a result, has made the nitrogen treatment more sustainable (Chan,T., 2003).

Some of the novel microbial nitrogen removal processes that have been developed are Single reactor system for High Ammonium Over Nitrite (SHARON) which involves part conversion of ammonium to nitrite, Anaerobic Ammonium Oxidation (ANAMMOX) process which involves anaerobic oxidation of ammonium and the Completely Autotrophic Nitrogen removal over Nitrite (CANON) process which involves nitrogen removal within one reactor under oxygen-limited conditions. There are other processes that have been developed such as Oxygen Limited Autotrophic Nitrification-Denitrification (OLAND) and a wetland based systems, all with high potential for nitrogen removal. (Browse M., Florante A., Gaspillo, Pag-asa D., & Auresenia J., 1996)

1.2.1 Conventional nitrification and denitrification

Conventional microbial nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification. The removal involves aerobic nitrification (i.e., the conversion of NH_4^+ to NO_2^- and further to NO_3^-) with molecular oxygen as the electron acceptor. The relevant reactions are as follows:

$$NH_4^+ + 1{,}5O_2 \rightarrow NO_2^- + 2H^+ + 2H_2O$$
$$NO_2^- + 0{,}5O_2 \rightarrow NO_3^-$$

The anoxic denitrification (i.e., the conversion of NO_3^- and NO_2^- to gaseous nitrogen) is accomplished with a variety of electron donors, including methanol, acetate, ethanol, lactate and glucose (Grabinska&Loniewska, 1991; Tam et al., 1992; Akunna et al., 1993).The anoxic denitrification involves the following reactions:

$$2NO_3^- + 10H^+ + 10e^- \rightarrow N_2^+ + 2OH^- + 4H_2O$$
$$2NO_2^- + 6H^+ + 6e^- \rightarrow N_2^+ + 2OH^- + 2H_2O$$

As nitrification and denitrification are carried out under different conditions and by different microorganisms, experience shows that these processes have to be separated in time or space to function effectively. The conventional

nitrification/denitrification reactions have been known for a long time (Winogradsky, 1890; Beijerinck & Minkman, 1910; Kluyver & Donker, 1926). During denitrification, the requirement of organic carbon is significant. For example, 2.47g of methanol is required per gram of nitrate nitrogen for complete denitrification (McCarty et al., 1969).

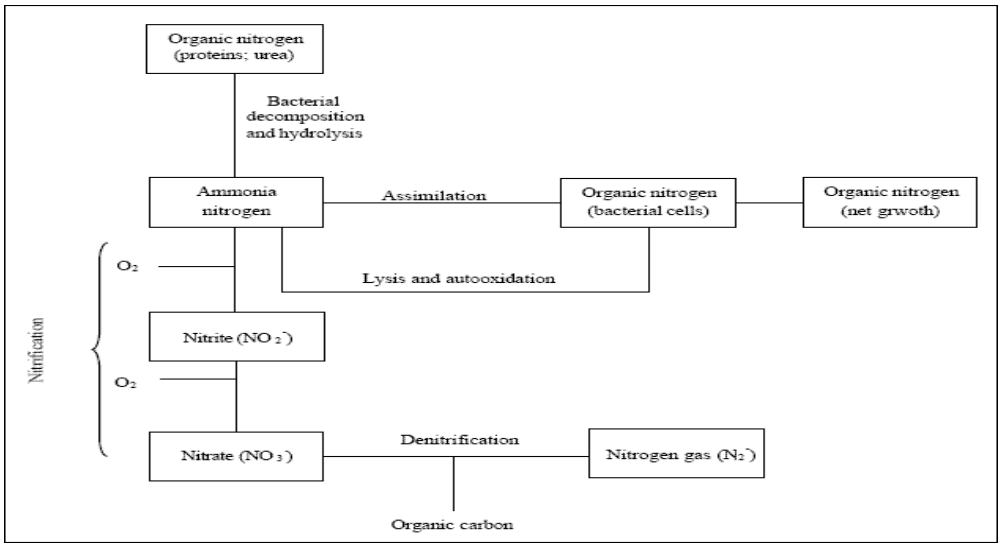

Figure 1.1 Conventional nitrification and denitrification

1.2.2 SHARON Process

The SHARON process (single reactor system for high ammonia removal over nitrite process) is a new process for biological nitrification. This process is operated without any biomass retention in a single aerated reactor at a relatively high temperature (35 ^{0}C) and pH (above 7) (Brouwer et al., 1996; Hellinga et al., 1997). The process involves partial nitrification of ammonium to nitrite, and this greatly reduces the expense of aeration. SHARON is the first successful process in which nitrification/denitrification with nitrite as an intermediate has been achieved under stable conditions (van Kempen et al., 2001). The SHARON process is especially suitable for the treatment of wastewater streams with high ammonium content, and the lower oxygen needs for the partial ammonium oxidation to nitrite allows important energy savings. The stoichiometry of the process (Equation) shows that

only 50% of the ammonium contained in the wastewater is oxidized when an equimolar ratio between ammonium and bicarbonate is provided. Moreover,since nitratation is not expected to occur, an additional reduction of 25% of the oxygen required to ensure compete nitrification is achieved (Mağrı , Coraminnas, Lopez , Campos , Balaguer ,& Colprim ,1996).

$$NH_4^+ + HCO_3^- + 0.75O_2 \rightarrow 0.5NH_4^+ + 0.5NO_2^- + CO_2 + 1.5H_2O$$

Nitrification

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \text{ (SHARON)}$$

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+ \text{ (Conventional)}$$

↑ 25% reduction

Denitrification

$$6\ NO_2^- + 3\ CH_3OH + 3\ CO_2 \rightarrow 3\ N_2 + 6\ HCO_3^- + 3\ H_2O \quad \text{(SHARON)}$$

$$6\ NO_3^- + 5\ CH_3OH + CO_2 \rightarrow 3\ N_2 + 6\ HCO_3^- + 7\ H_2O \quad \text{(Conventional)}$$

↑ 40% reduction

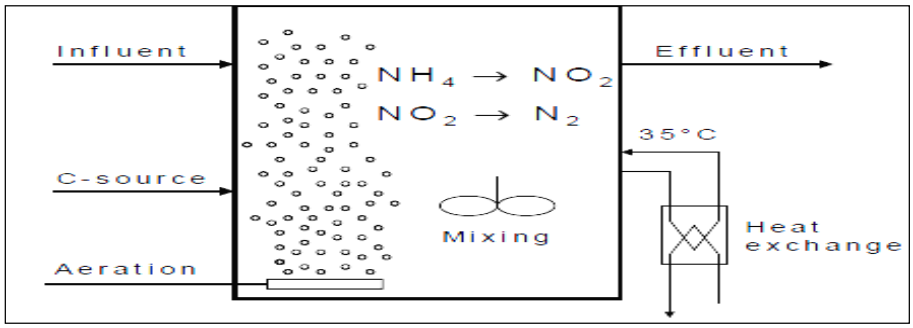

Figure 1.2 SHARON Process

SHARON is a very cost-effective treatment system for the total removal of nitrogen components from wastewater flow streams through nitrification/denitrification. The system is used for the treatment of municipal wastewater side streams from both dewatered digested primary sludge and waste activated biosolids to achieve high overall nitrogen removal. In addition it can be used to treat wastewater flows from sludge dryers and incinerators.

1.2.3 CANON Process

The interaction of aerobic and anaerobic ammonium oxidising bacteria under oxygen-limitation results in an almost complete conversion of ammonium to dinitrogen gas, along with small amounts of nitrate. A high loss of nitrogen has been reported in several systems with high ammonium loading and low organic carbon content of the wastewater (Helmer et al., 1999, 2001; Helmer & Kunst, 1998; Hıppen et al., 1997; Koch et al., 2000; Kuaı and Verstraete, 1998; Sıegrıst et al., 1998). The autotrophic conversion of ammonium into dinitrogen gas was defined microbiologically (Strous et al., 1997) and the process has been named CANON, an acronym for Completely Autotrophic Nitrogen-removal Over Nitrite (Dıjkman & Strous, 1999). If ammonium removal can be achieved in a single reactor, it would represent a very economical and efficient option for water treatment, especially for wastewater rich in ammonium but devoid of organic carbon (COD). Ammonium removal from wastewater is traditionally performed using oxic nitrification to nitrate, involving high aeration demands, followed by anoxic denitrification of the nitrate to nitrogen gas, in a separate tank. The CANON process is completely autotrophic, therefore avoiding COD addition, which is often required for the heterotrophic denitrification step in traditional systems (Brouwer et al., 1996; Hellinga et al., 1997).

1.2.4 DEPHANOX Process

The DEPHANOX process is atype of post denitrification with COD in influent preserved by mechanisms like as in a contact-stabilization activated sludge process (Lee , Nam, & Sikshin, 1996).

The innovative nutrient removal process scheme DEPHANOX proved to be very efficient because it maximises the utilisation of organic substrate for phosphorus and nitrogen removal. The process solves the competition for organic substrates among Poly-P organisms and denitrifiers as well as the problem of overgrowing of slow nitrifiers by faster organotrophs, typical of activated sludge (Bortone, Marsili Libelli, Tilche & Wanner; 1999).

1.2.5 ANAMMOX Process

The anammox process is the anoxic oxidation of ammoniumwith nitrite as electron acceptor (Graaf et al., 1996). The anammox process has attracted considerable attention in recent years as an alternative to conventional nitrogen removal processes, because the anammox process is a cost-effective and low energy alternate to the conventional biological nitrogen removal, which is typically achieved via sequential aerobic autotrophic nitrification and anoxic heterotrophic denitrification. In contrast to the conventional biological nitrogen removal processes, the anammox process requires less oxygen for nitrification and no external carbon source for denitrification, which leads to significant reduction of operational cost and energy (Dongen et al., 2001; Fux&Siegrist, 2004). Moreover, the anammox process produces little undesirable by-products such as greenhouse gases (e.g., N_2O) and low excess sludge. Despite these significant advantages, full-scale application of the anammox process was recognized to be limited primarily due to the slow growth rate and demand of nitrite as an essential substrate (Star et al., 2007).

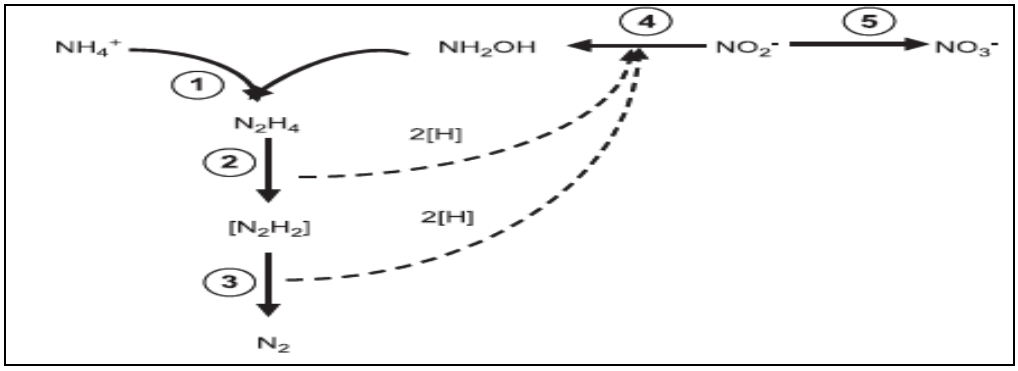

Figure 1.3 Anammox process

The Anammox is also a distinctive process, involving the oxidation of ammonium with nitrite as the electron acceptor to yield N_2 and NO_3^- under anoxic condition. Strous et al. reported that the stoichiometry of the anammox reaction based on mass balance over anammox enrichment culture was represented by the following equation.

$$NH_4^+ + 1.31NO^{-2} + 0.066HCO^{-3} + 0.13H^+ \rightarrow$$

$$1.02N_2 + 0.26\ NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

Initially anammox research was focused on the basic properties of the process and on providing evidence for its microbial nature and the principles of the nitrogen and carbon metabolism. It appears that the anammox process is based on energy conservation from anoxic ammonium oxidation with nitrite as the electron acceptor and hydrazine and hydroxylamine as the intermediates. Carbon dioxide is used as the main carbon source for growth (Shivaraman, 2003).

1.2.6 SBR Process

Enhanced nitrogen and phosphorus removal can be achieved in a sequencing batch reactor. Phosphorus release and some BOD_5 uptake take place during fill and anaerobic stir operation. Phosphorus uptake, BOD_5 oxidation, and nitrification occur under the aerobic cycle, Denitrification is achieved during anoxic stir and settling cycles (Metcalf &Eddy, Inc. 1991, WEF and ASCE, 1992).

Sequencing batch reactor (SBR) process utilizes a fill and draw reactor with complete mixing during the batch reaction step (after filling) and where the subsequent steps of aerotion and clarification occour in the same tank. All SBR systems have five steps in common. which are carried out in sequence as follows: (1)fill, (2)react (aeration), (3)settle (sedimantation/clarification), (4) draw (decant) and (5)idle (Metcalf &Eddy, WETR, 2004).

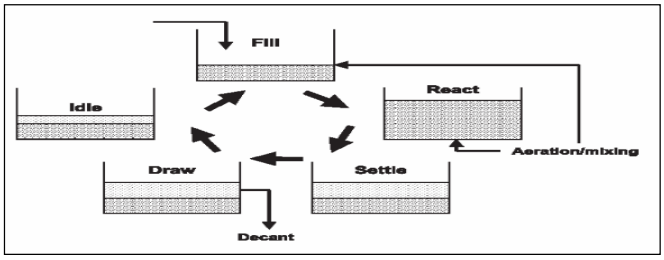

Figure 1.4 Sequencing batch reactor process

The SBR process has widespread application where mechanical treatment of small wastewater flows is desired. Because it provides batch treatment it is ideally suited for wide variations in flow rates, operation in the "fill and draw" mode prevents the "washout" of biological solids that often occurs with extended aeration systems.

Another advantage of SBR systems is that they require less operator attention yet produce a very high quality effluent (EPA,1993).

1.2.7 PHOREDOX Process

The three-stage Phoredox process is a very common method of performing nutrient removal since it requires the least amount of alterations to the typical conventional activated sludge plant. It is not the most efficient system, due to the nitrates in the Return Activated Sludge (RAS) inhibiting proper phosphorous release in the anaerobic zone. Facilities employing this $A^2/0$ process that are facing tighter permit limits than it can produce are forced to amend their process in order to meet the new limits, which results in additional capital costs (Water and Wastewater Asia January/February 2007).

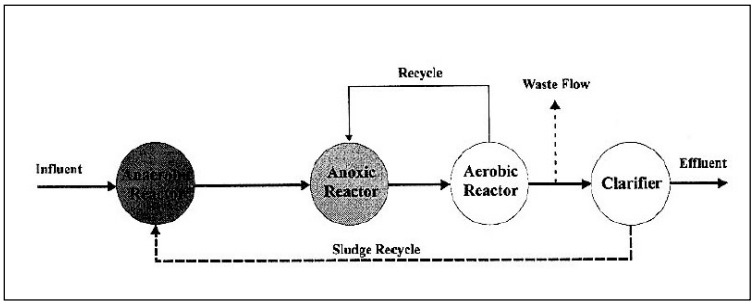

Figure 1.5 The three stage Phoredox process (Source: IWEM, 1994)

In the five-stage Bardenpho process, there are three locations that would benefit from an ORP measurement. The first, and most important application is in the first anoxic zone where a majority of the denitrification is taking place. Factors affecting the ORP of this zone are the amount of recycled aerobic water, the amount of anaerobic effluent from the first zone, along with the biological state of the bacteria from each zone. The ORP in this zone should be between -100 and100 mV, indicating a fairly neutral solution for the denitrification to take place (Water and Wastewater Asia January/February 2007).

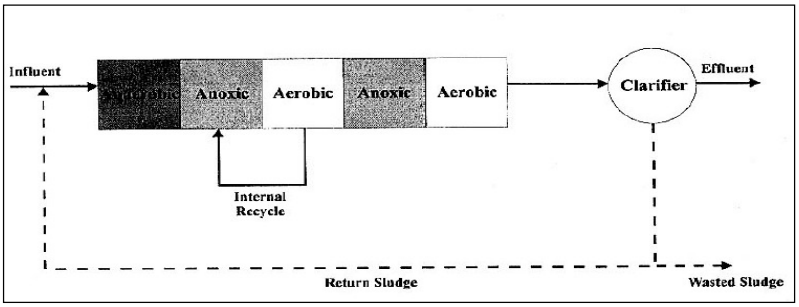

Figure 1.6 The Bardenpho Process Source**:** Bowker and Stensel (1990)

1.2.8 UCT Process

In the University of Cape Town (UCT) process for combined nitrogen and phosphorus removal, polyphosphate-accumulating bacteria will also be exposed to nitrate in the anoxic zone, i.e. an electron acceptor that may be utilized as well as the oxygen of the aerobic zone. (Ostgaard, Christensson, Lie, Jönsson and Welander, 1998). Both the return activated sludge and the aeration tank contents are recycled to the anoxic zone, and the contents of the anoxic zone are then recycled to the anaerobic zone (Ekama et.al., 1983).

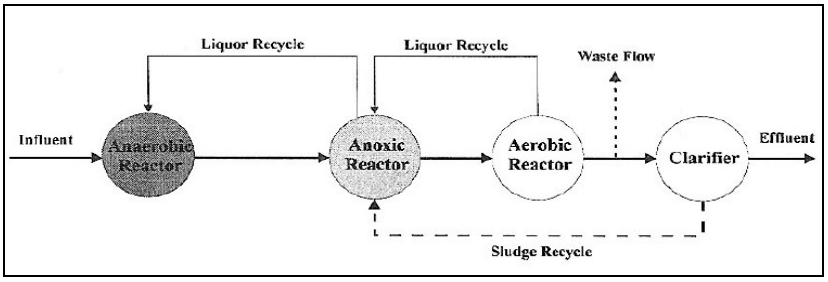

Figure 1.7 The UCT process (Source: IWEM, 1994)

1.2.9 VIP Process

The VIP (named for the Virginia Initiative Plant in Norfolk, Virginia) is similar to $A^2/0$ Process and UCT processes, except for the method of sludge recycle (Diagger et al., 1998).

The process, a unique type of biological nutrient removal, is an environmentally sound technique that eliminates much of the nutrients nitrogen and phosphorus from effluent. Less effluent discharged to waterways also means fewer nutrients released (Overstreet, 2003).

1.2.10 A^2/O Process

One of the widely used EPBR processes is the anaerobic, anoxic and oxide process, or in other terms, A^2O process. An A^2O process removes biological phosphorus along with simultaneous nitrification denitrification. In the process, ammonia will be transformed into nitrite and then nitrate (nitrification) in the aerobic tank, and the return supernatant in the aerobic tank will be returned to the anoxic tank to proceed with denitrification. On the other hand, phosphate is released in the anaerobic tank, and then uptaken excessively in the later aerobic tank. Thus, phosphorus and nitrogen removal can be achieved simultaneously in the A^2O process (Pai, Su & Leu, 2001).

1.2.11 UTA-Forth Worth Process

In 1908s a research team University of Texas at Arlington investigated a BNR system. This process simply incorporates an anoxic zone followed by an anaerobic in front of the aeration zone of the activated sludge process. Well-nitrified return activated sludge through the anoxic-anaerobic zones achieves good phosphorus release and denitrification. Significant phosphorus uptake is therefore achieved in the aeration basin (Qasim and Udomsinrod ,1986; Qasim et al., 1997).

1.2.12 PhoStrip Process

The proprietary PhoStrip process has a stripping tank in which a portion of the return sludge is diverted. Under anoxic/anaerobic condition, nitrogen is removed by denitrification and phosphorus is released into the liquid. The biological solids are separated and returned to the process. The phosphorus-rich supernatant is coagulated to precipitate phosphorus (Upton et al., 1993).

1.2.13 Orbal Process

The Orbal process typically consists of three concentric channels of aeration ditches operating in a series where the outside channel is maintained in an oxygen deficit condition, but a sizable amount of oxygen is delivered allowing simultaneous nitrification denitrification. Orbals or loop is designed for 80 percent total nitrogen removal. Higher removal rates (95 percent and more) are accomplished by recycling from the third channel back to the first (Envirex, 1989).

1.3 The Objective and Scope of the Study

In wastewater treatment, nitrogen is being considered a one of the essential parameter as it has significant adverse impacts on the environment. Anaerobic ammonia oxidation (ANAMMOX) is a novel process in which is a new powerful tool especially for strong nitrogenous wastewaters.

The main purpose of this thesis was to investigate optimum operational conditions for maximum NH_4-N and NO_2-N, NO_3-N removals in an UASB reactor and to detect the anaerobic ammonia removal throught anammox process in an UASB reactor. In the first step of the study, the effects of various influent COD, NO_2-N, NO_3-N and NH_4-N,concentrations on the removal of inorganic nitrogenous compounds were examined in the batch reactors. In the second step of the study, the removal of ammonia was researched via anammox process and the effects of different NH_4-N,NO_2-N, NO_3-N and COD concentrations on the removals of nitrogenous compounds was investigated in a continuous-fed UASB reactor throughout 42 weeks.

The main objectives of this study are summarized as follows:

1. To investigate the effect of different initial NH_4-N,NO_2-N, NO_3-N and COD concentration on nitrogen removal efficiency in batch experiments to obtain some results to be used throughout continous operation of the UASB reactor, to observe the anaerobic ammonia removal efficiencies throughout ANAMMOX process in UASB reactor.

2. To determine the ammonium, nitrit, nitrat and chemical oxygen demand (COD) removal efficiencies, total gas, methane gas, hidrogen sülfür gas productions and methane percentages in UASB reactor at increasing ammonium, nitrit, nitrat, and COD concentrations under different hydraulic retention times (4,4, 2,2, 2, 1,1 and 0,73 days).

3. To determine the most suitable operating conditions for maximum removals of NH_4-N and COD in different NH_4-N,NO_2-N, NO_3-N and COD in UASB reactor.

4. To determine the substrate (COD) and ammonium removal kinetics through continous operation of the UASB reactor.

CHAPTER TWO
LITERATURE REVIEW

2.1 Anammox

Anammox, an abbreviation for anaerobic ammonium oxidation, is a globally important microbial process of the nitrogen cycle. The bacteria mediating this process were identified only 20 years ago and at the time was a great surprise for the sceintific community. It takes place in many natural environments and anammox is also the trademarked name for an ammonium removal technology that has been developed by the Delft University of Technology (Wikipedia, 2010).

For a long time the general consensus was that ammonium could only be oxidised under aerobic conditions. The Austrian theoretical chemist Engelbert Broda was the first to recognise the possibility of anaerobic ammonium oxidation in 1977. The simultaneous removal of ammonium and production of nitrogen gas was observed in an industrial wastewater treatment in The Netherlands in 1986 (Wikipedia, 2010).

A number of studies on removal of nitrogen with anammox prosess are reported in literature. Strous et al.,(1998) estimated the stoichiometric parameters for the ANAMMOX microorganisms and obtained a yield value expressed as biomass produced per ammonia nitrogen reduced of 0.066 $mol(mol)^{-1}$, an ammonium consumption rate per biomass expressed as protein of 45 nmol mg^{-1} min^{-1} and a maximum specific growth rate of 0.0027 h^{-1}. This means a doubling time of at least 11 days (Dapena-Mora et al., 2004).

Microbial communities in the biological filter and waste sludge compartments of a marine recirculating aquaculture system were examined to determine the presence and activity of anaerobic ammonium-oxidizing (anammox) bacteria. The process has now been found in a range of environments including marine sediments, sea ice

The Anammox process performance was tested with synthetic wastewater in a completely stirred tank reactor (CSTR) by Güven et al., 2004. The reactor was operated for 511 days and fed with increasing amounts of ammonium and nitrite. In this period, an increase of ammonium and nitrite utilization rates were observed as a result of the increase of nitrogen loads in the influent. After 272 days, about 60 % of the biomass was removed from the reactor and the system was restarted. Throughout 511 days 90 % of the ammonium and more than 99 % of the nitrite were converted mainly to nitrogen (N_2) and nitrate.

The removal of nitrogen from an anaerobic digester effluent by combination of Sharon–Anammox was successfully tested on a pilot scale (3.6 m^3) for over half a year by Fux et al., 2002 (Mora, Campos, Corral, & Me´ndez, 2006).

The long-term stability of partially nitrification of swine wastewater digester liquor and the subsequent treatment by ANAMMOX process were also studied, and very stable nitrogen removal efficiency was obtained in 70 days at a nitrogen removal loading rate of 0.22 kg N/(m^3·day) (Yamamoto et al., 2008).

The anammox reactor type employed in Rotterdam was compared to other reactor types for the anammox process. Reactors with a high specific surface area like the granular sludge reactor employed in Rotterdam provide the highest volumetric loading rates. Mass transfer of nitrite into the biofilm is limiting the conversion of those reactor types that have a lower specific surface area. Now the first full-scale commercial anammox reactor is in operation, a consistent and descriptive nomenclature is suggested for reactors in which the anammox process is employed (Wouter R.L et al., 2006).

Van der Star et al., (2007) described the first full-scale Anammox reactor. The operation was compared with parameters previously reported in studies on laboratory scale. The maximum attained conversion of 9.5 kg N $m^{-3}d^{-1}$ was limited by the available influent load and was not a maximum volumetric conversion of the Anammox reactor.

A full-scale application of partial nitritation/Anammox process was successfully achieved in SBR reactor at the wastewater treatment plant in Strass, Austria (Innerebner et al., 2007).

According to another experimental study, the process kinetics for laboratory-scale anammox (anaerobic ammonium oxidation) upflow filter using synthetic wastewater as feed were investigated. The experimental unit consisted of a 2.0 L reactor filled with three-dimensional plastic media. The filterwas tested for different influent substrate concentrations and hydraulic retention time (HRT). The substrate loading removal rate was compared with prediction of Stover–Kincannon, second-order and the first-order substrate removal models. Upon approaching pseudo-steady-state condition, substrate ammonium or nitrite concentrations were increased from 280 to 462 mg N/L, while HRT was stepwise decreased from 14.4 to 2 h, with a concomitant increase in nitrogen loading rate (NLR) from 0.93 to 7.34 g/L day (Jin, Zheng; 2009).

Experimental studies were performed to evaluate the feasibility of granulation of Anammox microorganisms for biomass retention in up-flow reactors. Two experimental studies, one using a 6.4-L lab-scale reactor with synthetic medium and the other using a 200-L pilot-scale reactor with half-nitrified reject water from a sludge digester were conducted. The Anammox granules had a slightly lower density than the seed granules from the UASB process, but the size and other physical properties were comparable. The successful granulation of the Anammox microorganisms led to a stable nitrogen removal performance. The maximum nitrogen removal rate of the lab-scale reactor was observed to be 2.9 kg/(m^3·d) after 173 days of operation and that of the pilot-scale reactor was 6.4 kg/(m^3·d) after 12 months of operation (Imajo,Tokutomi & Furukawa, 2004).

2.2 Upflow Anaerobic Sludge Blanket

Anaerobic granular sludge bed technology refers to a special kind of reactor concept for the "high rate" anaerobic treatment of wastewater. The concept was initiated with upward-flow anaerobic sludge blanket (UASB) reactor. Upflow anaerobic sludge blanket (UASB) reactor is a popular anaerobic reactor for both high and low temperature (Dinsdale, et al.1997).

The UASB reactor concept was rapidly developed into technology, the first pilot plant was installed at a beet sugar refinery in The Netherlands (CSM suiker). Thereafter a large number of full-scale plants were installed throughout the Netherlands at sugar refineries, potato starch processing plants, and other food industries as well as recycle paper plants. The first publications on the UASB design concept appeared in Dutch language technical journals in the late 1970's and the first international publication appeared in 1980 (Lettinga et al. 1980).

Tay et al., (2001) studied that six upflow anaerobic sludge blanket (UASB) reactors were concurrently operated for 146 d to examine the effects of calcium on the sludge granulation process during start-up. Introduction of Ca^{2+} at concentrations from 150 to 300 mg/l enhanced the biomass accumulation and granulation process. The calcium concentration in the granules was nearly proportional to the calcium concentration in the feed, and calcium carbonate was the main calcium precipitate in the granules. The optimal calcium concentration was found to be between 150 and 300 mg/l when the influent COD concentration was kept at 4000 mg/l.

The COD and color removal efficiencies was investigated at increasing Congo red and Direkt Black 38 concentrations in a anaerobik (UASB)/aerobic (CSTR) sequential rector system. 46% COD removal efficiency was obtained at a Congo red concentrations of 4000 mg/l and a glucose-COD concentration of 3000 mg/l as co-substrate in anaerobic stage. 65% and 88% COD removal efficiencies was obtained in the aerobic and the total system effluents. The total removed color was found to be 99% (Işık & Sponza, 2003).

A pilot scale study was set up to investigate the principle design parameters of up flow anaerobic sludge blanket (UASB) reactors for treating wastewater of small communities in the tropical regions of Iran. A steel pipe with a diameter of 600 mm and a height of 3.6 m was used as the reactor in which a digestion and a 3-phase separator element had a volume of 0.848 and 0.17 m^3 respectively During the colder period the removal ratio of BOD_5, COD and TSS with an optimal hydraulic retention time of 8 hours and organic loading rate of 1.22 kg COD/m^3/day were 54, 46 and 53 percent respectively (Azimi & Zamanzadeh, 2004).

A combined upflow anaerobic sludge bed–activated sludge (UASB–AS) reactor system with consistently wasting of excess biomass was used to treat suspended-solids pre-settled piggery wastewater (COD: 2000 mg l^{-1}, total Kjeldahl nitrogen TKN : 400 mg l^{-1}, suspended solids : 250–400 mg l^{-1}) the combined system removed 95–97% of chemical oxygen demand (COD), 100% of TKN and 54–55% of total nitrogen (TN) (Huang, Wu & Chen, 2005).

Two lab-scale UASB reactors, one of which was inoculated with the mixture of anaerobic sludge and aerobic sludge, the other with river sediments, were started up, using the inorganic synthetic water containing ammonium and nitrite as influent. After 421 days' and 356 days operation respectively, the ammonium removal efficiencies in two reactors reached 94 % and 86 % respectively, the total nitrogen volumetric loading rates were 2.5 and 1.6 kg N/m^3.d (Yang Y. et al., 2006).

The effect of ferrous ion addition on the granularity of an upflow anaerobic sludge blanket (UASB) reactor was investigated. Two UASB reactors (R1 and R2) (35 °C; pH 7) were operated for 3 months at a 20-h hydraulic retention time (HRT) at organic loads from 1.4 to 10.0 g COD L−1 d−1. The addition of ferrous iron induced a stable and excellent COD conversion rate. Moreover, the addition of iron to the reactor R1 influent resulted in its steady accumulation in the granules. For low loading rates (<3 g COD L^{-1} d^{-1}), the COD digestion rate in each reactor was excellent (higher than 85%). However, when the COD loading rate was increased,

the COD digestion rate in reactor R2 in which iron was not supplied decreased (Vlyssides, Barampoutia & Maia, 2008).

The one-stage UASB reactor was operated in Palestine at a hydraulic retention time (HRT) of 10 h and at ambient air temperature for a period of more than a year in order to asses the system response to the Mediterranean climatic seasonal temperature fluctuation. Afterwards, the one-stage UASB reactor was modified to a UASB digester system by incorporating a digester operated at 35 °C. The achieved removal efficiencies in the one-stage UASB reactor for total, suspended, colloidal, dissolved and VFA COD were 54, 71, 34, 23%, and −7%, respectively during the first warm six months of the year, and achieved only 32% removal efficiency for COD total over the following cold six months of the year (Mahmoud, 2008).

A 450-dm^3 pilot-scale upflow anaerobic sludge blanket (UASB) reactor was used for the treatment of a fermentation-based pharmaceutical wastewater by B.K İnce, Yenigün & 0.İnce (2001). 94% COD removal efficiency was achieved in the UASB reactor at an organic loading rate (OLR) of 10.7 kg COD m^{-3} d^{-1}. Specific methanogenic activity (SMA) tests were, carried out to determine the potential loading capacity of the UASB reactor. The results showed that the sludge sample taken from the UASB reactor (OLR of 6.1 kg COD m^{-3} d^{-1}) had a potential acetoclastic methane production (PMP) rate of 72 cm^3 CH_4 g^{-1} VSS d^{-1}. When the PMP rate was compared with the actual methane production rate (AMP) of 67cm^3 CH_4 g^{-1} VSS d^{-1} obtained from the UASB reactor, the AMP/PMP ratio was found to be 0.94 which ensured that the UASB reactor was operated using its maximum potential acetoclastic methanogenic capacity.

2.3 UASB And Anammox

To apply the Anammox process to wastewater treatment, it is essential to develop a reactor configuration that is suitable for growing and accumulating the Anammox microorganism. Considering the characteristics of Anammox microorganisms noted above, attached-growth and granular-sludge reactors were considered to be suitable.

Among several types of reactors, UASB reactors have been successfully used for development and retention of high concentrations of anaerobic microorganisms that have slow growth rates and low yields. In addition, they have been used for treatment of various types of organic wastewaters (Lettinga et al., 1980, 1991).

Ni et al., (2008) studied that a mathematical model was developed to describe the anaerobic ammonium oxidation (ANAMMOX) process in a granular upflow anaerobic sludge blanket (UASB) reactor. ANAMMOX granules were cultivated in the UASB reactor by seeding aerobic granules. The granule-based reactor had a great N-loading resistant capacity. The model simulation results on the 1-year reactor performance matched the experimental data well. The yield coefficient for the growth and the decay rate coefficient of the ANAMMOX granules were estimated to be 0.164 g COD g^{-1} N and 0.00016 h^{-1}, respectively. With this model, the effects of process parameters on the reactor performance were evaluated. Results showed that the optimum granule diameter for the maximum N-removal should be between 1.0 and 1.3 mm and that the optimum N loading rate should be 0.8 kg N m^{-3} d^{-1}.

Beatriz et al., (2008) investigated that the anammox process, under different organic loading rates (COD), was evaluated using a semi-continuous UASB reactor at 37 ^{0}C. Three different substrates were used: initially, synthetic wastewater, and later, two different pig manure effluents (after UASB-post-digestion and after partial oxidation) diluted with synthetic wastewater.High ammonium removal was achieved, up to 92.1 ± 4.9% for diluted UASB-post-digested effluent (95 mg COD L^{-1}) and up to 98.5 ± 0.8% for diluted partially oxidized effluent (121 mg COD L^{-1}).

Waki, Tokutomi**,** Yokoyama and Tanaka, (2006) studied that to examine the applicability of the anaerobic ammonium oxidation (anammox) process to three kinds of low BOD/N ratio wastewaters from animal waste treatment processes in batch mode. A rapid decrease of NO_2^- and NH_4^+ was observed during incubation with wastewaters from AS and UASB/trickling filter and their corresponding control artificial wastewaters. This nitrogen removal resulted from the anammox reaction, because the ratio of removed NO_2^- and NH_4^+ was close to the theoretical ratio of the anammox reaction. Comparison of the inorganic nitrogen removal rate of the actual

wastewater and that of control artificial wastewater showed that these two kinds of wastewater were very suitable for anammox treatment. Incubation with wastewater from RW did not show a clear anammox reaction; however, diluting it by half enabled the reaction, suggesting the presence of an inhibitory factor. This study showed that the three kinds of wastewater from animal waste treatment processes were suitable for anammox treatment.

Ni B.J., et al.,(2010) studied the settling ability and community composition of the anammox granules which were cultivated in an upflow anaerobic sludge blanket (UASB) reactor seeded with aerobic granules. With this seed the startup period was less than 160 days at a NH_4^+-N removal efficiency of 94 % and a loading rate of 0.064 kg N per kgVSS per day.

Tran, Park, Cho, Kim and Ahn investigated to evaluate the development of the anammox process by the use of granular sludge selected from a digestion reactor as a potential seed source in a lab-scale UASB (upflow anaerobic sludge blanket) reactor system. The reactor was operated for approximately 11 months and was fed by synthetic wastewater. After 200 days of feeding with NH_4^+ and NO_2^- as the main substrates, the biomass showed steady signs of ammonium consumption, resulting in over 60 % of ammonium nitrogen removal.

Yang, et al., (2006) studied that two lab-scale UASB reactors, one of which was inoculated with the mixture of anaerobic sludge and aerobic sludge, the other with river sediments, were started up, using the inorganic synthetic water containing ammonium and nitrite as influent. After 421 days' and 356 days operation respectively, the ammonium removal efficiencies in two reactors reached 94% and 86% respectively, the total nitrogen volumetric loading rates were 2.5 and 1.6 kg $N/m^3.d$.

Tang, Zheng, Wang and Mahmood (2009) investigated the effect of organic matter on the nitrogen removal performance of anaerobic ammonium oxidation (Anammox) process in an upflow anaerobic sludge blanket (UASB) reactor fed with

nitrogen loading rate of 13.92 kg N m^{-3} day^{-1} at an HRT of 0.83 h. Mass balance showed that the heterotrophic denitrification prevailed in the UASB reactor, and became the dominant reactions when high influent COD/NO_2–N ratios of 2.92 were applied.

A combined process consisting of a short-cut nitrification (SN) reactor and an anaerobic ammonium oxidation upflow anaerobic sludge bed (ANAMMOX) reactor was developed to treat the diluted effluent from an upflow anaerobic sludge bed (UASB) reactor treating high ammonium municipal landfill leachate. The SN process was performed in an aerated upflow sludge bed (AUSB) reactor (working volume 3.05 L), treating about 50% of the diluted raw wastewater. The ammonium removal efficiency and the ratio of NO_2^--N to NOx^--N in the effluent were both higher than 80%, at a maximum nitrogen loading rate of 1.47 kg/(m^3·day). (Liu, Zuo, Yang Y., et al., 2009)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental System

3.1.1 Configuration of Upflow Anaerobic Sludge Blanket (UASB) Reactor

A schematic of the lab-scale sequential upflow anaerobic sludge blanket reactor used in this study is presented in Figure 3.1. The stainless-stell UASB reactor had an internal diameter of 90 mm and a height of 1000 mm with a volume of approximately 2.2 L was used in this study. Five evenly distributed sampling ports were installed over the top of the reactor. The influent feed was pumped using a peristaltic pump. The produced gas was collected via porthole in the top of the reactor. The operating temperature of the reactor was maintained constant at 37 ± 1^0C by placing to the UASB reactor a heater. A digital temperature probe located in the middle part of the second compartment provided the constant operation temperature.

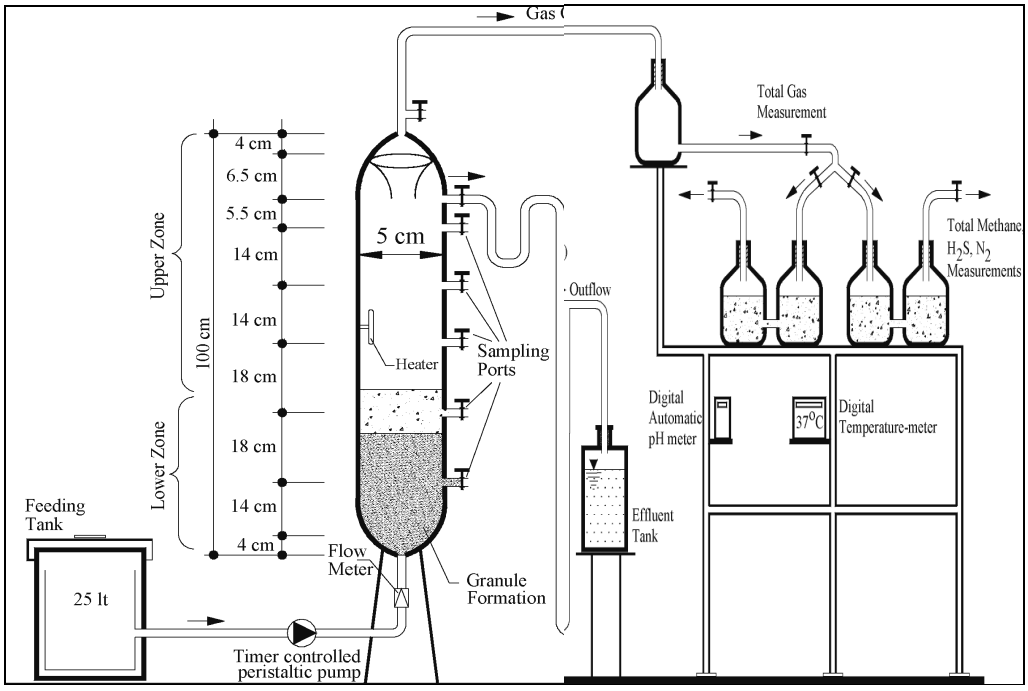

Figure 3.1 Schematic configuration of UASB reactor.

3.2 Operating Conditions

3.2.1 Operational Conditions for Batch Test

Four different sets of batch shake flask experiments were performed to investigate the optimum conditions for ammonia-nitrogen (NH_4-N) and COD removals with anammox reaction. NH_4^+-N, NO_3-N, NO_2-N and COD concentrations in the solution were measured thirtieth, forty-fifth and sixtieth days. Flask experiments were done in duplicates. All of the experiments were performed in 100 ml serum bottles in duplicates. The medium for the batch experiments was the same as the synthetic nutrient medium fed to the UASB reactor. The anammox biomass (11 ml) was inoculated in each bottle. Additionally, the magnetic stirrers were used to assure appropriate mixing of medium during the tests. Anammox activities were determined as NH_4^+ and NO_2^- consumption rates. The operating temperature of the batch shake flask experiments was maintained constant at 36 ± 1^0C. The pH of each sample was measured about 7,2 ± 0,1. The ORP values during the anoxic and anaerobic phases were approximately -50 mV and -165 mV, respectively.

The first sets of batch shake flask experiments had ten runs. In the first study, the effect of COD concentrations on the NH_4-N removal efficiencies by anammox process was investigated. After thirtieth days of operation nitrogen removal rate and COD removal efficiency were determined. Table 3.2 shows the operational conditions and the concentrations for run 1.

The second six sets were performed to investigate effects of the NO_3-N concentration on anammox activity, batch experiments were conducted with various initial NO_3-N concentrations during 45 days. Table 3.3 shows the operational conditions for run 2.

In the third steps of batch experiments six runs were performed to investigate NH_4-N removal efficiencies for different NH_4-N and concentrations during sixty days, Table 3.4 shows the operational conditions and the concentrations for run 3.

Furthermore, fourth sets of batch shake flask experiments were performed to investigate effects of the NO_2-N concentration on anammox activity, batch experiments were conducted with various initial NO_2-N concentrations. Table 3.5 shows the operational conditions and the concentrations for run 4.

Table 3.1 The Operational Conditions Used in These Tests

	Run 1	**Run 2**	**Run 3**	**Run 4**
Suspended Solids (SS mg/L)	500	500	500	500
Volatile Solids (VSS mg/L)	348	348	348	348
D.O (mg/l)	0.16	2.10	0.17	0.17
HRT (days)	45	45	45	45
ORP (mV)	-50	-100	-123	-165
Temperature (°C)	36	36	36	36
pH	7,17	7,4	7,14	7.7

Table 3.2 Operatinal Conditions for Influent NO_2-N, NH_4-N and NO_3-N and COD Concentrations in Run-1

	Serie-1	**Serie-2**	**Serie-3**	**Serie-4**	**Serie-5**	**Serie-6**	**Serie-7**	**Serie-8**	**Serie-9**	**Serie-10**
OLR kgCOD/m^3. d	0,272	0	0,272	0	0,272	0	0,272	0	0,27	0
COD (mg/L)	600	0	600	0	600	0	600	0	600	0
NH_4-N (mg/L)	133	134	133	121	128	121	138	132	128	124,5
NO_3-N (mg/L)	0,69	0,64	2	2	4,1	4,3	1	1,1	1	1,01
NO_2-N (mg/L)	136	134	134	132	133	136	40	44	246	248
COD/NH_4	4,5	-	4,5	-	4,6	-	4,35	-	4,6	-
NH_4/NO_3	192,7	193	66,5	60,5	31,2	28.1	138	120	128	122,7
NH_4/ NO_2	197,1	209	67	66	32,4	31,6	40	40	246	245
COD/NO_3	869,6	-	300	-	146.3	-	600	-	600	-
NO_2 /NO_3	191,3	209,3	67	66	32,4	31,6	40	40	246	245,5

Table 3.3 Operatinal Conditions for Influent NO_2-N, NH_4-N and NO_3-N and COD Concentrations in Run-2

	Series-1	**Series-2**	**Series-3**	**Series-4**	**Series-5**	**Series-6**
OLR (kg COD/m^{3}d)	0,272	0	0,272	0	0,272	0
COD (mg/l)	600	0	600	0	600	0
NH_4-N (mg/l)	135	131	132,1	129,1	133,3	134,2
NO_3-N (mg/l)	100	105,5	5,4	5,1	305	306
NO_2-N (mg/l)	200	206	207,7	203	200	201
COD/NH_4 **NH_4/NO_3** **NH_4/ NO_2** **COD/ NO_3** **NO_2 /NO_3**	4,4 1,35 0,675 6 2	- 1,35 0,635 - 1,95	4,5 24,5 0,637 111,1 38,46	- 25,2 0,635 - 39,8	4,5 2,3 0,665 1,96 0,65	- 2,3 0,66 - 0,65

Table 3.4 Operatinal Conditions for Influent NO_2-N, NH_4-N and NO_3-N and COD Concentrations in Run-3

	Series-1	**Series-2**	**Series-3**	**Series-4**	**Series-5**	**Series-6**
OLR (kgCOD/m^{3}d)	0,272	0	0,272	0	0,272	0
COD (mg/l)	600	0	600	0	600	0
NH_4-N (mg/l)	132	135	253	251	400	404
NO_3-N (mg/l)	1,02	1,21	1,3	1,25	1,21	1,22
NO_2-N (mg/l)	102	103	107	106	100	103
COD/NH_4 **NH_4/NO_2** **NH_4/NO_3** **COD / NO_3** **NO_2 /NO_3**	4,5 1,29 129,4 588,2 100	- 1,31 111,25 - 85,12	2,37 2.36 194,6 461,5 82,3	- 5 200,8 - 84,8	1,5 4 325,2 495 82,6	- 4 331,1 - 84,4

Table 3.5 Operatinal Conditions for Influent NO_2-N, NH_4-N and NO_3-N and COD Concentrations in Run-4

	Series-1	**Series-2**	**Series-3**	**Series-4**	**Series-5**	**Series-6**	**Series-7**	**Series-8**
OLR (kgCOD/m^{3}d)	0,272	0	0,272	0	0,272	0	0,272	0
COD (mg/l)	600	0	600	0	600	0	600	0
NH_4-N(mg/l)	135	135	131	132	128	132	119	131
NO_3-N (mg/l)	1	0,9	0,89	0,95	1,1	1	1,1	1,2
NO_2-N (mg/l)	100	103	20	23	208	211	412	425
COD/NH_4 **NH_4/NO_2** **NH_4/NO_3** **COD / NO_3** **NO_2 /NO_3**	4,44 1,35 135 600 100	- 1,31 150 - 114,4	4,5 6,5 147,1 674,1 22,4	- 5,73 139 - 24,2	4,6 0,61 24,2 545 189	- 0,62 132 - 211	5,04 0,28 108 545 374,5	- 0,3 109 - 354

3.2.2 Operating Conditions for Upflow Anaerobic Sludge Blanket (UASB)Reactor

A laboratory scale UASB reactor was operated with syntetic wastewater during 294 days in order to investigate the anaerobic ammonia removal efficiencies and the process kinetics at different hydraulic retention times (HRT). The COD concentratıons adjusted as between 300-1000 mg/L from 4,4 to 0,73 days by adjustıng the Up-Flow rates from to 0,5 to 3 l/d. This caused change of the organic loading rates (OLR) from 0.13 g/l day to 0.45 g/L day. Glucose was used as carbon source in this reactor. Sludge recycling was not done. NH_4-N loading rates varied between 0.025 g/L.day – 0.1 g/L.day and NO_3-N loading rates varied between 0.020 g/ L.day – 0.13 g/L.day. The NO_2-N loading rate varied between 0.020 g/L.day and 0.13 g/L.day. The operating temperature of the reactor was maintained constant at 35 ± 1 ^oC. The ORP values during the anoxic and anaerobic phases were approximately +5 mV and -50 mV, respectively. Table 3.6 shows the operational conditions in the UASB reactor.

Table 3.6 Operating Conditions for UASB Reactor

	RUN 1	**RUN 2**	**RUN 3**	**RUN 4**	**RUN 5**
COD/ NH_4-N RATIOS	2,72	4,52	5,44	10,88	5,44
COD concentration(mg/L)	600	300	1000	600	300
O.L(kg COD/m^3.d)	0,272	0,454	0,136	0,272	0,136
NH_4-N loading (g/L.day)	0,1	0,1	0,025	0,025	0,025
NO_2-N loading(g/L.day)	0	0,05	0,02	0,02	0,02
NO_3-N loading(g/L.day)	0,13	0,13	0,02	0,02	0,02
F/M (gKOİ/gUAKM.d)	0,037	0,11	0,04	0,145	0,113
SRT (DAY)	14,4	6,2	5,45	4,4	7,2
Up-Flow Rate (L/d)	0,5	1	1,1	2	3
Hydraulic Ret Time d	4,4	2,2	2	1,1	0,73
ORP(mV)	-33	-18	-50	-12	+5
TEMPERATURE ^0C	36,5	36,5	36,5	36,5	36,5

3.3 Sources of seed and feed

3.3.1 Sources of seed and feed in Continuous UASB reactor

Partially granulated anaerobic sludge was used as seed in the UASB reactor. The seed sludge was obtained from an anaerobic upflow anaerobic sludge blanket reactor containing acidogenic and methanogenic partially granulated biomass taken from the Pakmaya Yeast Beaker Factory in Izmir, Turkey. The volatile suspended solid (VSS) concentration of seed sludge in UASB reactor was adjusted as 27.000 mg VSS/L.

The synthetic wastewater was used in this experiment contained mineral media solution consisted of 737,2 mg/L of $NaHCO_3$, 300 mg/L of $CaCl_2.2H_2O$, 171,2 mg/L K_2HPO_4, 200 mg/L of $MgSO_4$, 4,64 mg/L of $FeCl_2$ and 6,25 mg/L of EDTA and trace element solution including 0,5 mg/L of $CoCl_2.4H_2O$, 0,5 mg/L of $MnCl_2.4H_2O$,500 mg/l of $CuSO_4.5H_2O$, of $Na_2MoO_4.2H_2O$, 500 mg/L of $NiCl_2.6H_2O$ 1000 mg/l of $Na_2SeO_3.5H_2O$, and of 500 mg/L of H_3BO_4. In experiments with different nutrient loading rates, synthetic wastewater used was composed of glucose carbon sources. Initial COD concentration varied between 300 and 1000 mg/L, while initial NH_4-N, NO_3-N and NO_2-N concentrations were between 2,5-100 mg/L, 25-130 and 0-50 mg/L, respectively. The anaerobic conditions were maintained by adding 667 mg/l of Sodium Thioglycollate (0.067 %) which is proposed between 0,01-0,2% (w/w) for maintaining the strick anaerobic conditions (Speece, 1996). The alkalinity and neutral pH were adjusted by addition of 5000 mg /L $NaHCO_3$.

3.3.2 Sources of seed and feed in batch reactor

11 ml of anaerobic sludge was added separately into all serum bottles. Then 0,9 ml glucose (1000 mg/L), 0,5 ml sodium thioglycollate (0.067 %) and 62,5 ml synthetic wastewater were added to the serum bottles. The total working volume in the serum bottle was 75 ml while the VSS concentration in serum bottles was 500 mg/L.

3.4 Analytical Methods Used in Experimental Studies

3.4.1 Dissolved Chemical Oxygen Demand (DCOD) Measurement

The dissolved COD was measured calorimetrically by using closed reflux method (APHA AWWA, 1992). Firstly the samples were centrifuged 10.0 min at 7000 RPM. Secondly, 2.5 ml samples were mixed with 1.5 ml 10216 mg/l $K_2Cr_2O_7$, 33.3 g/l $HgSO_4$ and 3.5 ml 18 M H_2SO_4 containing 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a heater with a temperature of 148°C for two hours. Finally, after cooling, the samples were measured at a wave-length of 600 nm with a Pharmacia LKB NovaPec II model spectrophotometer.

3.4.2 Gas Measurement

Gas productions were measured with liquid displacement method. The total gas was measured by passing it through a liquid containing 2% (v/v) H_2SO_4 and 10% (w/v) NaCl (Beydilli, Pavlosathis & Tincher, 1998). Methane gas was detected by using a liquid containing 3% NaOH to scrub out the carbon dioxide from the biogas (Razo-Flores et al., 1997). The methane gas percentage in biogas was also determined by Dräger Pac®Ex methane gas analyzer. The H_2S gas was measured using Dräger (Stuttgart, Germany) kits in a Dräger H_2S meter. H_2 gas was measured using (Dräger Pac®Ex) H_2 meter. N_2 gas was measured by discarding of the sum of $CH_4 + H_2S + H_2 + CO_2$ gases from the total gas.

3.4.3 Temperature and pH

Temperature and pH were measured by WTW model 340i multianalyzer in Fig 3.2.

Figure 3.2 WTW model 340i

3.4.4 Ammonium- Nitrogen (NH_4 -N), Nitrite-Nitrogen (NO_3-N) and Nitrate Nitrogen (NO_2-N) Analysis

Ammonium- Nitrogen (NH4 -N) (Merc cell kit # 14576), Nitrite-Nitrogen (NO3-N) (Merc cell kit # 973) and Nitrate Nitrogen (NO2-N) (Merc cell kit # 14723) were analyzed by using spectroquant cell test obtained from Merc. For photometric measurement, "Merc Photometer SQ 300" was used.

3.4.5 Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), Suspended Solids (SS) and Volatile Suspended Solid (VSS) Measurements

Biomass was measured as total suspended solid (TSS) and volatile suspended solid (VSS) in anaerobic reactors. Biomass in anaerobic tank was measured as mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Assays were performed according to Standard Methods for Examination of Water and Wastewater (APHA AWWA, 1992).

3.4.6 Sludge Retention Time (SRT, Θ_C)

At equilibrium condition, sludge withdrawn has to be equal to sludge produced daily. The sludge produced daily depends on the characteristics of the raw wastewater since it is the sum total of (i) the new VSS produced as a result of BOD removal, the yield coefficient being assumed as 0.1 g VSS/ g BOD removed, (ii) the non-degradable residue of the VSS coming in the inflow assuming 40% of the VSS are degraded and residue is 60%, and (iii) Ash received in the inflow, namely TSS-VSS mg/l. In none-recycled, at steady state conditions, the SRT in the UASB could be calculated using Eq (3.1). (http://nptel.iitm.ac.in/courses)

SRT:

$$\text{SRT} = \frac{V \times X}{(Q - Q_v) \times X_E + Q_w \times X_R} \qquad (3.1)$$

Where;

V= reactor volume, m^3

Q= influent flowrate, m^3/d

X_o=concentration of biomass in influent, g VSS/m^3

Q_w=waste sludge flowrate, m^3/d

X_e=concentration of biomass in effluent, g VSS/m^3

X_R=concentration of biomass in return line from clarifier g VSS/m^3 (Met&Calf Eddy, page:591)

3.4.7 Hydraulic Retention Time (HRT, Θc)

The another parameter is HRT which is given by Eq (3.2):

$$HRT=\frac{V}{Q} \tag{3.2}$$

Where;

V= reactor volume, m^3

Q= influent flowrate, m^3/d

3.4.8 Food to Microorganism (F/M) Ratio

The F/M ratio is defined as the rate of COD applied per unit volume of mixed liquor:

$$F/M \text{ (mg COD/mg VSS.day)} = \frac{Q \times S_0}{X \times V} \tag{3.3}$$

Where;

F/M= food to biomass ratio g COD /g VSS.d

S_o=influent COD concentration, g/m^3

V=reactor volume, m^3

X=biomass concentration in the reactor, g/m^3

3.4.9 Application of Substrate Removal Kinetics Models for UASB Reactor

One of the main aims of this thesis was to estimate the kinetic parameters of nitrogen removal. Due to this reason the UASB reactor was operated at five different HRTs to determine the kinetic constants of ammonium and nitrite removal through Anammox process.

3.4.9.1 Zero Order Reaction Kinetic

A zero-order reaction has a rate which is independent of the concentration of the reactant(s). Increasing the concentration of the reacting species will not speed up the rate of the reaction. Zero-order reactions are typically found when a material that is required for the reaction to proceed, such as a surface or a catalyst, is saturated by the reactants. The rate law for a zero-order reaction is as following Eq. (3.4).

$$r=k \tag{3.4}$$

Where;

r: the reaction rate,

k_0: the reaction rate coefficient with units of concentration/time

If, and only if, this zero-order reaction

$$S_t = S_0 - k_0 * t \tag{3.5}$$

Where;

k_0: zero order rate constant through substrate removal.

S_t: resisdual ammonium concentration at selected time (t) through substrate removal(mg/l)

S_0: ammonium concentration at the beginning of the substrate removal (mg/l)

t: time (day)

3.4.9.2 First Order Reaction Kinetic

A first-order reaction depends on the concentration of only one reactant (a unimolecular reaction). Other reactants can be present, but each will be zero-order. A first-order reaction is as following Eq. (3.6):

$$S_t = S_0 - e^{-k_1 * t} \quad (3.6)$$

K_1: the first order rate constant, which has units of 1/time.

S_t: resisdual ammonium concentration at selected time (t) through substrate removal(mg/l)

S_0: ammonium concentration at the beginning of the substrate removal (mg/l)

t: time (day)

3.4.9.3 Second Order Reaction Kinetic

A second-order reaction depends on the concentrations of one second-order reactant, or two first-order reactants. For a second order reaction, its reaction rate is given by Eq. (3.7):

$$\frac{1}{S_t} = \frac{1}{S_o} + k_2 * t \quad (3.7)$$

k_2: the second order rate constant, which has units of 1/mg.time.

S_t: resisdual ammonium concentration at selected time (t) through substrate removal (mg/l)

S_0: ammonium concentration at the beginning of the substrate removal (mg/l)

t: time (day)

3.4.9.4 Application of Monod Kinetic

For a UASB reactor with no biomass recycle, microbial and substrate mass balance can be expressed using Eq.3.8 and Eq.3.9. A microbial mass balance for the reactor can be described as follows:

(Microbial Change Rate) =
(microbial input rate) + (microbial growth rate) - (microbial death rate) - (microbial output rate) (3.8)

Mathematically, Eq (3.8) can be written as Eq (3.9).

$$\frac{d_x}{d_t} = \frac{Q}{V} * X_i + \mu * X_r - k_d * X - \frac{Q}{V} * X_e \quad (3.9)$$

V, Q, X_i, X_r, X_e are defined as the reactor volume (L), the flow rate (L/day), the concentration of biomass in the influent (g/L), the concentration of biomass in the reactor (g/L) and the concentration of biomass in the effluent (g/l). μ and k_d are specific growth rate (day^{-1}) and the endogenous decay coefficient (day^{-1}).

The concentration of biomass in the influent is very small and can be neglected ($X_i = 0$). Also, there is no change in the microbial mass at steady state conditions ($d_X/d_t = 0$). Therefore, Eq (3.9) can be written as Eq (3.10).

$$\mu - k_d = \frac{Q}{V} * \frac{X_e}{X_r} \quad (3.10)$$

Since no sludge wasting was applied in the anaerobic reactors, sludge retention time (SRT=θ_c) was calculated from the Eq (3.11) based on both MLVSS concentration into reactor and MLVSS concentration in the effluent of reactor.

$$\theta_c = \frac{V}{Q} * \frac{X_r}{X_e} \quad (3.11)$$

Equation (3.11) can be rearranged as follows:

$$\mu - k_d = \frac{1}{\theta_c} \quad (3.12)$$

Where; (μ-k_d) is the net specific growth rate, day^{-1}. Equation (3.13) indicates that the net microbial growth decreases as the sludge retention time (SRT=θ_c) increases. The relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod equation (3.13):

$$\mu = \frac{\mu_{max} * S}{K_s + S} \tag{3.13}$$

Eq (3.14) can be rearranged as follows.

$$\frac{\mu_{max} * S_i}{K_s + S_i} = \frac{1}{\theta_c} + k_d \tag{3.14}$$

$$\frac{\theta_c}{1 + \theta_c * k_d} = \frac{K_s}{\mu_{max}} * \frac{1}{S_i} + \frac{1}{\mu_{max}} \tag{3.15}$$

The value of maximum specific growth rate (μ_{max}) (day^{-1}) and half saturation concentration (K_s) (mg/l) could be determined by plotting the Eq (3.15). The value of μ_{max} can be calculated from the intercept of the straight line while Ks can be obtained from the slope of the line.

Substrate Mass Balance:

A substrate mass balance for the reactor can be described as Eq (3.13)

(Substrate Change Rate) =
(substrate input rate) - (substrate utilization rate) - (substrate output rate) (3.13)

Mathematically, Eq (3.16) can be written as Eq (3.17).

$$\frac{dS}{dt} = \frac{Q}{V} * S_i - (\mu - k_d) * \frac{X_r}{Y} - \frac{Q}{V} * S_e \tag{3.17}$$

ds/dt is defined as the rate of substrate removal (g/L day). S_i and S_e are influent substrate concentration (g/L) and the effluent substrate concentration (g/L), respectively. Y is defined the growth yield coefficient (mass cell produced mass substrate utilized) (g VSS/g COD).

At steady rate d_S/dt is 0. Thus, substrate balance at equilibrium can be rewritten as Eq (3.18).

$$\frac{(S_i - S_e)}{\theta_h} = (\mu - k_d) * \frac{X_r}{Y} \tag{3.18}$$

The equation given above can be reduced to equation (3.19)

$$\frac{S_i - S_e}{\theta_h} = \frac{X_r}{Y} * \left(\frac{1}{\theta_c} + k_d \right) \tag{3.19}$$

The kinetic parameters Y (g VSS / g COD), k_d can be obtained by rearranging Eq (3.19) as shown below:

$$\frac{(S_i - S_e)}{\theta_h * X_r} = \frac{1}{Y} * \left(\frac{1}{\theta_c} \right) + \left(\frac{1}{Y} \right) * k_d \tag{3.20}$$

The values of Y and k_d can determined by plotting $(1/\theta_c)$ versus $(S_i - S_c)/(X_r * \theta_h)$. The value of k_d can be calculated from the intercept of the straight line while Y can be obtained from the slope of the line.

3.4.9.5 Contois Kinetic Model

The relationship between specific growth rate and limiting substrate concentrations was given as follows (Contois, 1959).

$$\mu = \frac{\mu_{\max} * S}{\beta * X_r + S} \tag{3.21}$$

Where;

β is the contois kinetic parameter (g COD/g biomass).

By substituting Eq (3.21), instead of the Monod equation, into Eq (3.15) can be obtained Eq (3.22) can be obtained.

$$\frac{\theta_c}{1 + \theta_c * k_d} = \frac{\beta}{\mu_{\max}} * \frac{X_r}{S_i} + \frac{1}{\mu_{\max}} \tag{3.23}$$

Similarly, the values of μ_{max} and β can be obtained by plotting the Eq (3.23). The value of μ_{max} can be calculated from the intercept of the straight line and finally, β can be obtained from the slope of the line.

3.4.9.6 Grau Second- Order Multicomponent Substrate Removal Model

The general equation of a Grau second-order kinetic model is illustrated in Eq (3.24) (Grau, Dohanyas & Chudoba, 1975; Öztürk, Altinbas, Arikan & Demir, 1998)

$$\frac{ds}{dt} = k_s * X_r * \left(\frac{S_e}{S_i} \right)^2 \tag{3.24}$$

If Eq (3.24) is integrated and then linearilized, Eq (3.25) will be obtained:

$$\frac{(S_i * \theta_h)}{S_i - S} = \theta + \frac{S_i}{K_S * X} \tag{3.25}$$

If the second term of the right part of Eq (3.25) is accepted as a constant, the Eq (3.26) will be obtained.

$$\frac{(S_i * \theta_h)}{S_i - S_e} = b * \theta_h + a \qquad (3.26)$$

k_s is second-order substrate removal rate constant (L/day). If Eq (3.25) rearranged, Eq (3.26) will be obtained. This equation could be used to predict the effluent COD and NH_4-N concentrations.

$$S_e = S_i\left(1 - \frac{1}{(b + a/\theta_h)}\right) \qquad (3.27)$$

Where; a is equal S_i / (k_s *X) (day) and b are constant (dimensionless). (S_i-S_e)/S_e expresses the substrate removal efficiency and is symbolized as E (efficiency). S_e and S_i are effluent and influent COD concentrations (mg COD/L). X_e and X_i are effluent and influent NH_4-N Concentrations (mg COD/L). X_r is the average biomass concentration in the reactor (mg VSS/L). θ_h is hydraulic retention time (day).

3.4.9.7 Modified Stover-Kincannon Model

In this model, the substrate utilization rate is expressed as a function of the organic loading rate by monomolecular kinetic for biofilm reactors such as rotating biological contactors and biological filters. A special feature of Modified Stover-Kincannon model is the utilization of the concept of total organic loading rate as the major parameter to describe the kinetics of an anaerobic filter in terms of organic matter removal and methane production. A modified Stover-Kincannon model could be used for UASB reactor as follows (Yu, Wilson & Tay, 1998):

$$\frac{ds}{dt} = \frac{R_{max} * (Q * S_i / V)}{K_B + (Q * S_i / V)} \qquad (3.28)$$

Where; d*s*/d*t* is defined in Eq. (3.28):

$$\frac{ds}{dt} = \frac{Q}{V} * (S_i - S_e) \tag{3.29}$$

Eq (3.28) obtained from the linearization of Eq (3.29) as follows:

$$\frac{V}{Q * (S_i - S_e)} = \frac{K_B * V}{R_{\max} Q * S_i} + \frac{1}{R_{\max}} \tag{3.30}$$

If the maximum utilization rate (R_{max}) (g/Lday) and the saturation value constant (K_B) (g/L.day) values obtained for COD was substituted in Eq (3.30), Eq (3.31) and (3.32) could be used to predict the effluent COD concentrations, respectively. (QS_i/V) explain the organic loading rate (OLR) applied to the reactor. Q and V are the in flow rate (L/day) and the volume of the anaerobic reactor (L), respectively.

$$\frac{Q(S_i - S_e)}{V} = \frac{R_{\max}(QS_i / V)}{K_B + (QS_i / V)} \tag{3.31}$$

$$S_e = S_i - \frac{R_{\max} S_i}{K_B + (QS_i / V)} \tag{3.32}$$

CHAPTER FOUR
RESULT AND DISCUSIONS

4.1 Batch Studies

Several batch tests were performed to determine the effects of COD, NO_2-N, NO_3-N to Ammonia-N removals. These tests were performed at a temperature above 35°C to keep favorable conditions for growth of the Anammox bacteria for which 37°C is the optimal temperature (Egli et al., 2001) .The operating pH of each sample was measured about 7, 2 ± 1. The anaerobic conditions occurred in batch reactors. The ORP values during the anaerobic phases were approximately -50 mV and -247 mV. The operational conditions and the influent NO_2-N, NH_4-N and NO_3-N concentrations were illustrated in Tables 3.1 and 3.2, in the section "Materials and Methods" respectively.

4.1.2 Effect of COD Concentration on the NH_4-N Removal Efficiencies in Batch Reactor

In this study, the batch tests were divided into ten groups in order to recognize the significance of the presence of COD concentration on the NH_4-N removal efficiencies. The COD removal efficiencies throughout 10 series are illustrated in Figure 4.1 for OLRs varying between 0 and 0,271 kg COD/m^3 day. The maximum COD removal efficiency was obtained as 78 % in serie 5 when the COD/NH_4-N, NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N, COD/NO_3-N/NO_2-N and the NH_4-N/NO_3-N/NO_2-N ratios were 6/1,28=4,6, 1,33/0,041=32,4 ,1,28/1,33=0,96, 1,28/0,041=31,2 6/1,33/0,04=112,7 1,33/0,02/ 1,34=23,8 respectively. The COD yield was found between 68-69 % in series 1-3 in which the COD/NO_3-N/NO_2-N and the NH_4-N/NO_3-N/NO_2-N ratios were 6/1,34/0, 02=223,8 1,33/ 0,02/ 1,34=49,6 respectively.

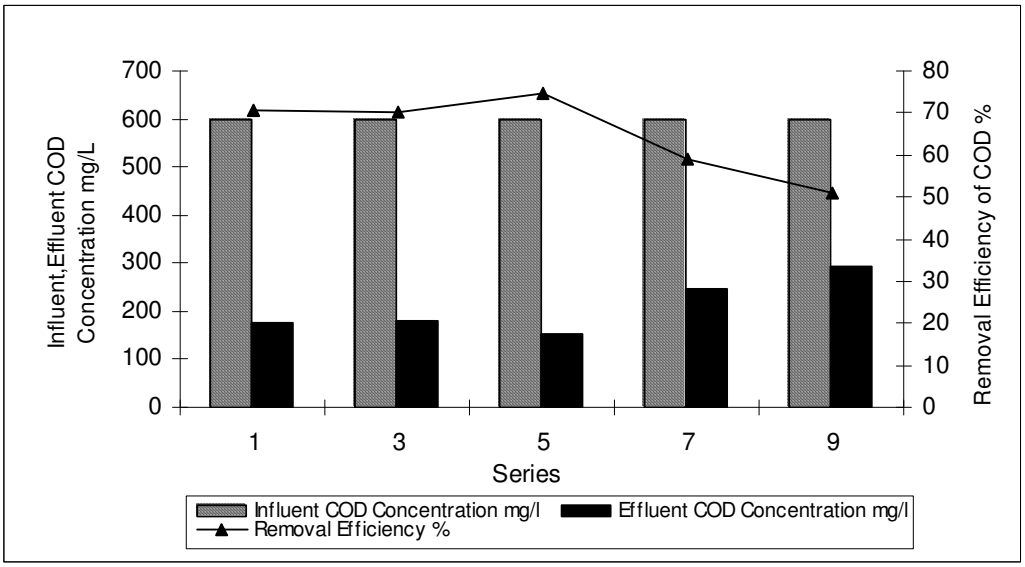

Figure 4.1 COD Removal Efficiencies in Ten Series throughout Batch Tests for Run-1

The effect of COD concentrations on NH_4-N removal efficiencies in batch reactor was shown in Figure 4.2. The results of this study showed that NH_4-N yields were not dependent to the COD concentrations. The NH_4-N removal efficiencies were >90% for all NH_4-N/ NO_3-N/ NO_2-N ratios with and without COD in series 3,4,5,6 with the exception of series 7 and 8. In these series 70% and 86% NH_4-N removal efficiencies were found, respectively. The maximum NH_4-N removal efficiencies were approximately 97% in series 5, 6 and 10. In this study it was found that when the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 128/4,1= 31,2 ,1,28/1,33 = 0,96 and 133/ 4,1=32,4, 121/4,3=28,1 121/136=0,89 and 136/4.3=31,6, 124/1,01=122,7 124/248=0,5 and 248/1.01=245,5 respectively, maximum NH_4-N removal was obtained.

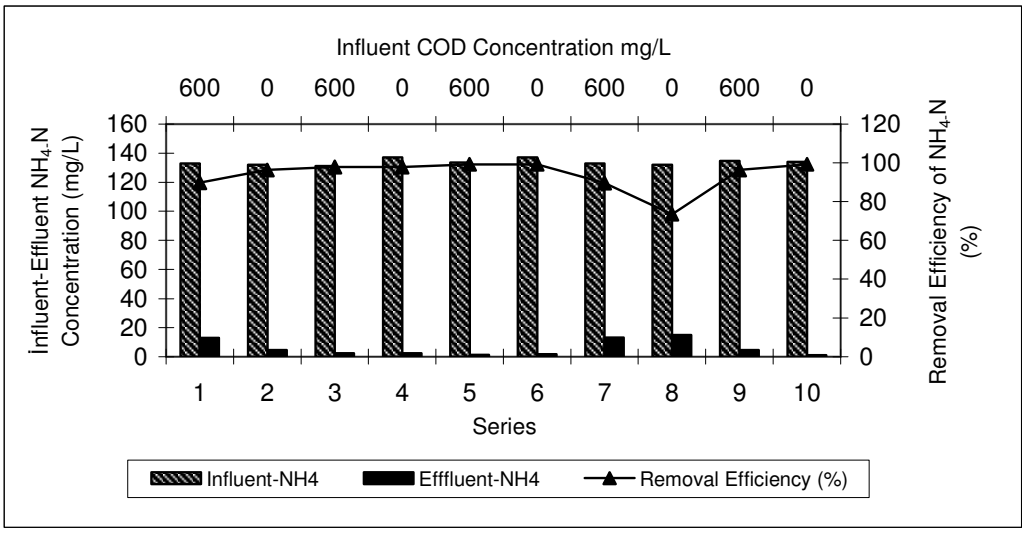

Figure 4.2 Removal Efficiencies of NH_4-N in Ten Series throughout Batch Tests for Run-1

Figures 4.3 and 4.4 showed the NO_2-N and NO_3-N removal efficiencies, respectively. The NO_2-N removal yields exhibited similar removals with NH_4-N removal yields because of the NH_4-N was removed throughout anammox process under anaerobic conditions when nitrite was used as the electron acceptor. After the 45 days of the experiment, NO_2-N removal yield was 96 % for all NH_4-N/NO_3-N/NO_2-N ratios with and without COD in series 3,4,5,6 with the exception of series 7 and 8. In the last two series 78 % and 85 % NO_2-N removal efficiencies were found, respectively. The maximum NO_2-N removal efficiencies were approximately 97 % in series 5, 6 and 10. In this study it was found that when the NH_4-N/NO_3-N, NH_4-N/NO_2-N, NO_2-N/NO_3-N ratios and the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 128/4,1=31,2 1,28/1,33=0,96 133/4,1=32,4 and 124/1,01=122,7 124/248=0,5 248/1.01=245,5 respectively, maximum NO_2-N removal was obtained. NH_4^+ is oxidized to gaseous N_2 using nitrite (NO_2^-) as electron acceptor. In this study the maximum total, nitrogen gas, hydrogen sulfide gas, methane gas productions were measured as 220 L/day, 172 L/day, 44 L/day ,3,2 L/day, respectively, while the methane percentage of the total gas was 20 % for the Run-1 (Table 4.1).

Table 4.1 Total, Dinitrogen, Hydrogen sulfide, Methane Gas Production and Methane Percantage in batch reactors for Run-1

	Serie -1	**Serie-2**	**Serie-3**	**Serie-4**	**Serie-5**	**Serie-6**	**Serie-7**	**Serie-8**	**Serie-9**	**Serie-10**
Total Gas	160	140	135	140	220	190	124	120	90	120
N_2 (L/d)	126,4	117,6	100,4	108,6	172,1	125,4	109,1	102	68,22	94,5
H_2S (L/d)	0	0	2,16	1,96	3,2	3,8	0	0	1,08	1,44
CH_4 (L/d)	33,6	22,4	32,4	29,4	55	60,8	14,88	18	20,7	24
%CH_4	21	16	24	21	20	32	12	15	23	20

Anammox is a distinctive process, involving the oxidation of ammonium with nitrite as the electron acceptor to yield N_2 and NO_3^- under anoxic condition. (Hsia et al., 2008) Strous et al., (1998) reported that the stoichiometry of the anammox reaction based on mass balance over anammox enrichment culture was represented by the following equation 4.1:

$$NH^+_4 + 1{,}31\ NO^-_2 + 0{,}066\ HCO^-_3 + 0{,}13\ H^+ \rightarrow 1{,}02N_2 + 0{,}26\ NO_3^- + 0{,}066CH_2O_{0,5}\,N_{0,15} + 2{,}03H_2O \quad (4.1)$$

According to the stochiometric equation 4.1 , in this study it was found that when the influent NH^+_4 and NO^-_2 concentrations were 130 mg/l and 140 mg/l, respectively, the effluent N_2 value was 0,89 mg/l in run -1.

After the exhaustion of NO_2–N, the ANAMMOX microorganisms could use NO_3–N as electron acceptor to yield dinitrogen gas. In this step of this experiment, the COD removal efficiency was about 60 % in serie 7. The data in this study showed that the COD could be utilized by denitrifying microorganisms to reduce nitrate to form dinitrogen gas throughout anammox process after NO_2-N was removed.

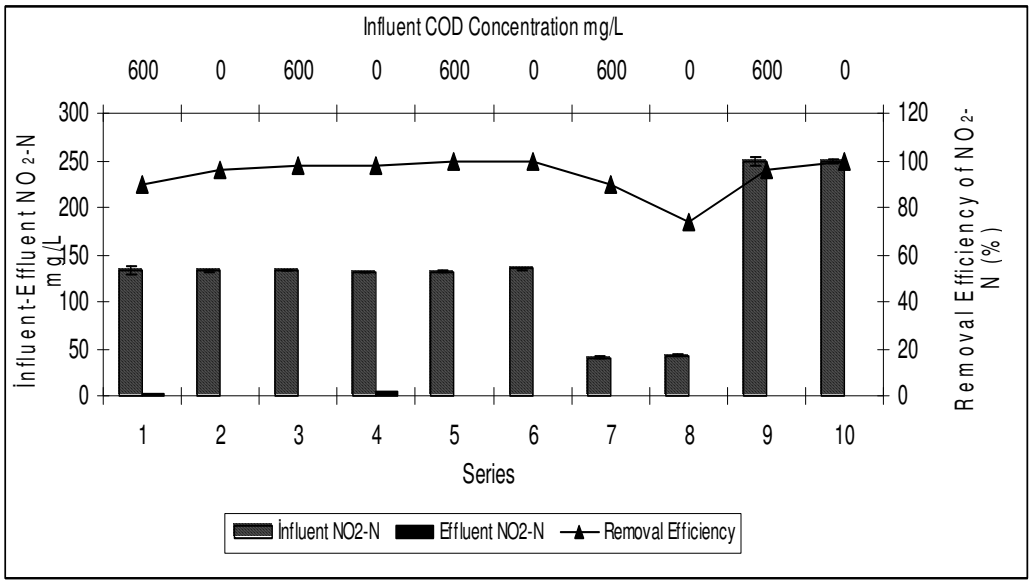

Figure 4.3 Removal Efficiencies of NO_2-N in Ten Series throughout Batch Tests for Run-1

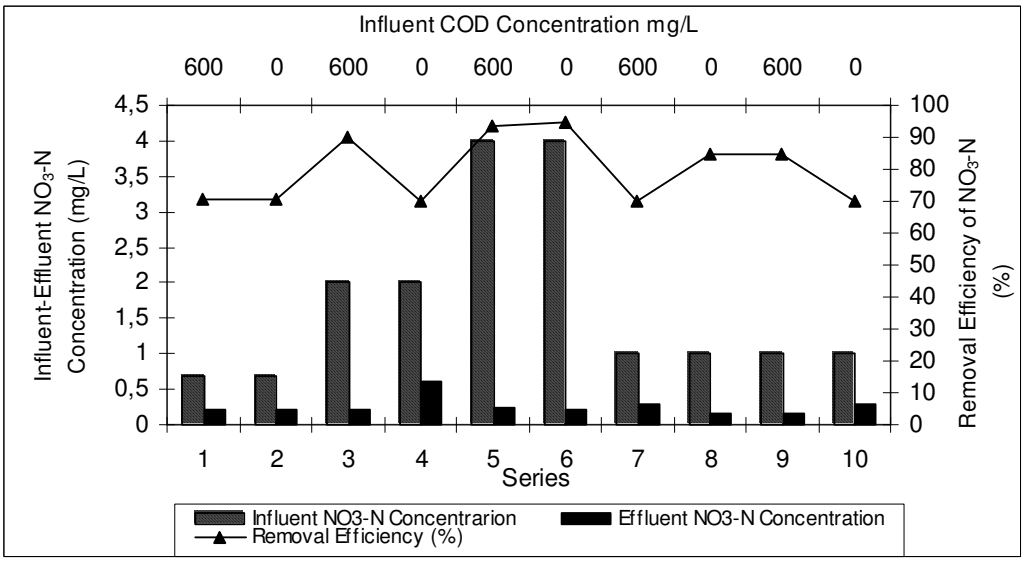

Figure 4.4 Removal Efficiencies of NO_3-N in Ten Series throughout Batch Tests for Run-1

In batch reactors the NH_4-N was removed via anammox process under anaerobic conditions when nitrite was used as the electron acceptor. During anammox process a part of ammonium was converted to nitrite and then the remaining ammonium and the formed nitrite was converted to dinitrogen gas by Anammox bacteria. These data suggested that the Anammox process was dominating in the system.

In a study performed by Chan T.Y.,(2003) 26 % of ammonium and 80 % nitrite removal efficiency was obtained in batch studies under anoxic conditions within 24 hours, at a COD loading of 10 g COD/L.d and at a influent NO_2-N concentration of 5-10 g/L. The ammonia yields obtained in the present study is higher than the ammonia yields obtained by Chan T.Y., (2003).

4.1.3 Effect of the various NO_3-N Concentration on the NH_4-N Removal Efficiencies in Bactch Reactor

Second series tests were divided into six groups in order to recognize the significance of the NO_3-N on the ammonia removals throughout anammox process in batch tests. The maximum COD removal efficiency was 92 % for serie 5 (Fig. 4.5). The effect of NO_3-N concentrations on NH_4-N removal efficiencies in batch reactor was shown in Figure 4.6. The results of this study showed that NH_4-N yields were not dependent to the NO_3-N concentrations. The NH_4-N removal yields were >98 % for all NH_4-N/ NO_3-N/ NO_2-N ratios with and without NO_3-N in series 2,3,4 and 6 with the exception of series 1 and 5 (Fig. 4.6). In these series 94 % and % 93 NH_4-N removal efficiencies were found, respectively. The maximum NH_4-N removal efficiencies were approximately 98% in series 2 and 6. In this study it was found that when the NH_4-N/ NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 1,35/1=1,35 1,35/2=0,675 and 2/1=2, respectively, a maximum NH_4-N removal was obtained (Fig. 4.6, Tab. 3.3)

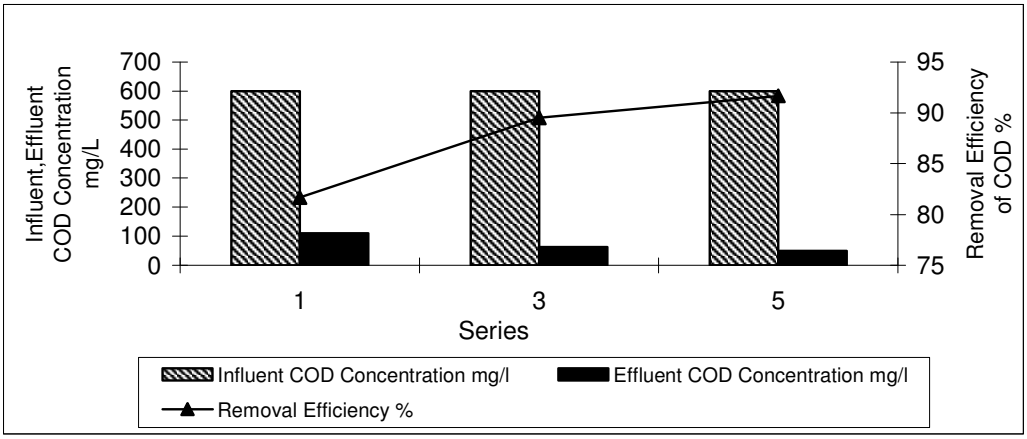

Figure 4.5 Removal Efficiency of COD Concentration in Six Series throughout Batch Tests for Run-2

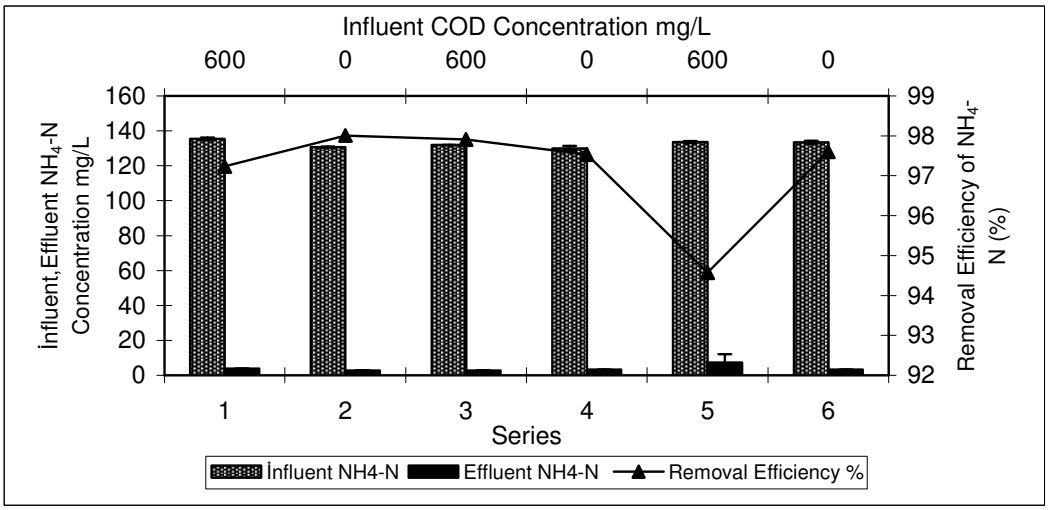

Figure 4.6 Removal Efficiency of NH_4-N in Six Series throughout Batch Tests for Run-2

The NO_2-N removal efficiencies were approximately 98 % except for in serie 4 (fig.4.7). The minumum NO_2-N removal efficiency was found at 90 % in serie 4 when the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 1,29/0,051=25,2, 1,29/2,03=0,63 2,03/0,051=39,8 respectively (See Table 3.3). The maximum NO_2-N removal efficiency was obtained as 100 % in series 5-6 when the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 1.33/3,05=0,43, 1,34/2,01=0,65, 2,01/3,06=0,65 respectively.

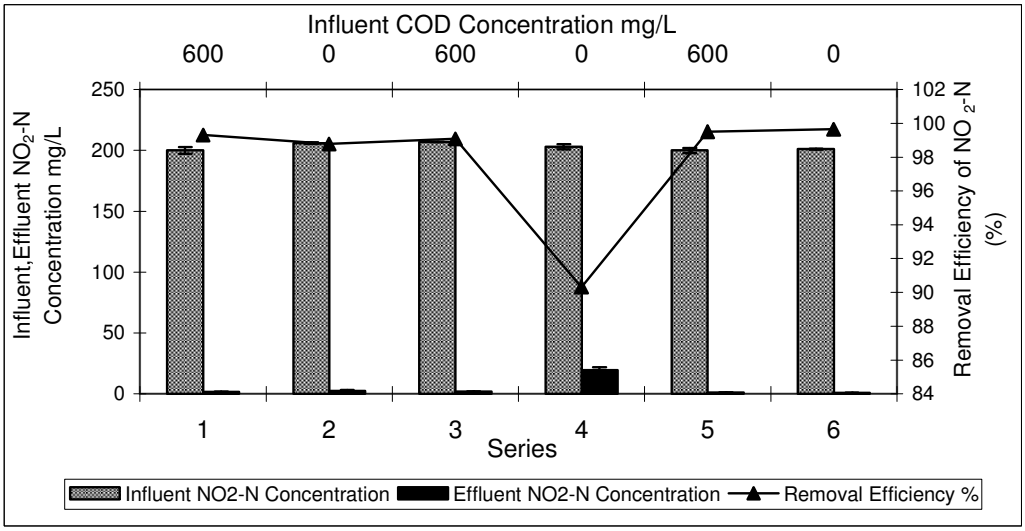

Figure 4.7 Removal Efficiency of NO_2-N in Six Series throughout Batch Tests for Run-2

NO_3^-–N was continuously reduced (Fig.4.8), which suggested that NO_3^-–N could be used as electron acceptor in the ANAMMOX reaction.

The influent for the Anammox process must be composed of NH_4^+-N and NO_2^--N in a ratio 1:1 and therefore only a partial nitrification of ammonium to nitrite is required. The modifications of some operational parameters like temperature, ammonium concentration, pH and solid retention time allows to achieve a partial nitrification with a final effluent only composed by NH_4^+-N and NO_2^--N at the right of the stoichiometric ratio. The system also managed well when there were operational variations in NH_4^+-N of the influent of NH_4^+-N and NO_2^-N in a 1:1 ratio without any modification in HRT When the molar HCO_3^-/NH_4^+ ratio is 1:1 approximately 50% of NH_4^+ is oxidised (Gal, Dosta, Loosdrecht, and Mata-Alvarez, 2006). In the present study this ratio was 1,32:1,02 for maximum (98 %) ammonia removal.

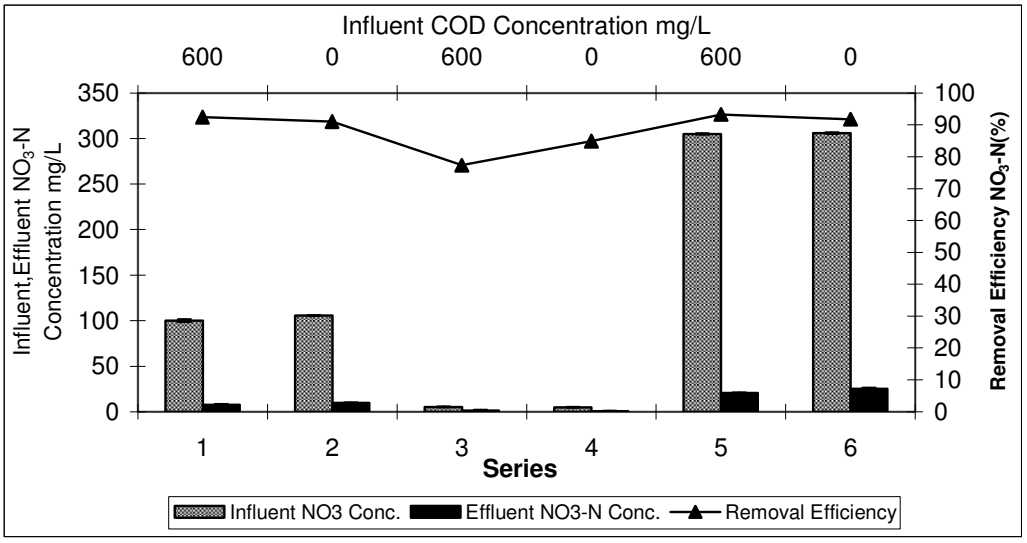

Figure 4.8 Removal Efficiency of NO_3-N in Six Series throughout Batch Tests for Run-2

The maximum total, nitrogen gas, hydrogen sulfide gas and methane gas productions were 180 L/day, 147,24 L/day, 28,8 L/day and 3,96 L/day while the methane percentage of the total gas was 16 %, respectively, for the Run-2 (Tab. 4.2).

Table 4.2 Total, Dinitrogen, Hydrogen sulfide, Methane Gas Production and Methane Percantage in batch reactors for Run-2

	Serie-1	**Serie-2**	**Serie-3**	**Serie-4**	**Serie-5**	**Serie-6**
Total Gas	140	117	135	120	180	190
N_2 (L/d)	110,6	96,6	108,64	92,88	147,2	135,85
H_2S (L/d)	0	1,755	2,06	1,92	3,96	2,85
CH_4 (L/d)	29,4	18,72	24,3	25,2	28,8	51,3
% CH_4	21	16	18	21	16	27

4.1.4 Effect of the various NO_2-N Concentration on the NH_4-N Removal Efficiencies in Batch Reactor

Third series tests were divided into eight groups in order to recognize significance of the NO_2-N on the removal of NH_4-N. The maximum COD removal efficiency was 92 %, respectively for these series (Fig. 4.9). The effect of NO_2-N concentrations on NH_4-N removal efficiencies in batch reactor was shown in Figure 4.10. The results of this study showed that NH_4-N yields were not dependent to the NO_2-N concentrations. The NH_4-N removal yields were >98 % for all NH_4-N/ NO_3-N/ NO_2-N ratios with and without COD in series 1,2,3,4,5,6,7 and 8. The maximum NH_4-N removal efficiencies were approximately 98 % in series 7 and 8 (Figure 4.10, Table 3.4). In this study it was found that when the NH_4-N/ NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratio were 1,19/0,011=108,1, 1,19/4,12=0,28 and 4,12/0,011=374,5 respectively, a maximum NH_4^--N removal was obtained.

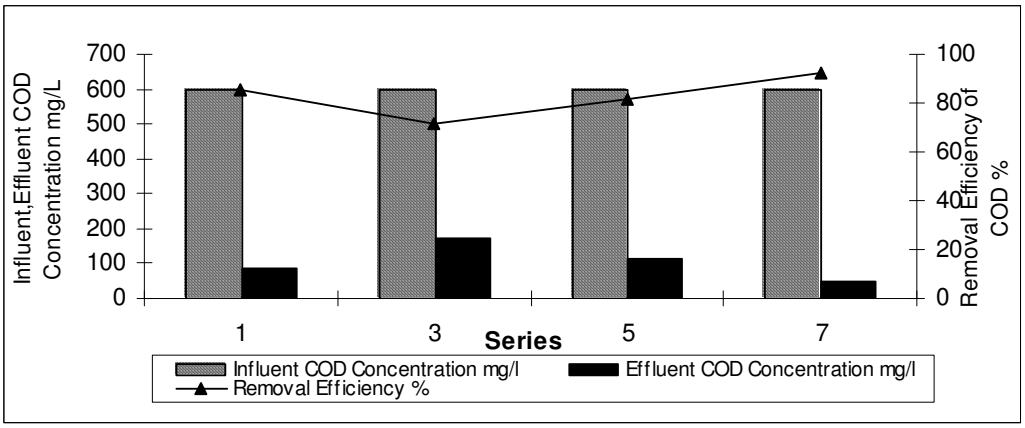

Figure 4.9 Removal Efficiency of COD Concentration in Six Series throughout Batch Tests for Run-3

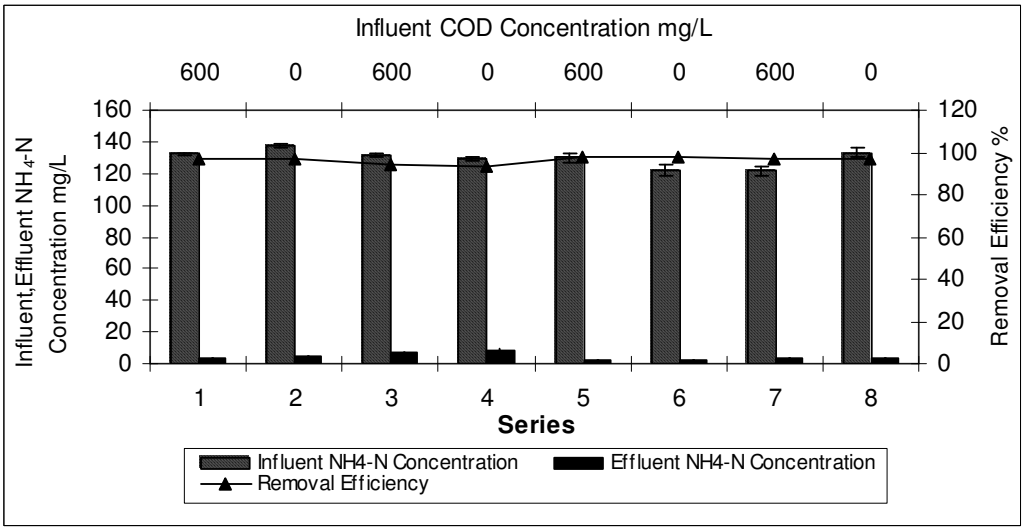

Figure 4.10 Removal Efficiency of Ammonium Nitrogen in Eight Series throughout Batch Tests for Run-3

The maximum NO_2-N removal efficiency was obtained as 100 % when the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 4,04/0,012=336,6, 4,04/1,23=3,28 and 1,23/0,013=94,6 (Figure 4.11 and Table 3.4).

NO_2^--N removal efficiencies and NH_4^+–N removal efficiencies exhibited similarities. Nitrite could be used as electron acceptor during nitrogen removal by anaerobic bacteria throughout anaerobic ammonium oxidation process (Strous et al., 1998). In the present study also similar results were obtained.

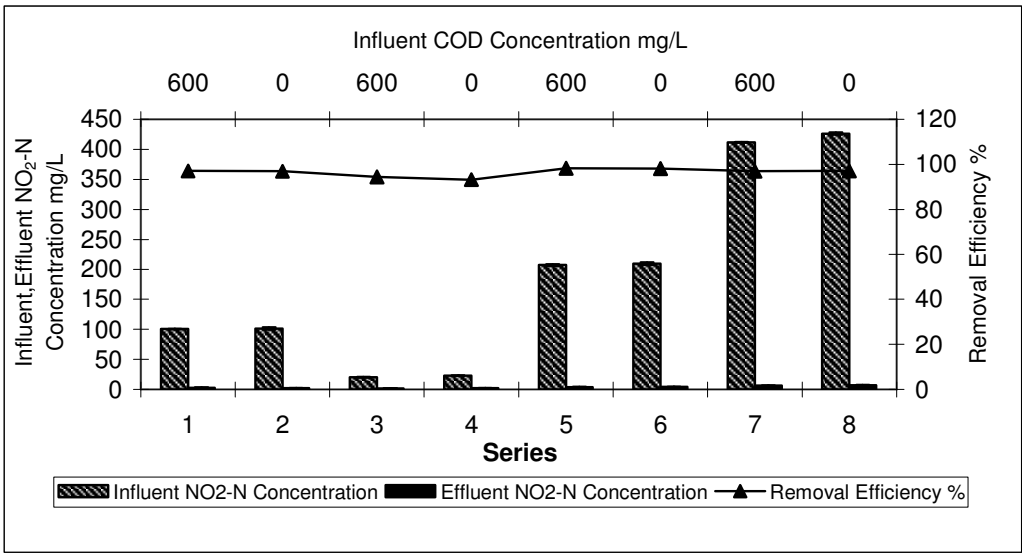

Figure 4.11 Removal Efficiency of Nitrite Nitrogen in Eight Series throughout Batch Tests for Run-3

In this study it was found that NO_3^-–N reduced after 45 days of batch operation in series 5, 7 and 8 as illustrated in Figure 4.12. The maximum NO_3-N removal efficiency was obtained as 58 % in serie-2 when the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 1,35/0,012=111,5, 1,35/1,03=1,3 and 1,03/0,012=85.8.

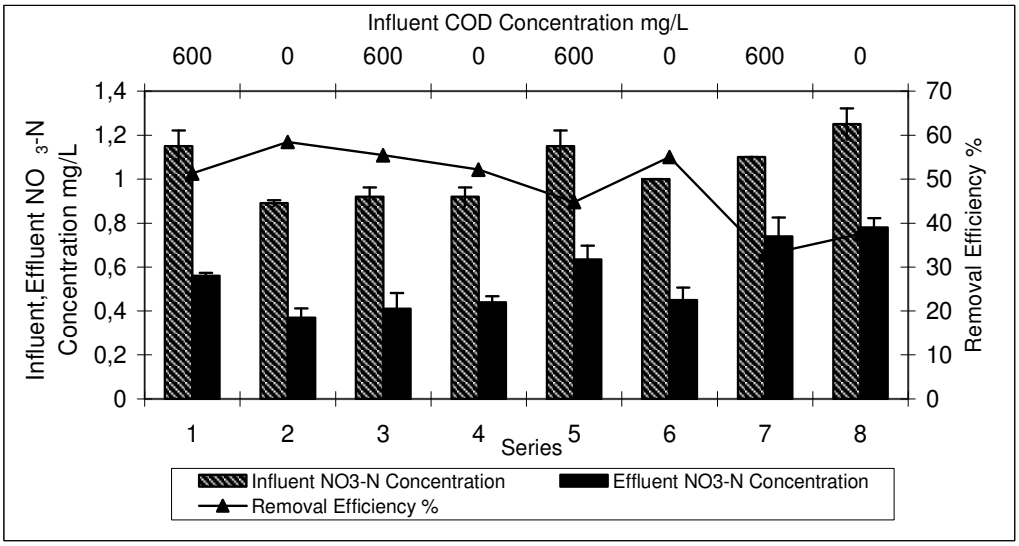

Figure 4.12 Removal Efficiency of Nitrate Nitrogen in Eight Series throughout Batch Tests for Run -3

The maximum total, nitrogen gas, hydrogen sulfide gas, methane gas, methane percentage were found as 190 L/day,139,3 L/day, 47,5 L/day ,3,2 L/day , 25 %, respectively, for the Run-3(Table 4.3).

Table 4.3 Total, Dinitrogen, Hydrogen sulfide, Methane Gas Production and Methane Percantage in batch reactors for Run-3

	Serie-1	**Serie-2**	**Serie-3**	**Serie-4**	**Serie-5**	**Serie-6**	**Serie-7**	**Serie-8**
Total Gas	120	**190**	135	130	110	130	50	62
N_2 (L/d)	92,88	**139,3**	108,64	97,5	90,3	100,1	41,68	54,5
H_2S (L/d)	1,92	**3,2**	2,06	0	2,1	0	1,32	0
CH_4 (L/d)	25,2	**47,5**	24,3	32,5	17,6	29.9	7	7,44
% CH_4	21	**25**	18	25	16	23	14	12

4.1.5 Effect of the various NH_4-N Concentration on the NH_4-N Removal Efficiencies in Batch Reactor

In this study, the batch tests were divided into six groups in order to recognize the significance of the influent NH_4-N concentration on NH_4-N removal efficiencies. The COD removal efficiencies throughout six series are illustrated in Figure 4.13 for OLRs varying between 0 and 0,271 kg COD/m^3 day. The COD removal efficiencies were approximately % 75 for the series containing COD. The effect of influent NH_4-N concentrations on NH_4-N removal efficiencies in batch reactor was shown in Figure 4.14. The results of this study showed that NH_4-N yields were dependent to the influent NH_4-N concentrations. The NH_4-N removal yields were >65% for all NH_4-N/ NO_3-N/ NO_2-N ratios with and without COD in series 1,2,3,4 with the exception of series 5 and 6 (Figure 4.14 and Table 3.5). In these series 65 % NH_4-N removal efficiencies were found. The maximum NH_4-N removal efficiencies were approximately 96 % in series 1 and 2. In this study it was found that when the NH_4-N/ NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were between 1,35/0,01=135, 1,35/1=1,35 and 1/0,01=100, respectively, maximum NH_4-N removal was obtained.

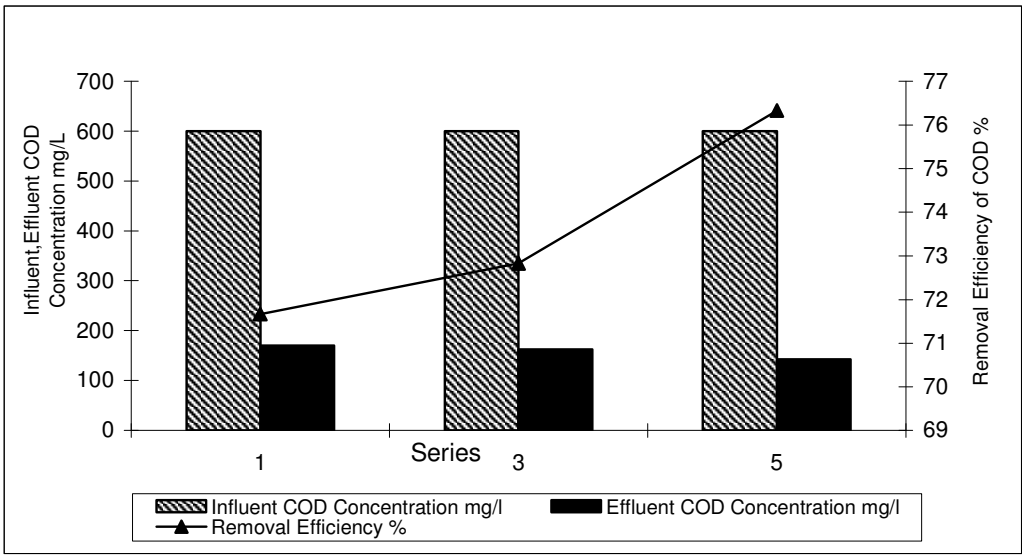

Figure 4.13 Removal Efficiency of COD Concentration in Six Series throughout Batch Tests for Run-4

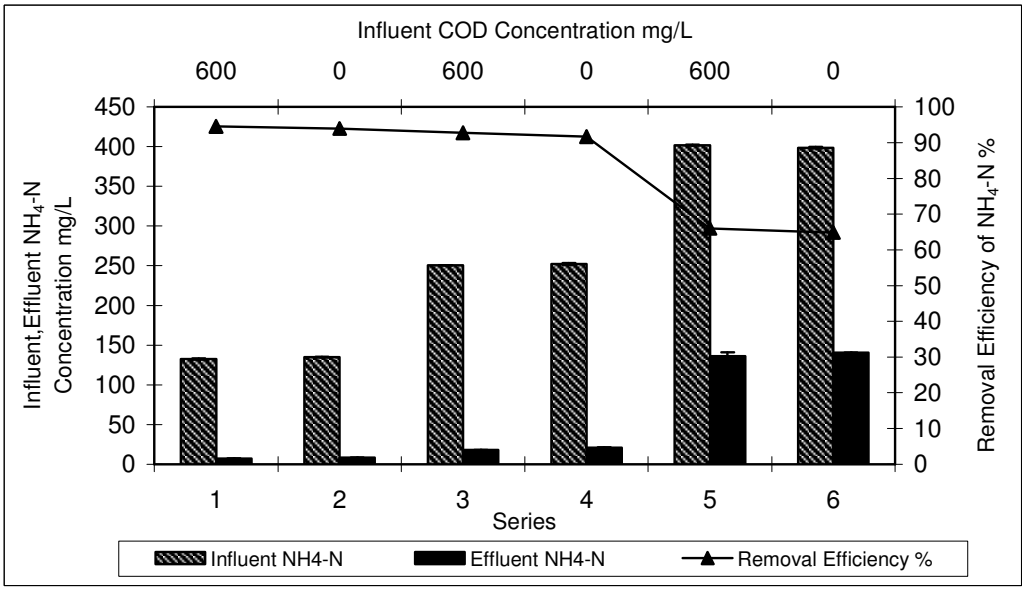

Figure 4.14 Removal Efficiency of NH_4-N in Six Series throughout Batch Tests for Run-4

The effect of influent NH_4-N concentrations on NO_2-N removal efficiencies in batch reactor was shown in Figure 4.15. The NO_2-N removal efficiency increased while the ammonium concentration was increased. The maximum NO_2-N removal efficiency was obtained as 99% in serie 6 when the NH_4-N/NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 4, 04/0,012=336,6, 4,04/1,03=3,92 1,03/0,012=84,4.

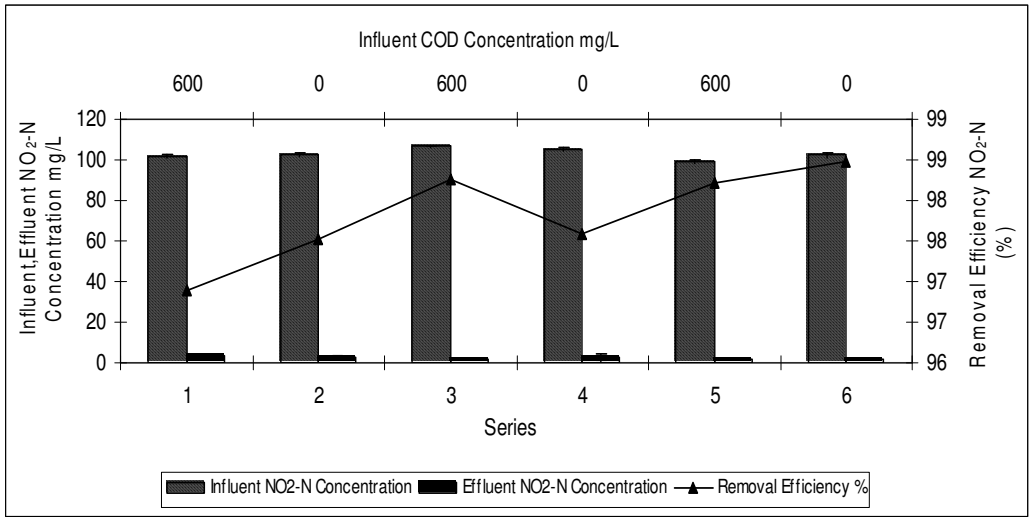

Figure 4.15 Removal Efficiency of NO_2-N in Six Series throughout Batch Tests for Run-4

The maximum NO_3-N removal efficiency was approximately % 60. The effect of influent NH_4-N concentrations on NO_3-N removal efficiencies in batch reactor was shown in Figure 4.16.

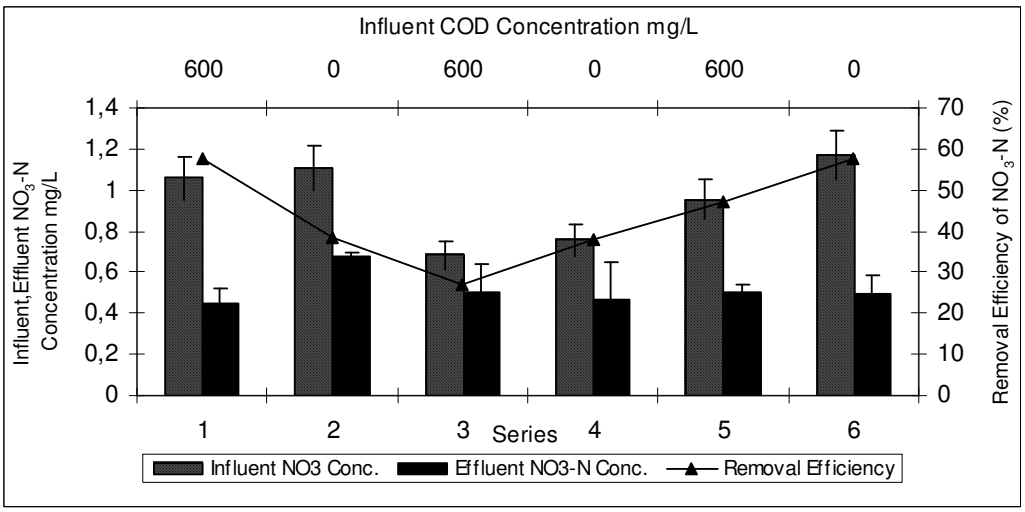

Figure 4.16 Removal Efficiency of NO_3-N in Six Series throughout Batch Tests for Run-4

The maximum total, nitrogen gas, hydrogen sulfide gas, methane gas, methane percentage were found as 110 L/day, 67,55 L/day, 30,8 L/day ,1,65 L/day , 28 %, respectively, for the Run-4.(Table 4.4)

Table 4.4 Total, Dinitrogen, Hydrogen sulfide, Methane Gas Production and Methane Percantage in batch reactors for Run-4

	Serie-1	**Serie-2**	**Serie-3**	**Serie-4**	**Serie-5**	**Serie-6**
Total Gas	70	53	75	50	62	110
N_2 (L/day)	55,3	43,4	60,2	43	52,3	67,55
H_2S (L/day)	0	3,2	1,96	0	2,2	1,65
CH_4 (L/day)	14,7	9,54	12,75	7	7,44	30,8
% CH_4	21	18	17	14	12	28

Consuquently, the batch tests results showed that the optimum NH_4-N/ NO_3-N, NH_4-N/NO_2-N and NO_2-N/NO_3-N ratios were 1:0,74, 1:1,48 and 1:1,05 respectively, for maximum COD, NH_4-N and NO_3-N removals.

The main product of anaerobic ammonium oxidation was N_2, but about 10% of the N in the feed (the total concentratıon of nitrite-N and ammonium-N) was converted to NO_3^-. The overall nitrogen balance Eq.(4.2) gave a ratio of NH_4^+ conversion to NO_2^- conversion of 1:1,31 (van de Graaf et al., 1996).

$$NH^+_4 + 1{,}31\ NO^-_2 + 0{,}0425\ CO_2 \rightarrow 1{,}045\ N_2 + 0{,}22\ NO^-_3 + 1{,}87\ H_2O + 0{,}09 OH^- + 0{,}0425\ CH_2O \quad (4.2)$$

(biomass)

Strous et al. (1998) estimated the ANAMMOX stoichiometry based on mass balance over ANAMMOX enrichment cultures, as presented in Eq.(4.3):

$$NH^+_4 + 1{,}31\ NO^-_2 + 0{,}066 HCO_3 + 0{,}13 H^+ \rightarrow 1{,}02\ N_2 + 0{,}26\ NO^-_3 + 2{,}03\ H_2O + 0{,}0425\ CH_2O_{0,5}N_{0,15} \quad (4.3)$$

Therefore the nitrate produced during the ANAMMOX process could also be used as electron acceptor to react with ammonium according to Eq. (4.3).

In the present study during the batch experiments, the optimum removal ratio of NH_4^+–N to NO_2–N and NO_3–N was found to be 1:1,48:0,5 while the optimum ratio of NH_4^+–N to NO_2^-–N was measured as 1:1,48 for maximum ammonia removal. This value was bigger than the ratio suggested by Strous et al. and van de Graaf et al. The results of this experiment accorded with Eq. (4.2) and (4.3) indicated the existence of the ANAMMOX reaction. COD was absent in the batch reactors when this optimum removal ratio was found.This result show that the presence of an organic compound (COD) was not necessary for ammonia removal with anammox process.

Howewer, in the batch reactors containing COD, the COD removal efficiency was >70 %. Based on calculation, it can be seen that COD could be utilized by denitrifying microorganisms to reduce nitrite and nitrate to form dinotrogen gas throughout denitrification process.

4.2 Continuous Studies

4.2.1 The Removal of Ammonium, Nitrite, Nitrate and COD in the UASB Reactor throughout Continuous Operation

The UASB reactor was operated during continuous studies more than 294 days. Table 4.5 shows the COD removal efficiencies in the UASB reactor. The long-term continuous operational results showed that the maximum removal efficiency of NH_4^+–N was 50% when the influent NH_4^+–N concentration was between 25 and 100 mg/L. The maximum NO_2^-–N removal efficiency was more than 98% when influent NO_2^-–N loading rate (NO_2-N concentration was between 20 and 130 mg/L) was between 0.020 g/l.day and 0.13 g/l.day in Run 1 at a HRT of 4,4 days. The maximum NO_3^-–N removal efficiency was between 85 and 95 % when the influent NO_3^-–N loading rates were between 0.020 g/l.day and 0.13 g/l.day (NO_3-N concentration was between 20 and 130 mg/L), respectively in Run 3. The maximum

COD removal efficiency was between 60 and 85 % when the influent COD loading rate was between 0.3 g/L.day and 1g/L.day, respectively (Fig.4.17,Tab 3.6). During anammox process, nitrite nitrogen was removed almost completely and the nitrate nitrogen was the main nitrogen form in the effluent of the UASB reactor. In the ANAMMOX reaction, the anaerobic granular sludge use NO_2^-–N as the electron acceptor and NH_4^+–N as the electron donor to yield the dinitrogen gas (Jianlong & Jing, 2004). Nitrogen gas production was obtained during continous studies in UASB reactor.

The anammox process, under different organic loading rates (COD), was evaluated by Molinuevo B., et al., (2009) using a semi-continuous UASB reactor at 37 ^{0}C. High ammonium removal was achieved up to 92.1 ± 4.9 % for diluted UASB-post-digested effluent (95 mg COD/L) and up to 98.5 ± 0.8 % for diluted partially oxidized effluent (121 mg COD/ L). This removal efficiency was higher than my studies.

In a study performed by Mora, Campos, Corral, Jetten & Méndez, (2004) an ANAMMOX gas-lift reactor and a sequential batch reactor (SBR) were operated during 200 days at nitrogen loading rates (NLRs) of 2.0 and 0.75 g l^{-1} per day, respectively. The efficiency in the nitrite (limiting substrate) removal was 99%. NO_2-N removal efficiency found in this study was similar to the aforementioned study.

Table 4.5 Influent and Effluent COD, NH_4-N, NO_3-N, NO_2-N Concentration in UASB Reactor

RUN	Inf. COD	Eff. COD	Removal Efficiency %	Inf. NH_4N	Eff. NH_4-N	Removal Efficiency %	Inf. NO_3-N	Eff. NO_3-N	Removal Efficiency %	Inf. NO_2-N	Eff. NO_2-N	Removal Efficiency %
Run-1	611	199	67	100	55,68	47,22	129,7	81,6	37,09	8	1	88
Run-1	614	95	85	92	89,2	3,80	124	16,1	87,06	2,475	2,15	13
Run-1	597	98	84	98	47,5	50,52	130,5	4,5	96,55	6,7	0,2	97
Run-1	580	92	84	95	73	25,00	120,5	3,3	97,30	0	1,5	0
Run-1	520	119	77	100	62,5	37,41	131	20,5	84,35	0	0,5	0
Run-1	477	80	83	100	45	54,73	131	17,2	86,85	0	0,45	0
Run-1	455	61	87	85	50	41,11	140,5	24,5	82,56	0,485	0,25	48
Run-1	650	145	78	82,4	72,8	11,89	145,5	61,5	57,73	0,05	5,45	0
Run-1	568	87	85	87,5	72	18,82	125,5	22,5	82,11	0,1	2,85	0
Run-1	571	112	80	81,25	72,25	12,05	131,5	5	96,23	0,05	0,15	0
Run-1	539	108	80	85	77,5	8,40	134,5	27,3	79,70	0,135	0,335	0
Run-2	1086	310	71	90	67,75	25,28	114,9	5,6	95,17	0,025	0,1	0
Run-2	994	478	51	84	73	14,34	122,35	3,1	97,45	52,5	1,035	98
Run-2	826	300	63	96,75	77,5	20,26	137,55	30,5	77,83	49,5	1,275	97
Run-2	1004	325	67	92	76	11,31	163,95	20	87,80	50	0,15	100
Run-2	1018	356	65	84	75	16,94	140,1	25,1	82,12	45,5	2,3	95
Run-2	996	335	66	91	75	21,81	144,9	10,1	93,03	50,5	1,35	97
Run-2	1004	665	33	71	55,25	14,62	140	12,6	91,04	47,75	0,15	100
Run-2	950	338	64	86	73,25	1,84	102,55	15,1	85,32	45,5	0,135	100
Run-2	1200	469	60	71	69	22,54	124,85	5,1	95,92	20,05	0,135	99
Run-2	1080	562	47	87,5	65,75	3,62	147,6	6,2	95,78	19,5	0,45	98
Run-2	1021	520	49	92,5	89	12,55	130,05	2,5	98,08	20,125	2,275	89
Run-3	348	151	56	95,25	81,75	6,19	140	6	95,71	17	0,135	99
Run-3	360	142	60	28,5	27	18,40	142,6	4,1	97,16	21,5	1	95
Run-3	300	159	47	27,5	22,3	26,15	15	2	87,00	20,5	1,545	92
Run-3	320	149	53	29	22	10,23	18,05	1,3	93,07	50,5	0,42	99
Run-3	410	220	46	29,6	26,5	4,79	22	0,9	96,14	50,5	0,775	98
Run-3	390	175	55	28,4	27,2	32,50	23,35	2,2	90,79	49,5	0,5	99
Run-4	610	170	72	26	27,1	47,89	16	1,3	91,88	49	0,5	99
Run-4	620	180	71	24	16	39,42	20,2	0,6	97,28	49	0,045	100
Run-4	595	240	73	27	14	49,45	18,1	1	94,48	50,5	0,25	100
Run-4	590	184	72	24	15	46,43	21	0,9	95,74	51	0,07	100
Run-5	375	195	68	25	16	40,04	23,05	0,7	96,96	64,5	0,045	100
Run-5	321	177	52	23	12	32,50	22	0,9	95,95	45,5	2,35	95
Run-5	340	135	44	28	15	25,24	23,05	0,7	96,92	50,75	0,5	99
Run-5	392	127	60	26	16	29,15	20,05	1,3	93,77	51,5	0,85	98
Run-5	405	208	57	25	17	20,79	21	1,5	92,86	53,5	0,5	99
Run-5	370	205	48	26,1	19,5	26,61	20,1	2	90,05	50,5	0,25	100
Run-5	321	169	44	25,8	18,7	51,96	22	3,1	86,14	47,5	0,34	99
Run-5	301	170	47	24,1	19,1	47,22	20,45	1,4	93,40	50	0,4	99
Run-5	406	228	43	24,8	18,25	3,80	45,15	5,1	88,70	51	0,41	99
Run-5	333	185	43	25	12	50,52	44,9	5,3	88,31	47,5	0,65	99

4.2.1.1 The Effect of Different Nitrite-Nitrogen (NO_2-N), Nitrate-Nitrogen (NO_3-N), Ammonium Nitrogen (NH_4-N) Concentratıons on the COD Removal Efficiencies in UASB Reactor

The effect of different nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonium nitrogen (NH_4-N) and COD concentrations on the COD removal efficiencies were recorded in UASB reactor throughuout 294 days of contiuous operation. Figure 4.17 shows the influent, effluent COD concentrations and the COD yields in UASB reactor. The COD removal efficiencies in this reactor varied between 70 and 90 % when the influent COD concentration was kept at 600 mg/L (at OLR's 0,272 kg COD/m^3.d) with glucose in run 1 at a HRT of 4,4 day. Then the COD removal efficiency decreased to 60 % and 35% at OLR's as high as 0,454 kg COD/m^3.d (NH_4-N, NO_2-N NO_3-N loading rates were approximately 0.1, 0.05, 0.13 g/L. day while the NH_4-N, NO_2-N NO_3-N concentrations were 100, 50 and 130 mg/L) (Table 3.6) in the same run 2. The maximum COD removal efficiency was obtained as %90 when the COD/NH_4-N/NO_3-N ratios were 6:1:1,3 in week of 2,3,5 and 9 at a HRT of 4.4 days.

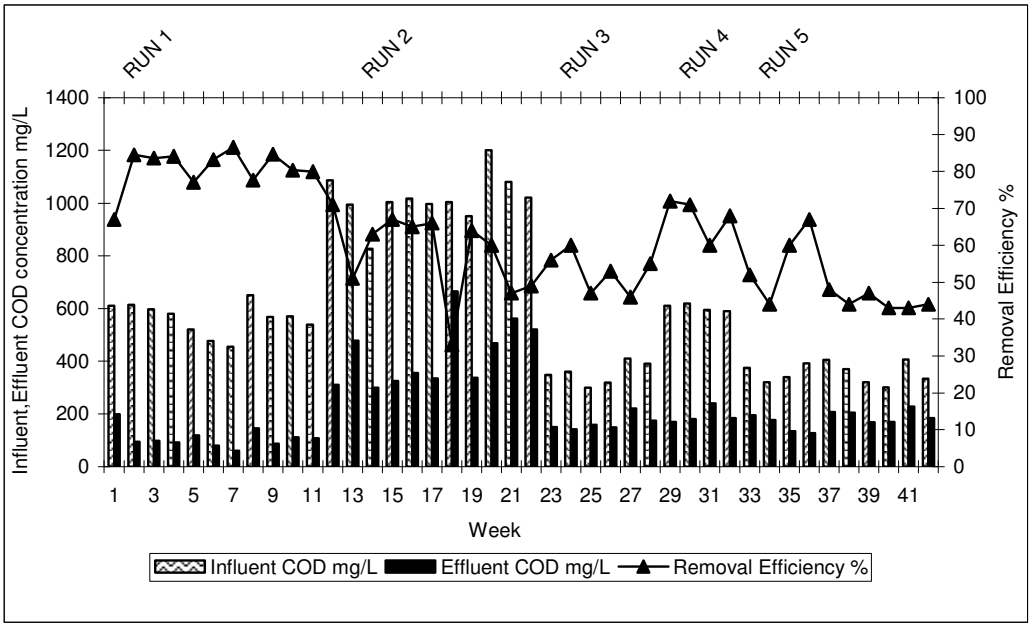

Figure 4.17 COD Removal Efficiency in UASB Reactor throughout Continuous Operation of UASB Reactor (COD: 300-1000 mg/L NH_4-N: 25-100mg/L NO_2-N:0-50mg/L NO_3-N: 20-130mg/L)

The operation of the UASB with NH_4-N was started at an influent NH_4-N concentration of 100 mg/L, and then ammonium nitrogen concentration was subsequently decreased from 100 mg/L to 25 mg/l in run 3 at a HRT of 2 day. The influent nitrite nitrogen concentration was zero in the first run, and then the nitrite nitrogen concentration was increased from 0 mg/l to 20 mg/L (at organic loading rates of 0,272, 0,454, 0,136, 0,272 and 0.136kg COD/m^3.d respectively).The nitrate nitrogen concentration was 130 mg/l in Run 1 and Run 2, after it decreased from 130 mg/l to 20 mg/l in Run 3, 4 and 5 respectively. The COD removal efficiency was measured as 30 and 40 % at twelfth and eighteenth weeks in run 2 when the COD/NH_4-N/NO_2-N/NO_3-N ratios were 10:1:0,5:1,3 at a HRT of 2,2 day(Figure 4.17, Table 3.6). The maximum COD removal efficiency was between 85-90 % when the COD/NH_4-N/NO_3-N ratios were 6:1:1,3 in the influent of the UASB reactor in run 1 at a HRT of 4,4 days. These COD removals could be attrıbuted together to the COD Removal solely in the UASB reactor and to the denitrification sınce “denitrıfication” process takes place in this run ın the UASB reactor. COD was used as an electronic acceptor during denitrification.The NO_3-N removals in this run was found as 90 % and is illustrated in figure 4.20.

The study performed by Jianlong & Jing, (2004) validates the existence of ANAMMOX reaction and investigate the performance of nitrogen removal by anaerobic granular sludge in an EGSB (expanded granular sludge bed) reactor. The influent NH_4^+–N concentration was 70–250 mg/L; the NO_2^-–N removal efficiency was more than 98% when the influent NO_2^-–N concentration was 70–250 mg/L in the aforementioned EGSB reactor. The COD removal efficiency was 84 % at an influent COD concentration of 500 mg/L in the EGSB reactor mentioned above. The COD removal efficiency found in this study is comparable higher than that the aforementioned study. The COD removal efficiency in this study was found as 85-90 % for an influent COD concentration of 600 mg/L.The maximum ammonium and NO_2-N removal efficieny was obtained 55% and 100 %. This results show that the UASB reactor is more efficient compared to the EGSB reactor.

An activated sludge process with partial nitrification (AS/PN) in combination with anaerobic ammonium oxidation (Anammox) process for treatment of seafood processing wastewater was developed and investigated by Lansam, Laohaprapanon, Annachhatre, (2008). The maximum COD removal efficiency was observed at 85 %. Maximum nitrogen removal rate for the Anammox process was found to be 0,6 kg N/ $m^{3.}$ d The COD removal efficiency found in this study is comparable higher than that aforementioned study. The maximum COD removal efficiency was measured as % 90 in this study.

4.2.1.2 The Effect of Different Nitrite-Nitrogen (NO_2-N), Nitrate-Nitrogen (NO_3-N), Ammonium Nitrogen (NH_4-N) and COD Concentration on the NH_4-N Removal Efficiencies in UASB Reactor

The effect of different nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and ammonium nitrogen (NH_4-N) concentrations on the NH_4-N removal efficiencies in UASB reactor was shown in Figure 4.18 (NO_2-N, NO_3-N concentration was between 0-50 and 20-130 mg/L, approximately).

The maximum NH_4-N removal efficiencies were found as 51 % and 55 % in when the influent COD concentrations were 300 mg/L and 600mg/l at NO_3-N, NO_2-N concentrations of 22, 131 and 47 mg/L, 0,1 mg/L, respectively in run 3 and 1 at a HRT of 2, 4,4 and 0,73 days. This result showed that high ammonium concentrations (25 and 100 mg/L) and high COD concentrations (300 and 1000 mg/L) reduced the ammonium removal efficiencies. On the other hand, high COD concentrations could inhibit the NH_4^+-N removal. The reason might be that the heterotrophic bacteria in the anaerobic granular sludge compete for nutrients with the autotrophic bacteria, which control the proceeding of ANAMMOX (Jianlong & Jing, 2004). The long-term continuous operational results showed that the maximal removal efficiency of NH_4^+–N was 55 % in run 1 at a HRT of 4,4 days.

In a study performed by Huang, Gao, Guo and Zhang (2009) a combined system consisting of an up-flow anaerobic sludge blanket (UASB) and anoxic/aerobic

bioreactor system was used to treat a mature landfill leachate with a high ammonia concentration and low ratio of COD/TN. After 160 days of a stable operation, the results indicated that organic matters were mainly removed by the UASB, while the ammonia was removed by shortcut nitrification in the anoxic/aerobic tank. The COD removal efficiency reached 50%-60 % at an organic loading rate (OLR) of 3.0 kg COD/ (m^3d). The nitrite accumulation rate and the NH_4^+-N removal efficiency were above 90 % and 85 % respectively when the reactor maintained at a low dissolved oxygen (DO) concentration (1.0-1.2 mg /L) and pH value (7.8-8.3) and temperature (25-30 ^{0}C) in the aerobic reactor.

The maximal removal efficiency of NH_4^+–N was found as 40 % when the influent NH_4^+–N concentration was 70–250 mg/L when it was used an EGSB reactor as the ANAMMOX reactor (Jianlong & Jing, 2004). This reactor was operated under anoxic conditions the ammonium removal efficiency found in this study is comparable higher than that aforementioned study.

Chan, (2003) 60 % ammonium removal efficiency and 80 % nitrite removal efficiency were achieved in a batch sequencing reactor. The ammonium removal efficiency found in my study is comparable lower than that the aforementioned study.

The ANAMMOX process was performed in an UASB reactor (working volume 8.5 L) using a mix of SN (mixture) reactor effluent and diluted with raw wastewater at a ratio of 1:1. The ammonium and nitrite removal efficiencies reached over 93% and 95%, respectively, after 70-day of continuous operation. (Liu, et al., 2010). In this study the ammonium removal efficiency is lower than that the aforementioned study.

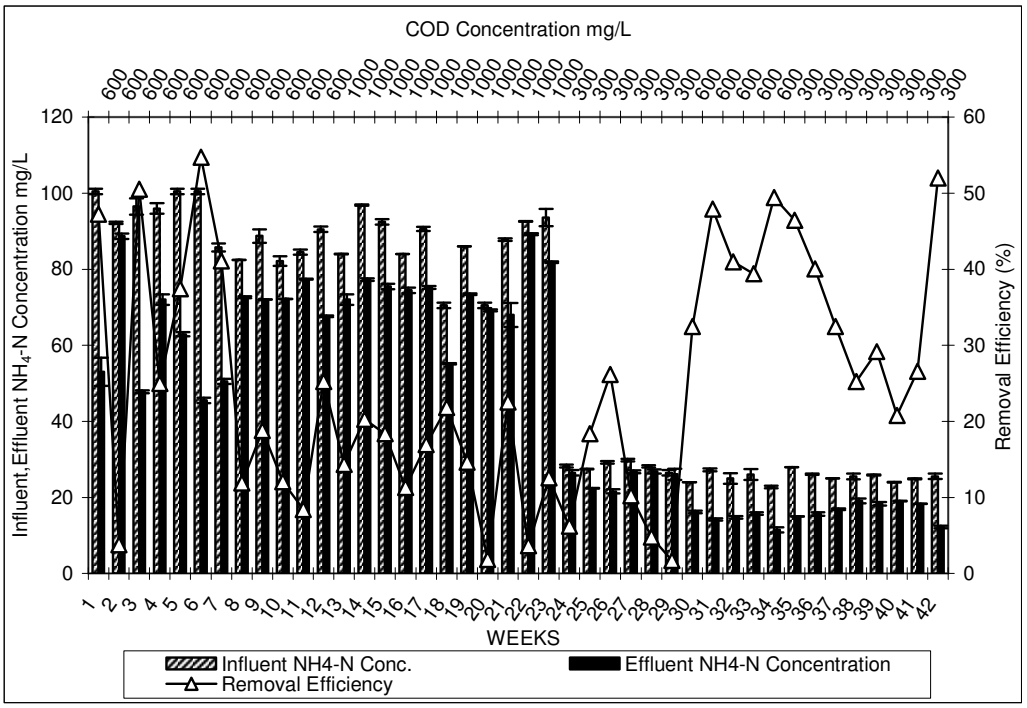

Figure 4.18 Ammonium Removal Efficiency in UASB Reactor throughout Continuous Operation of UASB Reactor (COD:300-1000mg/L, NH_4-N:25-100mg/L, NO_2-N:0-50mg/L, NO_3-N: 20-130mg/L)

4.2.1.3 The Effect of Different Nitrite-Nitrogen (NO_2-N), Nitrate-Nitrogen (NO_3-N), Ammonium Nitrogen (NH_4-N) and COD Concentrations on the NO_2-N Removal Efficiencies in UASB Reactor

Under anaerobic conditions, NO_2^-–N could be removed in UASB reactor. In the ANAMMOX reaction, the anaerobic granular sludge use NO_2^-–N as the electron acceptor and NH_4^+–N as the electron donor to yield the dinitrogen gas (Jianlong & Jing., 2004). The NO_2-N removal efficiency was 100 % when the the COD/NH_4-N/NO_3-N/NO_2-N ratios were between 20/2/2.6/1 and 15/1.25/1/2.5. (Figure 4.19, Table 3.6) in the influent at a HRT of 2.2 days in run 3. When the influent COD concentration was 1000 mg/L at a NO_3-N concentration of 130 mg/L, at a NH_4^+–N concentration of 100 mg/L, and at a NO_2-N concentration of 50 mg/L, the NO_2–N removal efficiency was about 86-98 % in the UASB reactor. When the influent COD concentration was 300 mg/L at a NO_3-N concentration of 20 mg/L, at a NO_2-N concentration of 20 mg/L and at a NH_4^+–N concentration of 25 mg/L, the NO_2^-–N removal efficiency was measured between 2 and 34 %. When the influent

COD concentration was 600 mg/L at a NO_3^--N concentration of 20 mg/L, at a NO_2-N concentration of 50 mg/L and at a NH_4^+–N concentration of 25 mg/L the NO_2^-–N removal efficiency was found as % 92-98. When the influent COD concentration was 300 mg/L at a NO_3-N concentration of 20 mg/L at a NO_2-N concentration of 50 mg/L and at a NH_4^+–N concentration of 25 mg/L, the NO_2^-–N removal efficiency was found as 96-100% in the UASB reactor.

The maximum NO_2-N removal efficiency was 100 % when the influent COD/NH_4-N/NO_3-N/NO_2-N ratios were between 20/2/2.6/1 and 15/1.25/1/2.5 in run 3,4 and 5 at a HRT of 2, 1,1 and 0,73 days.

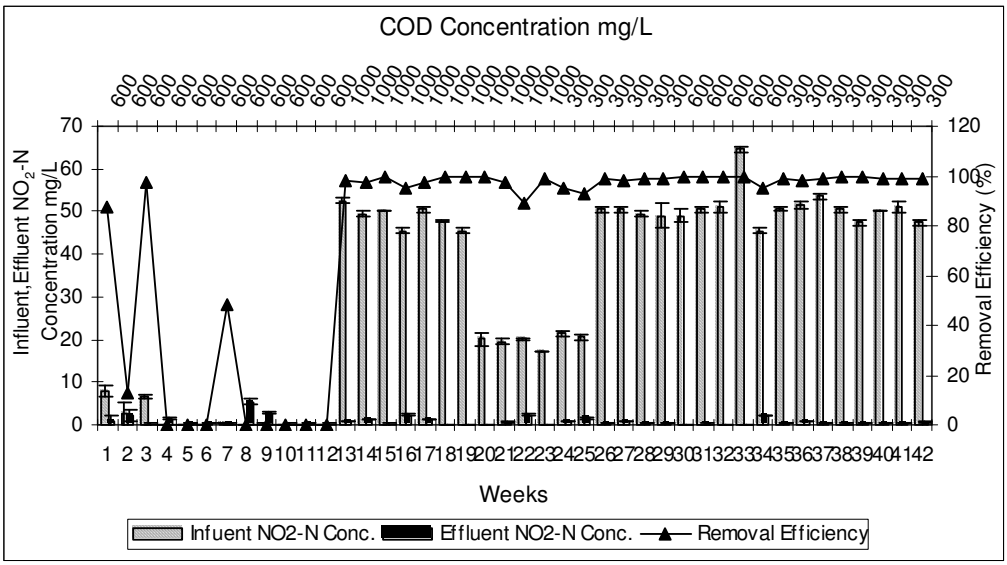

Figure 4.19 Nitrite Nitrogene Removal Efficiency in UASB Reactor throughout Continuous Operation (COD: 300-1000 mg/L NH_4-N: 25-100mg/L NO_2-N:0-50mg/LNO_3-N: 20-130mg/L)

The NO_2–N removal efficiency was more than 98% when the influent NO_2–N concentration was between 70 and 250 mg/L in an EGSB reactor in a study performed by Jianlong and Jing (2004).

The anammox process, under different organic loading rates (COD) was evaluated using a semi-continuous UASB reactor at 37 °C. Three different substrates were used: initially, synthetic wastewater, and later, two different pig manure effluents

(after UASB-post-digestion and after partial oxidation) and diluted with synthetic wastewater An EGSB reactor was operated under anoxic conditions and HRT was maintained as 1.20 days (B. Molinuevo et al., 2009). In this study 98.9% nitrite removal was achieved when the organic COD loads were 169 mg COD/L.d, 242 mg COD /L.d and 290 mg COD /L.d were applied, respectively. These ratios were found to be very close to the present study. In this study 100 % nitrite removal was achieved when the COD concentration were 454 mg COD/L.d 136 mg COD/L.d and 272 mg COD /L.d were applied, respectively .

4.2.1.4 The Effect of Different Nitrite-Nitrogen (NO_2-N), Nitrate-Nitrogen (NO_3N), Ammonium Nitrogen (NH_4-N) and COD Concentrations on the NO_3-N Removal Efficiencies in UASB Reactor

The effect of different nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonium nitrogen (NH_4-N) and COD concentrations on the NO_3-N removal efficiencies in UASB reactor was shown in Figure 4.20 (NH_4-N, NO_2-N, NO_3-N concentration was between 25-100, 0-50 and 20-130 mg/L, approximately)(Table 3.6). The NO_3-N removal yields were >80% for all NH_4-N/ NO_3-N/ NO_2-N ratios with different COD concentrations (300-1000 mg/L) during 42 weeks with the exceptions of first and eighth weeks in run 1 at a hrt of 4,4 days. In these weeks 40 % and 60 % NO_3-N removal efficiencies were found, respectively.In the first week, the optiumum conditions could not be satisfied, on the other hand, the pump failed in the eight weeks so that the system could not been fed. The maximum NO_3-N removal efficiencies were approximately 95 % in weeks 3, 4, 10, 12 and 13 in the UASB Reactor.

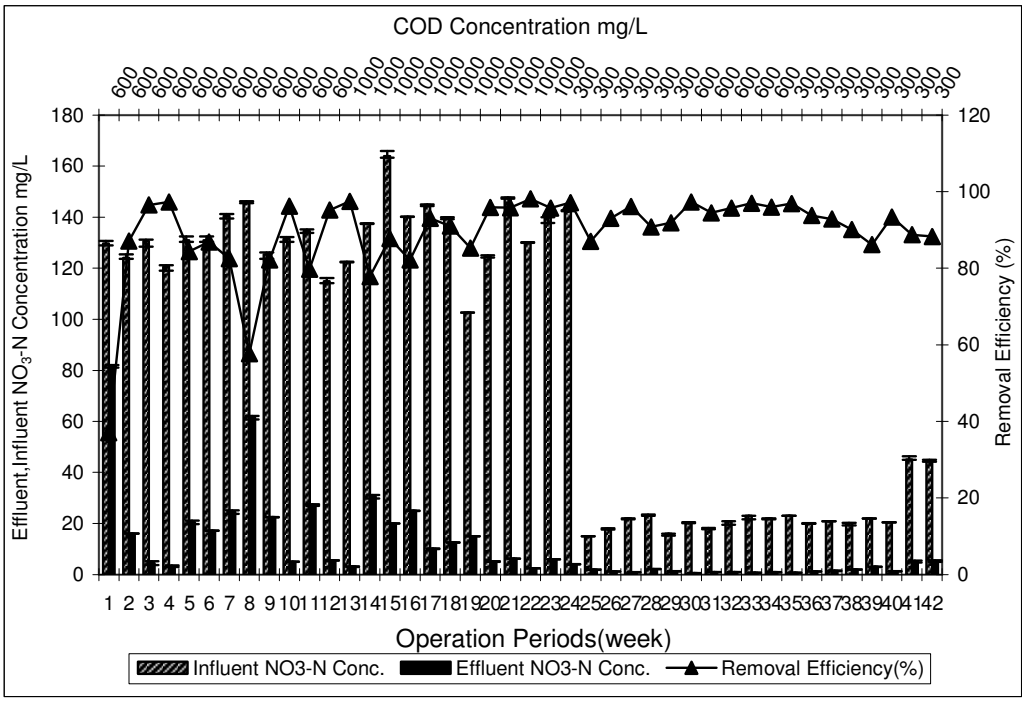

Figure 4.20 Nitrate Nitrogene Removal Efficiency in UASB Reactor throughout Continuous Operation (COD: 300-1000 mg/L NH_4-N: 25-100mg/L NO_2-N:0-50mg/LNO_3-N: 20-130mg/L)

The maximum total, nitrogen gas, hydrogen sulfide gas, methane gas and methane percentage were found as 154 L/day, 130,4 l/day, 1,96 L/day ,21,56 L/day , 14 %, respectively as illustrated in figure 4.21 for the continous studies.

According to the stochiometric equation 4.3, in this study, it was found that when the influent NH^+_4 and NO^-_2 concentrations were 25 mg/L and 50 mg/L, respectively, the maximum N_2 gas production was found to be 130 L/day in the effluent of the UASB.

$$NH_4 + NO_2 \rightarrow N_2 \qquad (4.3)$$

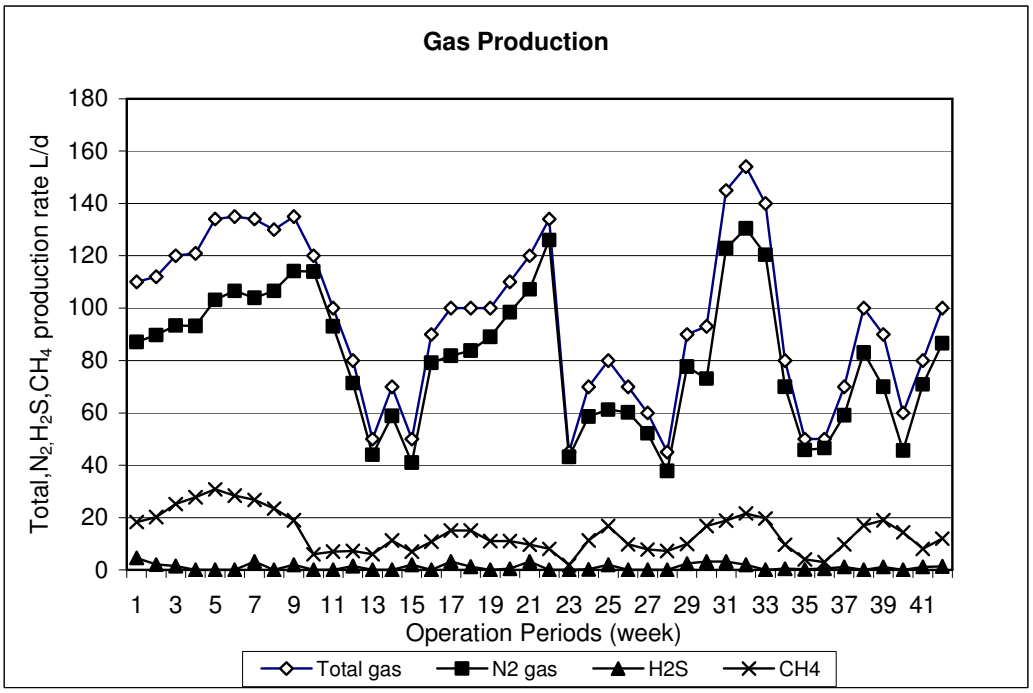

Figure 4.21 Total, Dinitrogen, Hydrogen sulfide, Methane Gas Production for Continous Studies

The NO_3-N was removed via anammox process under anaerobic conditions in UASB reactor which suggested that NO_3^-–N could be used as an electron acceptor in the anammox reaction as reported by Jianlong & Jing, (2004).

ANAMMOX microorganisms and integration of methanogenesis with simultaneous denitrification are researched and the disposal effect of high ammonia concentration in organic wastewater was reviewed by Wen-li, Yu-tao1, and Qi-xin (2009). After 106 days of continuous operation, the NO^-_3-N and the COD removal efficiencies were found to be 94% and 81%, respectively.

In this study, the results obtained showed that, denitrification and anammox process were occurring in the UASB reactor. NH_4-N was removed via anammox process under anaerobic conditions with nitrite was used as the electron acceptor. During anammox process a part of ammonium was converted to nitrite and then the remaining ammonium and the formed nitrite was converted to dinitrogen gas by Anammox bacteria.

Anammox and denitrification always occurred simultaneously showing that both processes could coexist in the same environment. So, environmental conditions (COD, nitrite, nitrate, ammonium, pH, and temperature) have to be controlled to get a good balance between anammox and denitrification communities (Molinuevo, García , Karakashev, and Angelidaki, 2008).

In the Anammox process, ammonium is converted with nitrite as electron acceptor in a ratio 1:1,32, respectively, to dinitrogen gas (Strous et al., 1998)

4.2.2 Effect of Hydraulic Retention Time (HRT) on The Performance of UASB Reactor

4.2.2.1 Effect of HRTs on the COD, NH_4-N, NO_2-N and NO_3-N Removal Efficiency in UASB Reactor

The effect of hydraulic retention times (HRTs) on the COD, NH_4-N ve NO_3-N removal efficiencies was shown in Figure 4.22. 90% COD removal efficiency was obtained at a HRT of 4,4 days in UASB reactor. When the HRT was decreased from 4,4 days to 2,2 days, the COD removal efficiency decreased from 90 % to 50 %, respectively. Lan, Kumar, Wang and Lin, (2010) investigated the simultaneous partial nitrification, anammox and denitrification (SNAD) process in a sequential batch reactor (SBR) and the influence of hydraulic retention time (HRT) on the SNAD process. Around 96 % NH_4-N removal and 87 % COD removal were observed at 9 d HRT. Kuscu & Sponza, (2009) found that as the HRT decreased from 10,38 days to 2,5 days the COD removal efficiencies in the anaerobic and anaerobic/aerobic reactor effluents decreased from 94 % to 92 % and from 98 % to 97%, respectively.

The maximum NH_4-N removal efficiency was obtained as 55 % at a HRT of 4,4 days in UASB reactor. When the HRT was decreased from 4,4 days to 2,2 days, the NH_4-N removal efficiency decreased from 55 % to 20 %, respectively. When the HRT was decreased from 2 days to 1,1 days, the NH_4-N removal efficiency increased from 20 % to 50 % respectively. The maximum NO_2-N,NO_3-N removal efficiencies was obtained as 100 % and 98 % at a HRT of 2,2 day and 4.4 day, respectively, in UASB reactor.

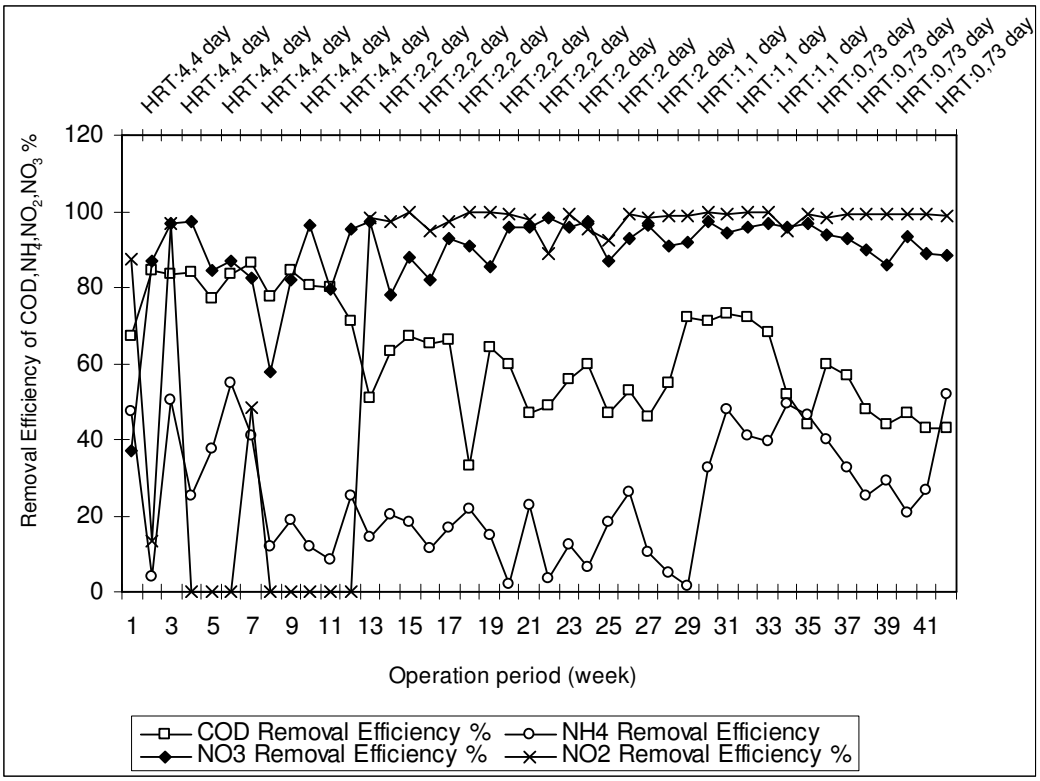

Figure 4.22 Effect of HRTs on the COD, NH_4-N ve NO_3-N Removal Efficiency in UASB Reactor

4.2.3 Determination of Kinetic Constants

The process kinetics for a laboratory-scale anammox-UASB reactor (anaerobic ammonium oxidation) was investigated using a synthetic wastewater as feed. The UASB reactor was tested for different influent substrate concentrations and hydraulic retention time (HRT). Determination of kinetic constant of an UASB reactor is a useful tool to be able to describe and predict the performance of the anaerobic system for anaerobic ammonia oxidation. Therefore in this study, the kinetic constant of the

UASB treating ammonium was evaluated according to the experimental data at five HRTs. In order to determine the most suitable biokinetic model in the UASB reactor treating ammonium and COD, some kinetic models such as Monod, Grau second-order, contois kinetic and Modified Stover-Kincannon models were applied to the experimental results obtained from the continuous operation. The interpretions of the models and the kinetic constants were performed in this step.

4.2.3.1 Determination of Kinetics Constant Through Anaerobic Degradation of COD in UASB Reactor at Decreasing HRTs

In order to obtain the kinetic coefficient for different kinetic models the UASB reactor was operated with synthetic wastewater containing at different COD concentration of 300-1000 mg/L at five different HRTs.

4.2.3.1.1 Monod Kinetic Model. Five steady state set datas were used to determine the kinetic constants for Monod Model. Figure 4.23 was plotted from the Eq 3.21 (See chapter 3.4.8.1) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (gVSS/gCOD) and endogenous decay coefficient (k_d) (day^{-1}) values calculated from the intercept and the slope of the straight line are illustrated in Figure 4.23 with regression coefficient of R^2=0.3853, (y=0,2811x-0,0002) for COD. Y and k_d values was calculated as 287,7 g VSS /g COD and 0,0007 day^{-1}, respectively. The values of maximum specific substrate utilization rate (μ_{max}) (mgCOD/mgVSS.day) and half saturation concentration (K_S) (mg/L) for COD was determined from Figure 4.24 using Eq (3.15). (μ_{max}) and (K_S) for COD were calculated as 1,3033 day^{-1} and 0.029 mg/L, respectively with regression coefficient of R^2=0.5345, (y=0,9893x+ 1,4127). The Y value found for COD removal showed that the this kinetic parameter was found to be not significant for the limit data suggested for COD removal by heterogens and methanogens in anaerobic reactors (Metcalff & Eddy, 2001). The low k_s value also indicated that there is no any organıc carboneous compound limitation based on COD in the UASB reactor while the low level of kd (0,0007 d^{-1}) mentioned that the death of the heterotropic bacteria are not significant in the UASB reactor.

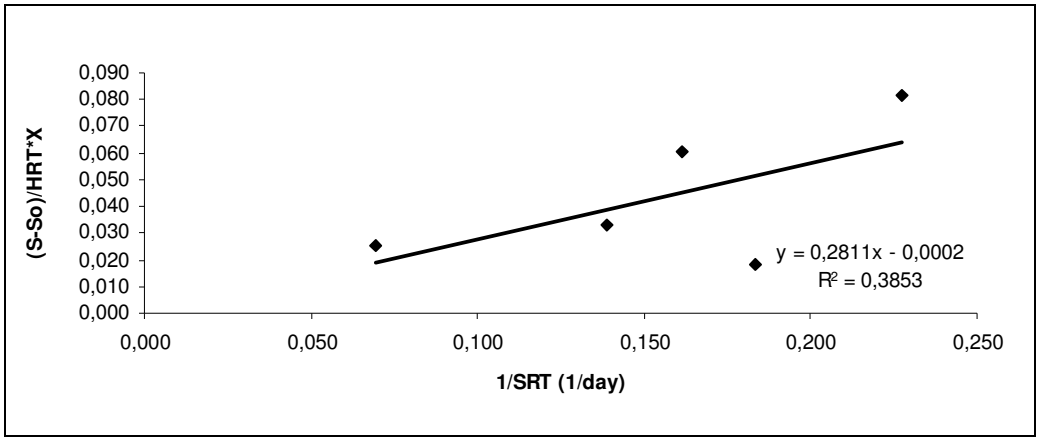

Figure 4.23 Determination of yield coefficient (Y) and death rate constant (k_d) values for COD.

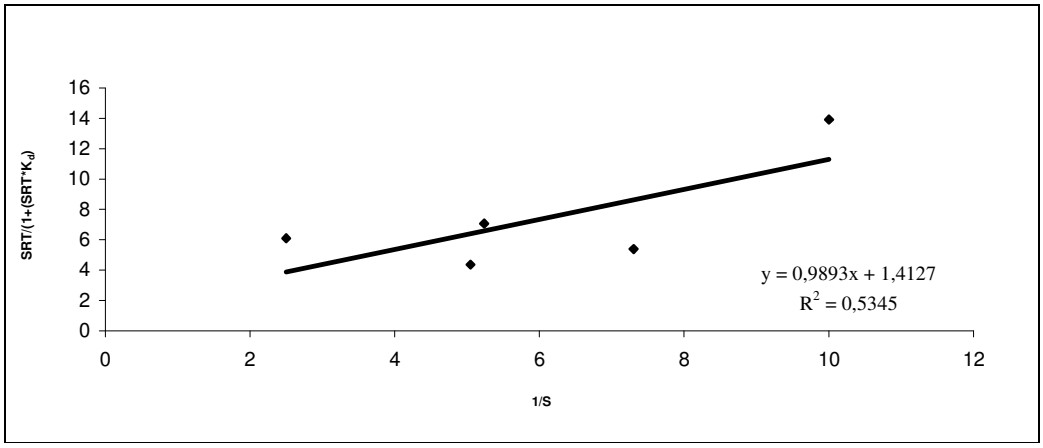

Figure 4.24 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_S) values for COD.

4.2.3.1.2 Grau Second-Order Multicomponent Substrate Removal Model. In order to determine a (S_i/k_s*X) (day), b (dimensionless) and second order substrate removal rate constant (k_s) (day-1), Equation 3.33 were plotted in Figure 4.25. The values of a and b were calculated from the intercept and slope of the straight line on graph. The values of a, and b were found to be 1,0282 day and 1,005 (dimensionless) with a regression coefficient of R^2=0.93, (y=1,0282x+ 1,006) for COD. Second order multicomponent substrate removal rate constant (k_s) was calculated as 0.0089 L/day from the equation $a=S_i/(k_s.X)$, indicating the substrate removal for each unit of microorganism depends on second order substrate removal rate constant (k_s).

If a and b kinetic constants were substituted in 3.33, the effluent COD concentrations could be predicted using this equations, for Grau Second Order model. The Effluent substrate concentration or substrate removal efficiency is related to influent substrate concentration and Grau Second-order kinetic constant.

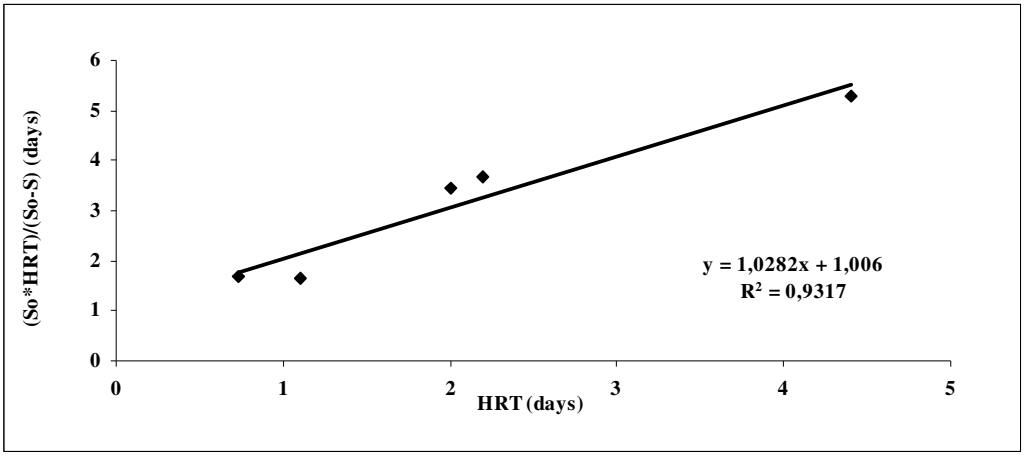

Figure 4.25 Determination of kinetic constants (a,b and ks) for COD for Grau second order multicomponent substrate removal model.

4.2.3.1.3 Modified Stover-Kincannon Model. Figure 4.26 shows the graph plotted between reciprocal of total removed organic loading removal rate, $[V/(Q*(S_i-S_e))]$, against to the reciprocal of total organic loading rate, $V/(Q*S_i)$ using Eq (3.36). Since the pilot of $[V/(Q*(S_i-S_e))]$ versus $V/(Q*S_i)$ was found to be linear, linear regressions were used to determine the intercept ($1/R_{max}$) and the slope (K_B/R_{max}). Saturation value constant (K_B) (g/L*day) and maximum utilization rate (R_{max}) (g/L*day) for COD was calculated from the line plotted on graph given in Figure 4.26. K_B and R_{max} was found as 0,804 g COD/L*day and 1,006 g COD/L*day, respectively with high regression coefficient (R^2=0,83; y=1.2509x+1,2423) for COD. Indicating the substrate removed by microorganisms during time and the maximum substrate removed by the anaerobic organisms versus time, respectively. The kinetic coefficients calculated from the Stover-Kincannon kinetics are more significant.

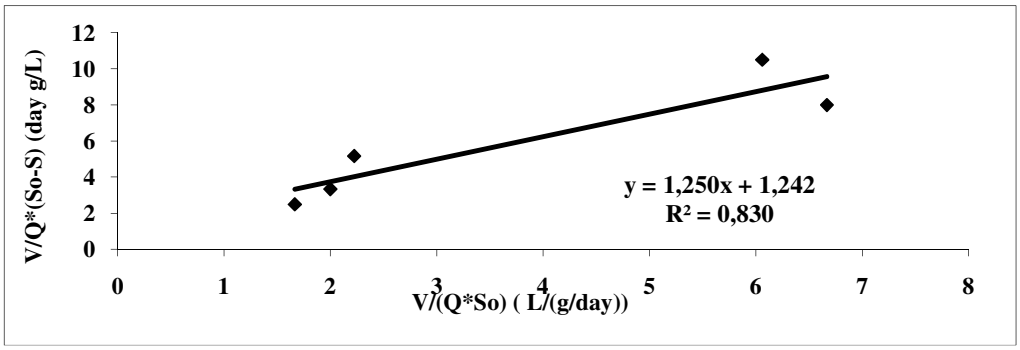

Figure 4.26 Determination of kinetic constants (K_B and R_{max}) for COD Modified Stever Kincanon substrate removal model.

4.2.3.1.4 Contois Kinetic Model. Figure 4.27 was plotted from the Eq 3.29 for determining the values of μ_{max} and β in this model. Maximum specific grow rate (μ_{max})(day^{-1}) and kinetic constant (β) (g COD/g biomass) values calculated from the intercept and the slope of the straight line illustrated in Figure 4.27 with regression coefficient of R^2=0.476, (y= 0,04887x+44,644) for COD. μ_{max} and β values was calculated as 0,62 day^{-1} and 0,126 (dimensionless), respectively. Maximum specific grow rate μ_{max} is low so this model is not suitable.

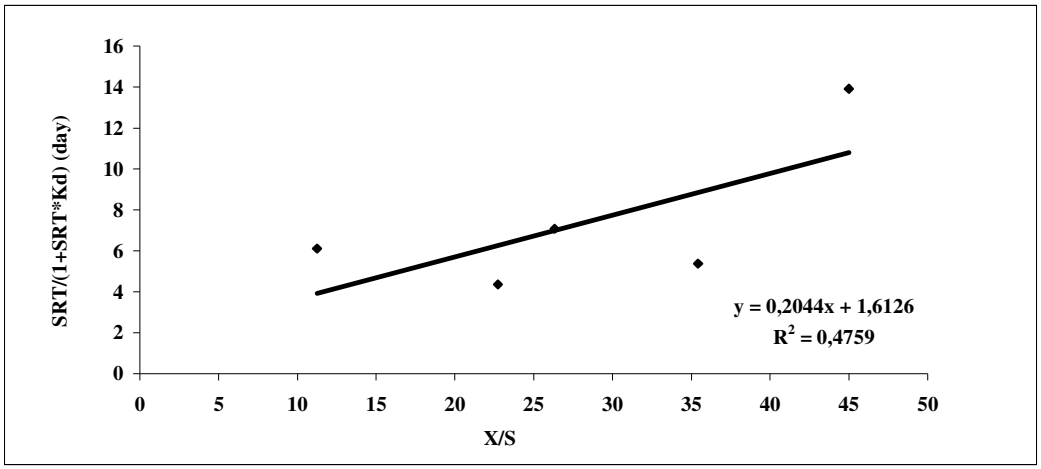

Figure 4.27 Determination of kinetic constants (μ_{max} and β) in Contois Kinetic Model for COD

4.2.3.2 Determination of Kinetics Constant through anaerobic degradation of Ammonium in UASB reaktor at decreasing HRTs

In order to obtain the kinetic coefficient for different kinetic models the UASB reactor was operated with synthetic wastewater containing at different ammonium concentration of 25-100 mg/L at five different HRTs**.** The process kinetics was investigated for laboratory-scale anammox (anaerobic ammonium oxidation) UASB reactor using synthetic wastewater as feed. The experimental unit consisted of a 2.0 L reactor filled. The UASB reactor was tested for different influent substrate concentrations and hydraulic retention time (HRT). Upon approaching pseudo-steady-state condition, substrate ammonium concentrations were decreased from 100 to 25 mg N/L, while the HRT was stepwise decreased from 4.4 to 0.73 d. Based on calculations, Stover–Kincannon model and second-order "Grau" model were found to be the appropriate models to describe the anaerobic ammonia oxıdation via anammox process in UASB reactor. According to Stover–Kincannon model, the maximum total substrate removal rate constant (R_{max}) and saturation value constant (K_B) were suggested as 0.09 g N/L day and 0.025 g N/L day, respectively. Since the Stover–Kincannon model gave high correlation coefficient (95.61%), this model may be used in predicting the behavior or design the anammox UASB reactor.

4.2.3.2.1 Monod Kinetic Model. Five steady state set datas were used to determine the kinetic constants for Monod Model. Figure 4.28 was plotted from the Eq 3.21 (See chapter 3.4.8.1) for determining the values of Y and k_d in this model. Growth yield coefficient (Y) (gVSS/g NH_4-N) and endogenous decay coefficient (k_d) (day^{-1}) values calculated from the intercept and the slope of the straight line are illustrated in Figure 4.28 with regression coefficient of R^2=0.0269, (y=-0,0035x+ 0,0031) for NH_4-N. Y and k_d values was calculated as 287,7 g VSS /g NH_4-N and 0,88 day^{-1}, respectively. The values of maximum specific substrate utilization rate (μ_{max}) (mg NH_4-N /mg VSS.day) and half saturation concentration (K_S) (mg/L) for NH_4-N was determined from Figure 4.27 using Eq (3.15). (μ_{max}) and (K_S) for NH_4-N were calculated as 0.008 day^{-1} and 0.079 mg/L, respectively with regression coefficient of R^2= 0.4961, (y=-0,1113x+12,604). The Y value found for NH4-N removal showed

that the this kinetic parameter was found to be not significant for the limit data suggested for NH_4-N removal by heterogens and methanogens in anaerobic reactors (Metcalff & Eddy pg.584,2001). The low k_s value also indicated that there is no any organıc carboneous compound limitation based on NH_4-N in the UASB reactor while the low level of kd (0,88 day^{-1}) mentioned that the death of the heterotropic bacteria are not significant in the UASB reactor.

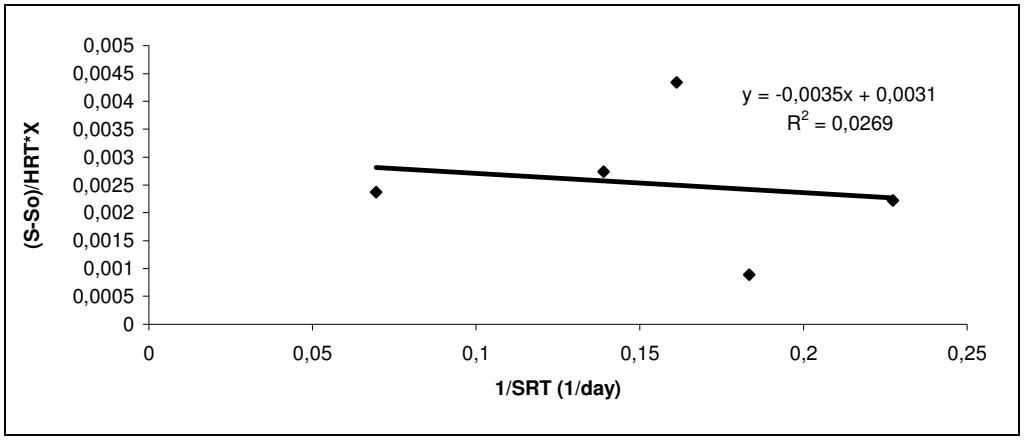

Figure 4.28 Determination of yield coefficient (Y) and death rate constant (k_d) values for NH_4-N

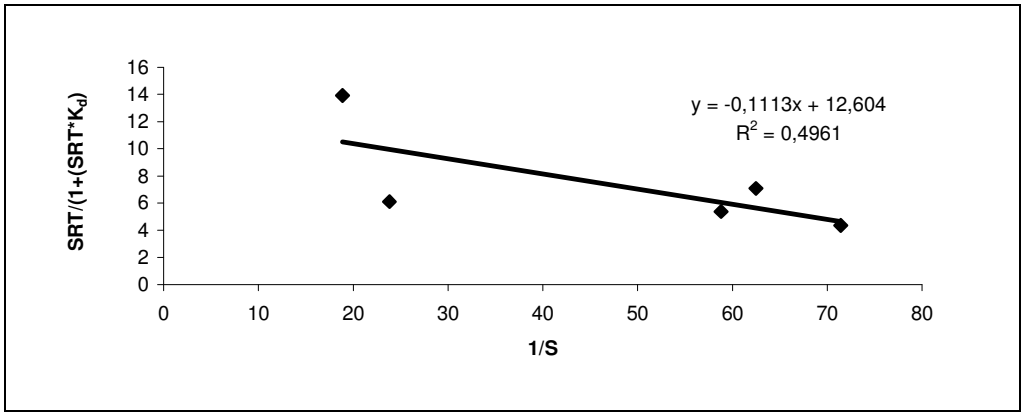

Figure 4.29 Determination of maximum specific substrate utilization rate (μ_{max}) and half saturation concentration (K_S) values for NH_4-N

4.2.3.1.2 Grau Second-Order Multicomponent Substrate Removal Model. In order to determine a (S_i/k_s*X) (day), b (dimensionless) and second order substrate removal rate constant (k_s) (l/day), Equation 3.33 were plotted in Figure 4.30. The values of a and b were calculated from the intercept and slope of the straight line on graph. The

values of a and b were found to be 2,004 day and 0,7168 (dimensionless) with a regression coefficient of R^2=0.91, (y= 2,0042x+ 0,7168) for NH_4-N. Second order multicomponent substrate removal rate constant (k_s) was calculated as 0.095 L/day from the equation $a=S_i/(k_s.X)$, indicating the substrate removal for each unit of microorganism depends on second order substrate removal rate constant (k_s). The maximum substrat removal rate konstant k_s will be increased as the NH_4-N removal efficiency increase. The regression coefficient is higher in Stover-Kincannon model compared to Grau second order model. Furthermore, the kinetic konstants determined in Grau model are more meaningful than those observed in Stover Kincannon model.

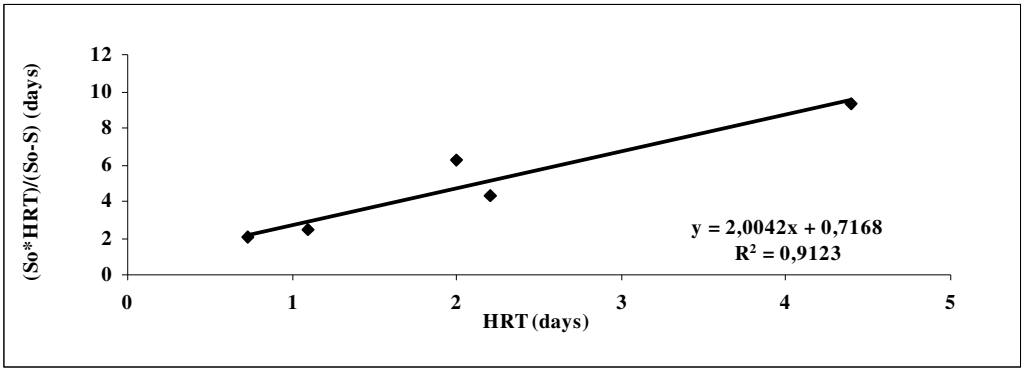

Figure 4.30 Determination of kinetic constants (a, b and ks) for NH_4-N for Grau second order multicomponent substrate removal model.

4.2.3.2.3 Modified Stover-Kincannon Model. Figure 4.31 shows the graph plotted between reciprocal of total removed organic loading removal rate, $[V/(Q*(S_i-S_e))]$, against to the reciprocal of total organic loading rate, $V/(Q*S_i)$ using equation (3.36). Since the pilot of $[V/(Q*(S_i-S_e))]$ versus $V/(Q*S_i)$ was found to be linear, linear regressions were used to determine the intercept ($1/R_{max}$) and the slope (K_B/R_{max}). Saturation value constant (K_B) (g/L*day) and maximum utilization rate (R_{max}) (g/L*day) for NH_4-N was calculated from the line plotted on graph given in figure 4.29. K_B and R_{max} was found as 0,09 g NH_4-N /L*day and 0,025 g NH_4-N /L*day, respectively with high regression coefficient (R^2=0,9561; y=3,5418x-38,969) for NH_4-N.

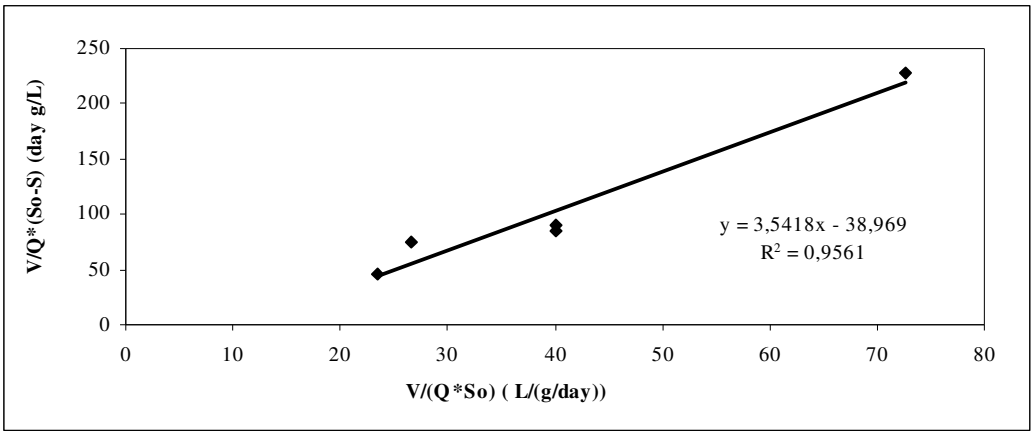

Figure 4.31 Determination of kinetic constants (R_{max} and K_B) in Stover Kincannon model for NH_4-N

4.2.3.2.4 Contois Kinetic Model. Figure 4.32 was plotted from the equation 3.29 for determining the values of μ_{max} and β in this model. Maximum specific grow rate (μ_{max})(day^{-1}) and kinetic constant (β) (g NH_4-N /g biomass) values calculated from the intercept and the slope of the straight line illustrated in figure 4.30 with regression coefficient of R^2=0.4961, (y= -0,0247x+12,604) for NH_4-N. μ_{max} and β values was calculated as 0,079 day^{-1} and 0,001 (dimensionless), respectively.

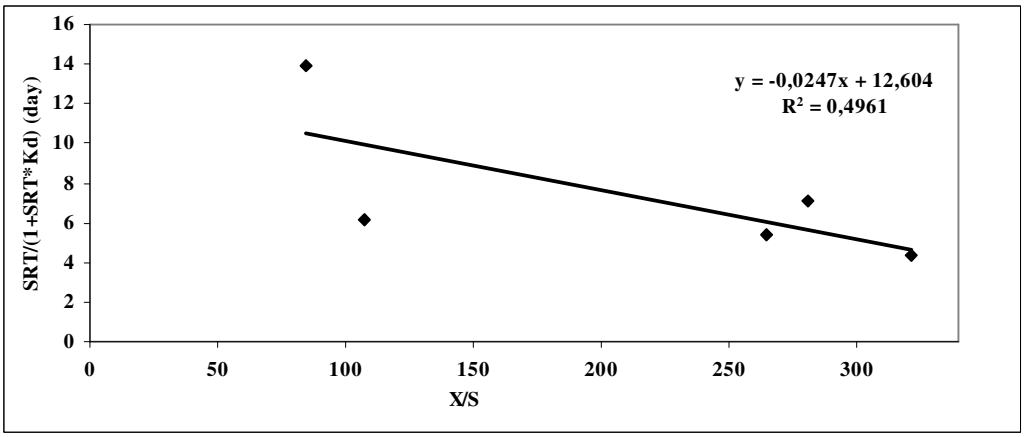

Figure 4.32 Determination of kinetic constants (μ_{max} and β) in Contois Kinetic Model for NH_4-N

4.2.3.3.5 Zero-order Reaction Kinetic. The value of k_o was obtained as 10,6 (mg/L.day) from the slope of the line by plotting (S_0−S)/HRT versus S in equation. (3.2). Figure 4.33 shows that the correlation coefficient of the plot was 0.7804. The high value of the coefficient (R^2) clearly indicates that zero-order kinetics can be applied with fair degree of precision.

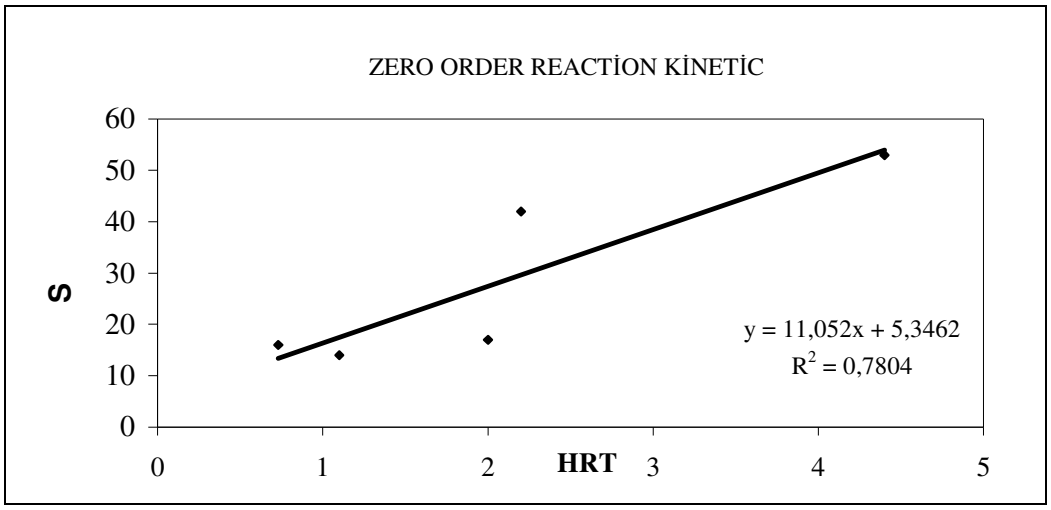

Figure 4.33 Zero-order kinetics model plot. S is the substrate (ammonium) concentrations.

4.2.3.3.6 First-order Kinetic Models. The value of k_1 was obtained 0,87 (L/day) from the slope of the line by plotting (S_0-S)/HRT versus S in Eq. (3.5). Fig.4.35 shows that the correlation coefficient of the plot was 0.7301. The low value of the coefficient (R^2) clearly indicates that first-order kinetics cannot be applied with fair degree of precision.

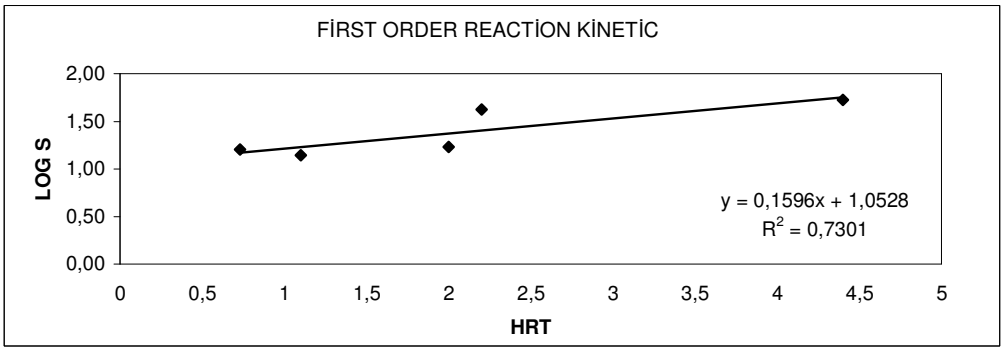

Figure 4.34 First-order kinetics model plot. S is the substrate (ammonium) concentrations.

4.2.3.3.7 Second-order Kinetic Models. Data used for second-order kinetic model are given in Table 4.5. Using figure 4.33 k_2 was obtained 0,002 (L/mg.day) with correlation coefficient of 0.68.

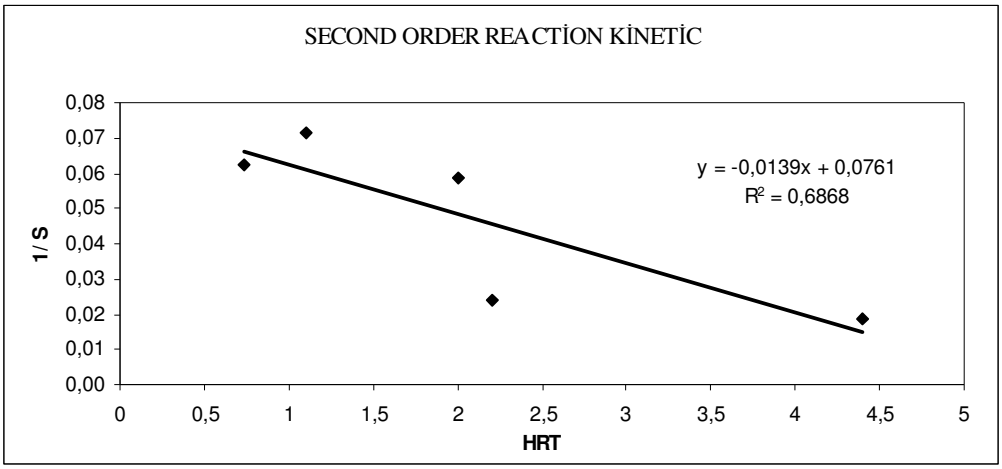

Figure 4.35 Second-order kinetics model plot. S is the substrate (ammonium) concentrations.

4.2.3.2.5 Evaluation of the Kinetic Models Thought Anammox Process of Ammonium in UASB Reactor.

All kinetic coefficients calculated from the models are summarized in Table 4.6 with regration coefficients. The kinetic data showed that Grau second order model was more appropriate model than other models for predicting the performance of the lab scale UASB reactor when the regression coefficients and kinetic coefficients were compared with each other.

The regression coefficients for ammonium under five different HRTs were higher in Grau second order model (R^2=0.95), Stover Kincannon kinetic model (R^2=0.91), Monod kinetic model (R^2=0.026) and compared to Contois kinetic model (R^2=0.4961). As second-order "Grau" model and Stevor Kincannon model, gave high correlation coefficients (95.61% and 91.65 %, respectively). Based on calculations, Stover–Kincannon model and second-order "Grau" model were found to be the appropriate models to describe the UASB reactor. According to second-order "Grau" model k_s was 0,0089 l/day, this value was low, on the other hand, according to Stover Kincannon model, the maximum total substrate removal rate constant (R_{max}) were suggested as 0.025 g.N/L.day. The kinetic coefficients calculated from the Stover-Kincannon kinetics are more significant.

Stevor Kincannon model may be used in predicting the behavior or design of the anammox UASB reactor.

The process kinetics for laboratory-scale anammox (anaerobic ammonium oxidation) upflow filter using synthetic wastewater as feed were investigated by Cun and Ping (2009). Based on calculations, Stover-Kincannon model and second-order "Grau" model were found to be the appropriate models to describe the upflow filter. As Stover-Kincannon model and second-order model gave high correlation coefficients (97.9% and 98.6%, respectively), according to Stover–Kincannon model, the maximum total substrate removal rate constant (U_{max}) and saturation value constant (K_B) were suggested as 12.4 and 12.0 g N/L day, respectively. These coefficients and kinetic coefficients higher than in this study.

Table 4.6 Kinetic parameters of UASB reactor treating ammonium

Kinetic models	Kinetic paramaters	Values	(R^2)
Monod	$Y(mg_{VSS}/mg_{NH4-N})$	287,7	0,0269
	k_d (day^{-1})	0,88	
	μ_{max} (day^{-1})	0,008	0,4961
	k_s (mg/L)	0,079	
Grau Second Order	k_s (l/day)	0,0089	0,9561
	a (day)	2,004	
	b (dimensionless)	0,7168	
Modified Stover-Kincannon	K_B (g NH_4-N /L.day)	0,09	0,9123
	R_{max} (g NH_4-N /L.day)	0,025	
Contois	μ_{max} (day^{-1})	0,079	0,4961
	β(g COD/g biomass)	0,001	
Zero Order Rection Kinetic Model	k_o (mg/L.day)	10,6	0,78
First Order Rection Kinetic Model	k_1 (L/day)	0,87	0,73
Second Order Reaction Kinetic Model	k_2 (L/mg.day)	0,02	0,68

Although the regression coefficient obtained in the Stover- Kincannon model (R^2=98) is high the saturation value constant (K_B) found according this kinetic is high. The kinetic constants (k_s 0,0089 l/day) found in the Grau kinetic models were found to be meaningfull. Therefore, it can be concluded that the ammonium is removed according to the Grau kinetic model with NH_4-N as substrate under anaerobic conditions in UASB reactor.

CHAPTER FIVE
CONCLUSIONS

5.1 Conclusions

In this study, nitrogen removal from synthetic wastewater with anammox process in batch rectors and in an UASB reactor was examined. The Experimental results showed that high ammonia concentration could be removed by anammox process. The concluding remarks from this study can be given as follows:

In the present study during the batch experiments, the optimum removal ratio of NH_4^+–N to NO_2–N and NO_3–N was found to be 1:1,48:0,5 while the optimum ratio of NH_4^+–N to NO_2^-–N was measured as 1:1,48 for maximum ammonia removal (98 %).

The effect of COD on the ammonia removal was found to be not significant in batch reactors when the optimum removal ratio aforementioned was found for 95 % removal. This result shows that the presence of an organic compound (COD) was not necessary for ammonia removal with anammox process.

In batch reactors containing COD, the COD removal efficiency was >70 %. Based on calculation, it can be seen that COD could be utilized by denitrifying microorganisms to reduce nitrite and nitrate to form dinitrogen gas throughout denitrification process. During the batch experiments, the maximum total, nitrogen gas, hydrogen sulfide gas, methane gas productions were measured as 220 L/day, 172 L/day, 44 L/day ,3,2 L/day, respectively, while the methane percentage of the total gas was 20 % in Run-1.

In the present study during the continuous studies, the maximum NH_4-N removal efficiencies were found as 51 % , 55 % in when the COD concentrations were 300 mg/L , 600 mg/l at NO_3-N, NO_2-N concentrations of 22 ,131, and 47 mg/L, 0,1 mg/L, respectively in the UASB reactor. The maximum COD removal efficiency was between 85-90 % when the COD/NH_4-N/NO_3-N ratios were 6:1:1,3 in the influent of

UASB reactor the HRT of 2 and 4,4 days. The maximum NO_3-N removal efficiencies were approximately 95 % in weeks 3, 4, 10, 12 and 13 in UASB Reactor. The maximum NO_2-N removal efficiency was 100 % when the influent COD/NH_4-N/NO_3-N/NO_2-N ratios were between 20/2/2.6/1 and 15/1.25/1/2.5 during continuous studies in the UASB reactor.

The maximum total, nitrogen gas, hydrogen sulfide gas, methane gas, methane percentage were found as 154 L/day, 130,4 l/day, 1,96 L/day 21,56 L/day, 14%, respectively in figure 4.21 for the continuous studies

Results obtained showed that, denitrification and anammox process occurrs in the UASB reactor. NH_4-N was removed via anammox process under anaerobic conditions with nitrite was used as the electron acceptor. During anammox process a part of ammonium was converted to nitrite and then the remaining ammonium and the formed nitrite was converted to dinitrogen gas by Anammox bacteria.

Based on calculations, Stover–Kincannon and second-order "Grau" models were found to be the appropriate models to describe the ammonia removal from the UASB reactor. According to Stover–Kincannon model, the maximum total substrate (COD) removal rate constant (R_{max}) and saturation value constant (K_B) were suggested as 0.09 g N/Land 0.025 g N/L day, respectively. As Stover–Kincannon model and high correlation coefficients (95.61% and 78.04 %, respectively), this models may be used in predicting the behavior or design the anammox UASB.

Anammox and denitrification always occurred simultaneously showing that the processes could coexist in the same environment. So, environmental conditions (COD, nitrite, nitrate, ammonium, pH, and temperature) have to be controlled to get a good balance between anammox and denitrification communities.

5.2 Recommendations

In the future, the anammox process in an UASB reactor could be used to remove the ammonia and the other inorganic inorganic compounds from different industrial wastewaters.

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